Metabolism lecture slides

These lecture slides have been developed for undergraduate university courses. They are free for everyone to use.

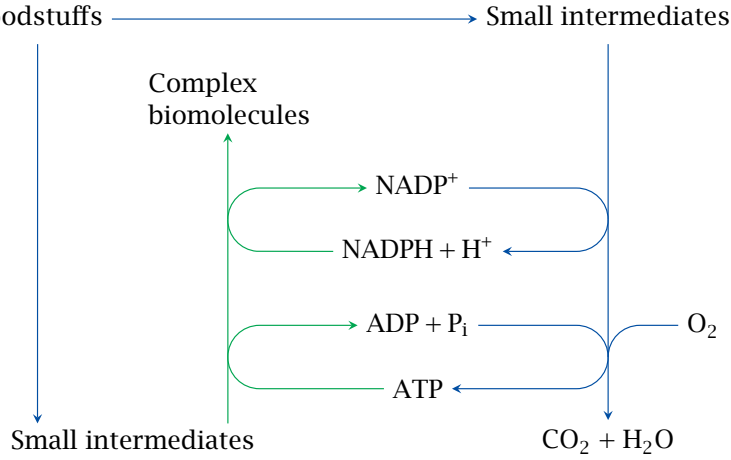
Please see `mpalmer.heresy.is/webnotes/Metabolism` for updates, PowerPoint versions of these slides, and lecture notes.

Introduction

Significance of metabolism in medicine

- hereditary enzyme defects
- diabetes, atherosclerosis, gout
- antimetabolites in the chemotherapy of cancers and infections
- inactivation and elimination of xenobiotics and drugs

Catabolic and anabolic reactions



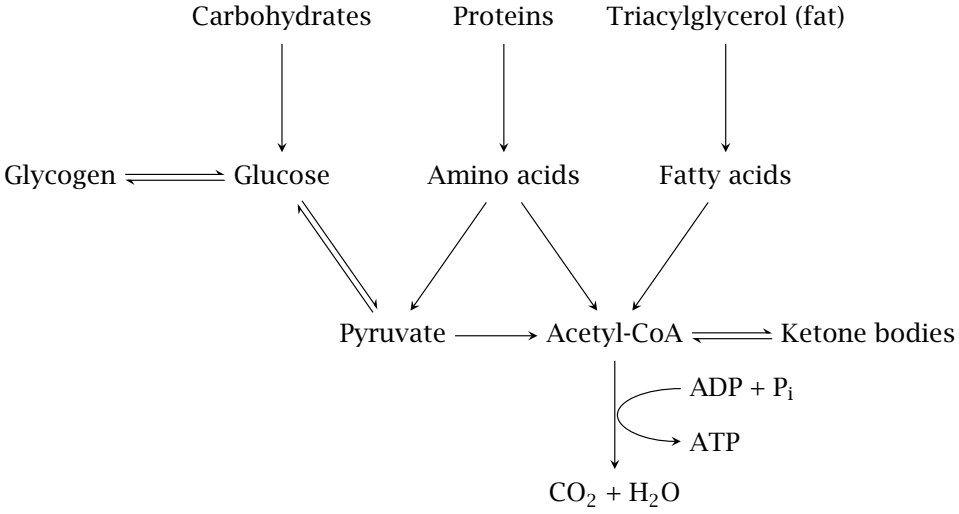
Diversity of metabolism: pathways in plants and bacteria

Pathway	Organisms
photosynthesis	plants and cyanobacteria
nitrogen fixation	specialized soil bacteria
oxidation or reduction of inorganic minerals	archaebacteria
acid- and gas-producing fermentations	anaerobic bacteria

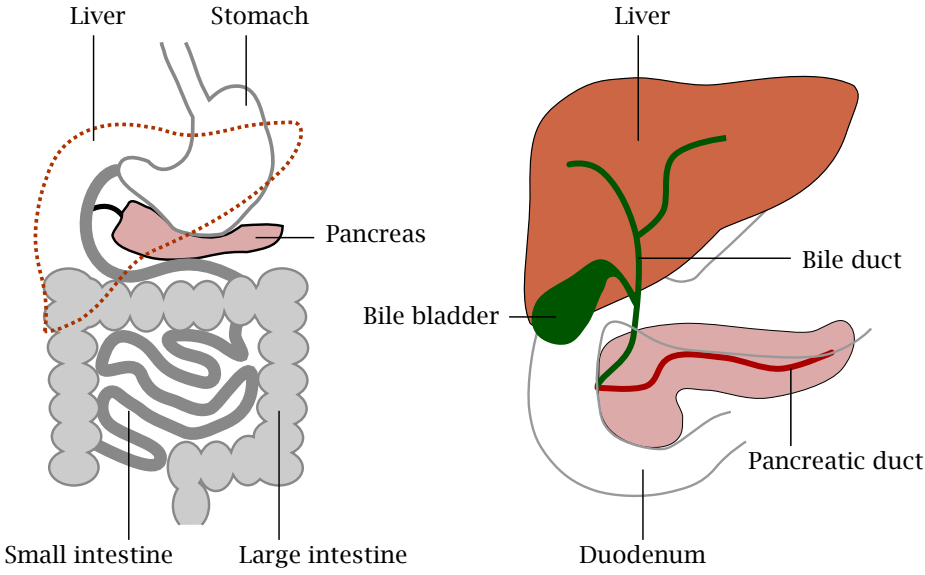
Types of foodstuffs

- carbohydrates
- protein
- fat
- nucleic acids

Breakdown of foodstuffs: Overview



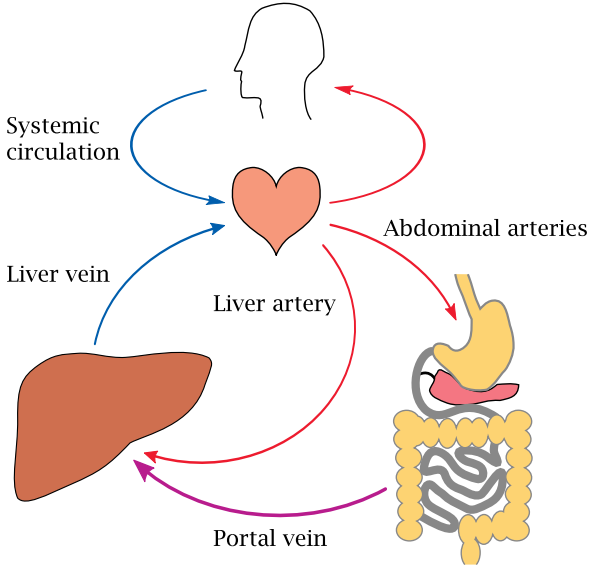
Functional anatomy of the digestive system



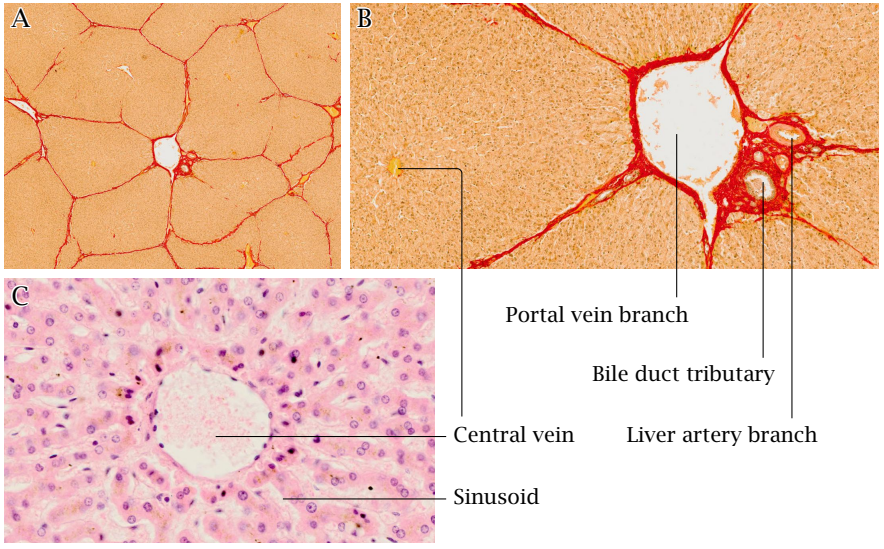
Intestinal organs: functional overview

Organ	Function
stomach	killing of microbes contained in the food; protein denaturation
small intestine	breakdown of macromolecules to small molecules, uptake of the latter
large intestine	fluid and ion reuptake
pancreas	production of digestive enzymes and of hormones
liver	production of bile; metabolic homeostasis

The portal circulation

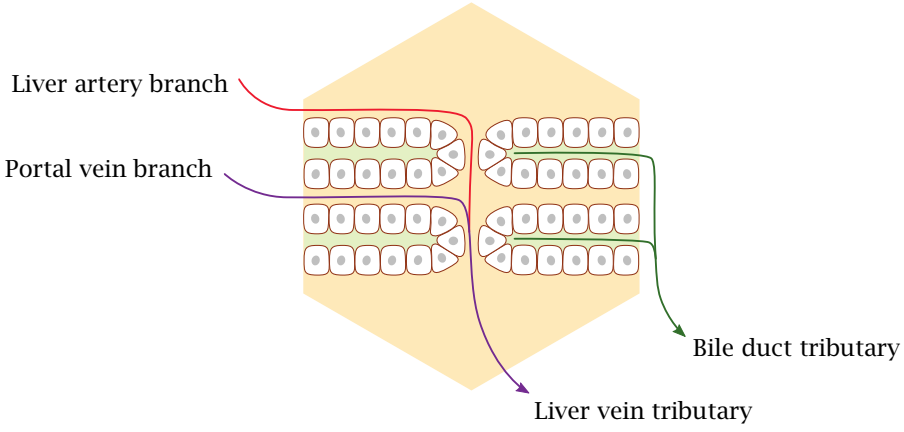


Liver tissue structure



A–C reproduced with permission from `pathorama.ch`.

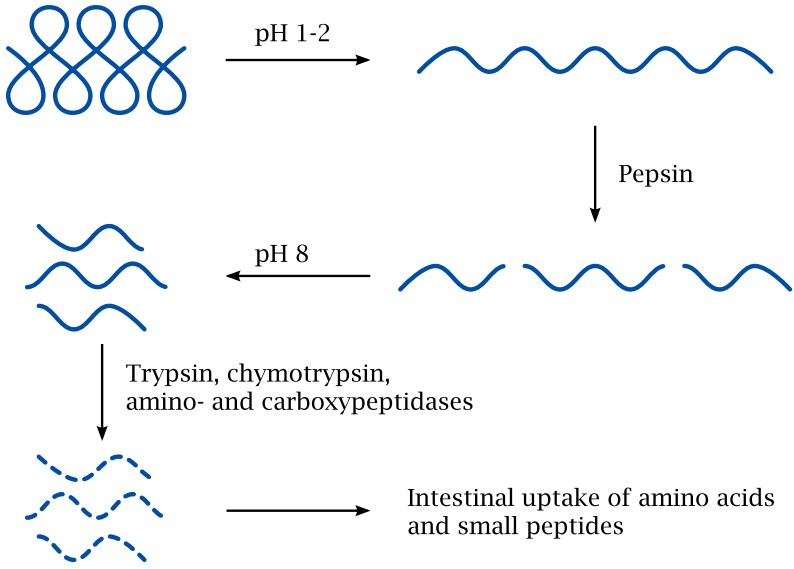
Blood flow and bile flow within the liver lobule



The stomach: functions of gastric acid

- HCl, pH 1–2
- secreted by specialized cells in the mucous membrane (parietal cells)
- kills germs contained in food; patients with lack of gastric acid are at increased risk of intestinal infection
- denatures food proteins and makes them accessible to cleavage by proteases

Gastric acid and pepsin in protein digestion



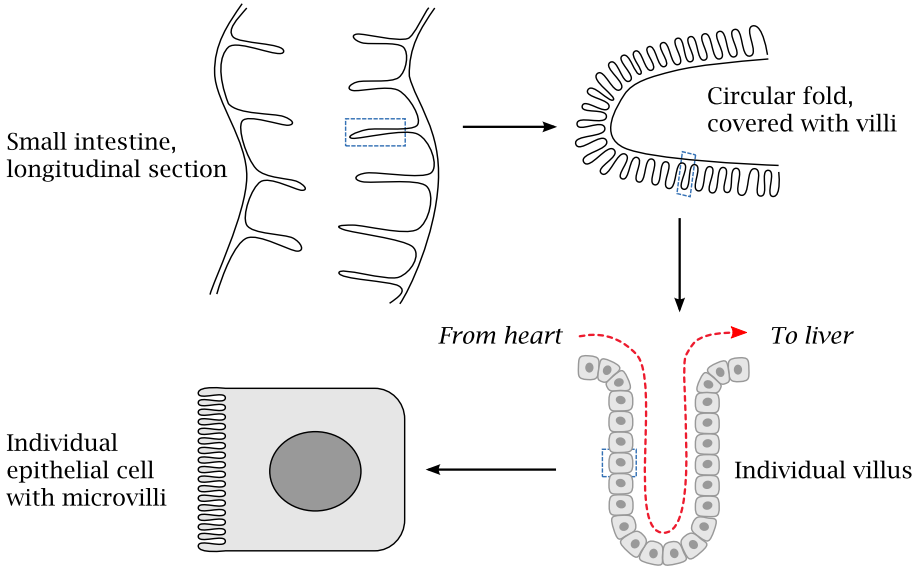
Function of the exocrine pancreas

- secretion of digestive enzymes
  - amylase
  - proteases, peptidases
  - lipases
  - DNAse, RNAse
- secretion of sodium bicarbonate to neutralize gastric acid

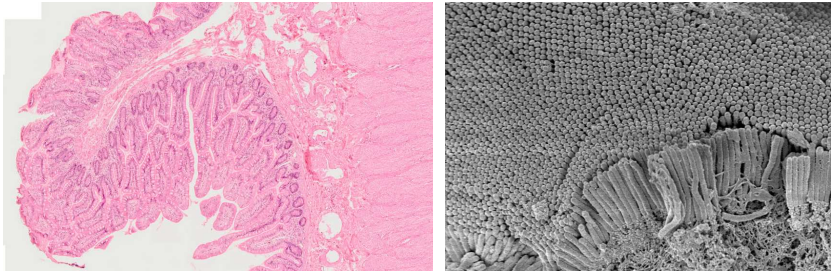
Roles of bile in digestion

- Bile acids solubilize triacylglycerol and make it accessible to pancreatic lipase
- Bicarbonate contributes to the neutralization of gastric acid

The small intestine



Microscopic structure of the small intestine



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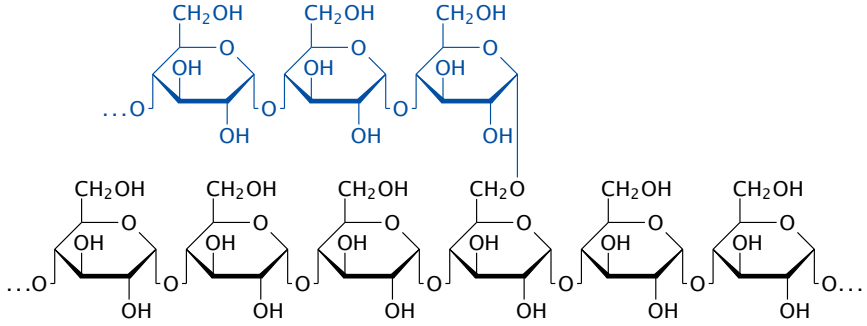
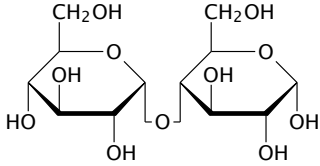
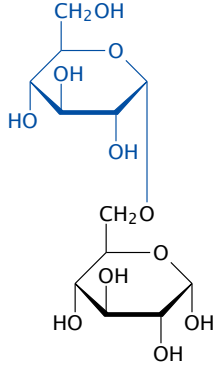
Amylose and amylopectin are polymers of α-D-glucose



Amylase breaks down starch to maltose and isomaltose

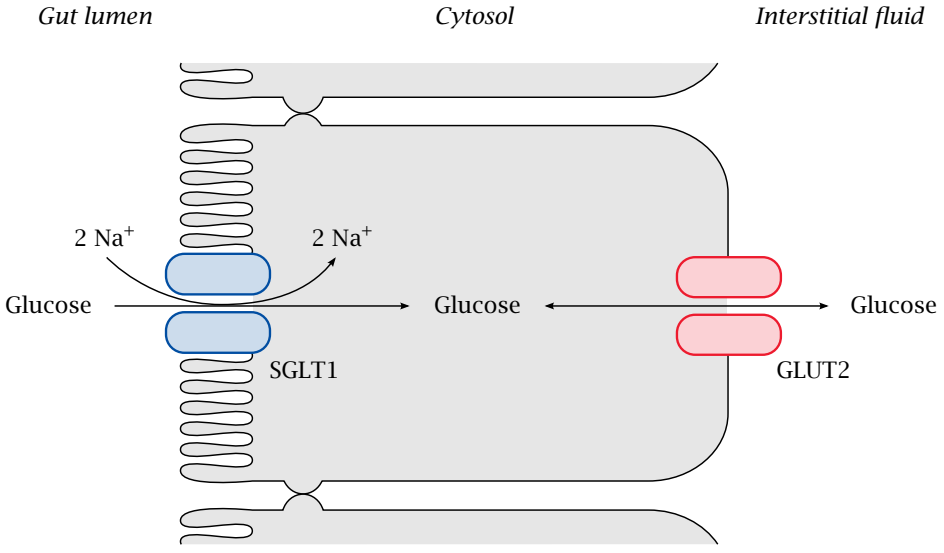


Maltose



Isomaltose

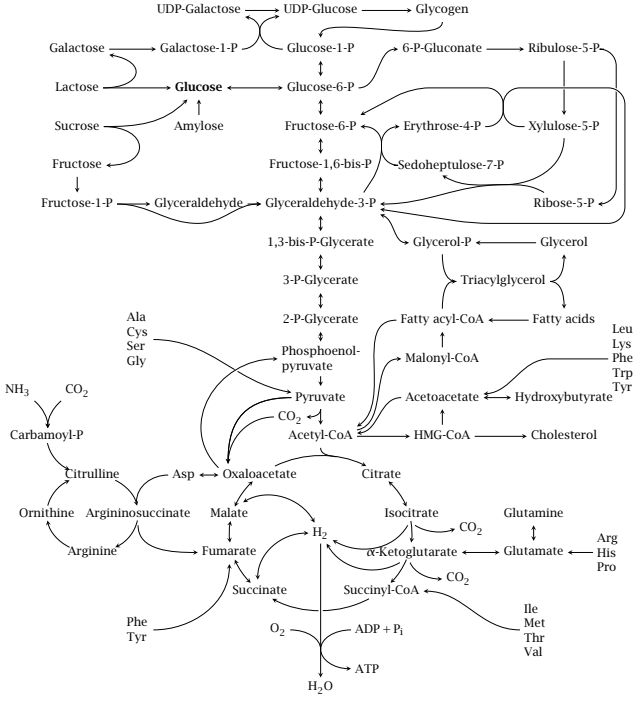
Mechanism of glucose uptake from the gut



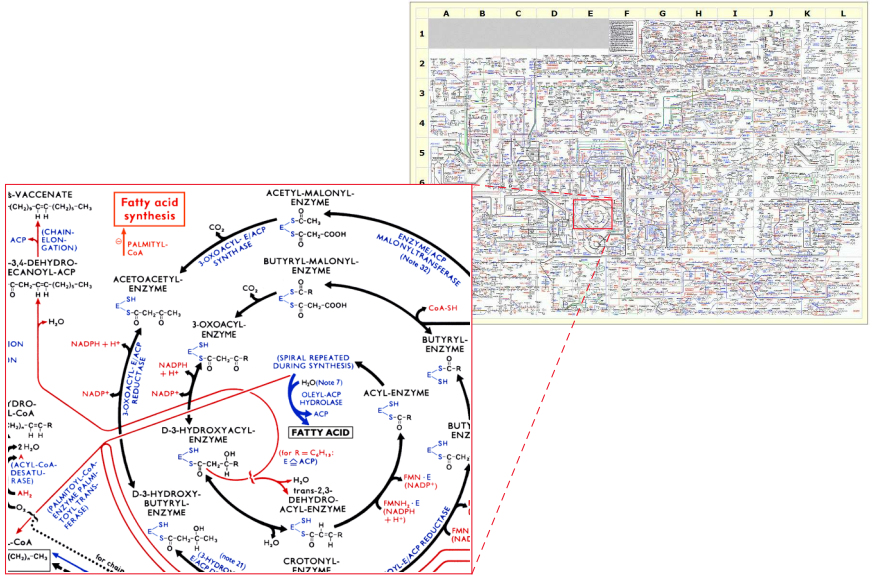
The large intestine

- Anaerobic milieu—99% of all bacteria in the large intestine are strict anaerobes
- Bacteria degrade non-utilized foodstuffs, reducing osmotic activity of gut content
- Mucous membrane recovers water and electrolytes
- Bacterial metabolism releases potentially toxic products (e.g. ammonia), which are taken up and inactivated by the liver

A metabolic map for Chem 333

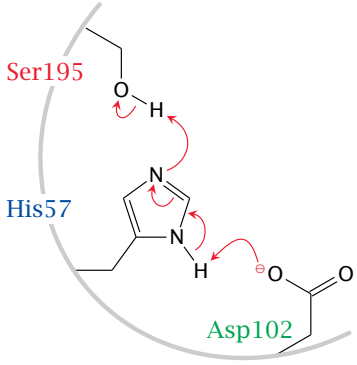


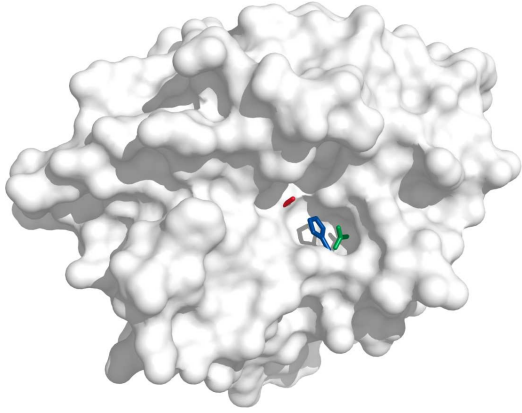
A more realistic metabolic map



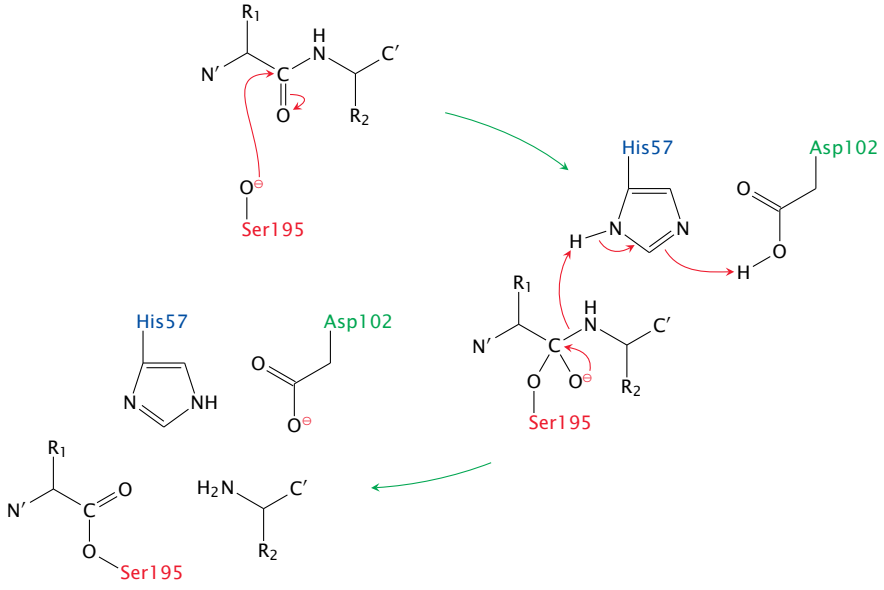
Refresher

The “catalytic triad” in the active site of chymotrypsin





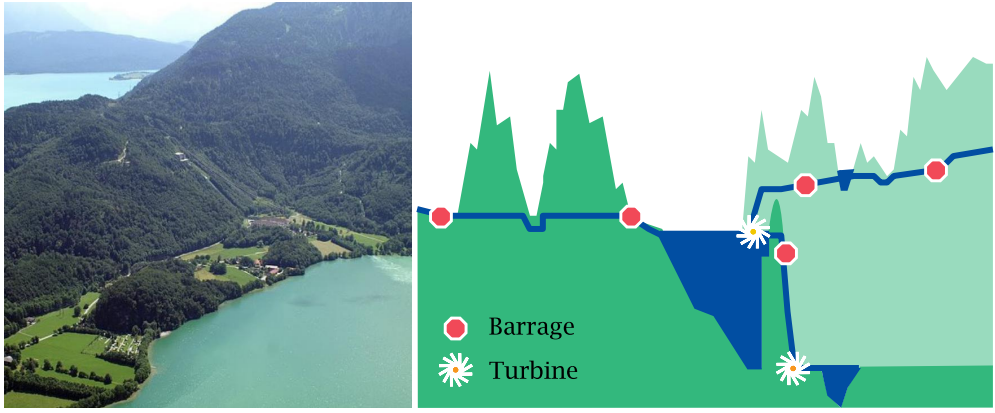
The catalytic mechanism of chymotrypsin



IUBMB classification of enzymes

Enzyme class	Catalyzed reactions
oxidoreductases	catalyze redox reactions, frequently involving one of the coenzymes NAD^+, $NADP^+$, or FAD
transferases	transfer functional groups between metabolites, e.g. a phosphate from ATP to a sugar hydroxyl group
hydrolases	catalyze hydrolysis reactions, such as those involved in the digestion of foodstuffs
lyases	perform elimination reactions that result in the formation of double bonds
isomerases	facilitate the interconversion of isomers
ligases	form new covalent bonds at the expense of ATP hydrolysis

A simile: the Walchensee–Kochelsee hydroelectric power system



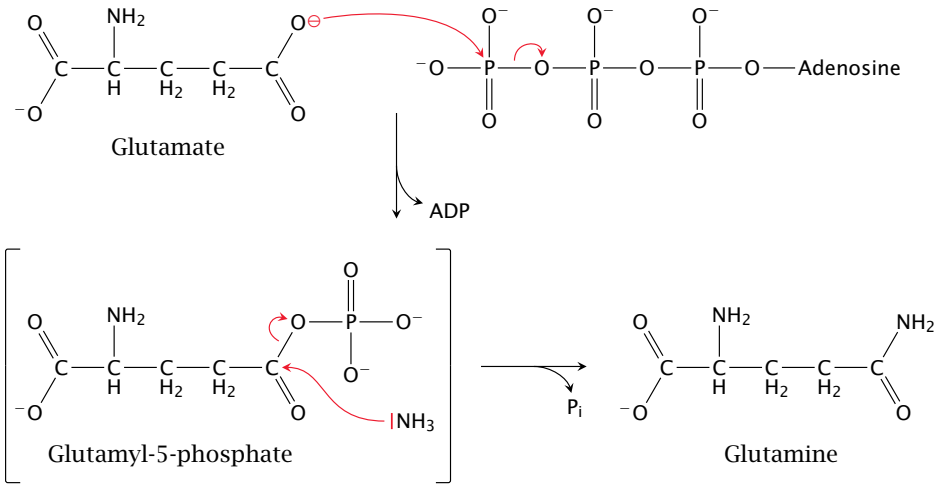
Analogies in the simile

Hydroelectric system	Metabolic pathway
altitude	energy
difference in altitude between lakes	energy difference between metabolites (ΔG)
height of ridge between lakes	ΔG^* of uncatalyzed reaction
tunnels	enzymes
tunnel barrages	regulatory switches of enzymes

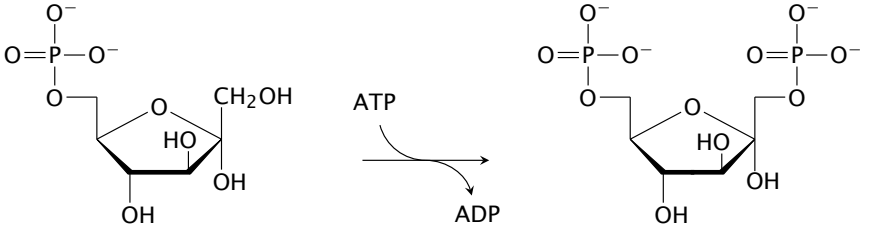
Discrepancies in the simile

Hydroelectric system	Metabolic pathway
all tunnels work the same way	enzymes have different catalytic mechanisms
potential energy determined by one parameter: altitude	free energy of metabolites depends on two parameters: ΔH and ΔS
water always collects at the bottom	molecules partition between lower and higher energy levels

The catalytic mechanism of glutamine synthetase



The phosphofructokinase reaction



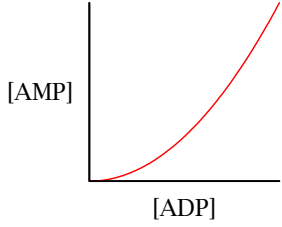
Fructose-6-phosphate

Fructose-1,6-bisphosphate

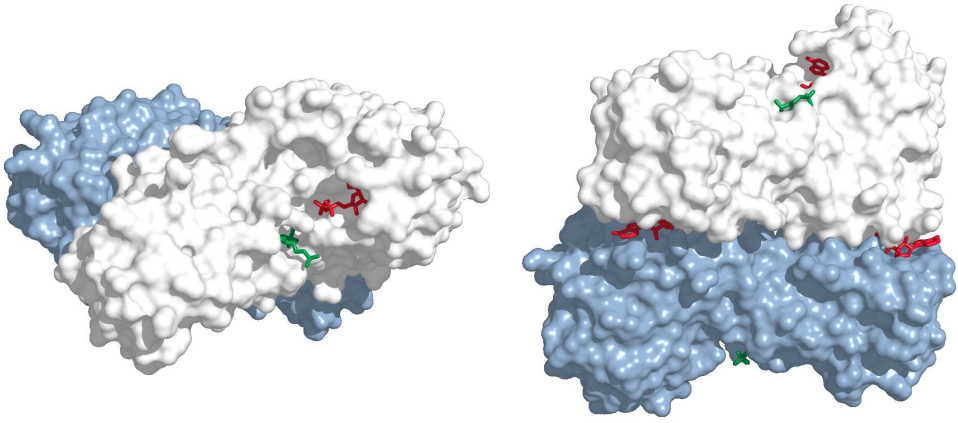
The adenylate kinase reaction equilibrates AMP, ADP and ATP

$$2\mathrm{ADP} \leftrightarrow \mathrm{ATP} + \mathrm{AMP}$$

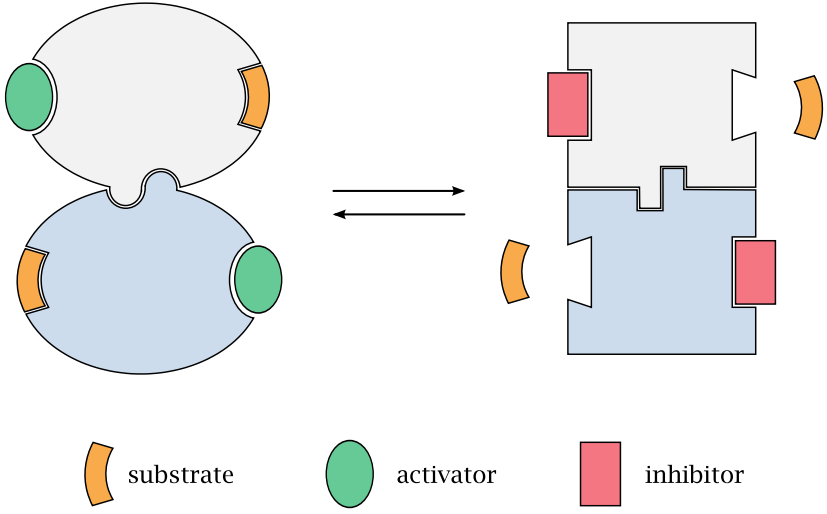
$$K = \frac{[\mathrm{ATP}]\,[\mathrm{AMP}]}{[\mathrm{ADP}]^2} \iff [\mathrm{AMP}] = [\mathrm{ADP}]^2 \frac{K}{[\mathrm{ATP}]}$$



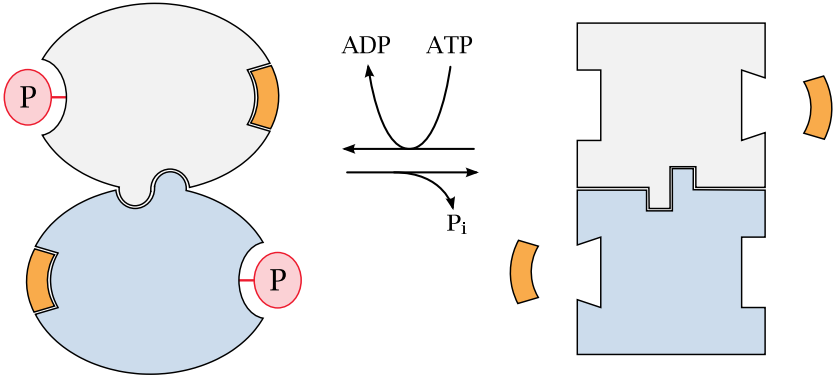
Allosteric regulation of phosphofructokinase by AMP



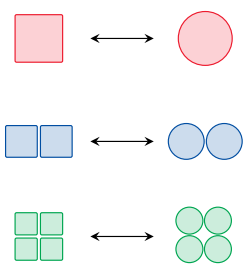
How allosteric regulation works

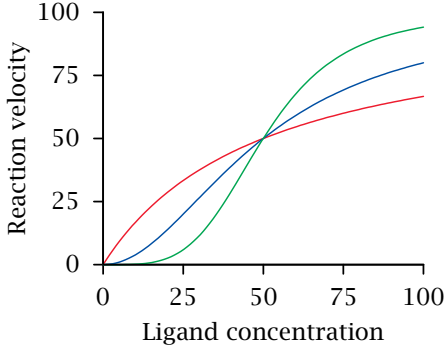


Enzyme regulation by protein phosphorylation

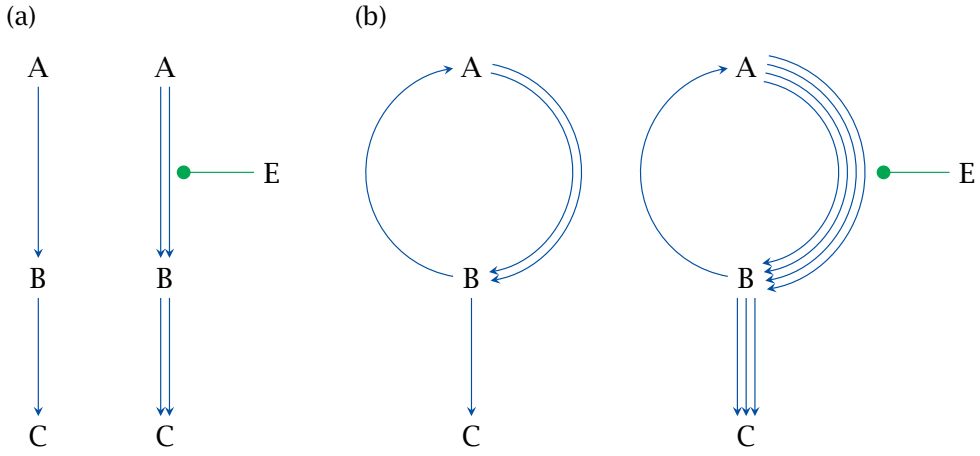


Oligomeric enzymes behave cooperatively





Substrate cycles can amplify molecular regulation mechanisms



Regulation of enzyme molecule abundance

- transcriptional induction
- accelerated mRNA degradation
- ubiquitin ligation, followed by proteolytic degradation

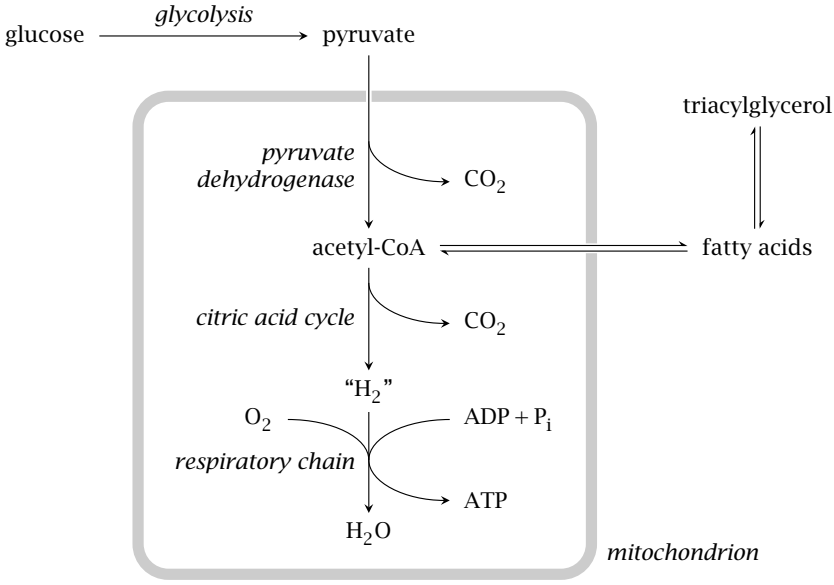
Chapter 3

Glycolysis

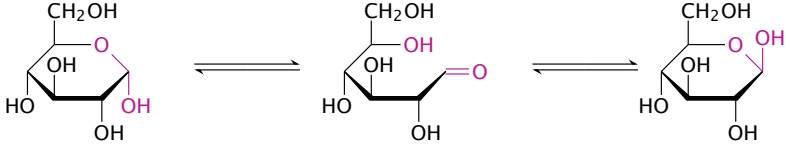
Overview of glucose metabolism

Pathway	Function
glycolysis, citric acid cycle, respiratory chain	complete degradation of glucose for ATP production
hexose monophosphate shunt	degradation of glucose for regeneration of NADPH
glycogen synthesis and degradation	short-term glucose storage
gluconeogenesis	synthesis of glucose from amino acids, lactate, or acetone

The place of glycolysis in glucose degradation

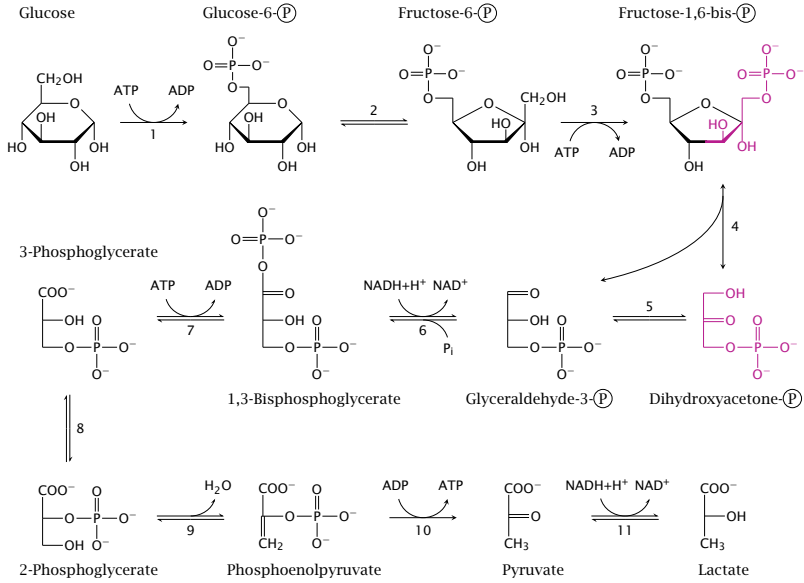


Alternate structures of D-glucose

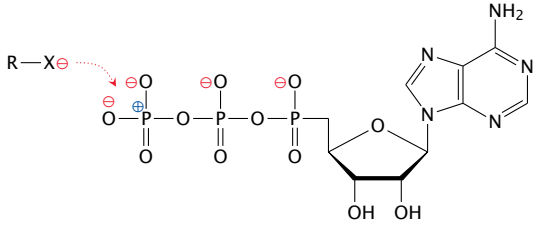


α-D-Glucose Aldehyde form β-D-Glucose

Reactions in glycolysis

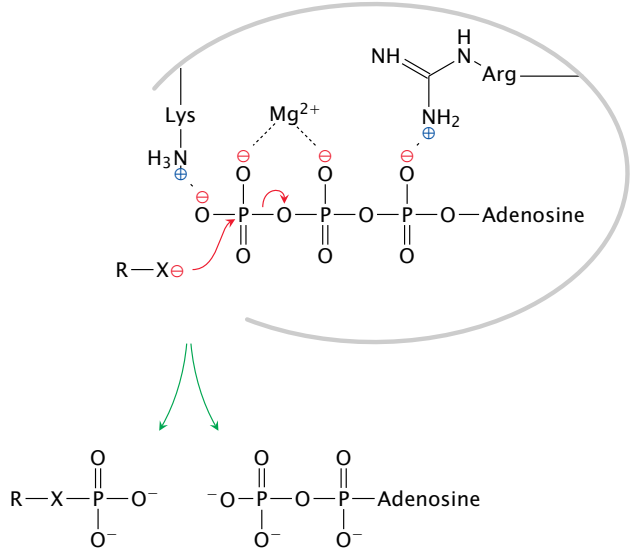


The phosphate groups in ATP are shielded from nucleophilic attack

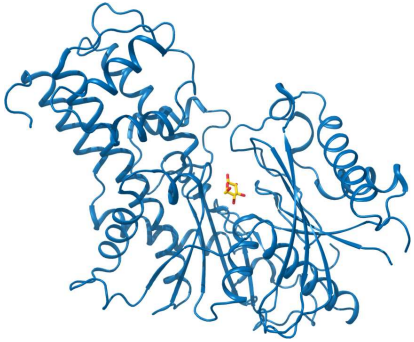


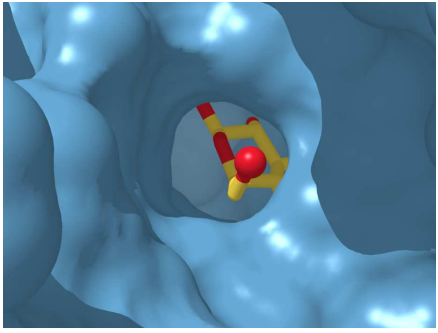
. . . and therefore, phosphate group transfer needs assistance from enzymes.

The catalytic mechanism of hexokinase

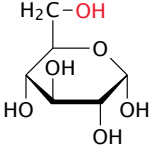


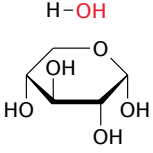
Hexokinase envelopes its substrates to prevent ATP hydrolysis





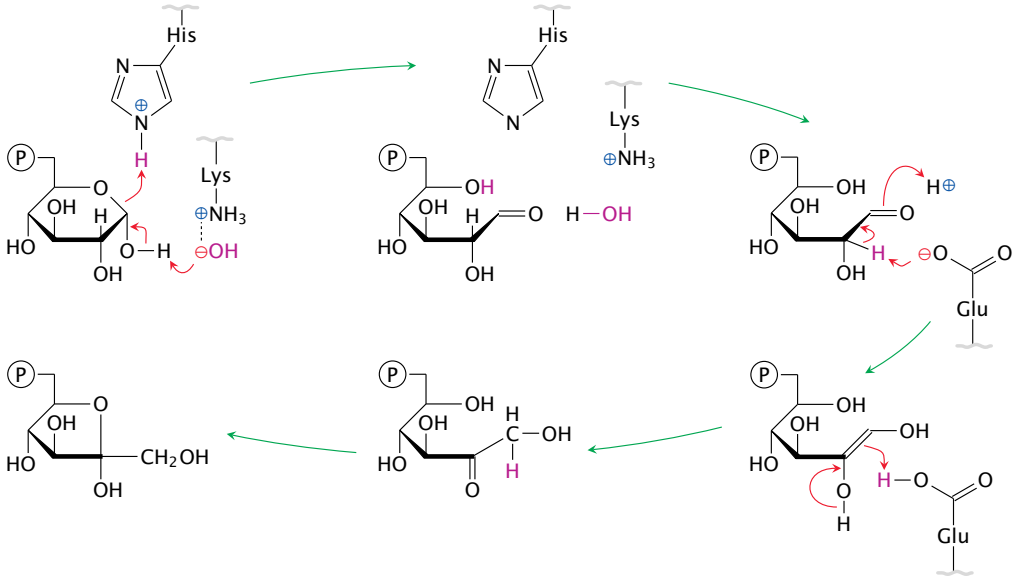
Glucose



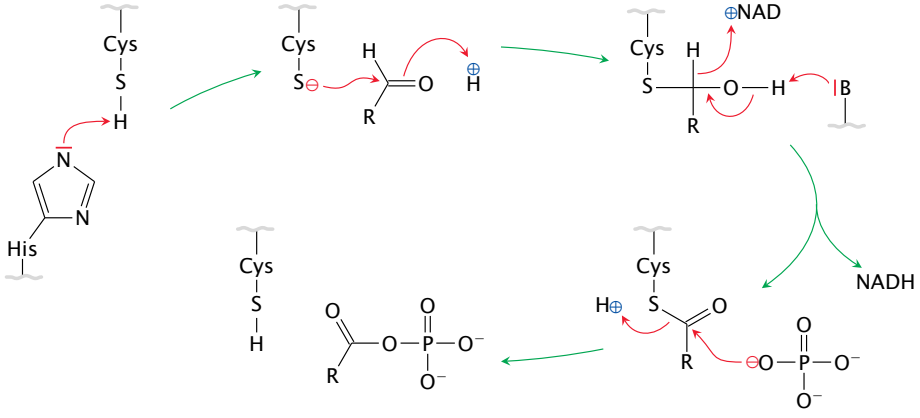


Xylose

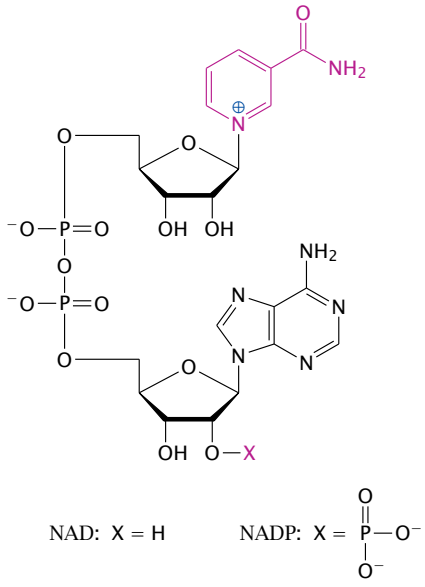
Phosphohexose isomerase performs acid-base catalysis

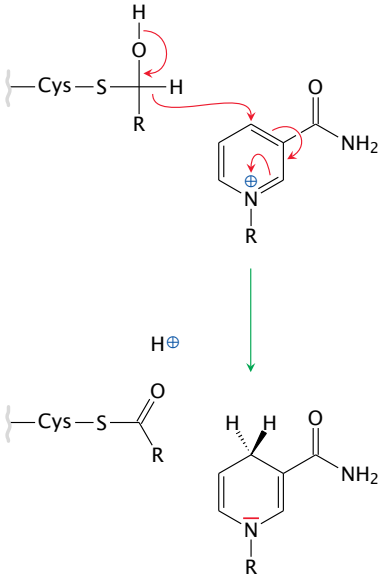


Glyceraldehyde-3-phosphate dehydrogenase carries out covalent catalysis

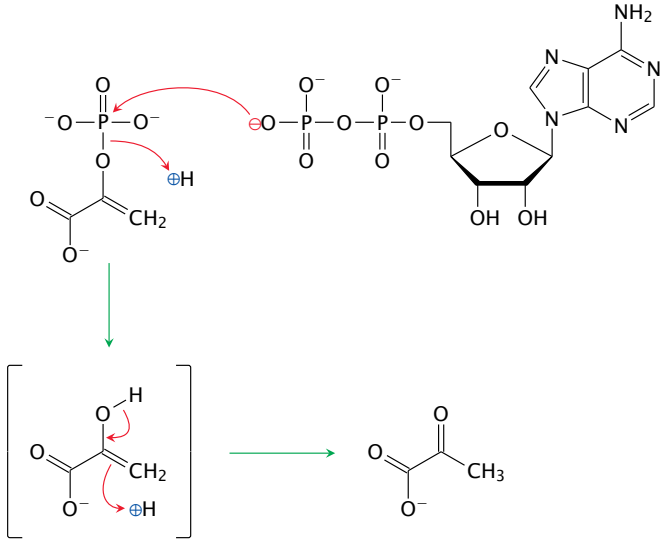


Structure and redox chemistry of NAD^+ and $NADP^+$





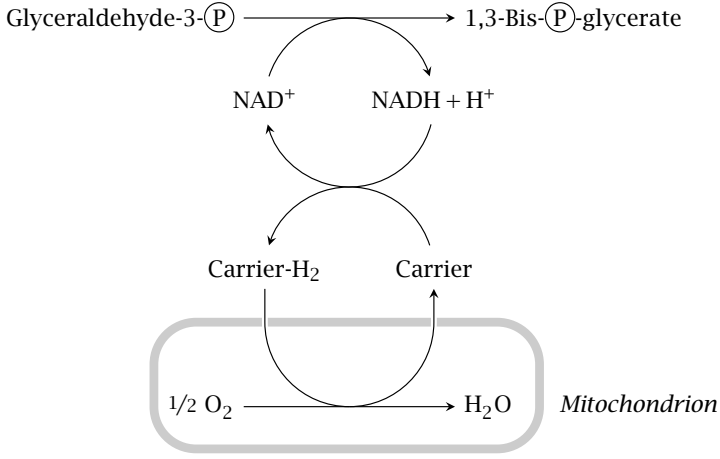
Pyruvate Kinase



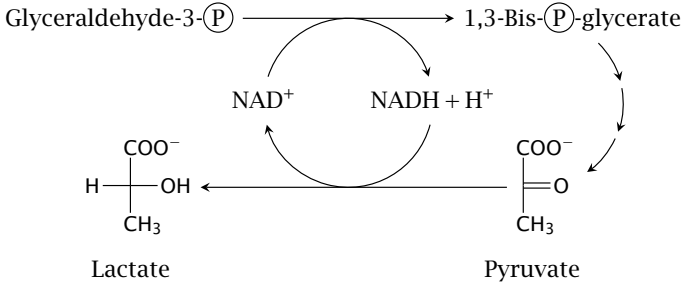
Energy-rich functional groups in substrates of glycolysis

- the enolphosphate in PEP
- the carboxyphosphate in 1,3-bisphosphoglycerate
- the thioester in the active site of glyceraldehyde-3-dehydrogenase

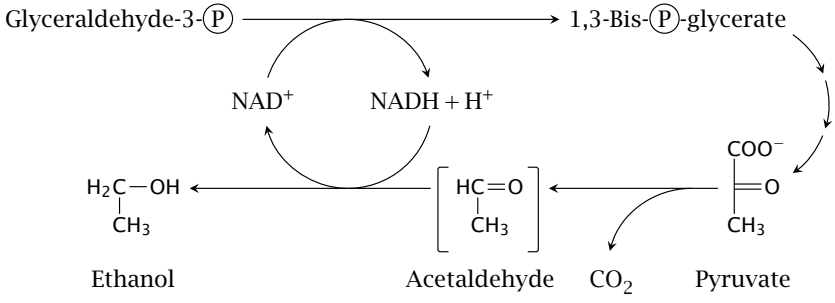
Regeneration of cytosolic NAD^+ under aerobic conditions



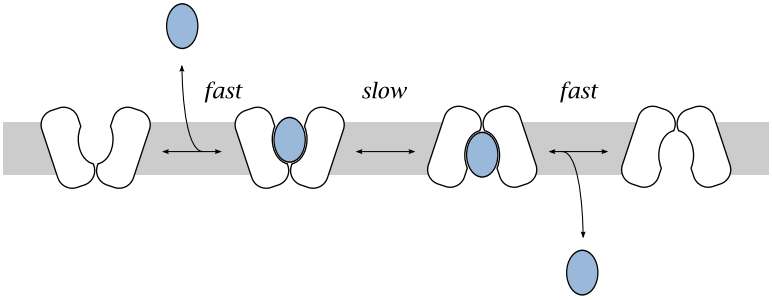
Under anaerobic conditions, NAD^+ is regenerated by lactate dehydrogenase



Ethanolic fermentation in yeast serves a dual purpose

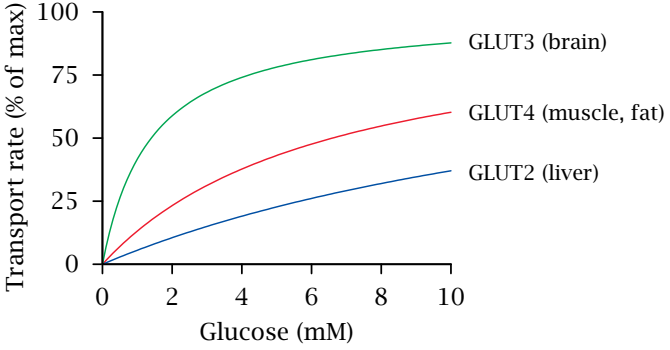


Kinetics of glucose transport by facilitated diffusion

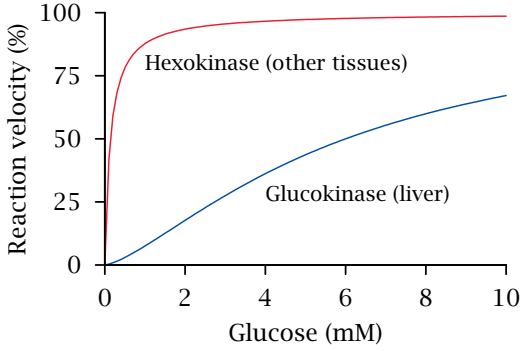


$$V_{\text{transport}} = V_{\max} \frac{[S]}{K_{\text{M}} + [S]}$$

GLUT transporters in different tissues vary in their affinity for glucose



Reaction velocities of hexokinase and glucokinase



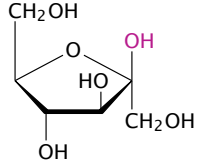
Chapter 4

Catabolism of sugars other than glucose

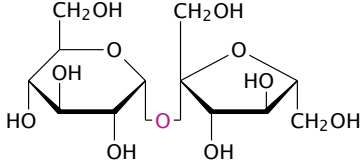
Dietary sugars other than glucose

Trivial name	Composition	Source
lactose (milk sugar)	disaccharide of glucose and galactose	milk
sucrose	disaccharide of glucose and fructose	sugar cane, sugar beet, other fruits
fructose	monosaccharide	various fruits
sorbitol	sugar alcohol	fruits; semisynthetic
ribose, deoxyribose	monosaccharides	nucleic acids

Degradation of fructose and sucrose

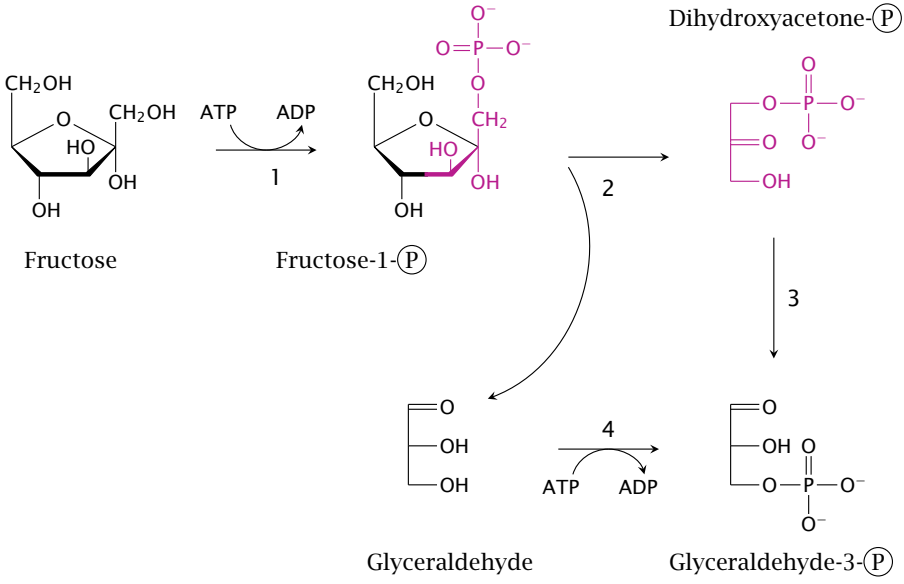


fructose

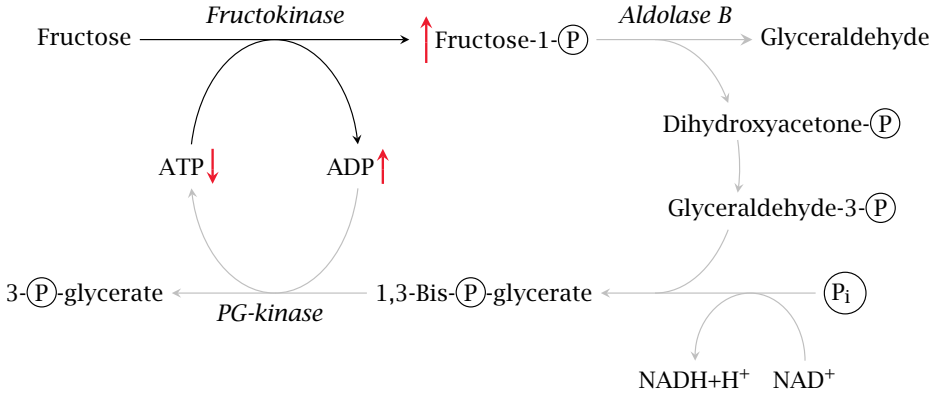


α-D-glucosyl-(1,2)-β-D-fructoside (sucrose)

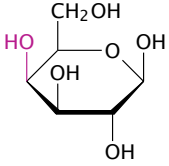
The fructolysis pathway



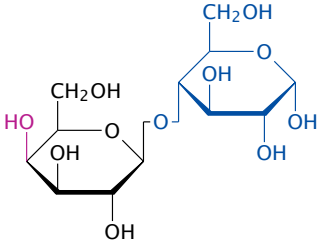
Fructose intolerance



Lactose and galactose

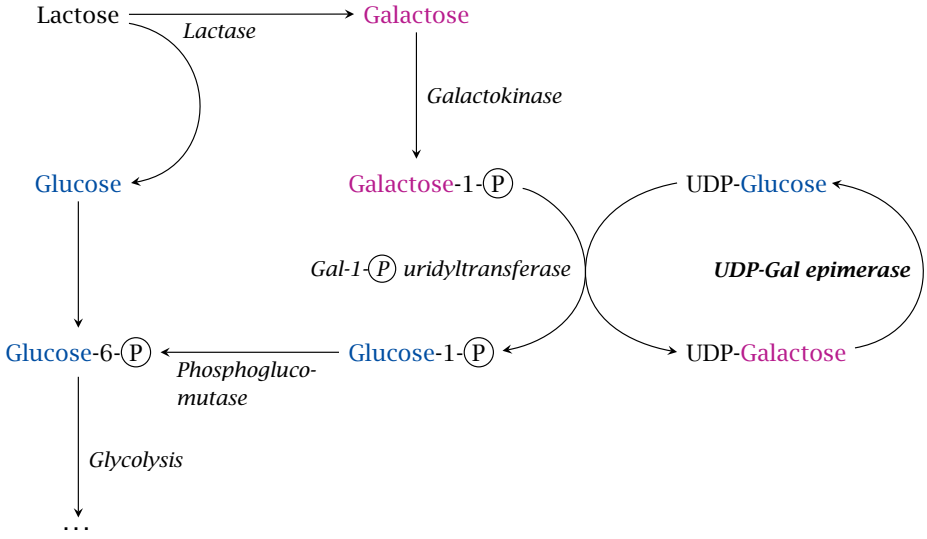


β-D-galactose

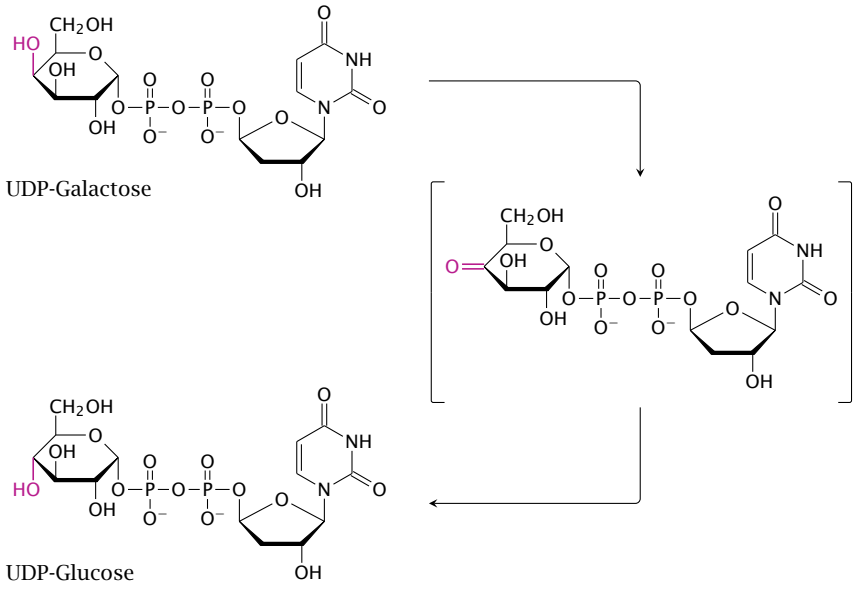


β-D-galactosyl-(1 → 4)-D-glucoside (lactose)

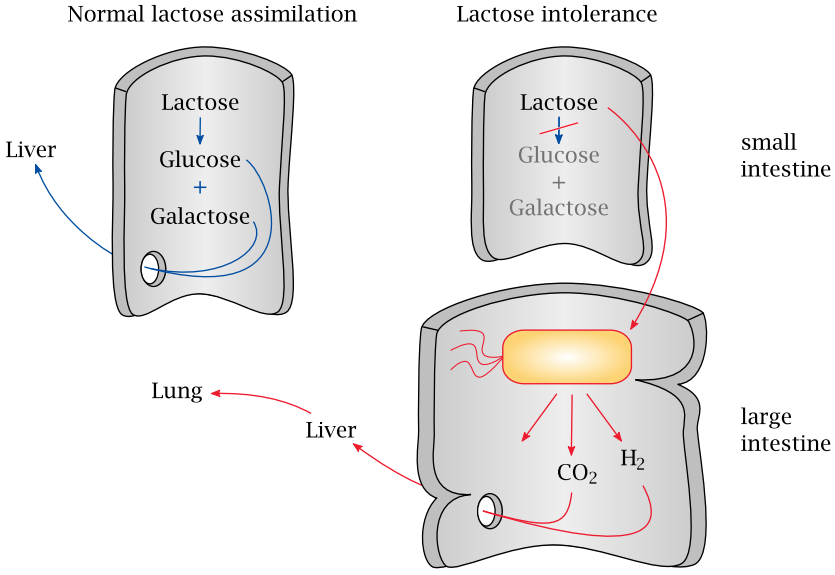
The Leloir pathway for galactose utilization



Mechanism of UDP-galactose epimerase



Lactose intolerance

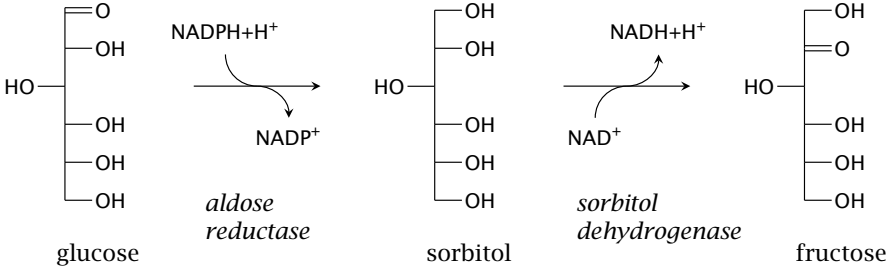


Galactosemia

Type	Enzyme deficiency	Accumulating metabolites
I	galactose-1-phosphate-uridyltransferase	galactose, galactose-1-phosphate, galactitol, galactonate
II	galactokinase	galactose, galactitol
III	UDP-galactose epimerase	galactose-1 phosphate, UDP-galactose

- sorbitol pathway

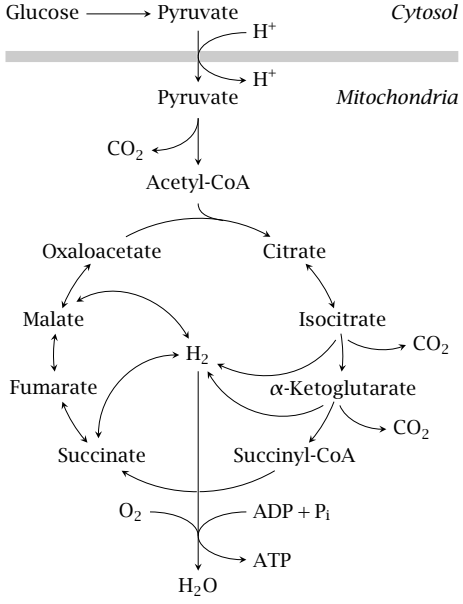
Sorbitol is an intermediate of the polyol pathway



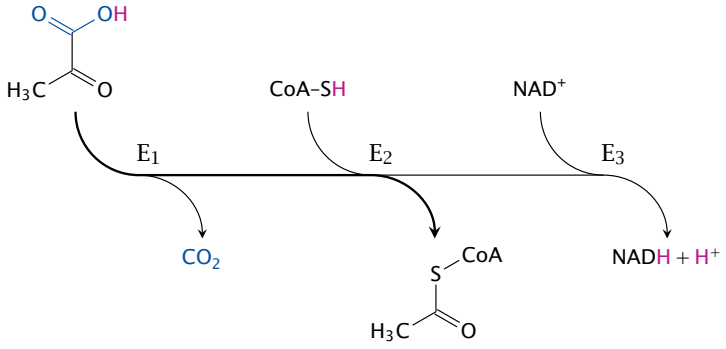
Chapter 5

Pyruvate dehydrogenase and the citric acid cycle

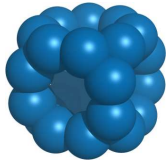
Pyruvate degradation occurs in the mitochondria



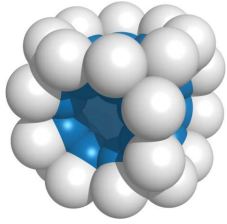
The PDH reaction occurs in three successive steps that are catalyzed by three different subunits



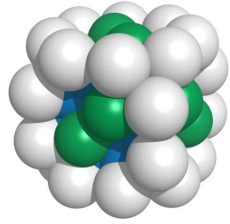
The structural organization of the *E. coli* PDH complex



E_2

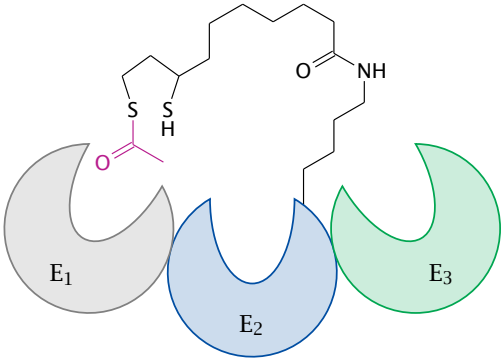


$E_2 + E_1$



$E_2 + E_1 + E_3$

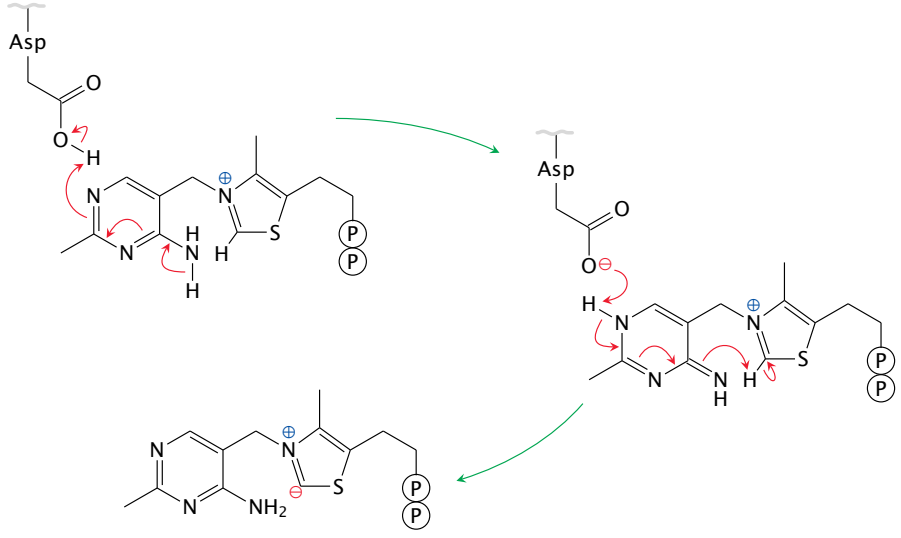
A lipoamide tether guides the substrate from one active site to the next



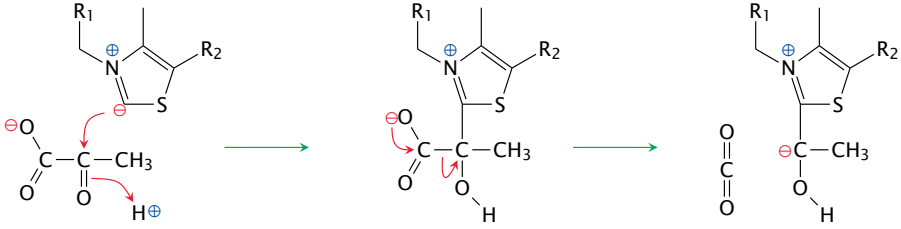
The pyruvate dehydrogenase reaction involves multiple coenzymes

Coenzyme	Subunit	Role in catalysis
thiamine pyrophosphate	E_1	provides a carbanion for nucleophilic attack on the substrate
lipoamide	E_2	transfers substrate to coenzyme A, retains hydrogen
flavin adenine dinucleotide (FAD)	E_3	transfers H_2 from lipoamide to NAD^+

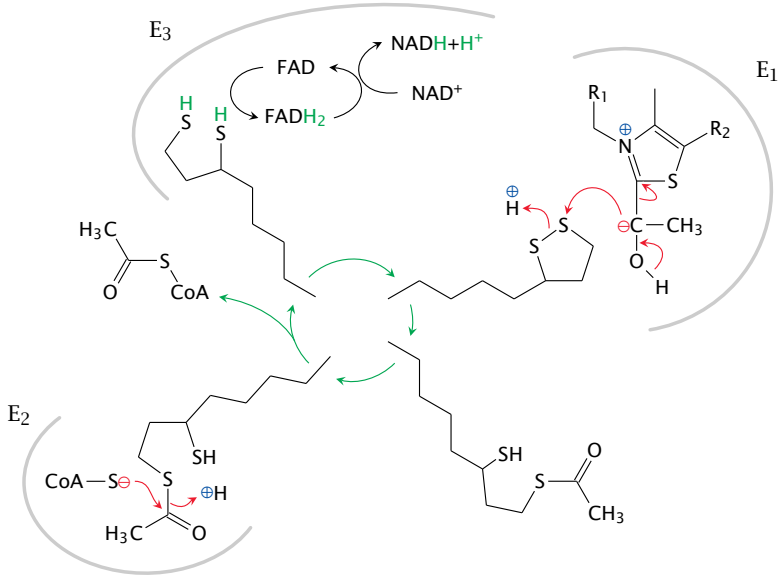
Thiamine pyrophosphate forms a carbanion



Decarboxylation of pyruvate by E_1



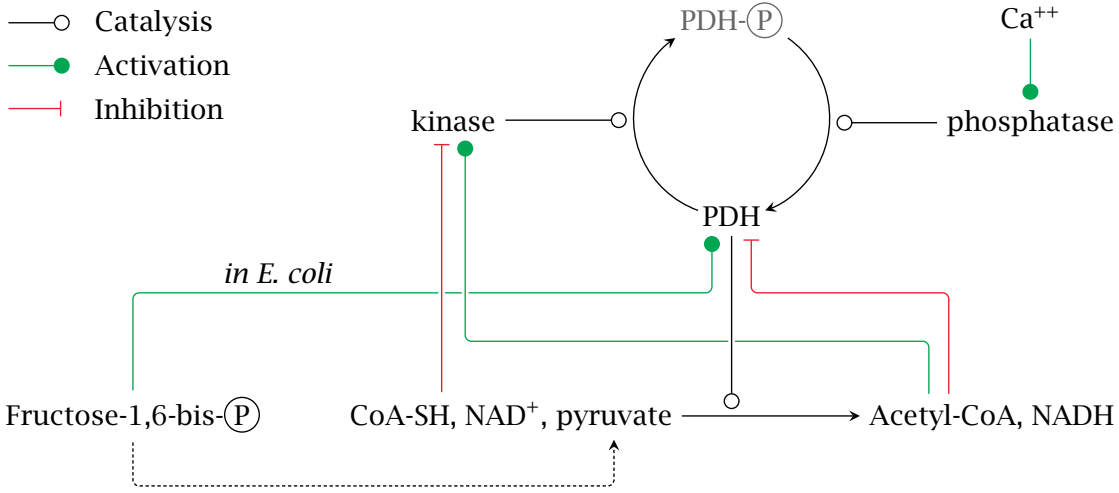
Release of acetyl-CoA and disposal of hydrogen



Alternate metabolic destinations of pyruvate

1. Conversion to acetyl-CoA by PDH for complete degradation or for synthesis of fatty acids and cholesterol
2. Carboxylation to oxaloacetate, for use in gluconeogenesis or in the citric acid cycle
3. Synthesis of amino acids, e.g. transamination to alanine
4. Reduction to lactate

Regulation of PDH by allosteric effectors and by phosphorylation



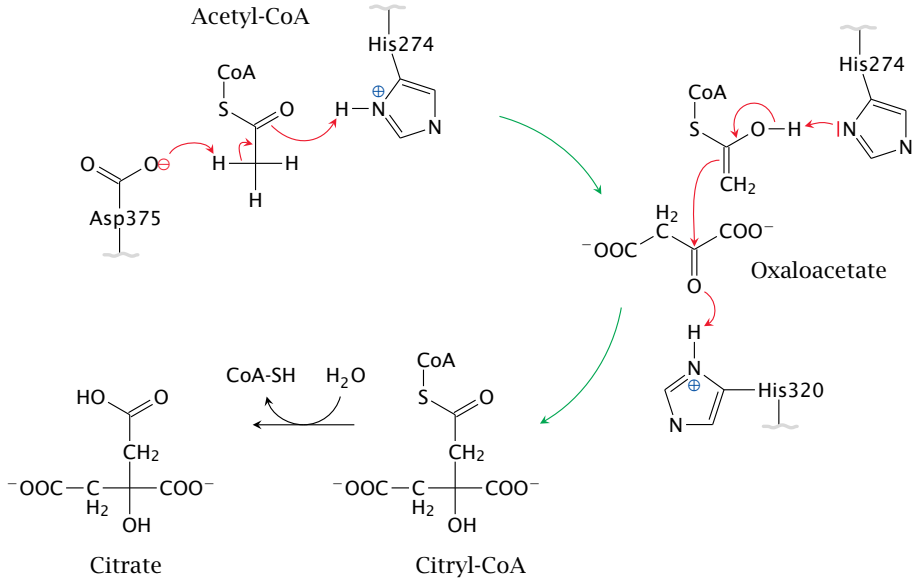
The overall reaction of the TCA cycle: does it add up?

$$CH_3COOH \longrightarrow 2\,CO_2 + 4\,H_2$$

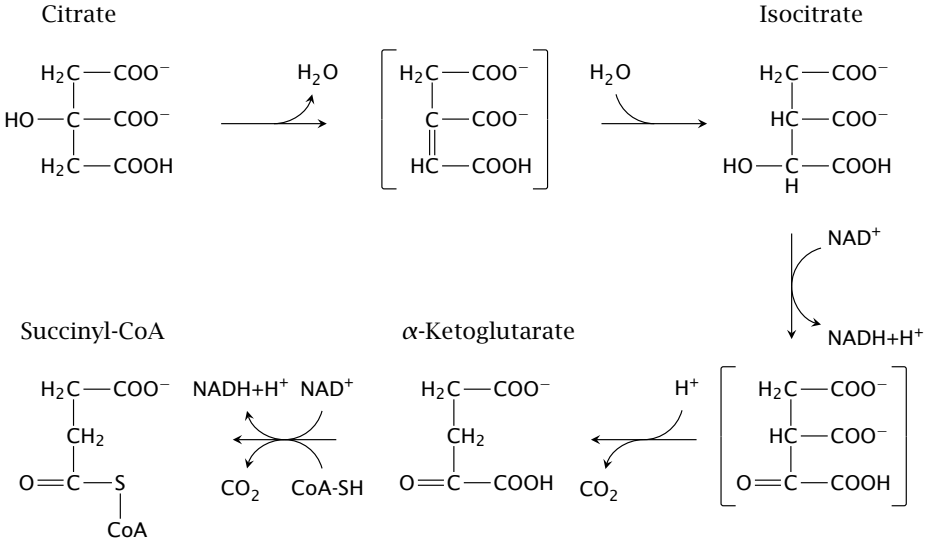
$$2\,H_2O + CH_3COOH \longrightarrow 2\,CO_2 + 4\,H_2$$

- cycle

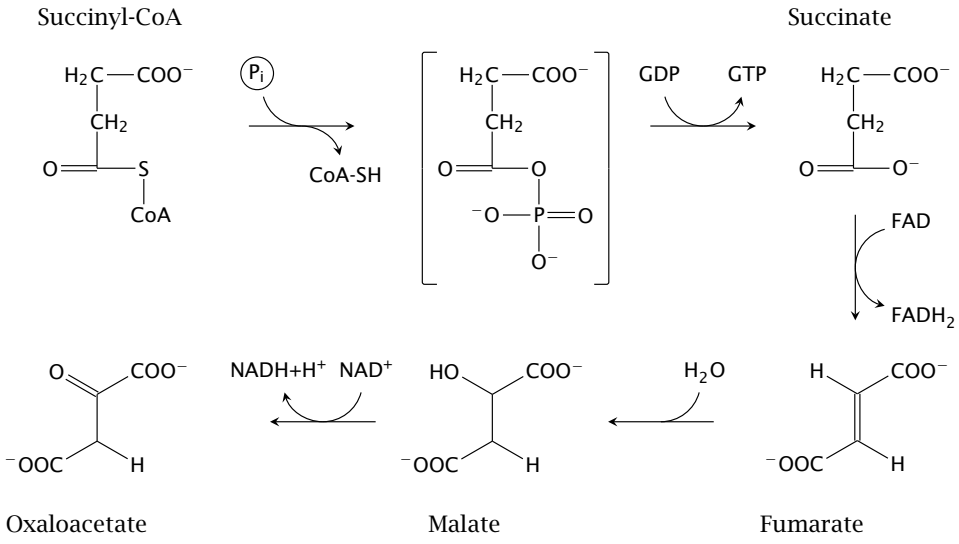
The citrate synthase reaction



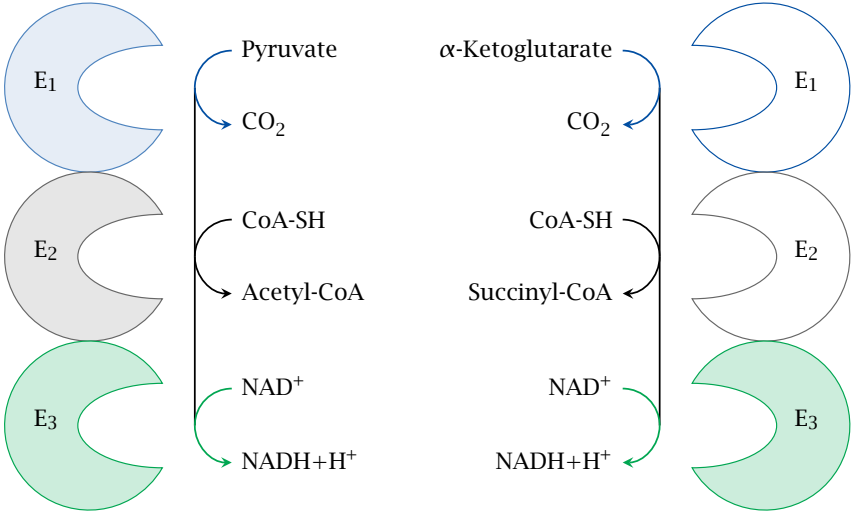
Reactions in the TCA cycle: from citrate to succinyl-CoA



Reactions in the TCA: from succinyl-CoA to oxaloacetate



α-Ketoglutarate dehydrogenase resembles PDH



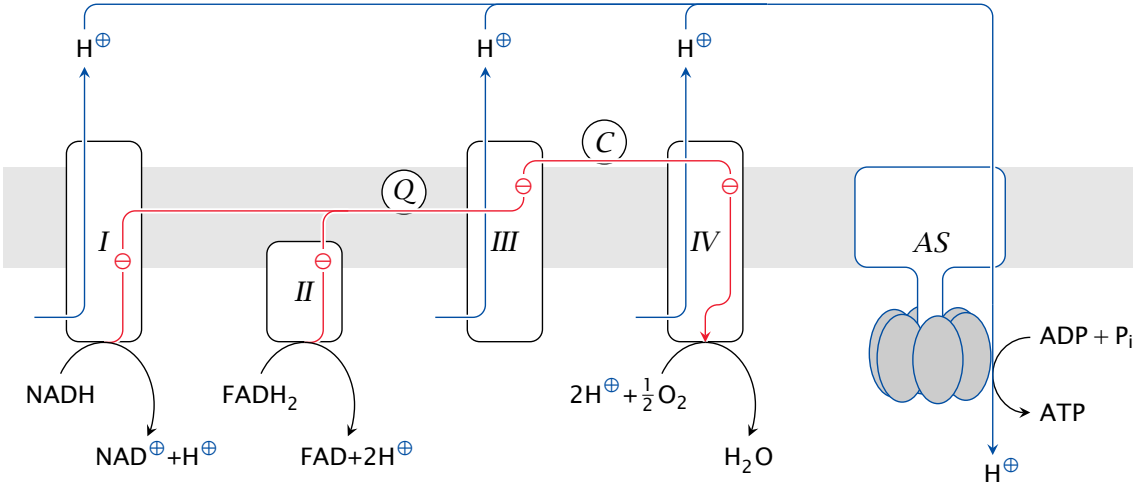
Regulation of the citric acid cycle

- ATP and NADH inhibit isocitrate dehydrogenase
- NADH inhibits α-ketoglutarate dehydrogenase
- High levels of NADH will lower the oxaloacetate concentration, which limits citrate synthase activity

Chapter 6

The respiratory chain

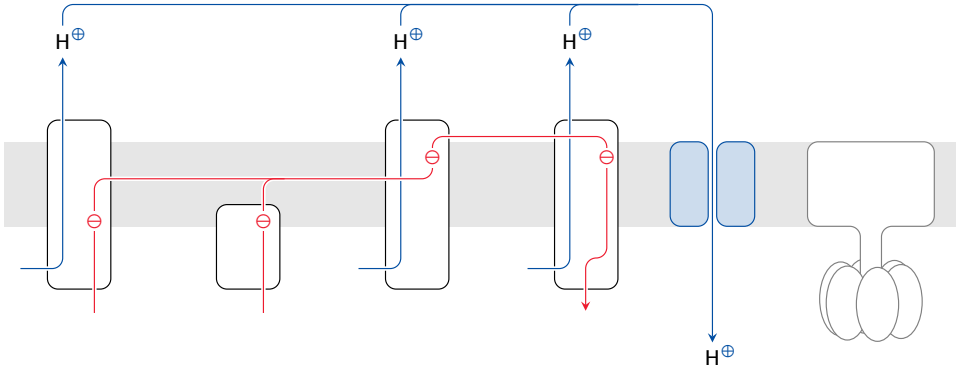
Overview of the respiratory chain



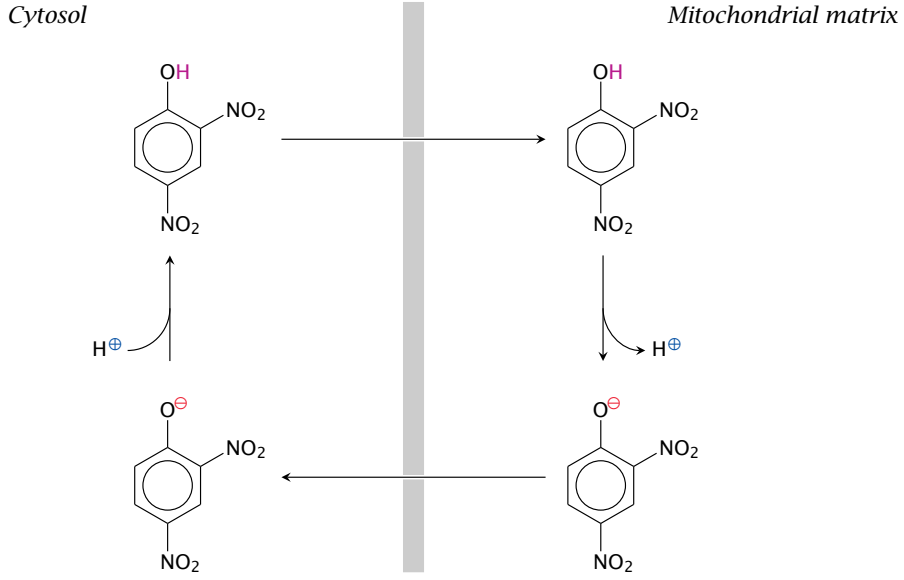
Functional stages in the respiratory chain

1. H_2 is abstracted from NADH+H^+ and from $FADH_2$
2. The electrons obtained with the hydrogen are passed down a cascade of carrier molecules located in complexes I–IV, then transferred to O_2
3. Powered by electron transport, complexes I, III, and IV expel protons across the inner mitochondrial membrane
4. The expelled protons reenter the mitochondrion through ATP synthase, driving ATP synthesis

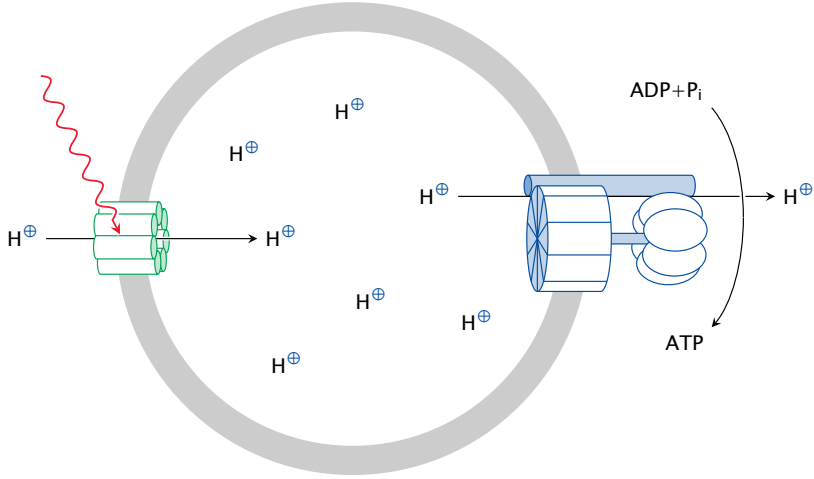
Uncoupling proteins dissipate the proton gradient



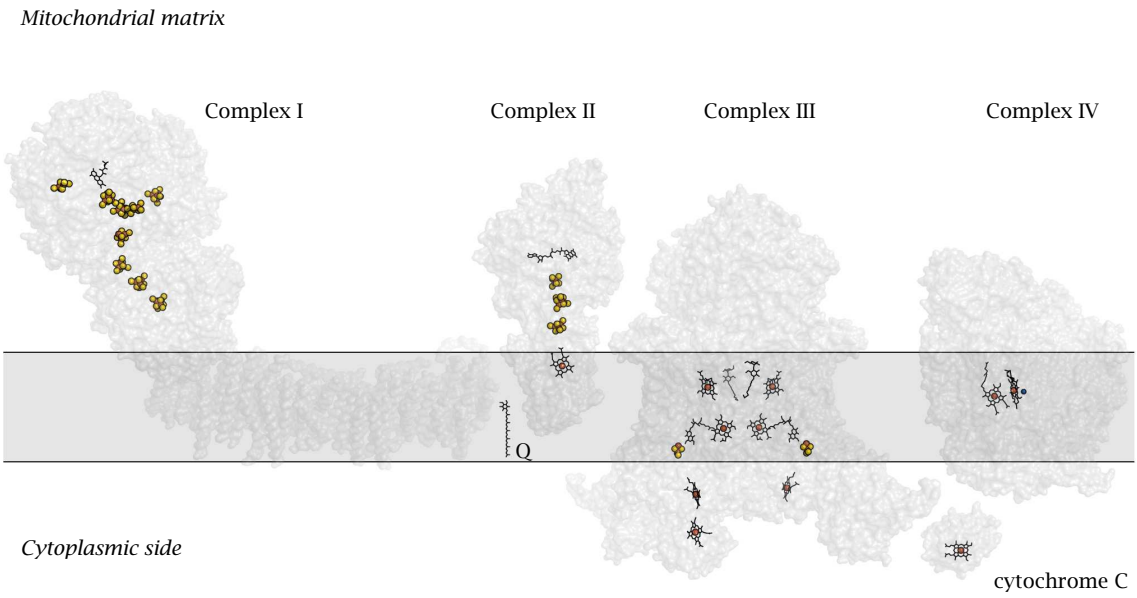
The uncoupling action of dinitrophenol



The Racker experiment: bacteriorhodopsin can drive ATP synthase

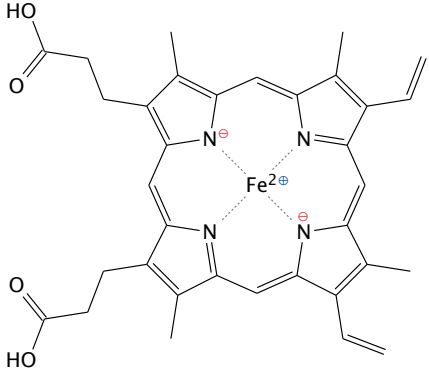


Molecules in the electron transport chain

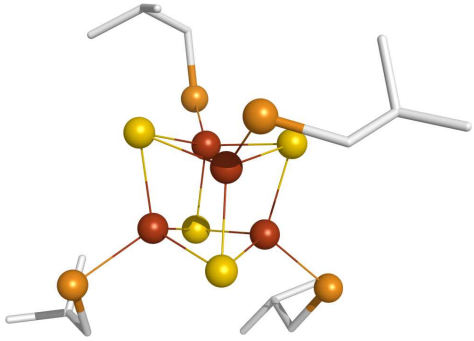


Iron-containing redox cofactors

Heme

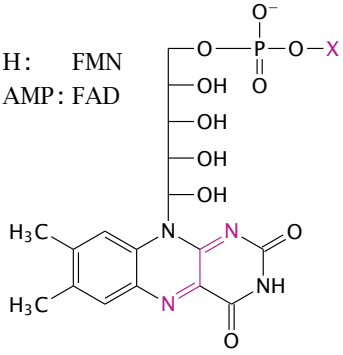


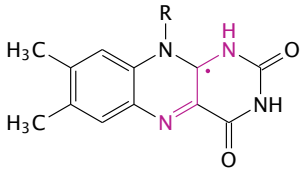
Iron-sulfur cluster



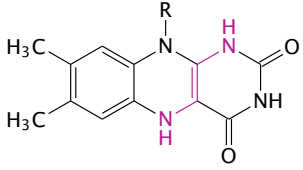
Flavin-containing redox cofactors

X = H: FMN
X = AMP: FAD



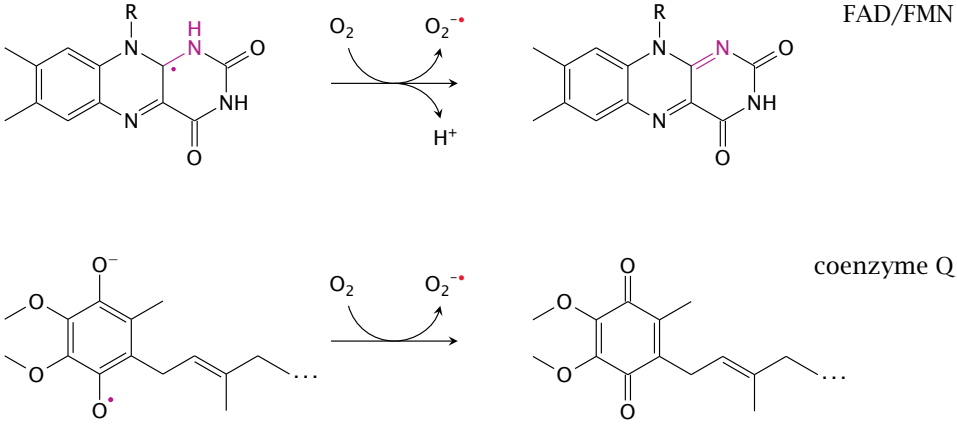


FMNH/FADH

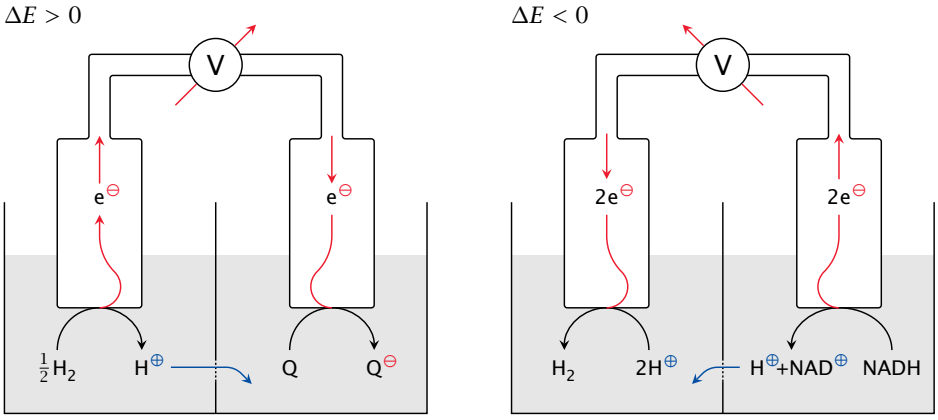


$FMNH_2/FADH_2$

The respiratory chain generates reactive oxygen species as by-products



Redox reactions can be compartmentalized to produce a measurable voltage



Energetics of electron transport

- Each electron transfer step along the chain is a redox reaction: the first cofactor is oxidized and the second one is reduced
- In a redox reaction, electrons flow spontaneously if the reduction potential increases in the forward direction ($\Delta E > 0$)
- Redox reactions, like other reactions, proceed spontaneously if their free energy is negative ($\Delta G < 0$)

$\longrightarrow$ How is the reduction potential of a redox reaction related to its free energy?

The redox potential (ΔE) is proportional to the free energy (ΔG)

$$\Delta G \equiv \frac{\text{energy}}{\text{moles (number of molecules)}}$$

$$\Delta E \equiv \frac{\text{energy}}{\text{charge transferred}}$$

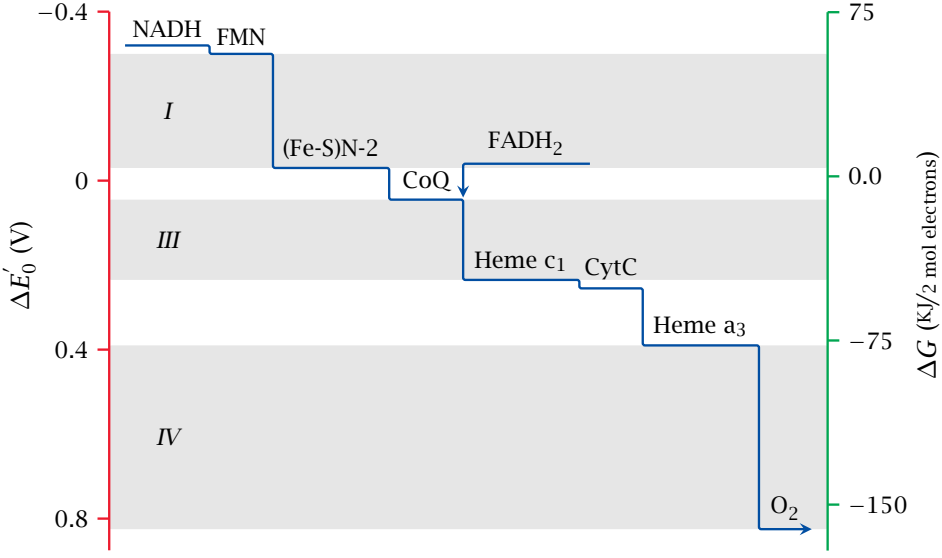
$$\Delta G = \frac{\text{energy}}{\text{charge transferred}} \times \frac{\text{charge transferred}}{\text{moles}}$$

$$\Delta \text{G} = \Delta E \times \frac{\text{charge transferred}}{\text{moles}}$$

therefore

$$\Delta G = -\Delta E \times n \times \text{F}$$

Redox potentials and free energies in the respiratory chain



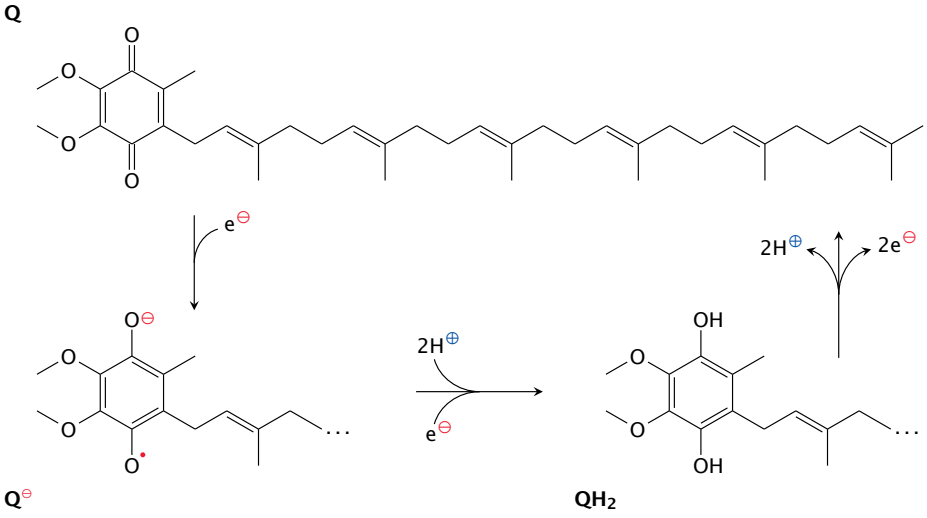
The first two redox steps in complex I

$$\mathrm{NADH + H^+ + FMN \longrightarrow NAD^+ + FMNH_2}$$

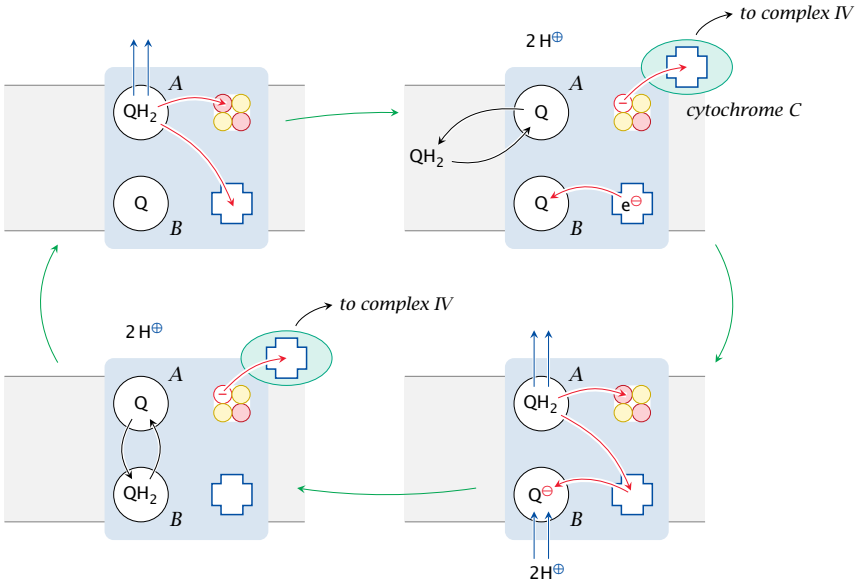
$$\mathrm{FMNH_2 + Fe^{III} - S \longrightarrow FMNH^{\bullet} + H^+ + Fe^{II} - S}$$

$$\mathrm{FMNH^{\bullet} + Fe^{III} - S \longrightarrow FMN + H^+ + Fe^{II} - S}$$

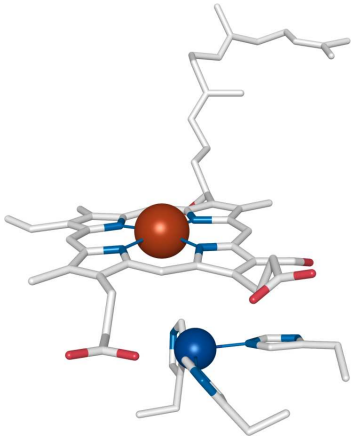
The reduction of coenzyme Q involves protons and electrons

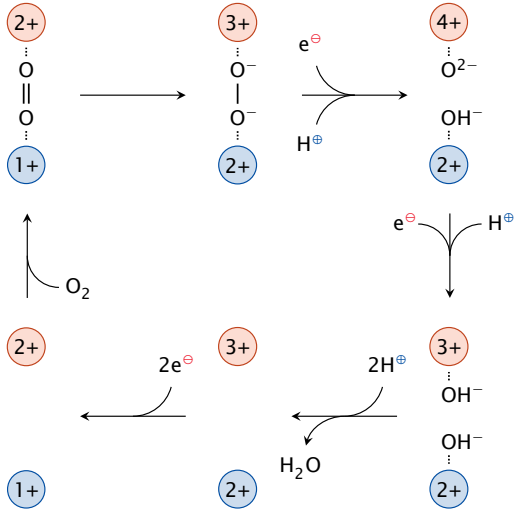


The Q cycle (criminally simplified)



Reduction of oxygen by cytochrome C oxidase (complex IV)

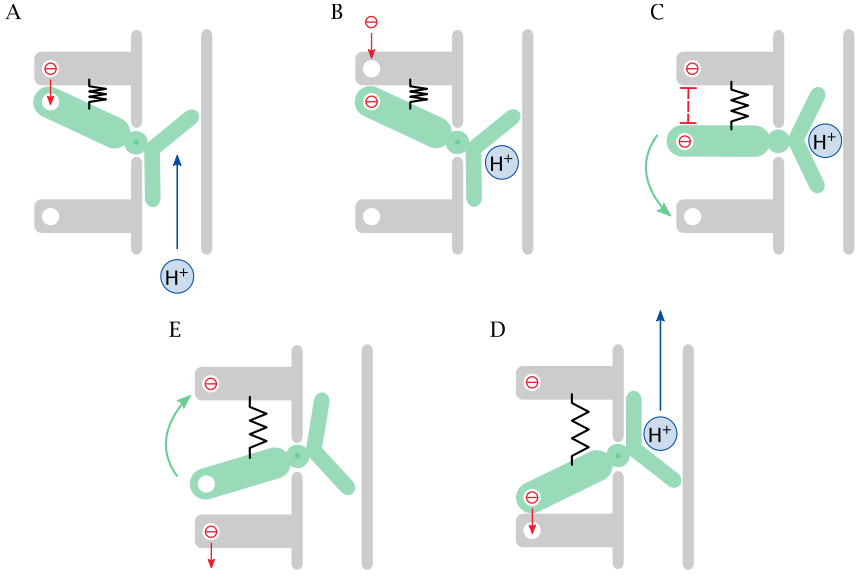




How is electron transport linked to proton pumping?

- Some redox steps in the ETC are coupled to proton binding and dissociation, which may occur at opposite sides of the membrane. Example: Coenzyme Q cycle at complex III
- Redox steps that do not involve hydrogen directly need a different mechanism in order to contribute to proton pumping. Example: Sequence of iron-sulfur clusters and hemes in complex IV

Linking electron movement to proton pumping: A conceptual model

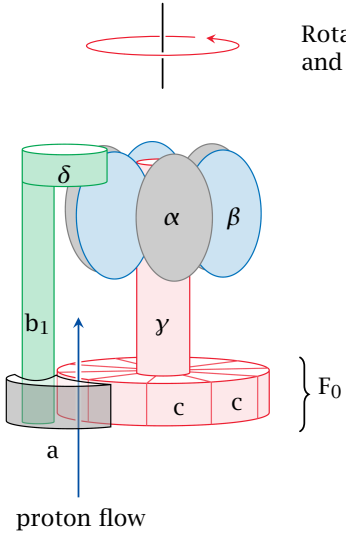


Proton pumping creates both a concentration gradient and a membrane potential

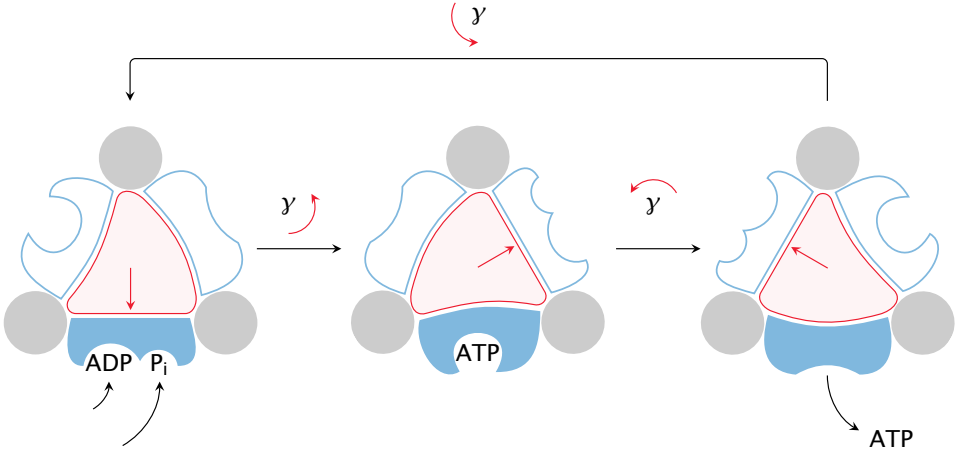
$$\Delta G_{\text{concentration}} = RT \times \ln K = 6 \, \frac{\text{kJ}}{\text{mol}}$$

$$\Delta G_{\text{potential}} = \Delta\psi \times n \times \text{F} = 15 \, \frac{\text{kJ}}{\text{mol}}$$

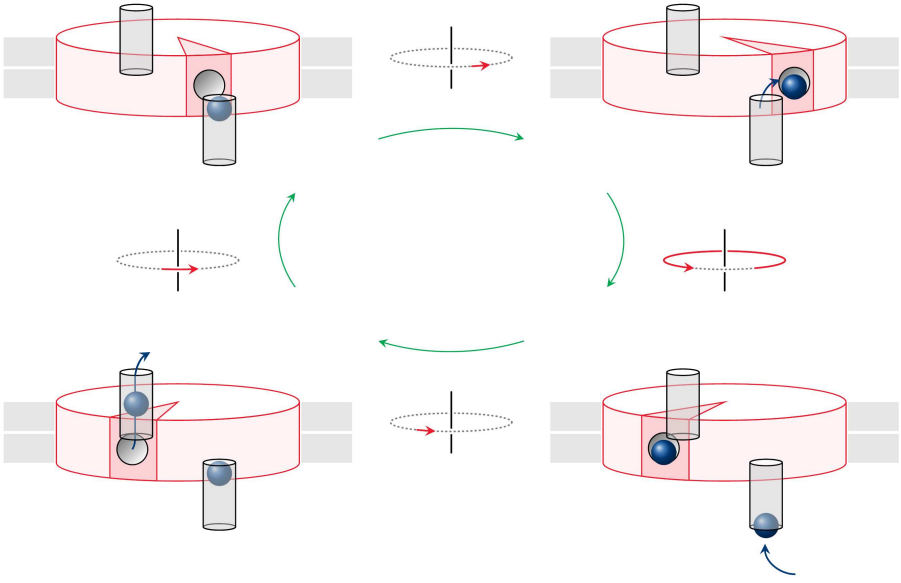
Structure of ATP synthase



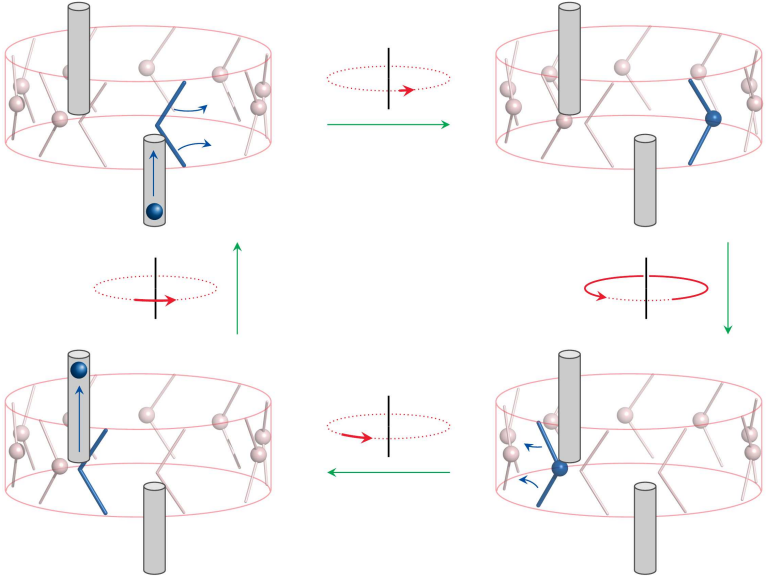
The binding-change model of ATP synthase catalysis



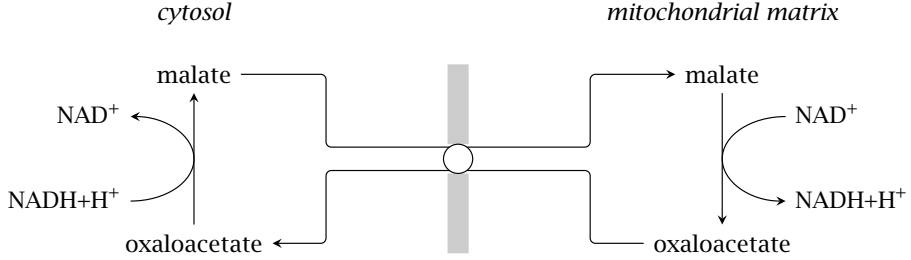
How does proton flux drive ATP synthase?



Proton flux causes *c* chains to rotate *within* the F_0 disk

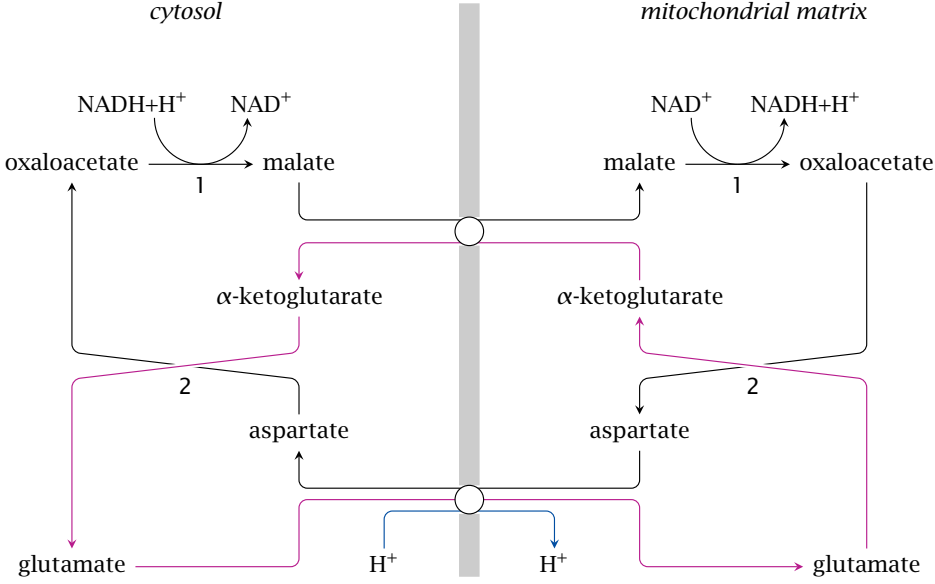


A hypothetical malate-oxaloacetate shuttle

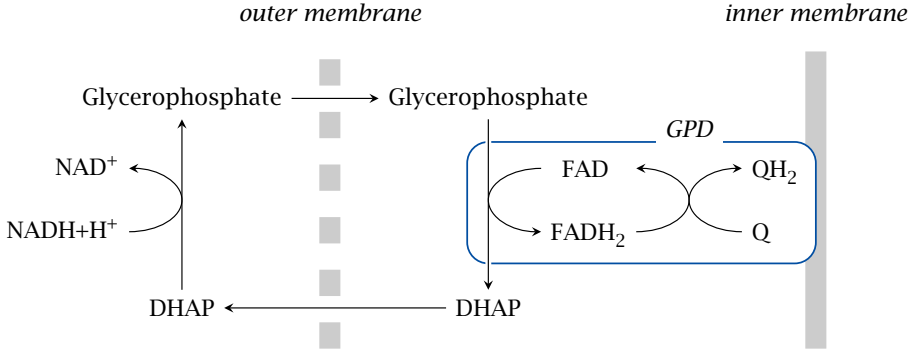


- shuttles overview

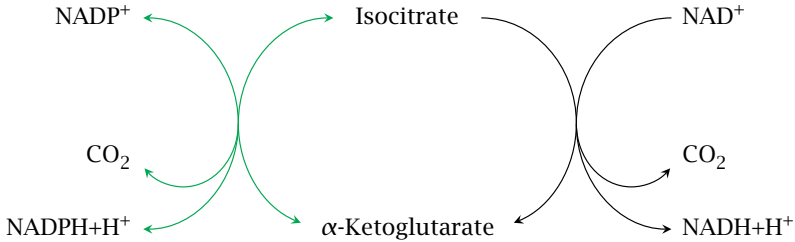
The malate-aspartate shuttle



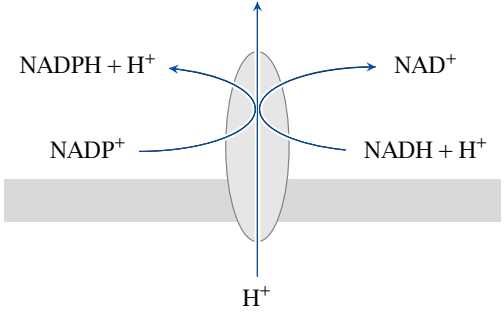
The glycerophosphate shuttle



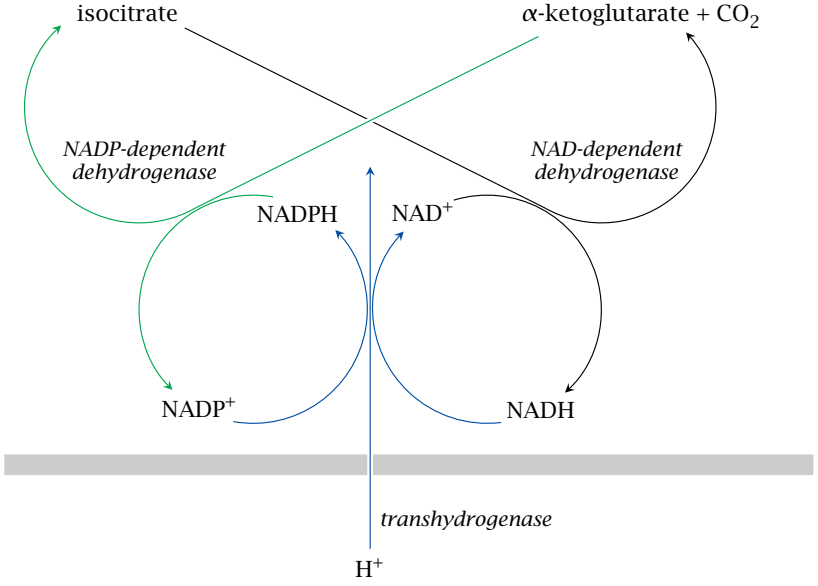
The two mitochondrial isocitrate dehydrogenases



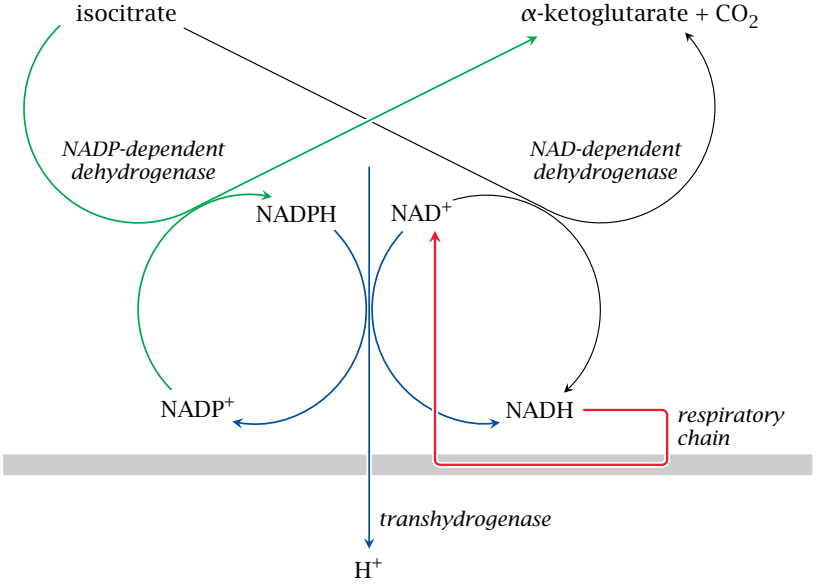
Nicotinamide nucleotide transhydrogenase couples hydrogen transfer with proton transport



At rest, transhydrogenase and the two isocitrate dehydrogenases form a futile cycle



When ATP demand is high, transhydrogenase turns into an auxiliary proton pump



Theoretical ATP yield per molecule of glucose completely oxidized

Quantity	Intrinsic value	Per glucose
Accrued hydrogen		10 NADH, 2 $FADH_2$
Protons ejected	10 per NADH, 6 per $FADH_2$	112
Proton-powered ATP synthase revolutions	10 protons per revolution	11.2
ATP from ATP synthase	3 per revolution	33.6
ATP from glycolysis		2
GTP from TCA cycle		2
Total		37.6

Processes other than ATP synthesis that are powered by the proton gradient

- Nicotinamide nucleotide transhydrogenase
- Uncoupling proteins; proton leak
- Secondary active transport:
  - ATP^{4-}/ADP^{3-} antiport
  - phosphate/H^+ symport
  - amino acid/H^+ symport
  - pyruvate/H^+ symport

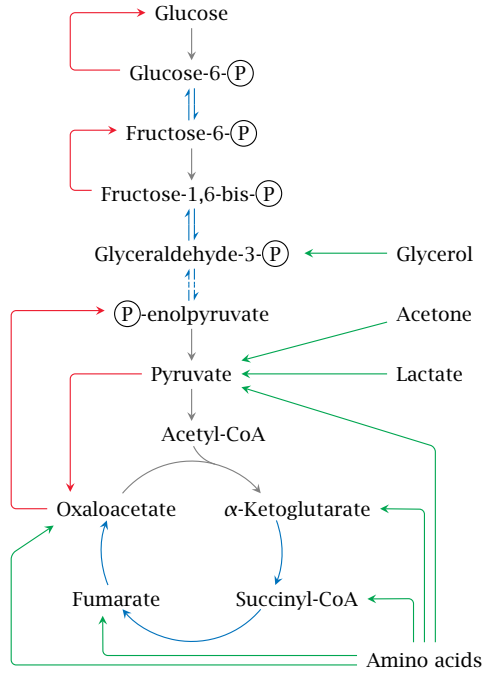
Chapter 7

Gluconeogenesis

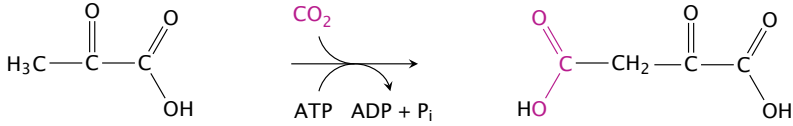
Glucose is an indispensable metabolite

- The brain requires at least ~50% of its calories in the form of glucose
- Red blood cells exclusively subsist on glucose
- Glucose is a precursor of other sugars needed in the biosynthesis of nucleotides, glycoproteins, and glycolipids
- Glucose is needed to replenish NADPH, which supplies reducing power for biosynthesis and detoxification

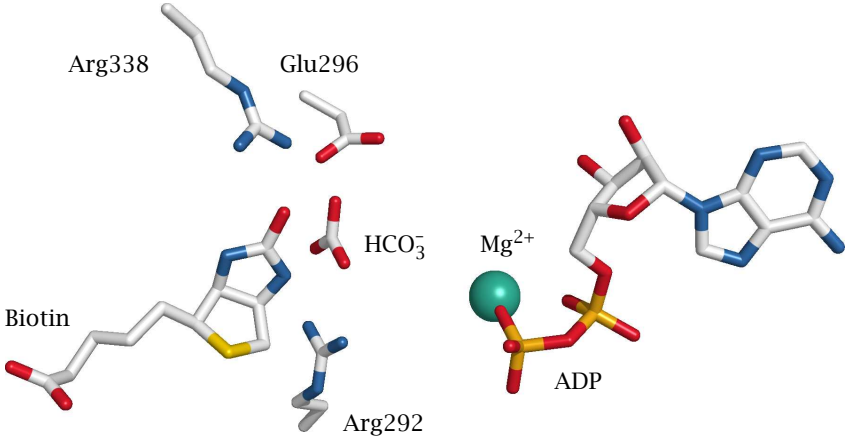
Overview of gluconeogenesis



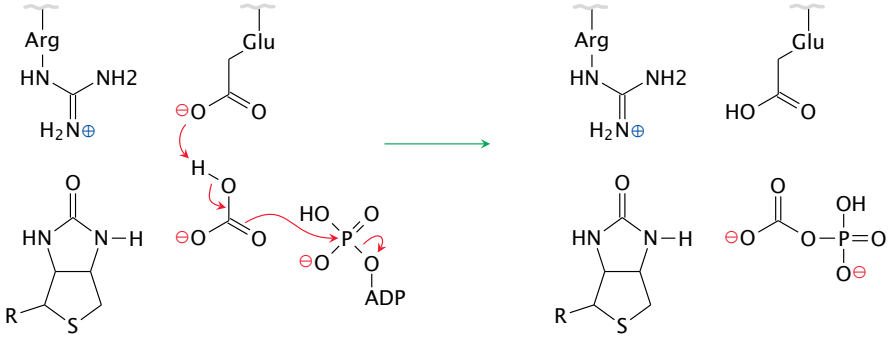
The pyruvate carboxylase reaction



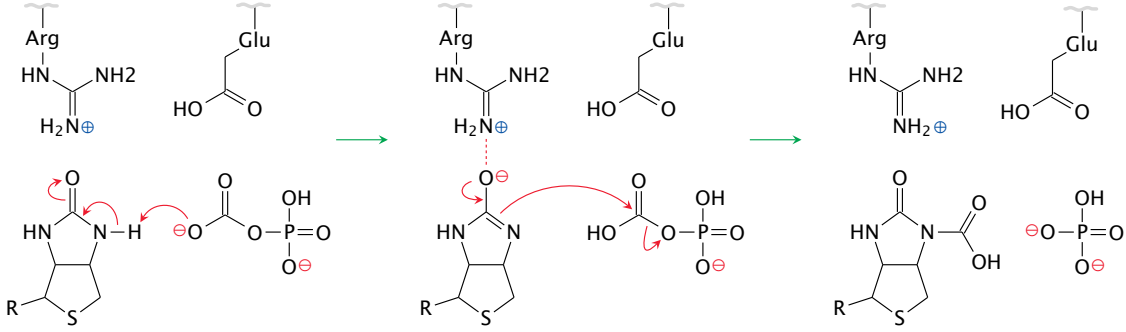
The active site of *E. coli* biotin carboxylase



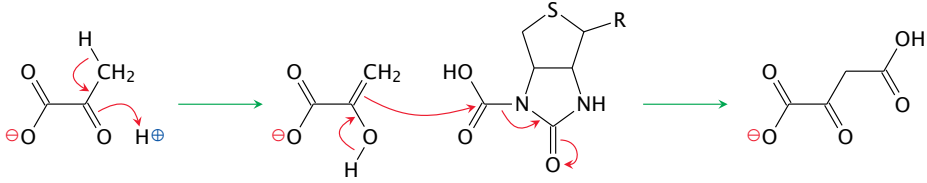
Activation of bicarbonate



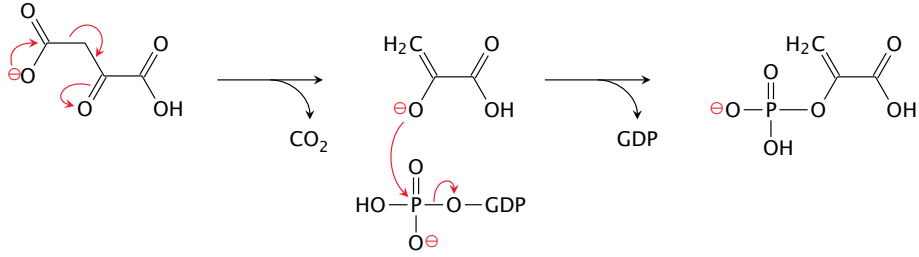
The carboxylation of biotin



The carboxylation of pyruvate



The phosphoenolpyruvate carboxykinase reaction



Fructose-1,6-bisphosphatase and glucose-6-phosphatase

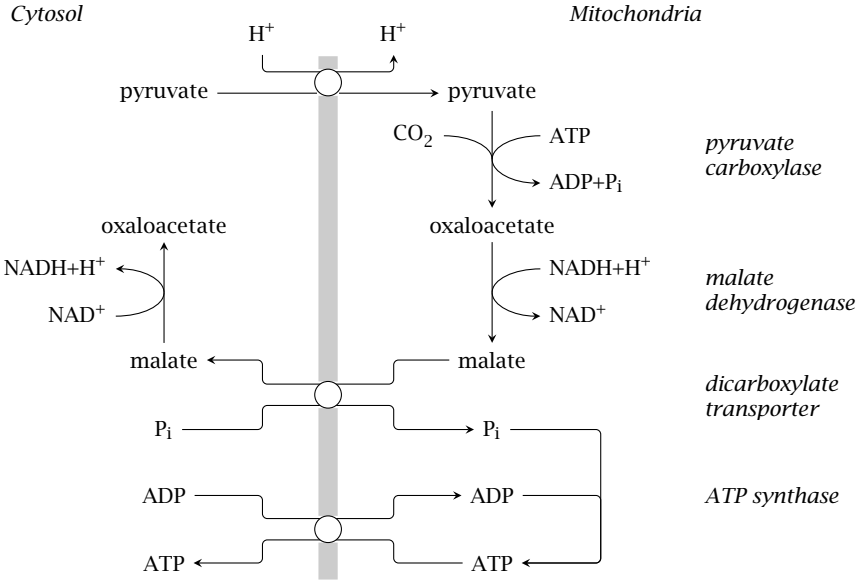
$$\text{Fructose-1,6-bisphosphate} + H_2O \longrightarrow \text{Fructose-6-phosphate} + P_i$$

$$\text{Glucose-6-phosphate} + H_2O \longrightarrow \text{Glucose} + P_i$$

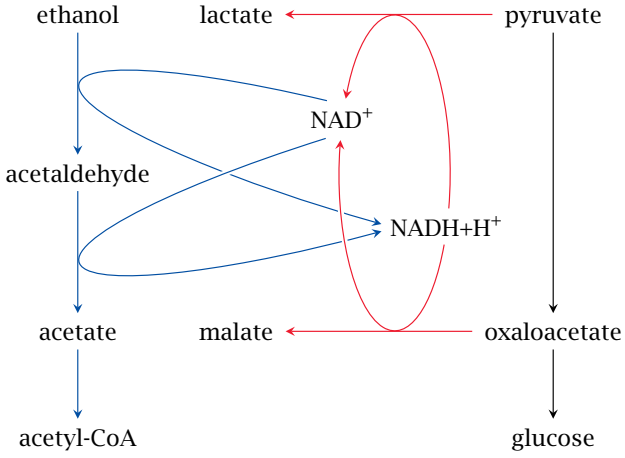
Energy balance of gluconeogenesis

Reaction			ATP/GTP input
2 pyruvate	$\longrightarrow$	2 oxaloacetate	2
2 oxaloacetate	$\longrightarrow$	2 PEP	2
2 3-Ⓟ-glycerate	$\longrightarrow$	2 1,3-bis-Ⓟ-glycerate	2
Total			6

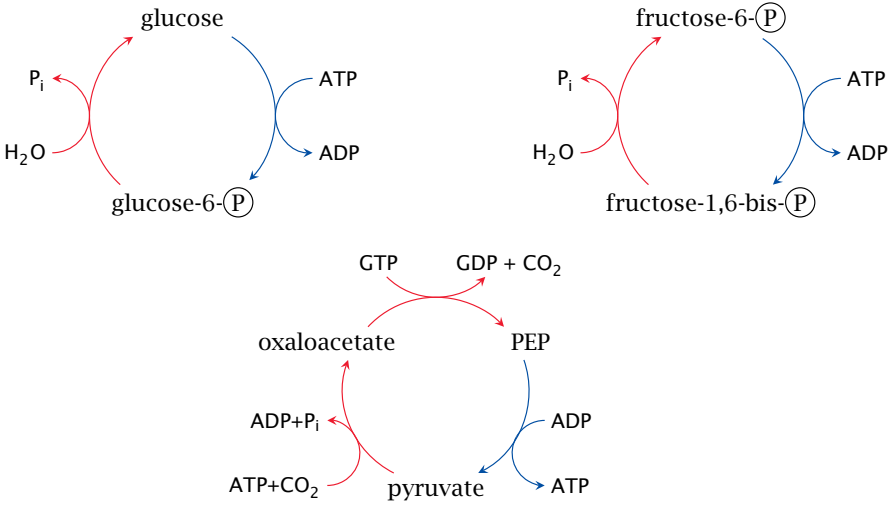
Mitochondrial substrate transport in gluconeogenesis



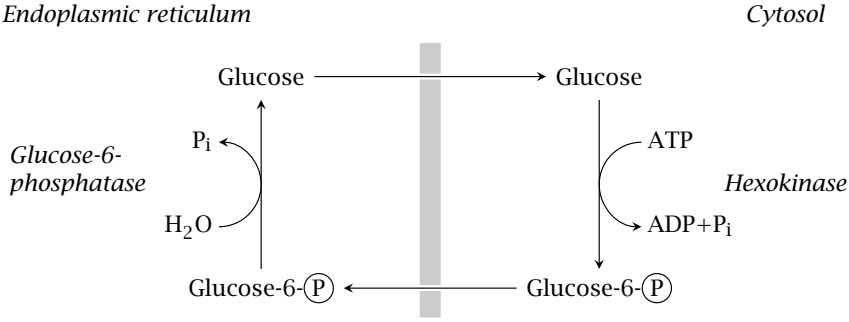
Ethanol degradation inhibits gluconeogenesis



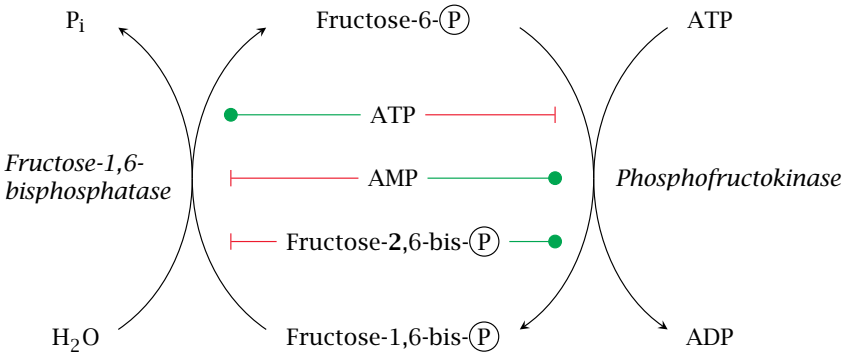
Simultaneous activity of glycolysis and gluconeogenesis creates futile cycles



Glucose phosphorylation cycling involves two separate compartments

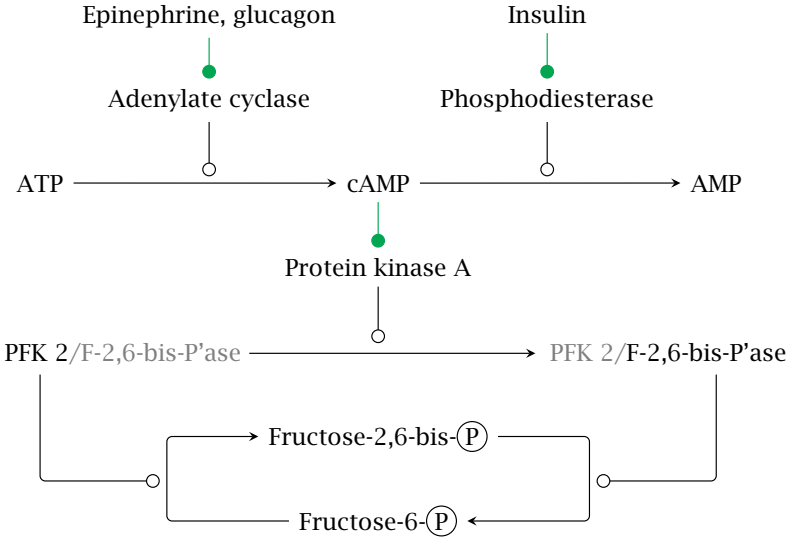


Allosteric regulation limits fructose-6-phosphate phosphorylation cycling

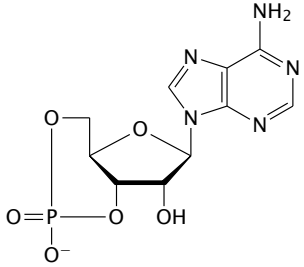


- substrate cycling

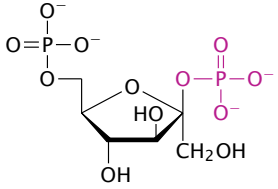
The level of fructose-2,6-bisphosphate is controlled by hormones



The secondary messengers cAMP and fructose-2,6-bisphosphate



cyclic 3′,5′-AMP (cAMP)



fructose-2,6-bisphosphate

Regulation of pyruvate kinase

- allosteric activation by fructose-1,6-bisphosphate
- allosteric inhibition by ATP and alanine
- inhibition by PKA-mediated phosphorylation

Chapter 8

Glycogen metabolism

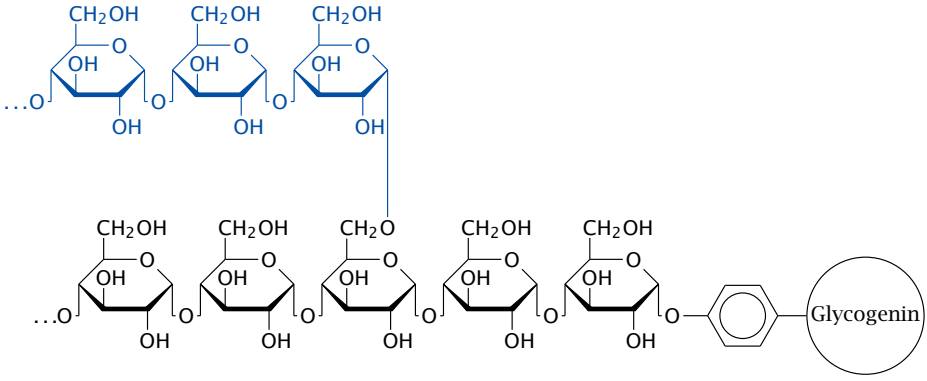
Why store glucose in polymeric form?

- The osmotic pressure is governed by the gas equation:

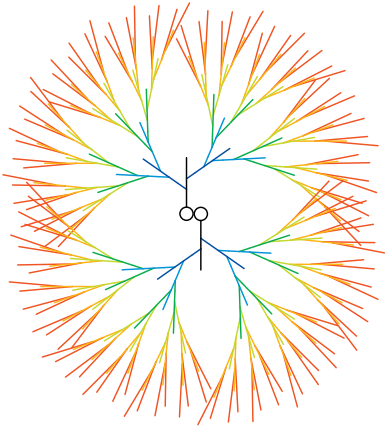
$$pV = nRT \iff p = \frac{n}{V}RT$$

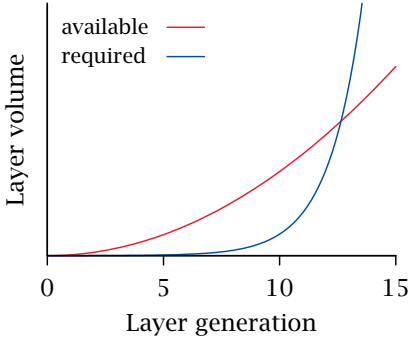
- Glycogen amounts to 10% of the liver's wet weight, equivalent to 600 mM glucose
- When free, 600 mM glucose would triple the osmotic activity of the cytosol—liver cells would swell and burst
- Linking 2 (3, ...) molecules of glucose divides the osmotic effect by 2 (3, ...), permitting storage of large amounts of glucose at physiological osmolarity

Covalent structure of glycogen



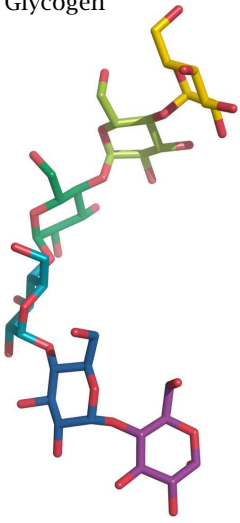
The size of glycogen particles is limited by crowding in the outer layers



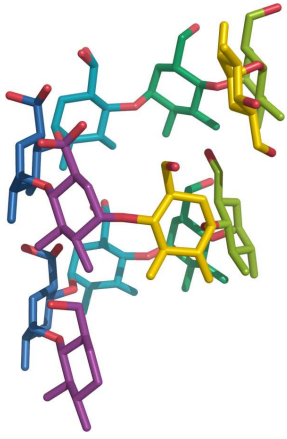


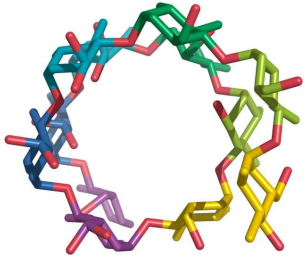
Glycogen is more loosely packed and more soluble than amylose

Glycogen



Amylose





Life cycle of glycogen

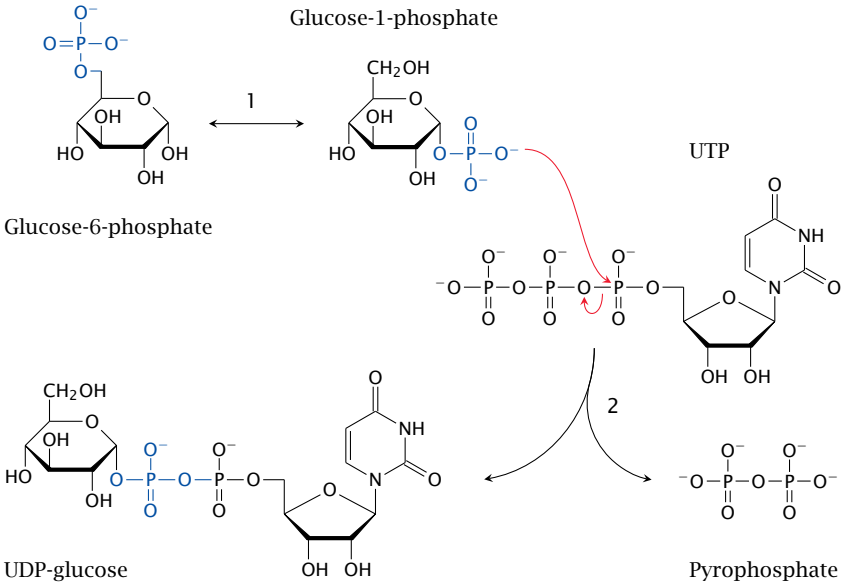
Synthesis:

1. synthesis of an activated precursor, UDP-glucose, by UTP:glucose-1-phosphate uridylyltransferase
2. initiation of glycogen synthesis by glycogenin
3. introduction of branches by branching enzyme
4. chain elongation by glycogen synthase
5. repeat steps 3 and 4

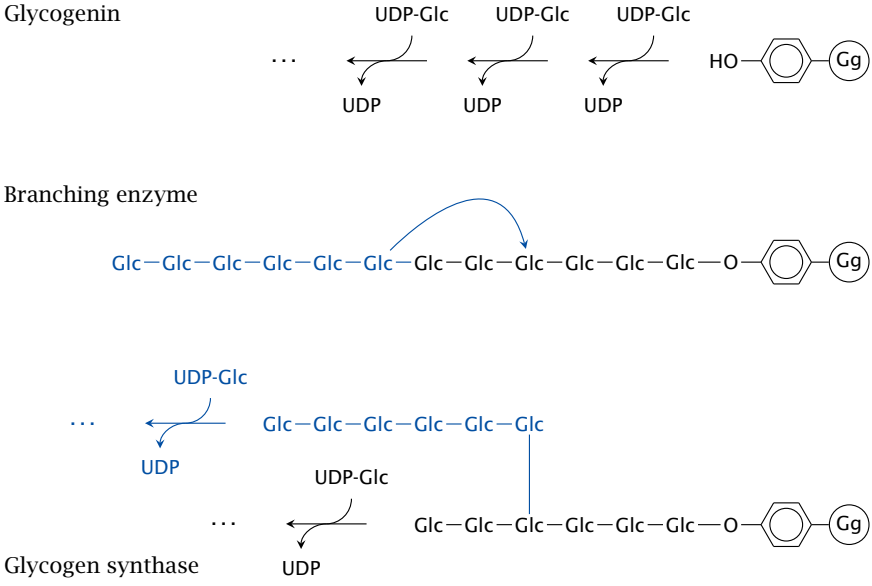
Degradation:

1. depolymerization of linear strands by phosphorylase
2. removal of branches by debranching enzyme
3. repeat steps 1 and 2

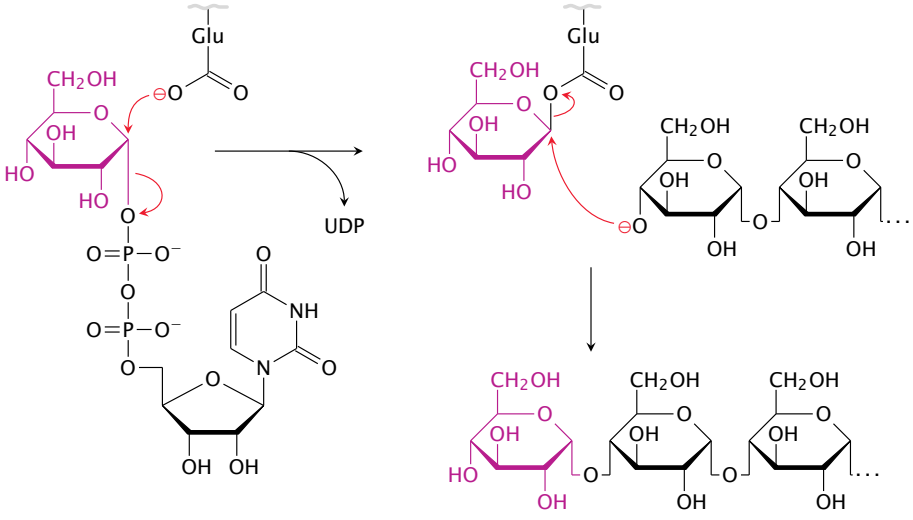
Activation of glucose for glycogen synthesis



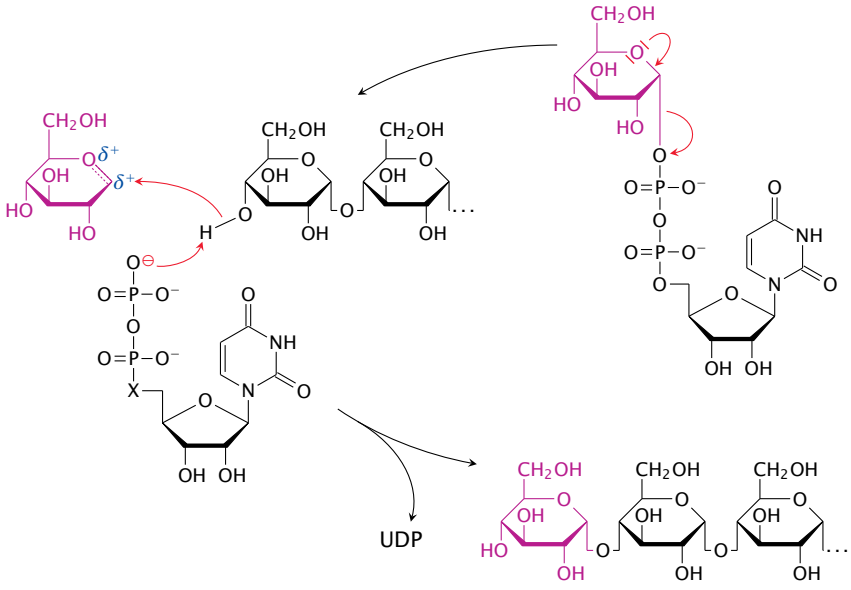
Overview of glycogen synthesis



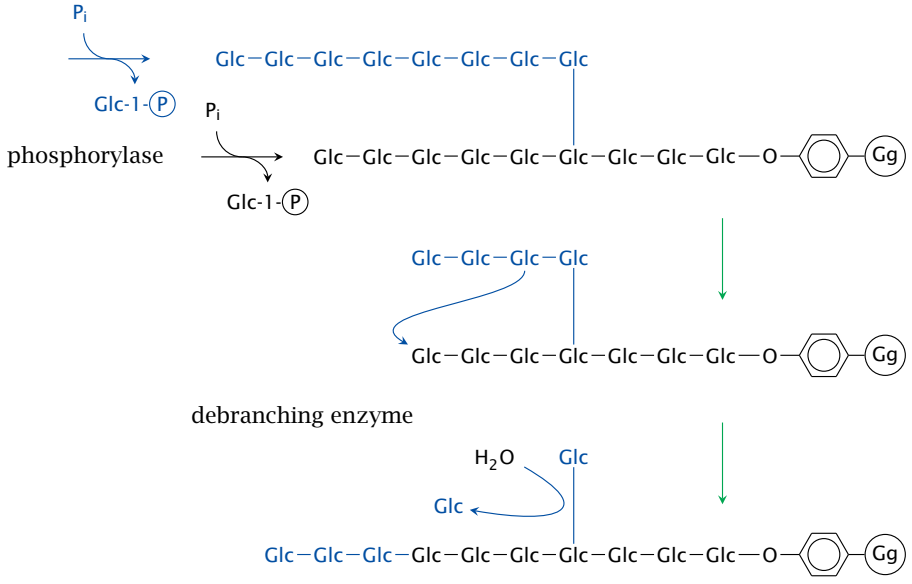
A hypothetical reaction mechanism of glycogen synthase



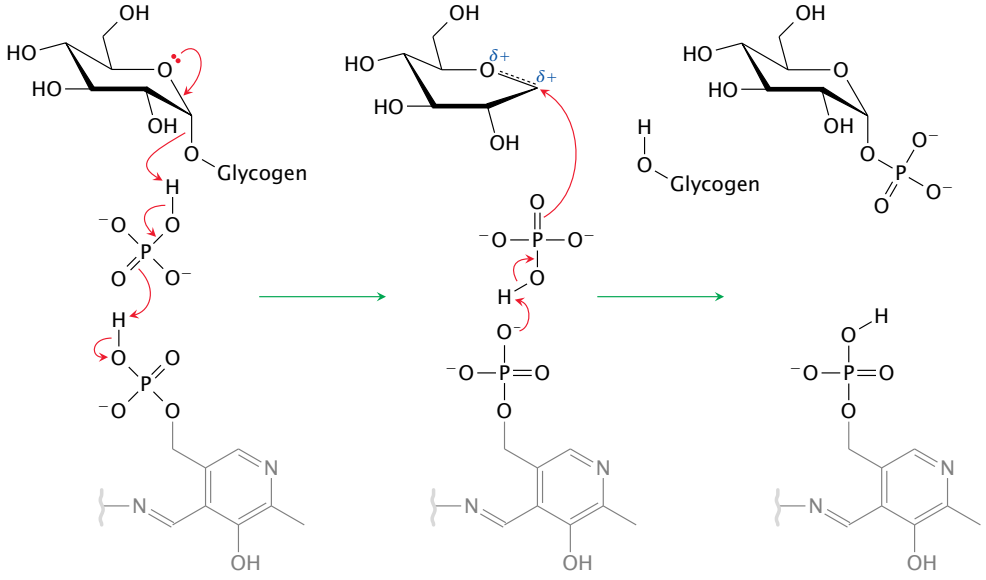
An alternative glycogen synthase mechanism



Overview of glycogen degradation



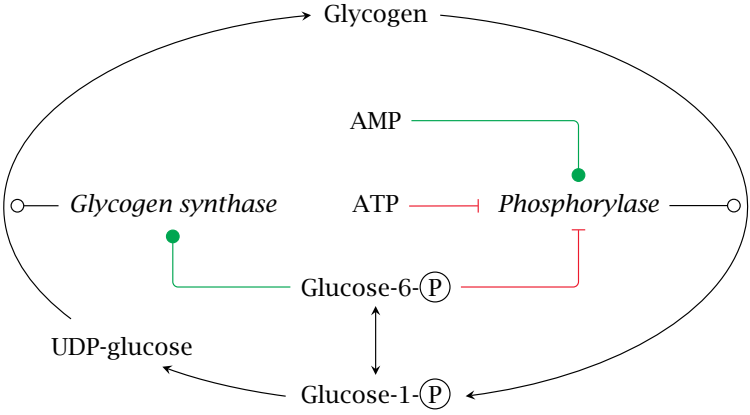
The reaction mechanism of phosphorylase



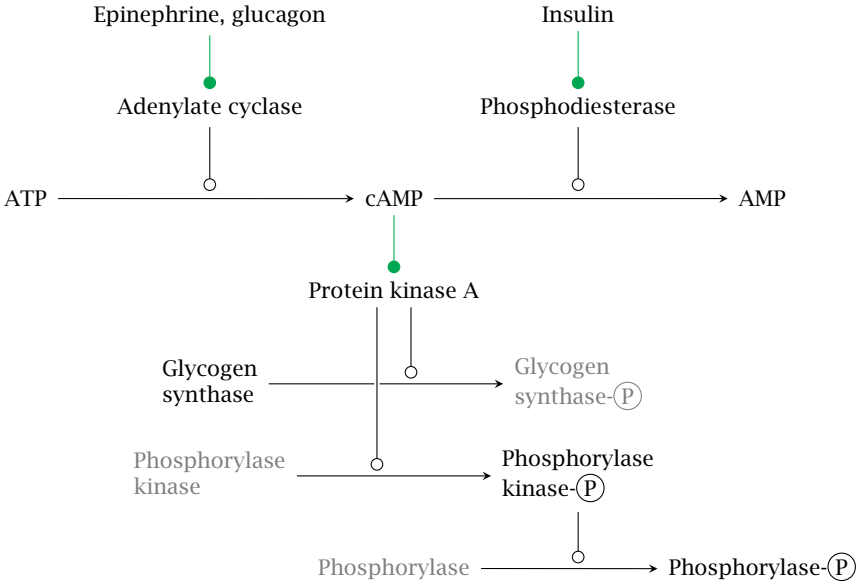
Lysosomal glycogen disposal

- concerns a minor fraction of glycogen
- key enzyme: acid maltase; enzyme defect causes slow but inexorable glycogen accumulation
- possible role: disposal of structurally aberrant glycogen particles that have become “tangled up” during repeated cycles of glucose accretion and depletion

Allosteric regulation of glycogen synthase and phosphorylase



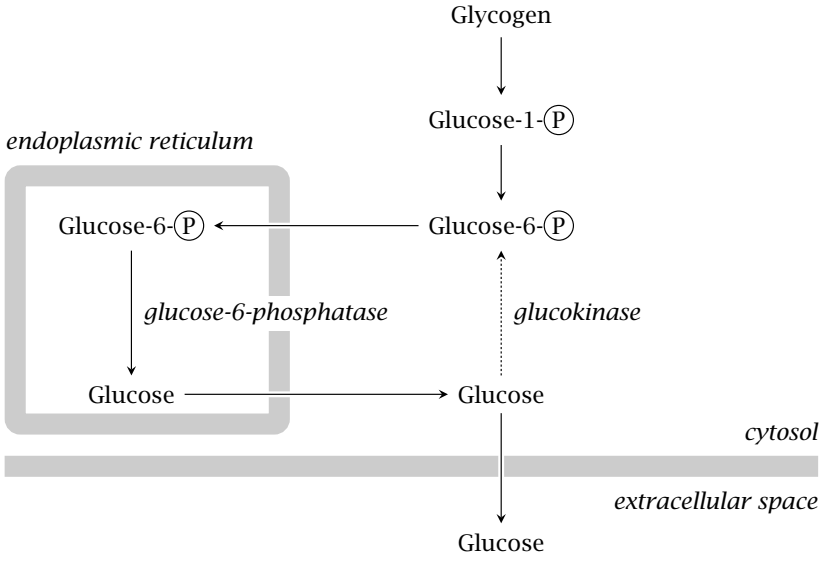
Hormonal control of glycogen metabolism



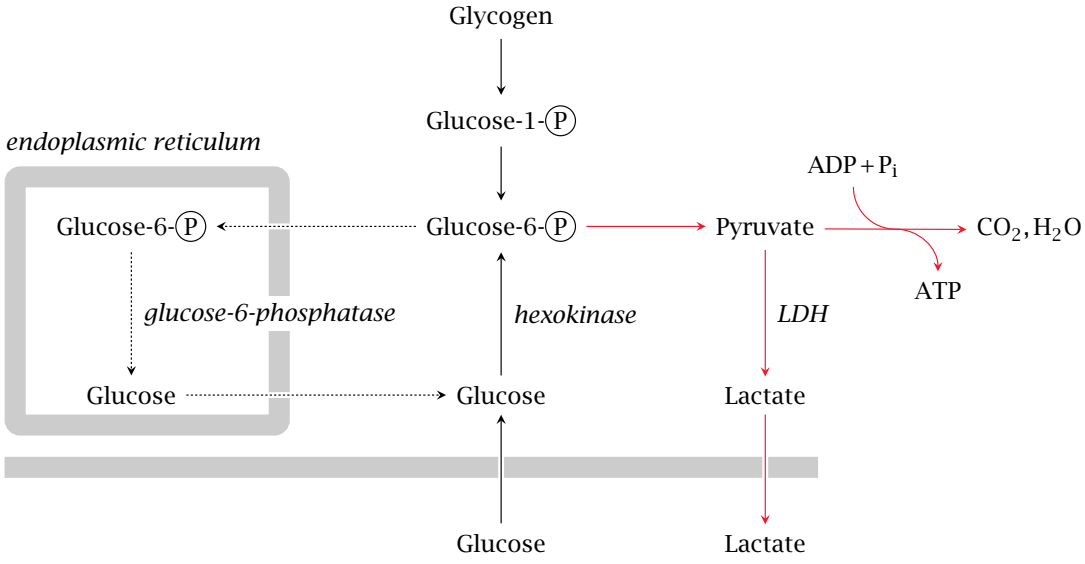
Regulatory differences between liver and muscle phosphorylase

	Liver enzyme	Muscle enzyme
Inhibition by glucose	+	−
Activation by Ca^{2+}	−	+
Activation by AMP even when unphosphorylated	−	+

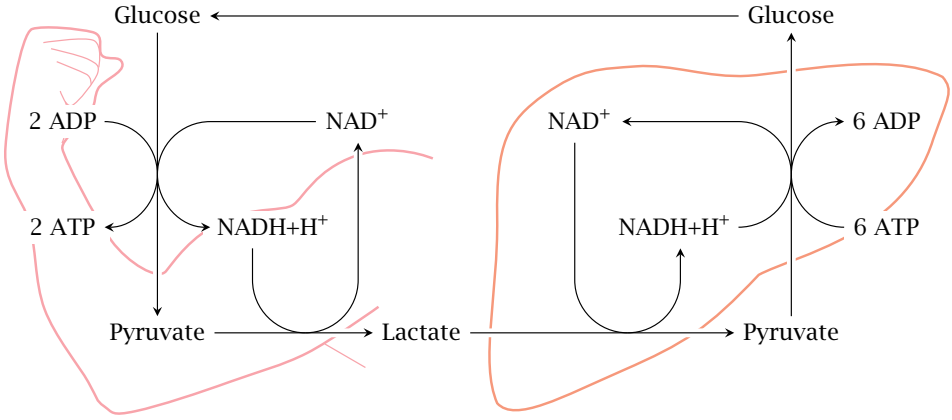
Liver glycogen utilization



Muscle glycogen utilization



The Cori cycle



Glucose-6-phosphatase deficiency (von Gierke disease)

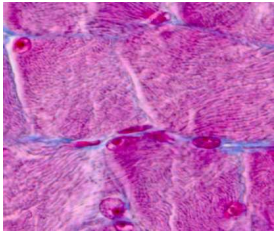
Biochemical defect:

- glucose-6-phosphate formed in gluconeogenesis or glycogen degradation cannot be converted to free glucose
- glucose cannot be exported from liver and kidney cells

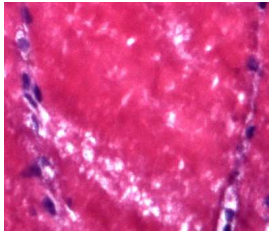
Clinical manifestations:

- glycogen builds up in liver and kidneys (organ enlargement and functional impairment)
- severe hypoglycemia
- lactic acidosis
- hyperlipidemia
- hyperuricemia

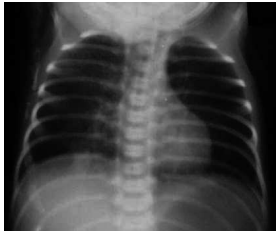
Acid maltase deficiency (Pompe disease)



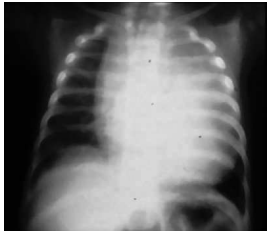
Normal skeletal muscle (transverse section)



Glycogen aggregates in Pompe disease



Infant chest X-ray, normal heart



Infant with Pompe disease, distended heart

Muscle phosphorylase deficiency (McArdle's disease)

- Deficient glycogen breakdown inhibits rapid ATP replenishment
- Patients experience rapid exhaustion and muscle pain during exertion
- Liver phosphorylase and blood glucose homeostasis remain intact

▸ muscle glycogen utilization

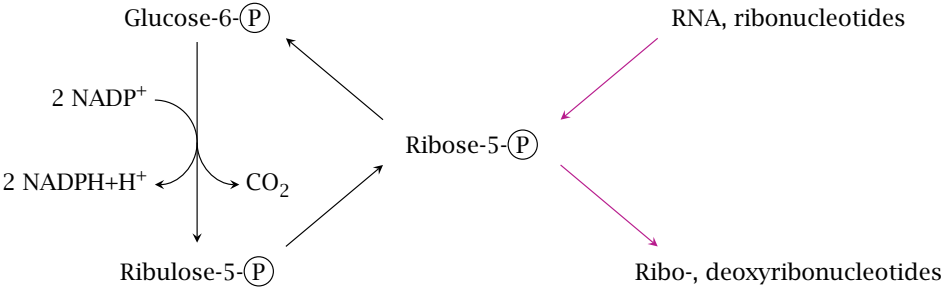
Lafora disease

- deficiency for laforin, a glycogen phosphatase
- accumulation of hyper-phosphorylated glycogen (Lafora bodies)
- patients develop epilepsy, dementia

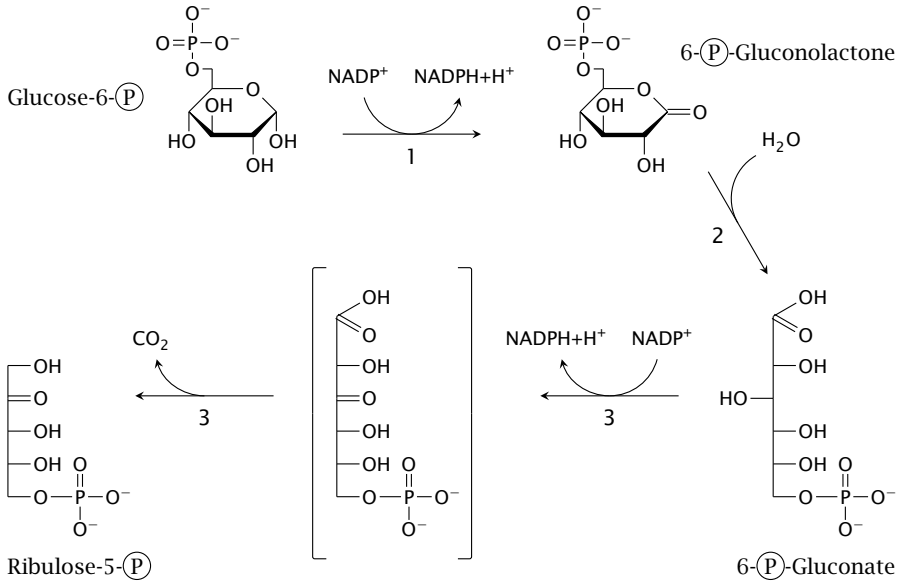
Chapter 9

The hexose monophosphate shunt

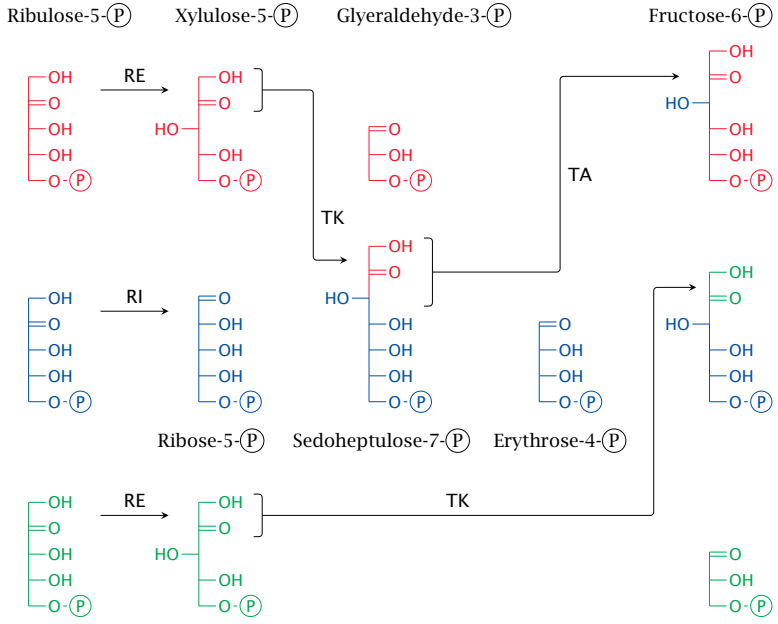
The hexose monophosphate shunt: Overview



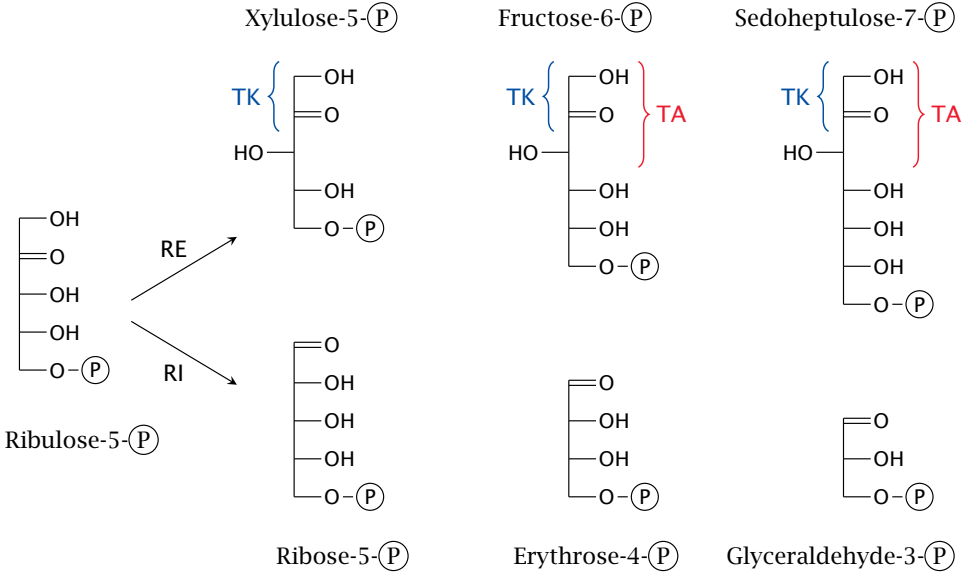
Reactions in the oxidative stage



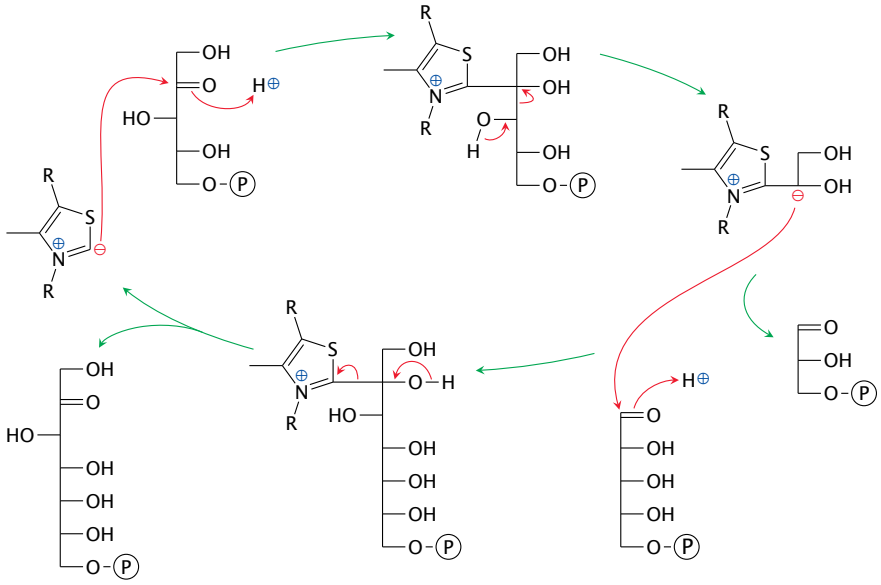
Reactions in the sugar shuffle stage



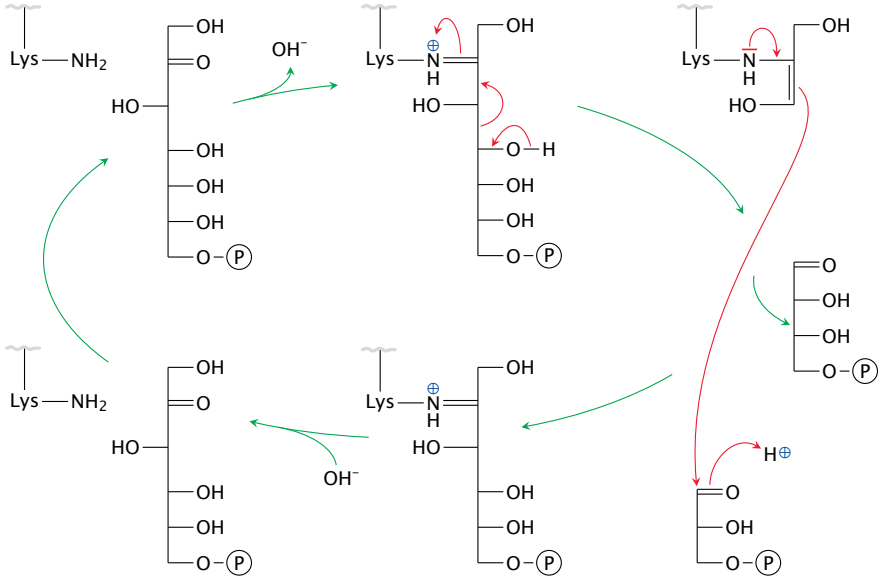
Ketoses and aldoses in the HMS



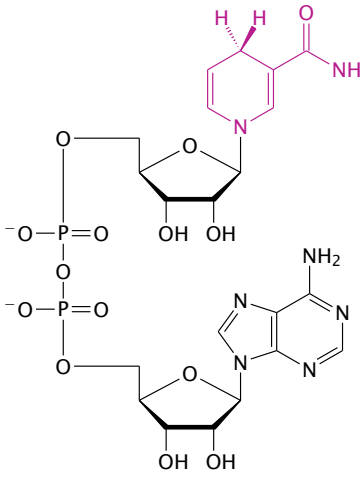
The mechanism of transketolase

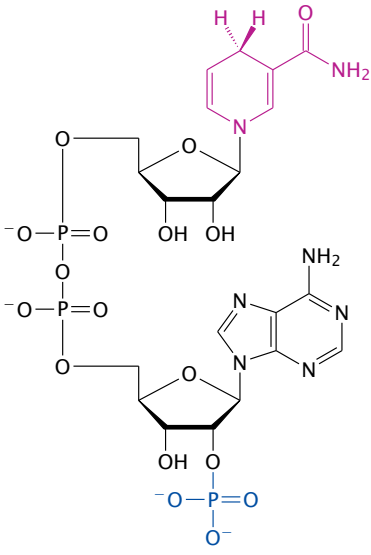


The mechanism of transaldolase

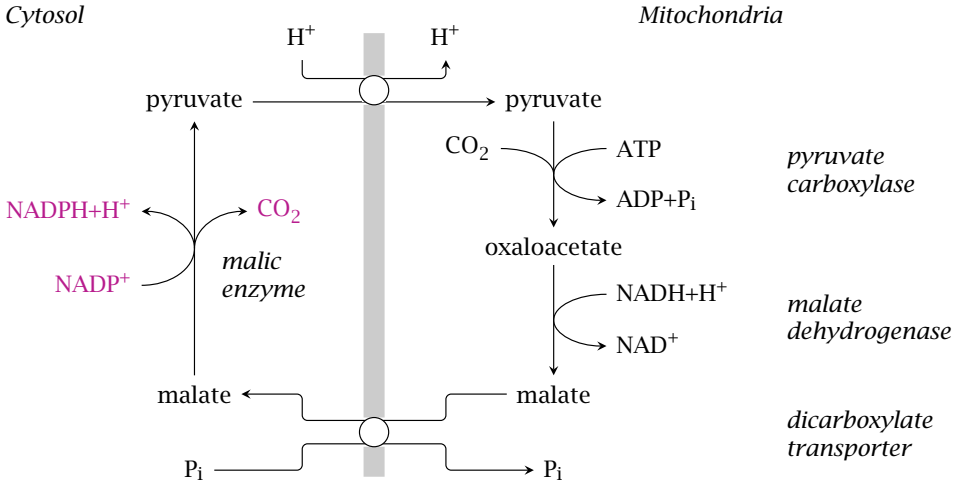


Why do we need both NADH and NADPH?

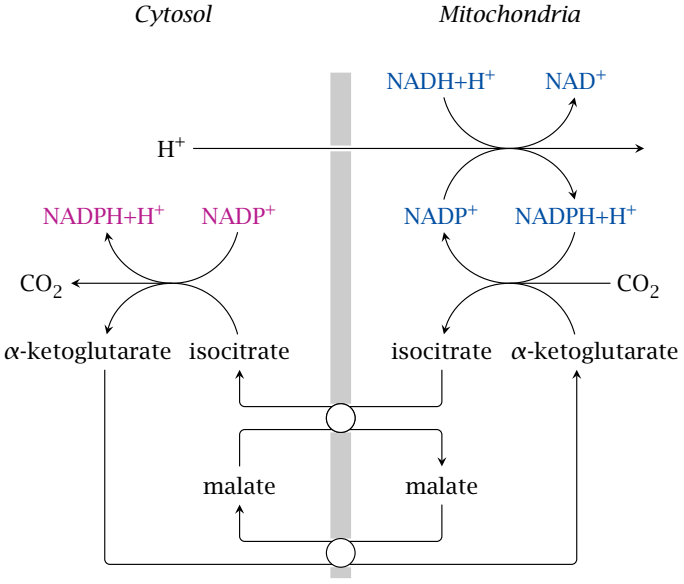




NADPH generation by malic enzyme



NADPH generation by transhydrogenase and NADP-linked isocitrate dehydrogenase



nicotinamide nucleotide transhydrogenase

isocitrate dehydrogenase

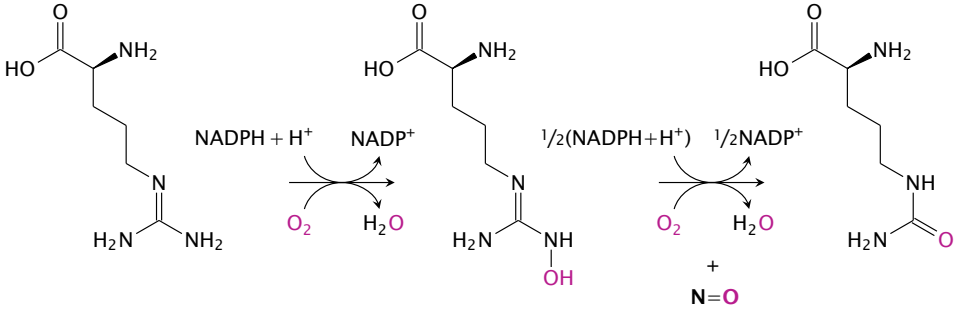
citrate/isocitrate carrier

oxoglutarate carrier

Uses of NADPH

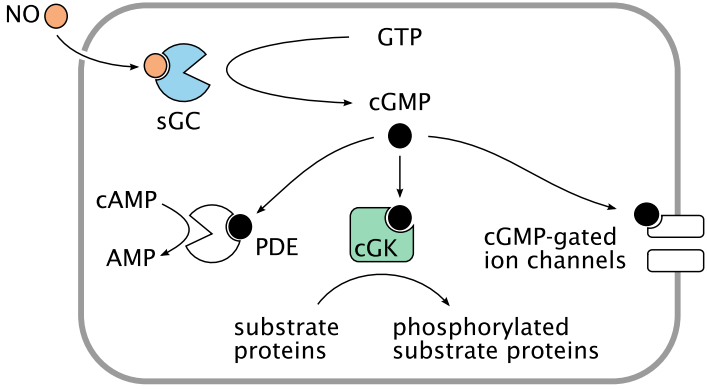
1. synthesis of fatty acids and cholesterol
2. fixation of ammonia by glutamate dehydrogenase
3. oxidative metabolism of drugs and poisons by cytochrome P450 enzymes
4. generation of nitric oxide and of reactive oxygen species by phagocytes
5. *scavenging* of reactive oxygen species that form as byproducts of oxygen transport and of the respiratory chain

The nitric oxide synthase reaction

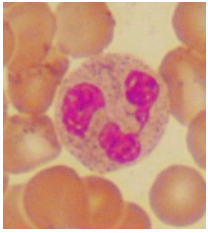


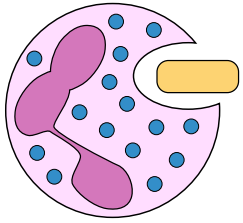
arginine N-hydroxyarginine citrulline

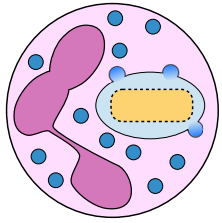
Signaling effects of nitric oxide



Phagocytes use NADPH to generate reactive oxygen species





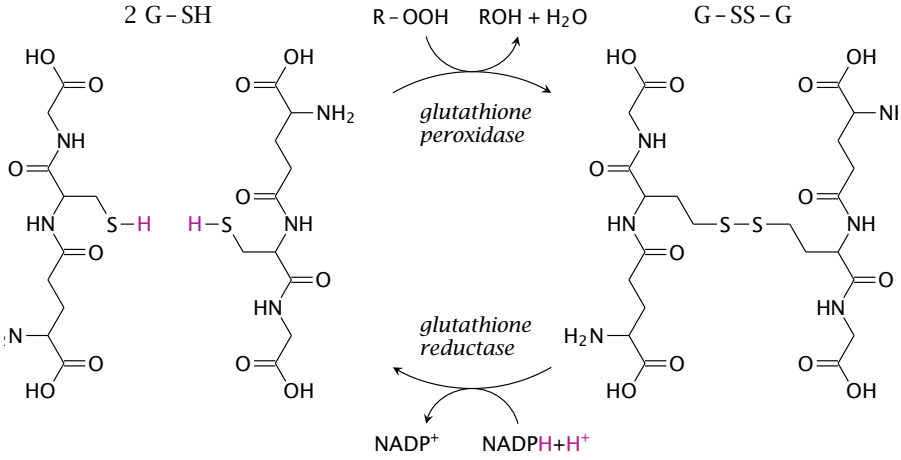


$$2O_2 + \text{NADPH} \xrightarrow{\textit{NADPH oxidase}} 2O_2^- + \text{NADP}^+ + H^+$$

$$2O_2^- + 2H^+ \xrightarrow{\textit{Superoxide dismutase}} H_2O_2 + O_2$$

$$H_2O_2 + Cl^- \xrightarrow{\textit{Myeloperoxidase}} OCl^- + H_2O$$

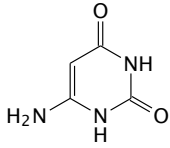
Scavenging of reactive oxygen species requires NADPH, too



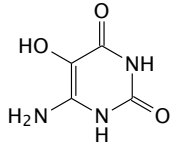
Glucose-6-phosphate dehydrogenase deficiency

- most patients are healthy most of the time—hemolytic crises occur upon exposure to drugs or diet components that cause enhanced formation of ROS
- manifest in red blood cells because these cells lack protein synthesis—no replacement of deficient protein molecules
- affords partial protection against malaria—similar to sickle cell anemia and other hemoglobinopathias
- X-chromosomally encoded—males more severely affected

Vicia faba and favism

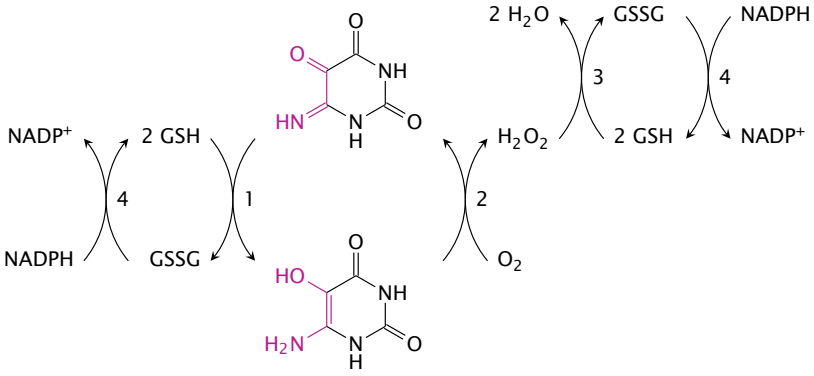


Divicine

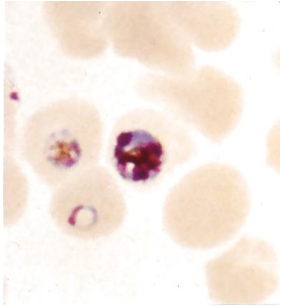


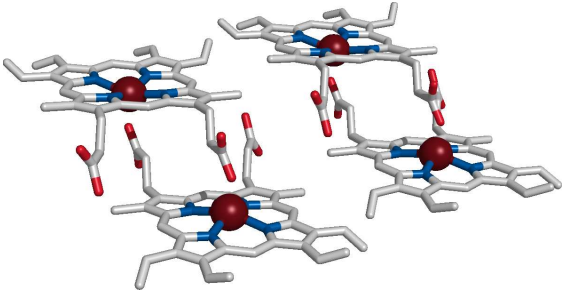
Isouramil

Redox cycling of isouramil

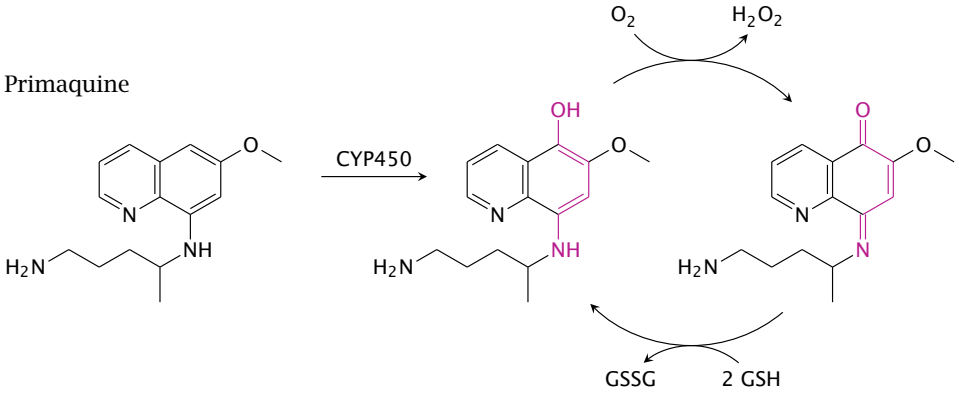


Malaria parasites detoxify heme by crystallization





Primaquine and glucose-6-phosphate dehydrogenase deficiency



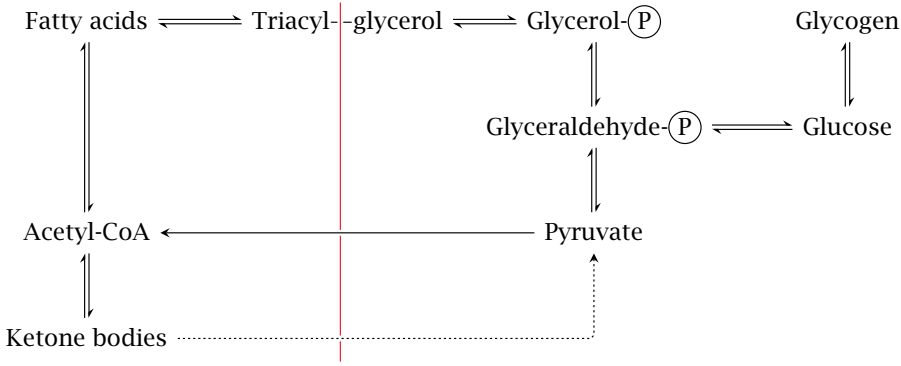
Chapter 10

Triacylglycerol metabolism

Foodstuffs and their energy contents

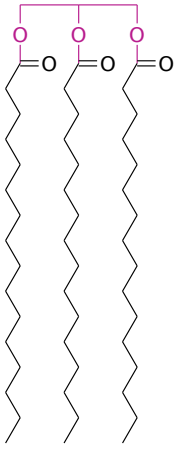
Foodstuff	Energy (kcal/g)
protein	4
carbohydrates	4
triacylglycerol	9
alcohol	7

Carbon pools in carbohydrate and fat metabolism

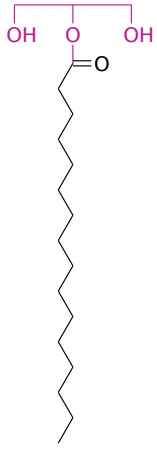


Triacylglycerol and its cleavage products

Triacylglycerol

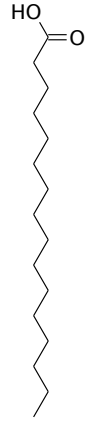


Monoacylglycerol

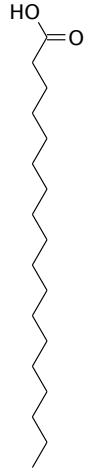


Fatty acids

C16:0



C18:0



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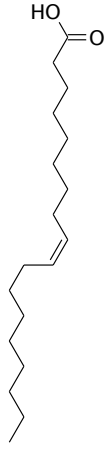
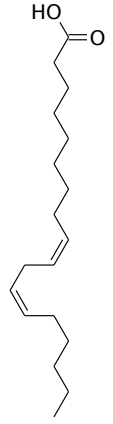
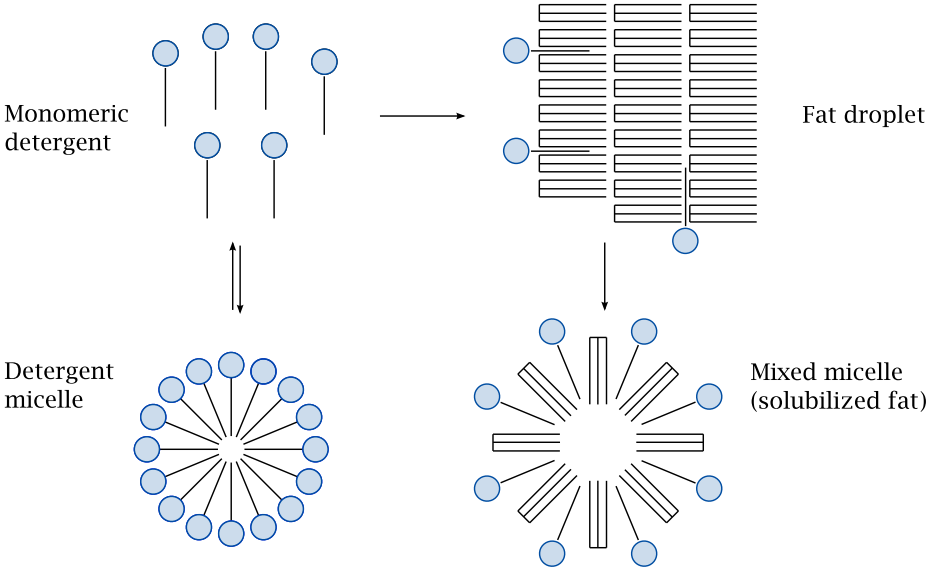


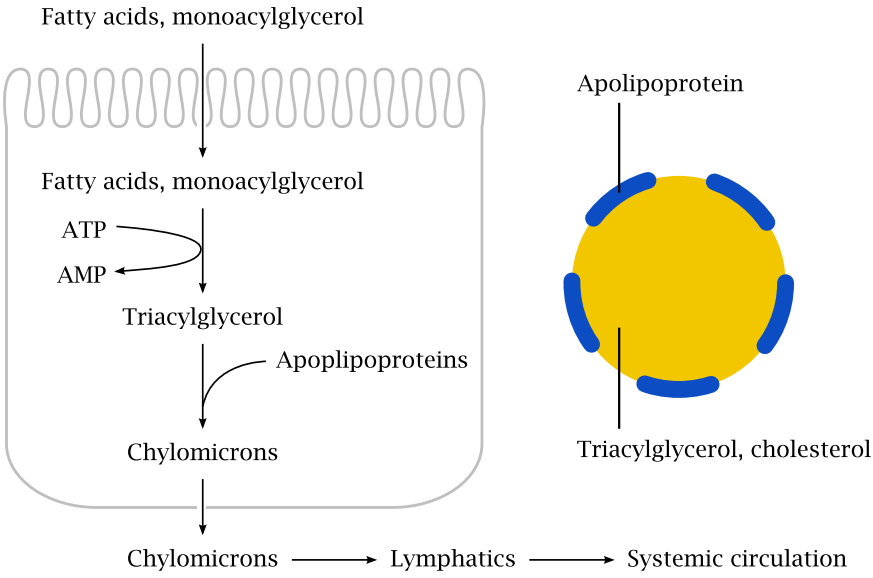
C18:2



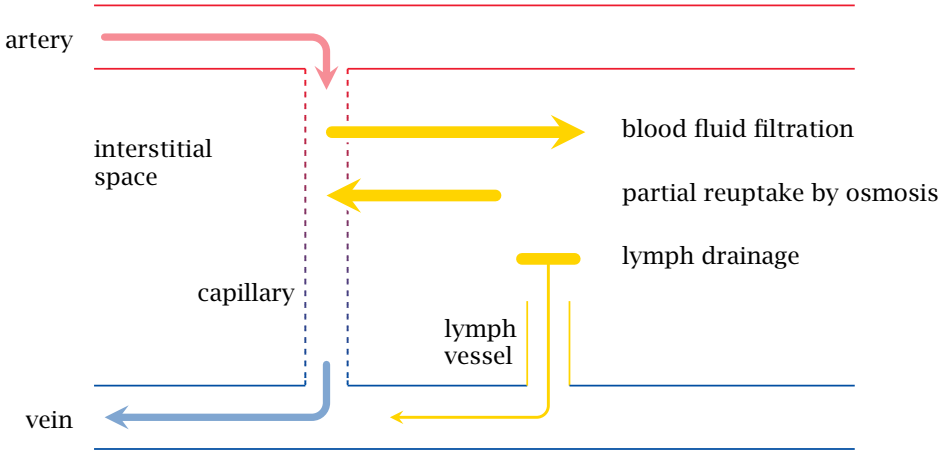
Solubilization of fat by detergents



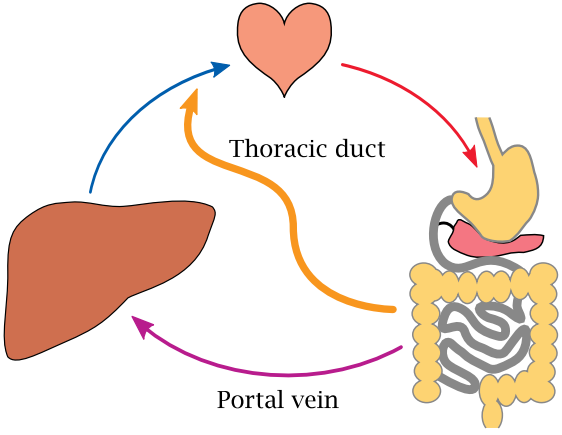
Uptake and re-packaging of digested fat in the small intestine



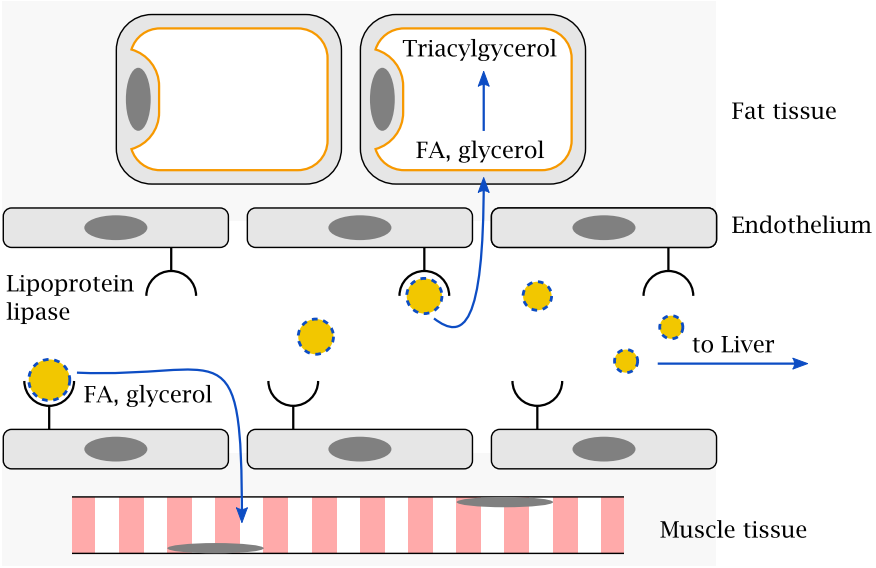
The lymphatics drain excess fluid from the interstitial space



Chylomicrons are drained from the intestine through the lymphatics, bypassing the liver



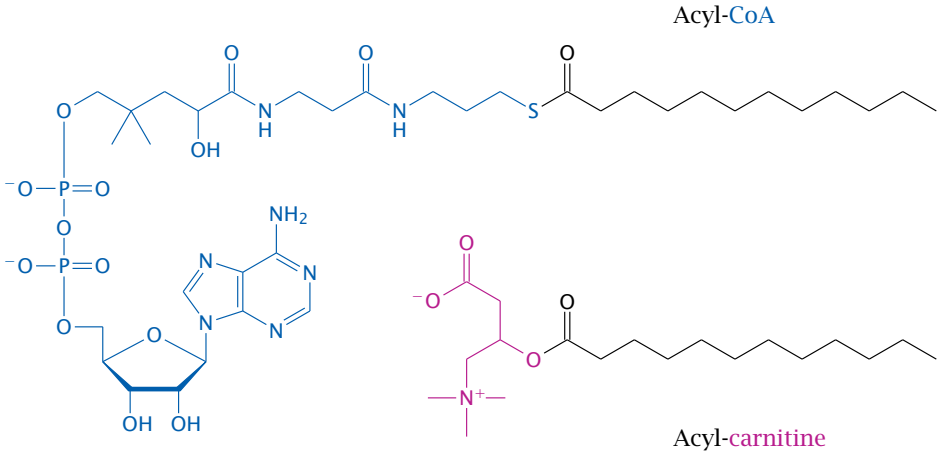
Lipoprotein lipase extracts triacylglycerol from chylomicrons



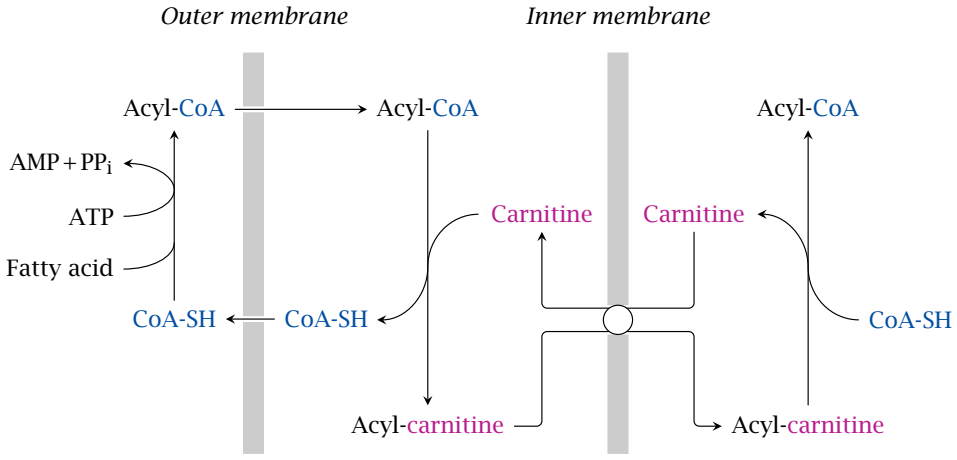
Medium-chain fatty acids

- contain less than 12 carbon atoms
- low content in most foods, but relatively high (10–15%) in palm seed and coconut oil, from which they are industrially prepared
- triglycerides with medium chains are more soluble and more rapidly hydrolyzed by gastric and pancreatic lipase
- not efficiently re-esterified inside intestinal cells; systemic uptake mostly as free fatty acids
- reach mitochondria by diffusion, without prior activation to acyl-CoA and acyl-carnitine

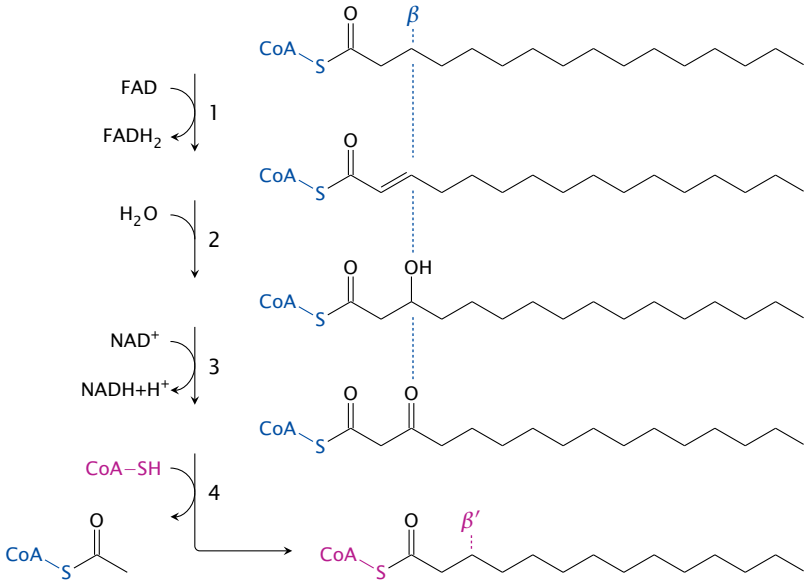
Two activated forms of fatty acids



Activation of fatty acids and transport to the mitochondrion



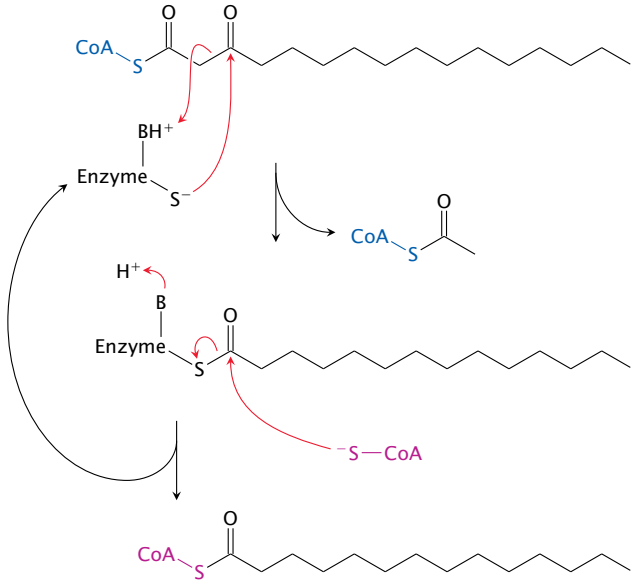
Reactions in β-oxidation



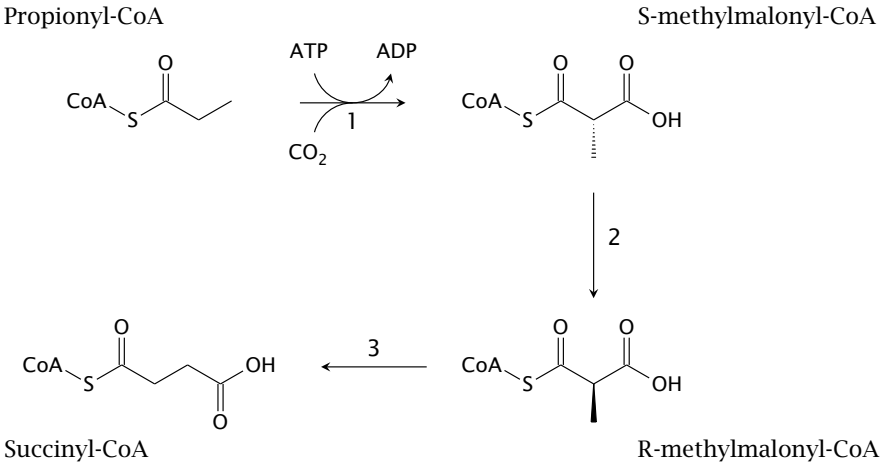
Shared reaction patterns in β-oxidation and TCA cycle

Enzyme	Reaction	Cosubstrate	TCA cycle pendant
acyl-CoA dehydrogenase	dehydrogenation of CH_2-CH_2 bond	FAD	succinate dehydrogenase
enoyl-CoA hydratase	hydration of $CH_2=CH_2$ bond	H_2O	fumarase
hydroxyacyl-CoA dehydrogenase	dehydrogenation of CH – OH bond	NAD^+	malate dehydrogenase

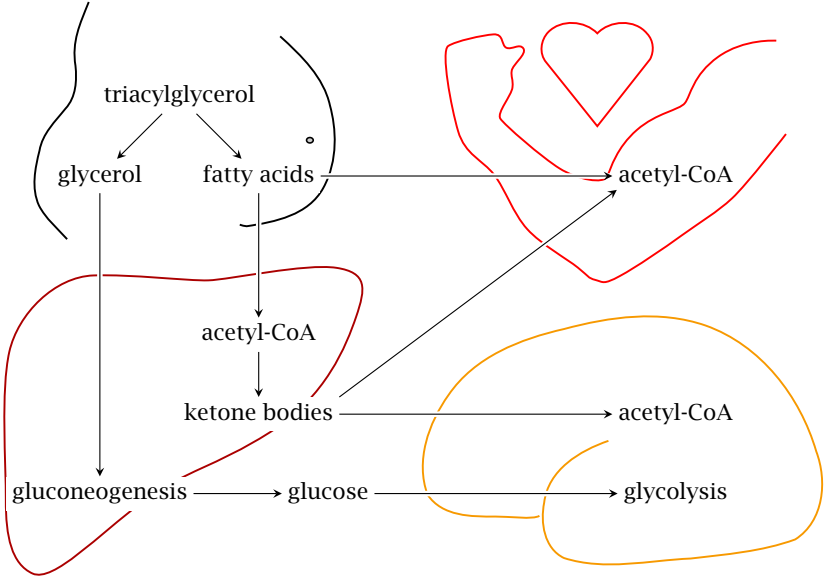
The reaction mechanism of thiolase



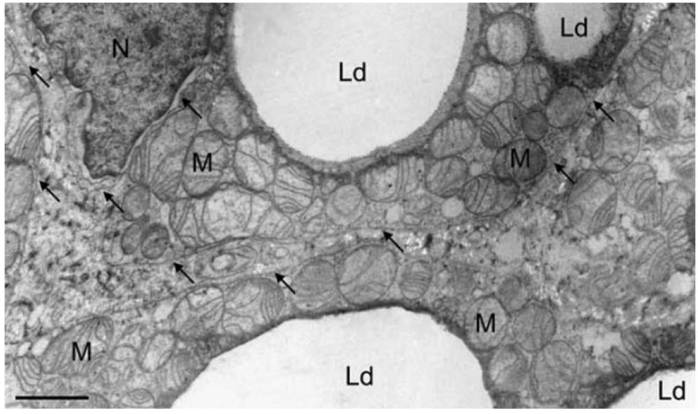
Utilization of propionate



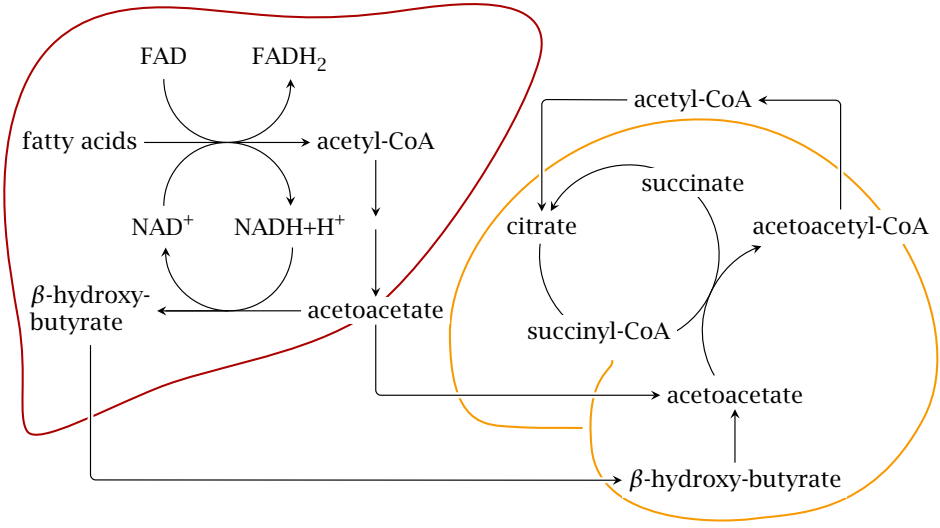
Organ relationships in triacylglycerol utilization



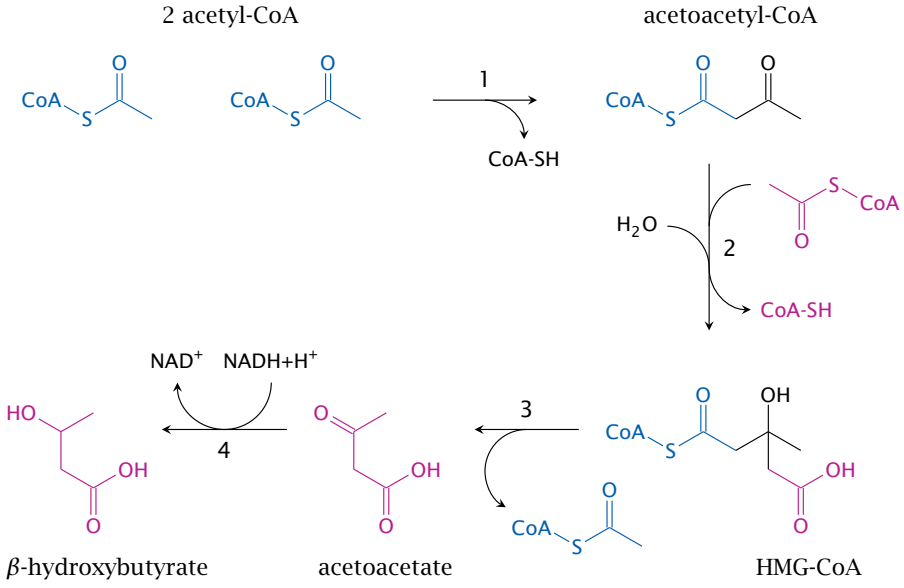
Brown fat tissue



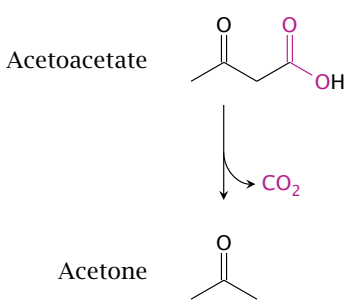
Ketone body metabolism

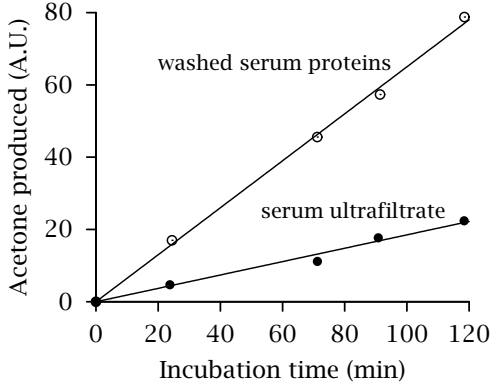


Synthesis of acetoacetate and β-hydroxybutyrate

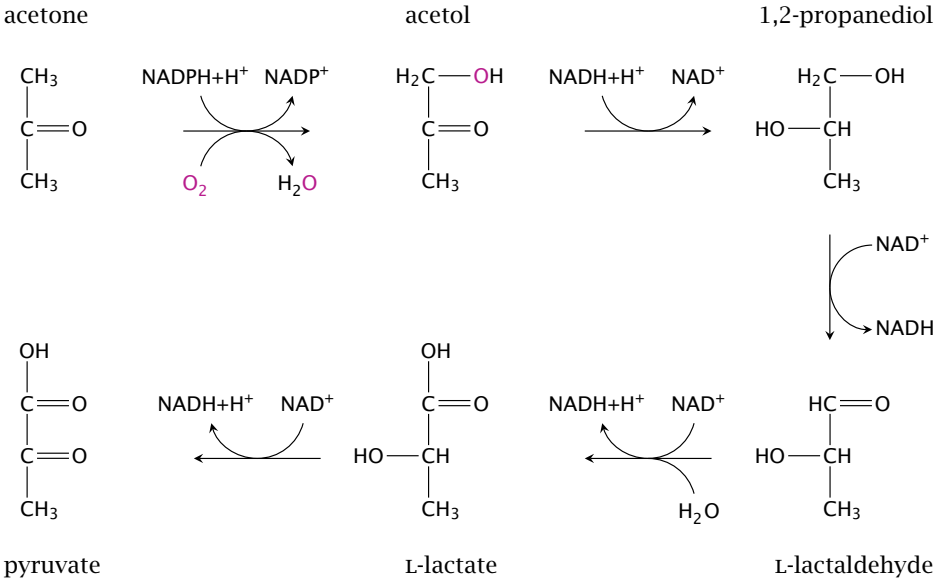


Decarboxylation of acetoacetate





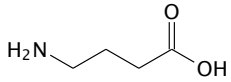
Acetone can serve as a precursor for gluconeogenesis

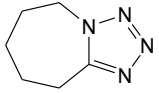


- carbon pools

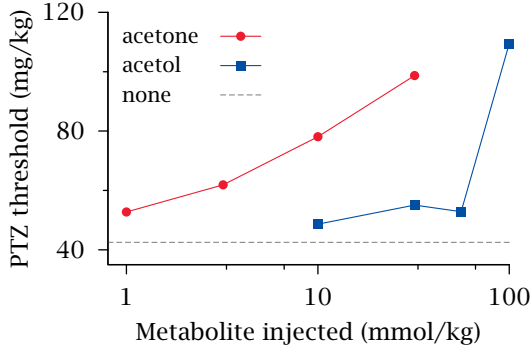
Anticonvulsant effects of acetone and acetol

γ-Aminobutyric acid (GABA)





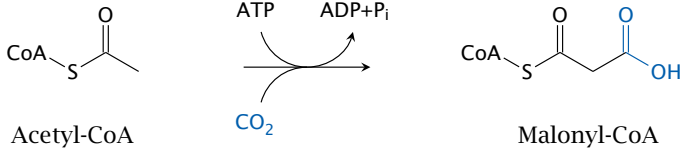
Pentylenetetrazole (PTZ)



Fatty acid synthesis

- carried out mostly by one large cytosolic enzyme (fatty acyl synthase)
- uses acetyl-CoA, which is activated by carboxylation
- reducing power provided by NADPH
- final product: palmitate (hexadecanoate)
- elongation and desaturation carried out by dedicated enzymes in the ER

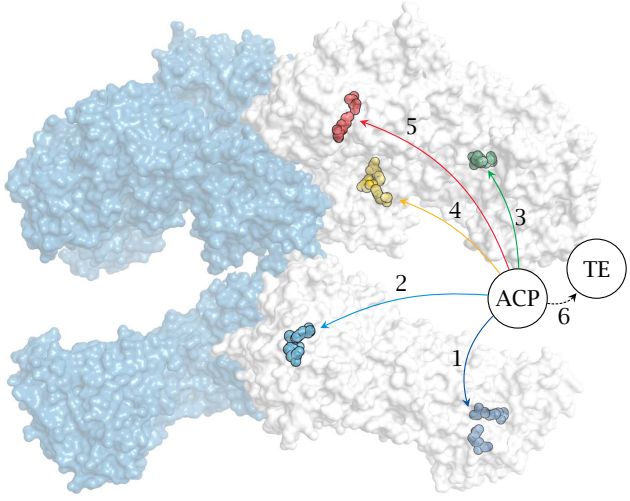
The acetyl-CoA carboxylase reaction



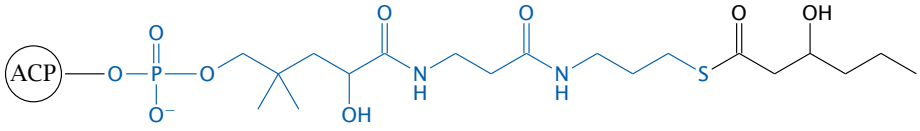
Acetyl-CoA

Malonyl-CoA

The structure of fatty acid synthase

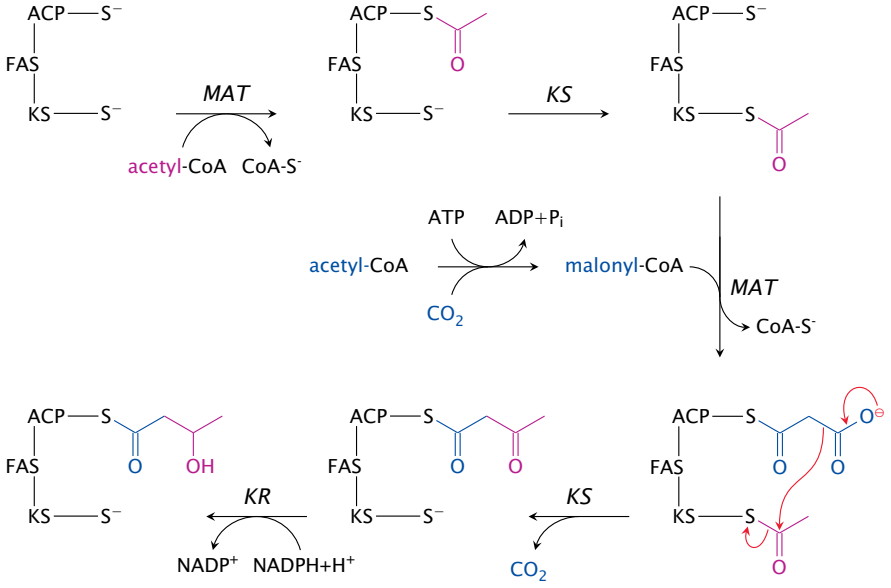


Phosphopantetheine acts as a flexible tether in acyl carrier protein

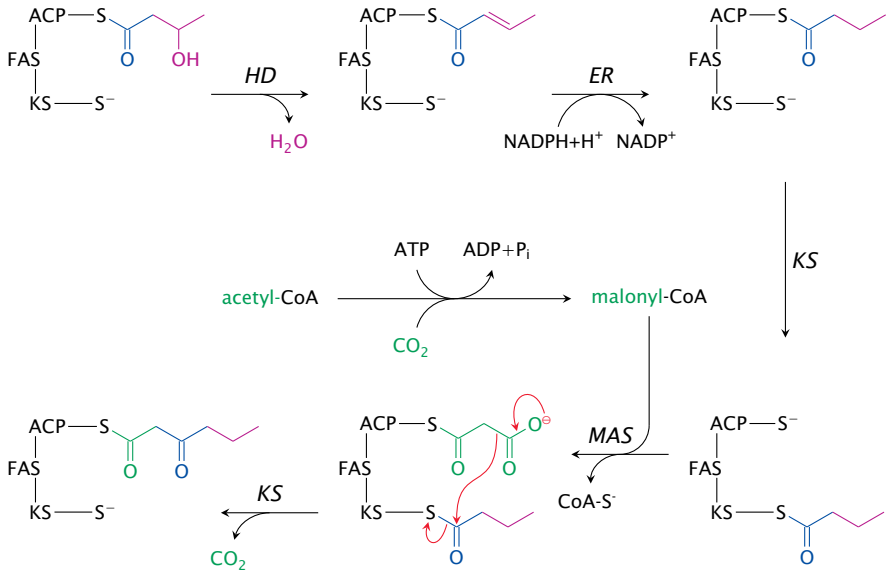


- acyl-CoA

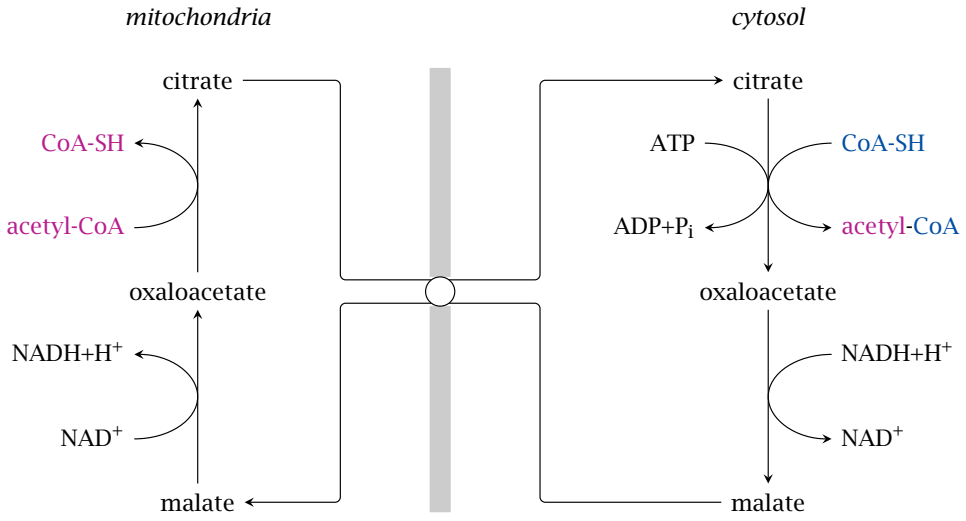
Fatty acid synthase reactions (1)



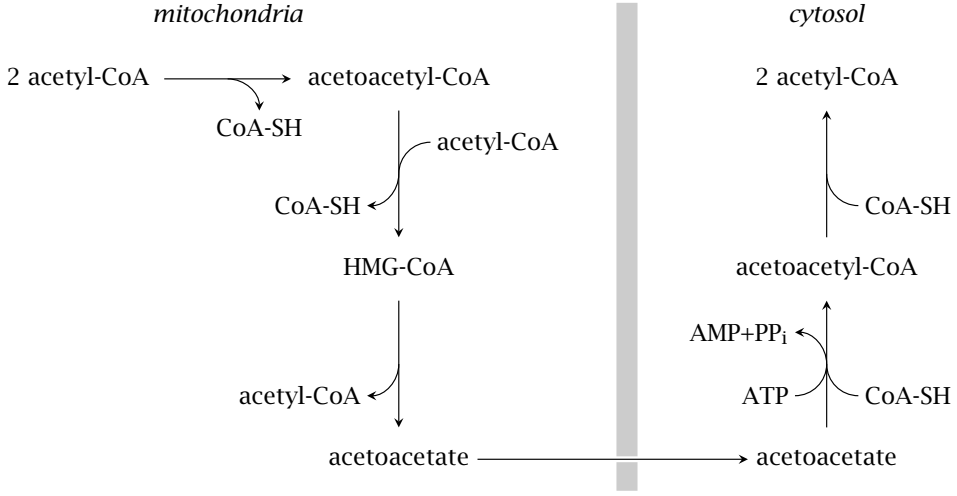
Fatty acid synthase reactions (2)



Mitochondrial export of acetyl-CoA via citrate



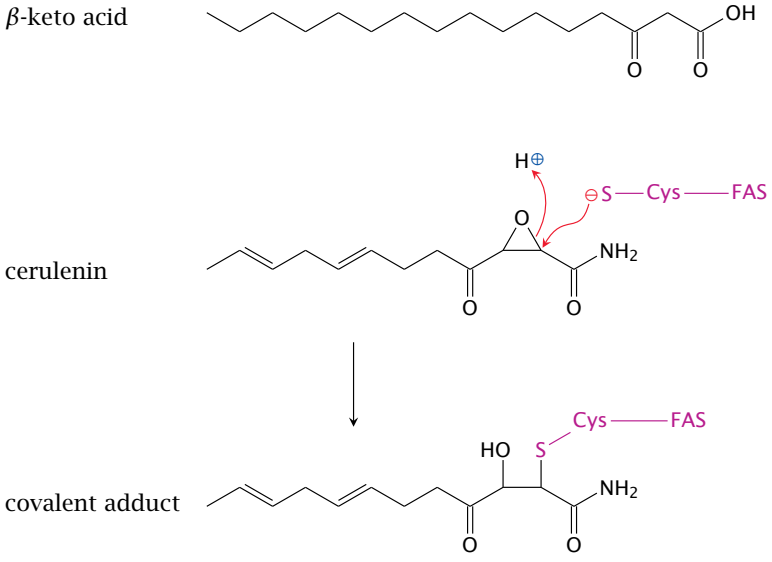
Mitochondrial export of acetyl-CoA via acetoacetate



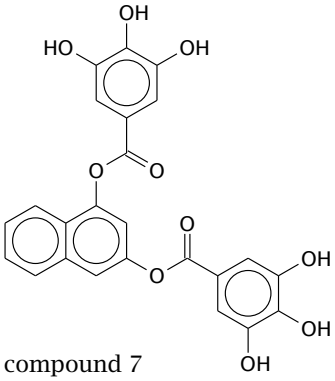
Elongation and desaturation of fatty acids

- elongases reside in mitochondria and endoplasmic reticulum
- chemistry of elongation similar to β-oxidation in mitochondria, similar to fatty acid synthase in the ER
- desaturases occur in the ER, introduce double bonds at various positions
- double bonds are created at least 9 carbons away from the ω end—ω-3 fatty acids cannot be formed in human metabolism and are therefore *essential*

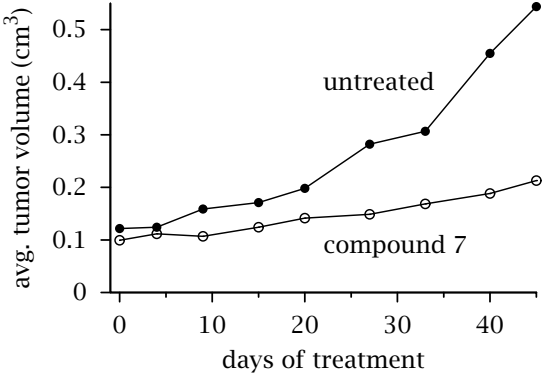
Cerulenin, an antibiotic that irreversibly inhibits fatty acid synthase



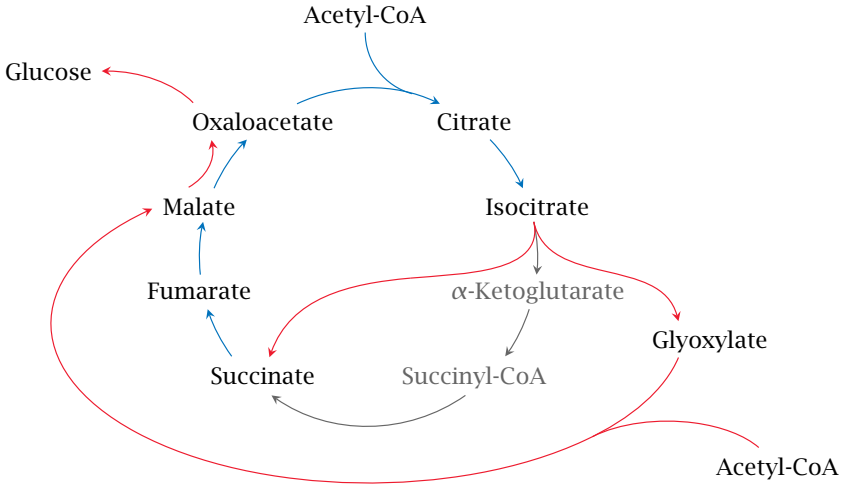
Fatty acid synthase inhibition slows tumor growth in mouse experiments



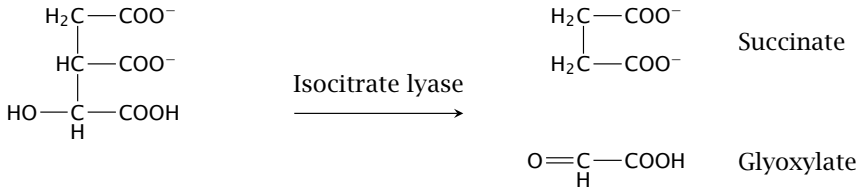
compound 7

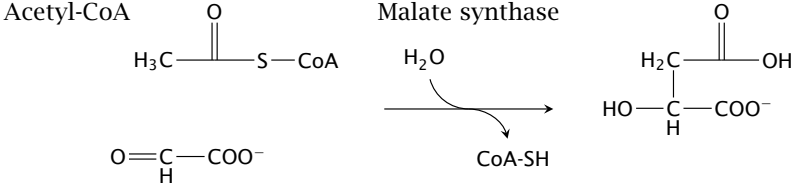


The glyoxylate cycle



Reactions in the glyoxylate cycle





Chapter 11

Cholesterol metabolism

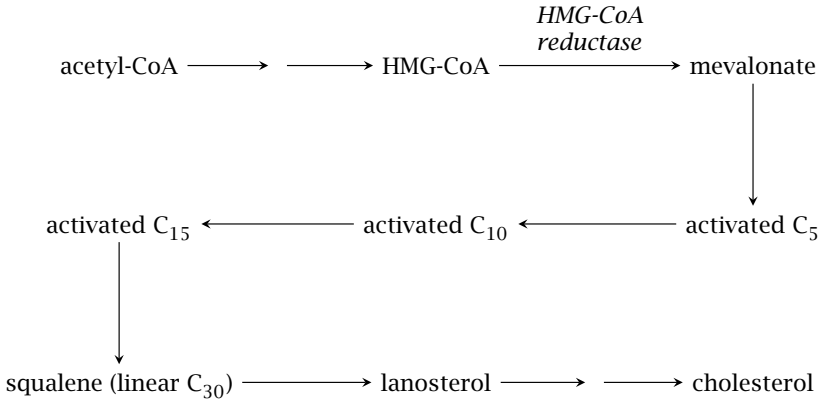
Biological significance of cholesterol

- Cholesterol is an essential lipid constituent of cell membranes
- Cholesterol is a precursor of steroid hormones and of bile acids
- Intermediates of cholesterol biosynthesis are required to make vitamin D and for posttranslational modification of membrane proteins
- High plasma cholesterol promotes atherosclerosis

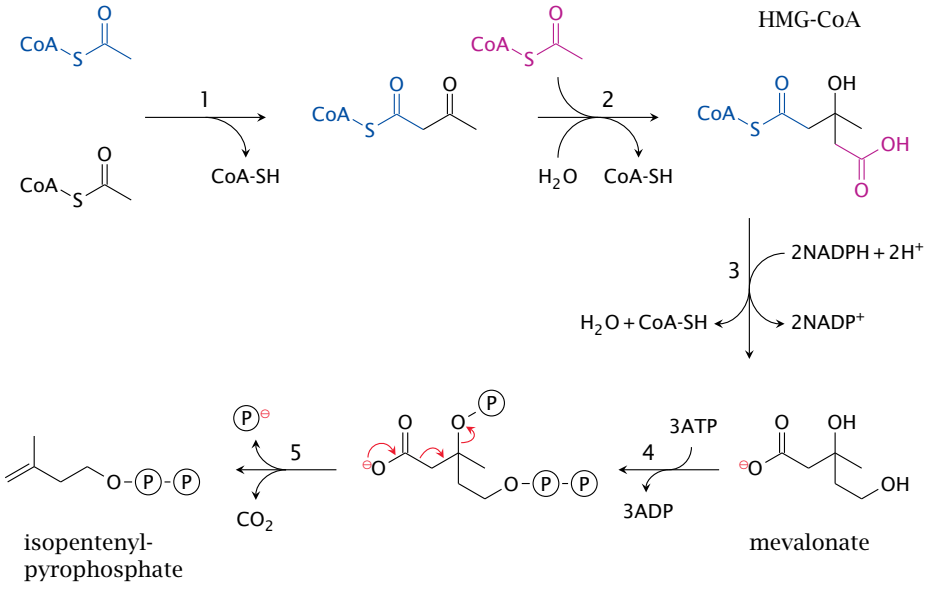
Processes that determine the cholesterol balance

- intestinal uptake of dietary cholesterol
- *de novo* cholesterol synthesis
- synthesis of steroid hormones from cholesterol
- synthesis of bile acids from cholesterol, and their biliary secretion
- biliary secretion of surplus cholesterol in unmodified form

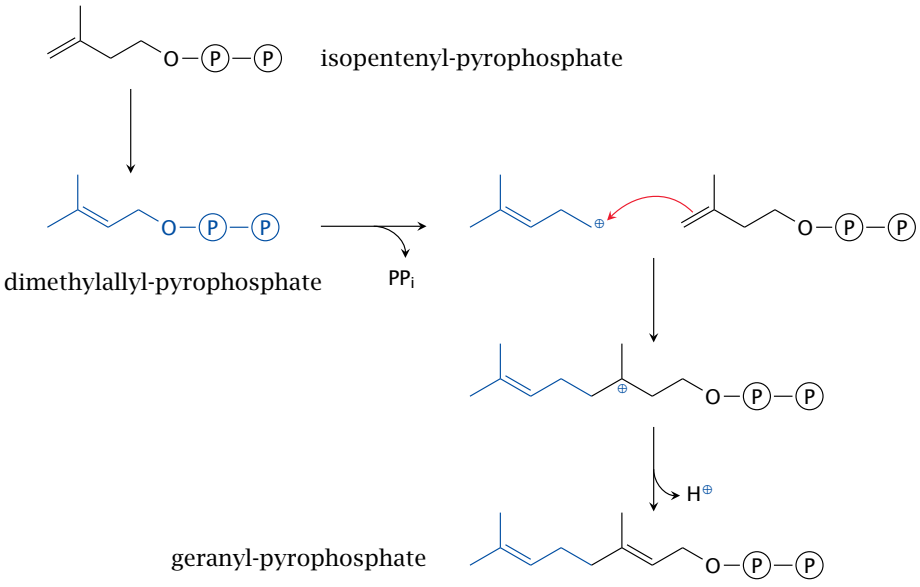
Overview of cholesterol synthesis



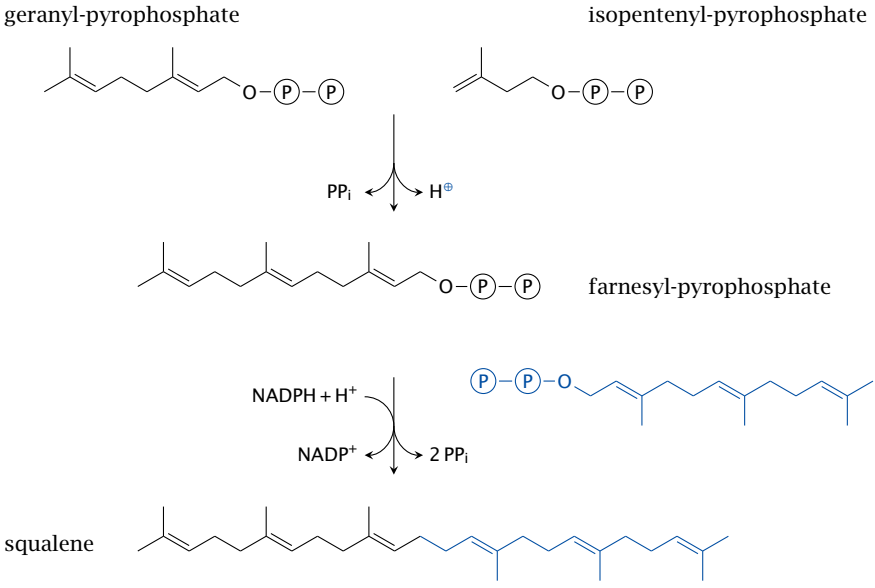
Initial activation steps in cholesterol synthesis



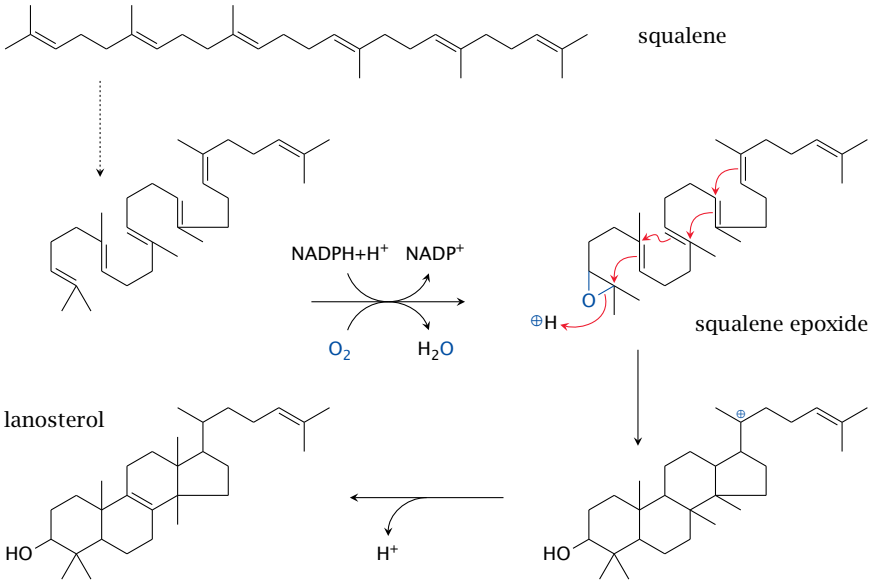
Formation of a C_{10} intermediate



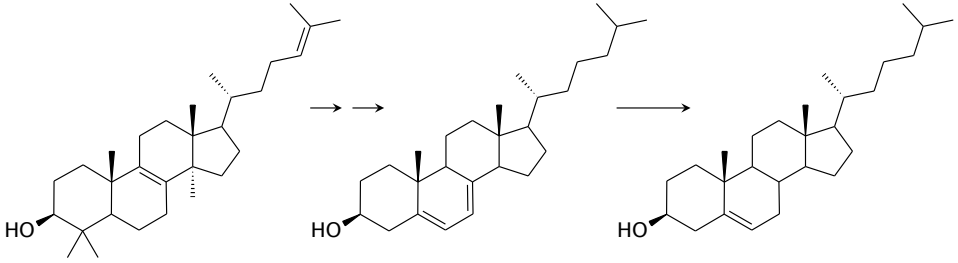
Formation of C_{15} and C_{30} intermediates



Squalene cyclization yields the first sterol intermediate

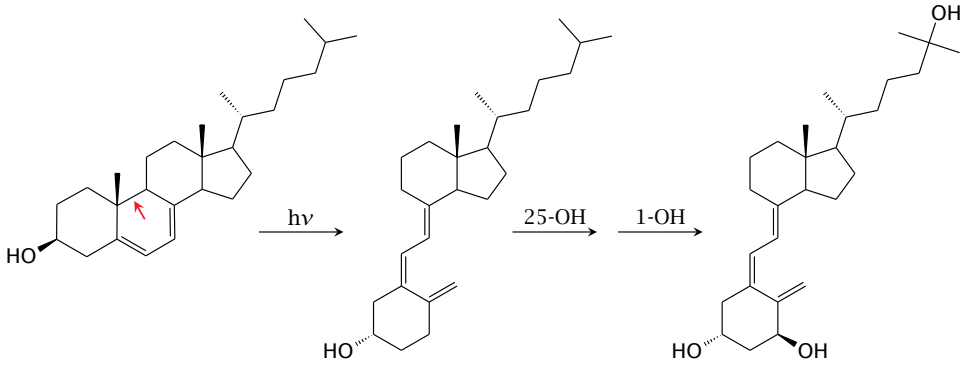


Demethylation, desaturation and saturation steps convert lanosterol to cholesterol



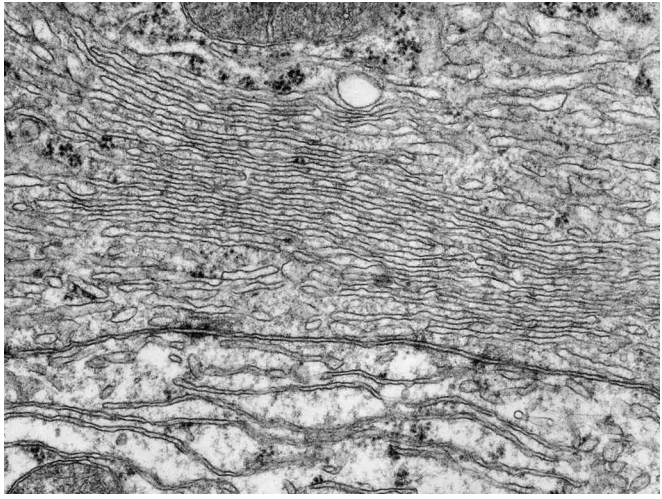
lanosterol 7-dehydrocholesterol cholesterol

UV-dependent synthesis of cholecalciferol



7-Dehydrocholesterol Cholecalciferol 1,25-Dihydroxycholecalciferol

Sterol metabolism occurs in the smooth endoplasmic reticulum



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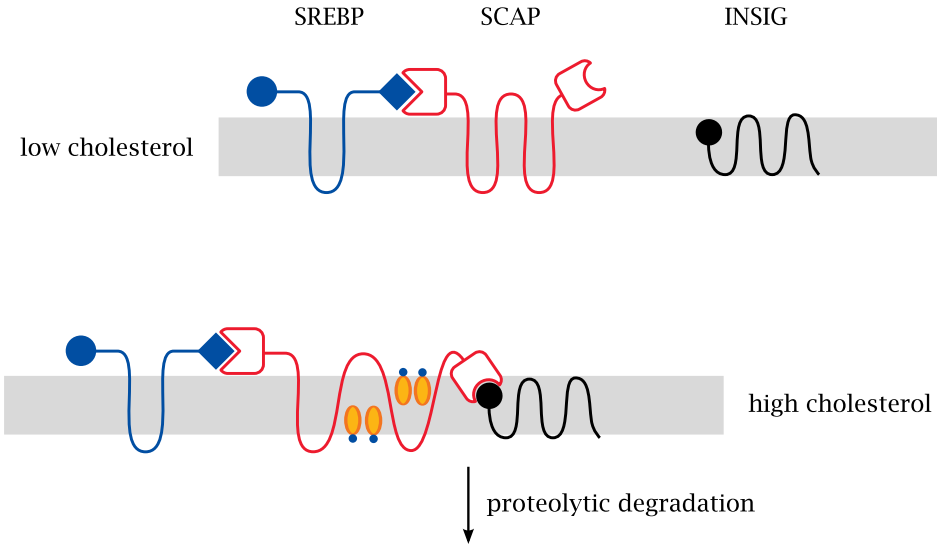
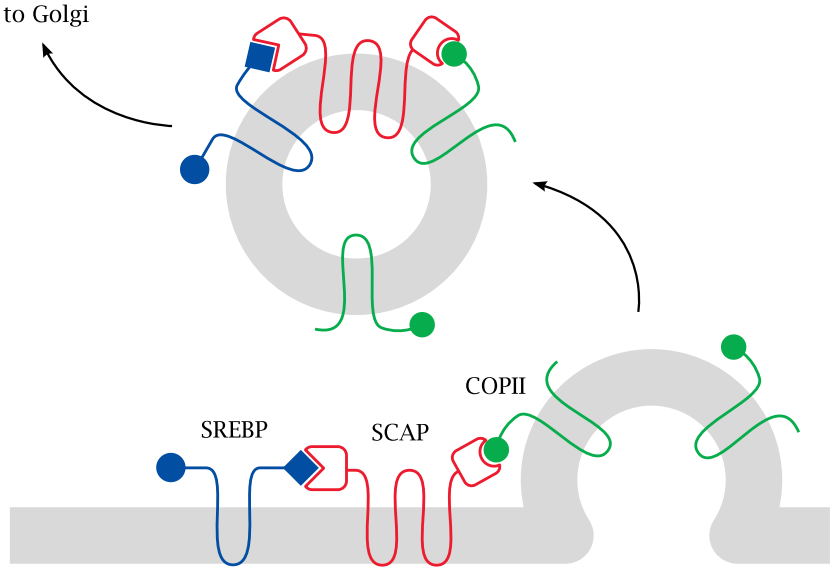
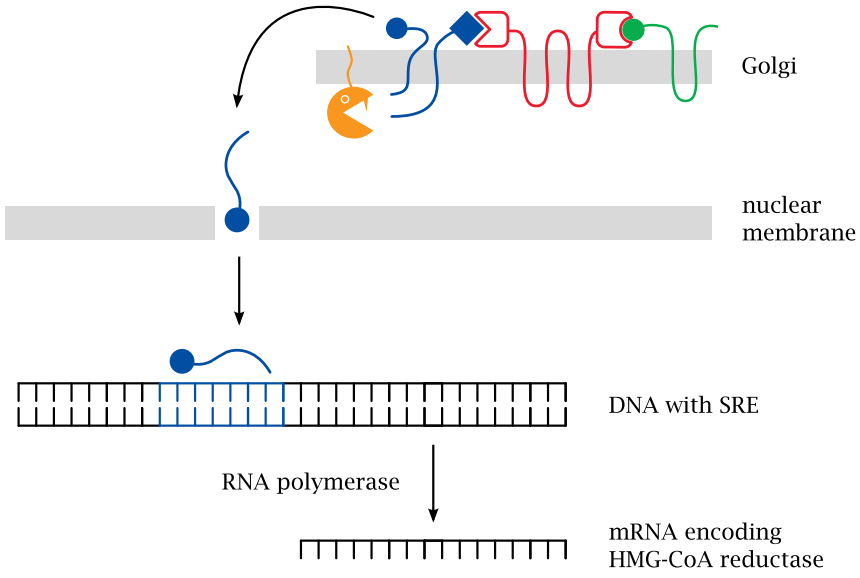
Transcriptional regulation of cholesterol synthesis starts in the endoplasmic reticulum



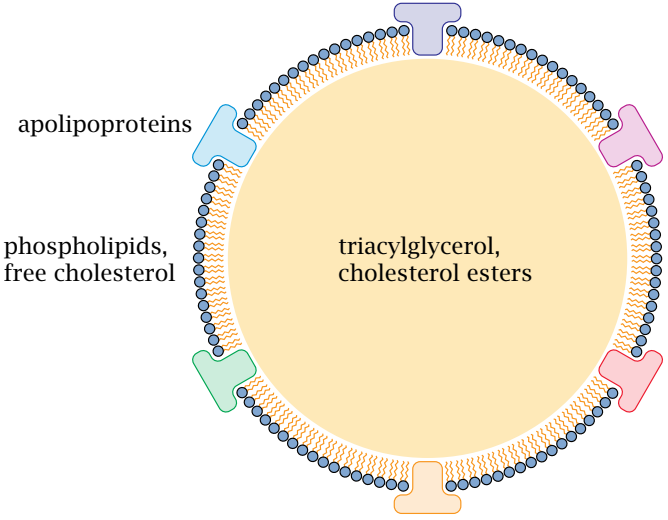
When cholesterol is low, SREBP is sorted to the Golgi apparatus



Proteolytic cleavage in the Golgi releases SREBP



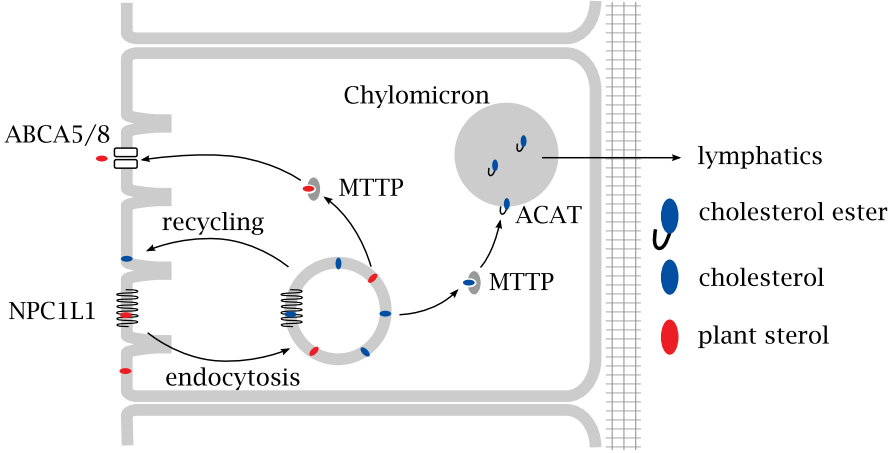
Lipoprotein structure



Classification of plasma lipoproteins

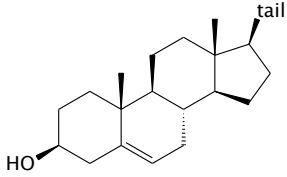
	Chylomicrons	VLDL	LDL	HDL
Density (g/ml)	0.95	0.95–1.0	1.02–1.06	1.06–1.12
Origin	small intestine	liver	liver	liver
Function	distribute dietary TAG and cholesterol	distribute TAG from liver	distribute cholesterol from liver	return excess cholesterol to liver
Predominant lipid species	TAG	TAG	cholesterol	phospholipids, cholesterol

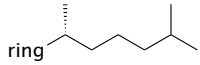
Two membrane proteins control the uptake of sterols from the intestine



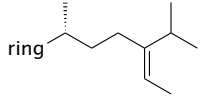
▸ Chylomicron drainage

Plant sterol structures

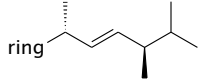




cholesterol



avenasterol



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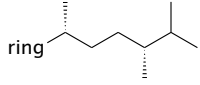
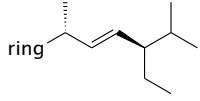
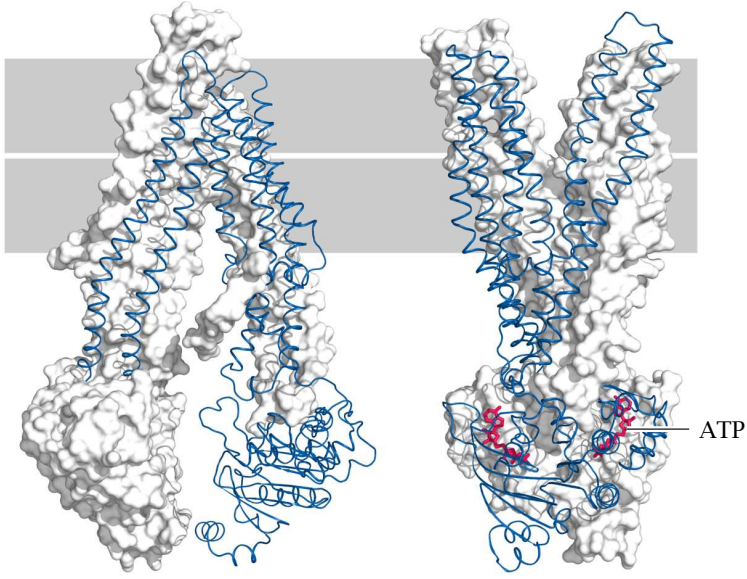


campesterol

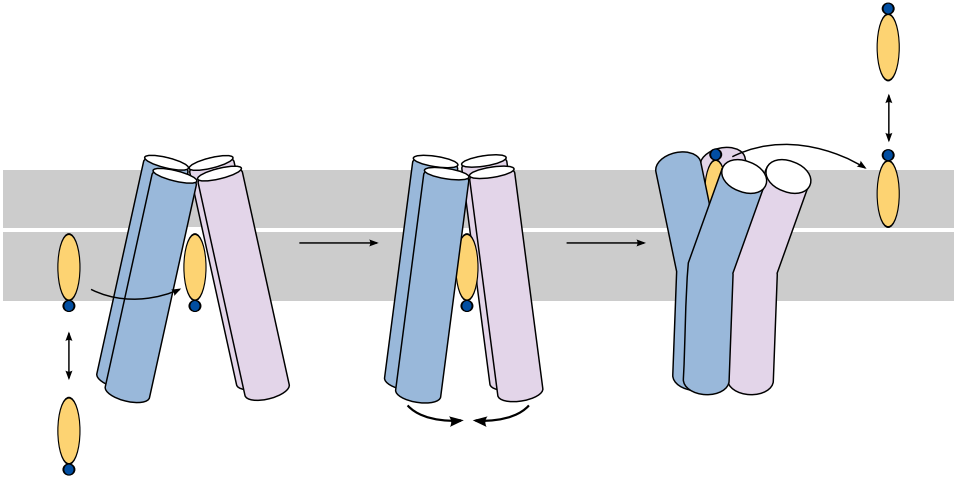


stigmasterol

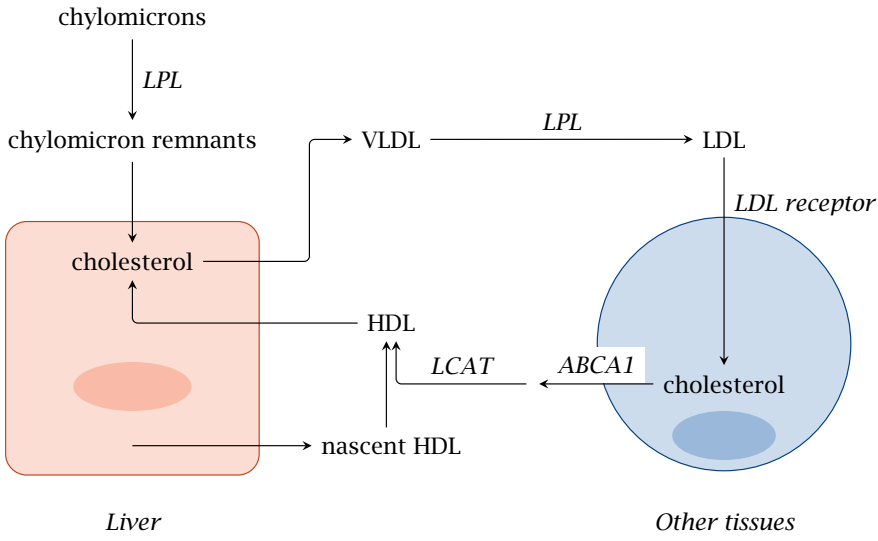
Structures of ABC transporters in the inward-open and outward-open conformations



ABC transporters induce substrate “flip-flop” across the membrane



Transport of cholesterol between the liver and peripheral tissues



Stages of cholesterol transport

Dietary cholesterol

- Packaged into chylomicrons, which turn into chylomicron remnants through triacylglycerol extraction by lipoprotein lipase
- Chylomicron remnants are taken up by the liver

Liver cholesterol (from diet, or endogenously synthesized)

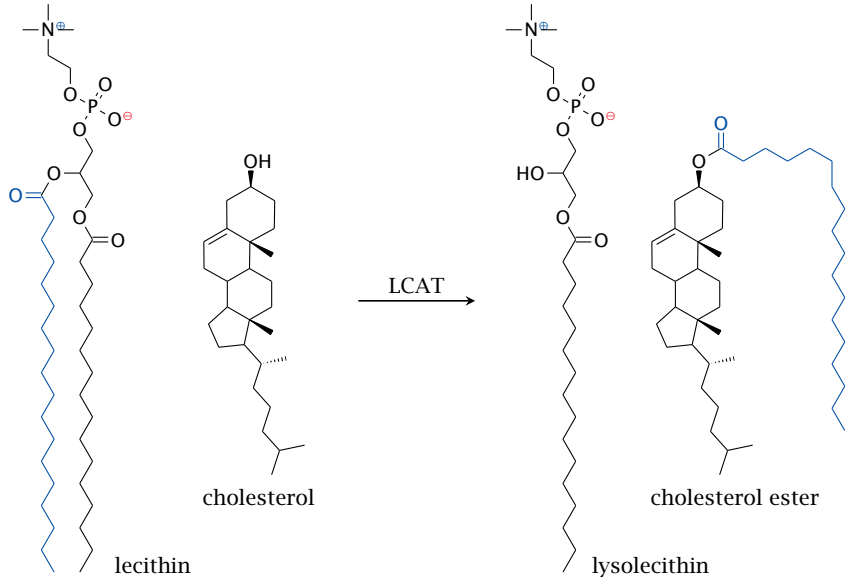
- Packaged into VLDL
- Lipoprotein lipase turns VLDL into IDL and then LDL
- LDL is taken up through receptor-mediated endocytosis in peripheral tissues

Cholesterol transport (ctd.)

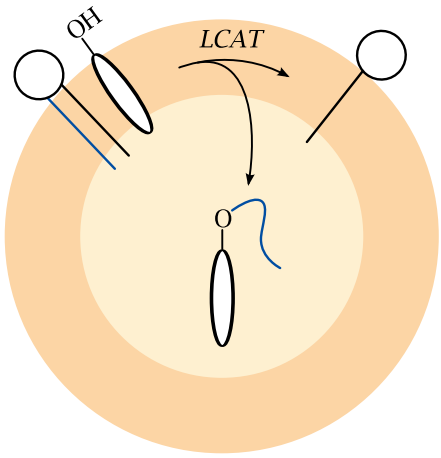
Cholesterol in peripheral tissues

- HDL is produced in liver and intestines as an empty carrier for cholesterol (containing mainly phospholipid and apo A-1)
- HDL binds to cells in periphery (including in vascular lesions) and takes up surplus cholesterol
- Cholesterol-laden HDL is taken up into the liver by endocytosis, cholesterol is recycled

The lecithin-cholesterol acyltransferase (LCAT) reaction

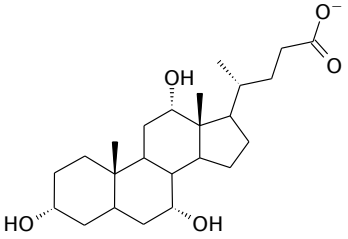


Cholesterol esters can be stored inside lipoprotein particles

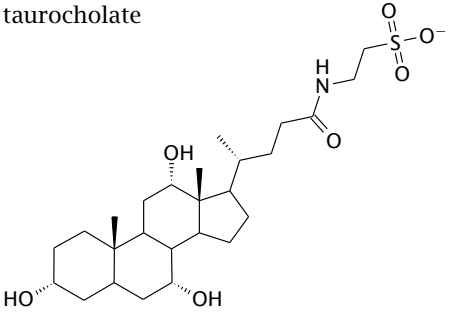


Bile acids are derived from cholesterol

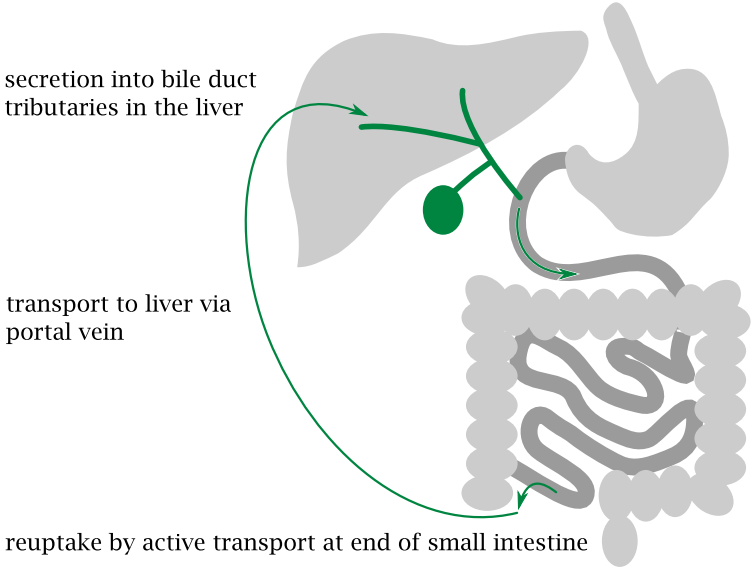
cholate



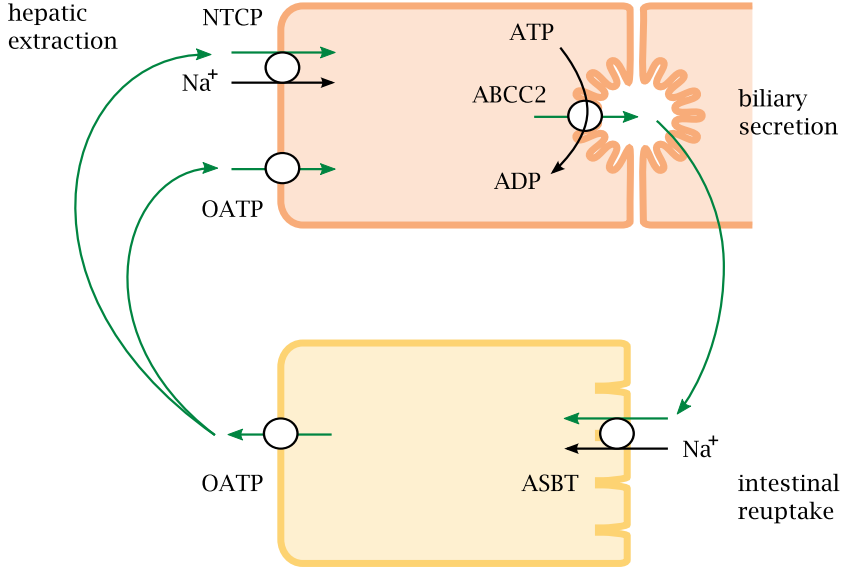
taurocholate



Bile acids undergo enterohepatic cycling



Bile acid cycling involves multiple transport proteins



A deficient ABCC2 transporter causes Dubin-Johnson syndrome

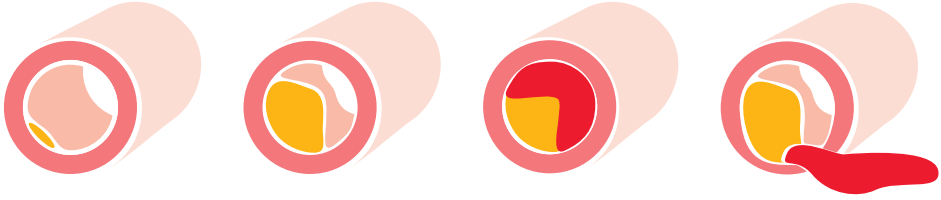
- impaired excretion of bile acids $\longrightarrow$ cholesterol precipitates in the bile $\longrightarrow$ bile stones
- impaired excretion of bilirubin $\longrightarrow$ jaundice
- impaired excretion of many drugs $\longrightarrow$ potential drug toxicity

Is atherosclerosis a metabolic disease?

> *. . . it is important to remember that the best documented initiating factor is still hypercholesterolemia . . . additional factors should be considered in the context of how they relate to the processes initiated by hypercholesterolemia.*

Daniel Steinberg, "Atherogenesis in perspective: Hypercholesterolemia and inflammation as partners in crime", Nature Medicine 8:1211 (2002).

Macroscopic appearance of atherosclerotic lesions



fatty streak
(asymptomatic)

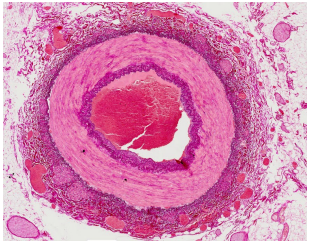
advanced lesion
(subacute occlusion)

thrombus
(acute occlusion)

ruptured lesion
(hemorrhage)

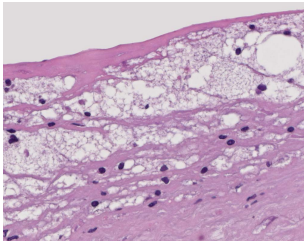
Microscopic appearance of atherosclerotic lesions

A



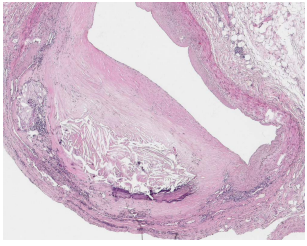
Normal artery

B



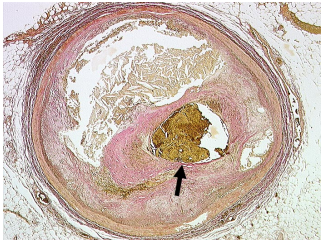
Foam cells in early lesion

C



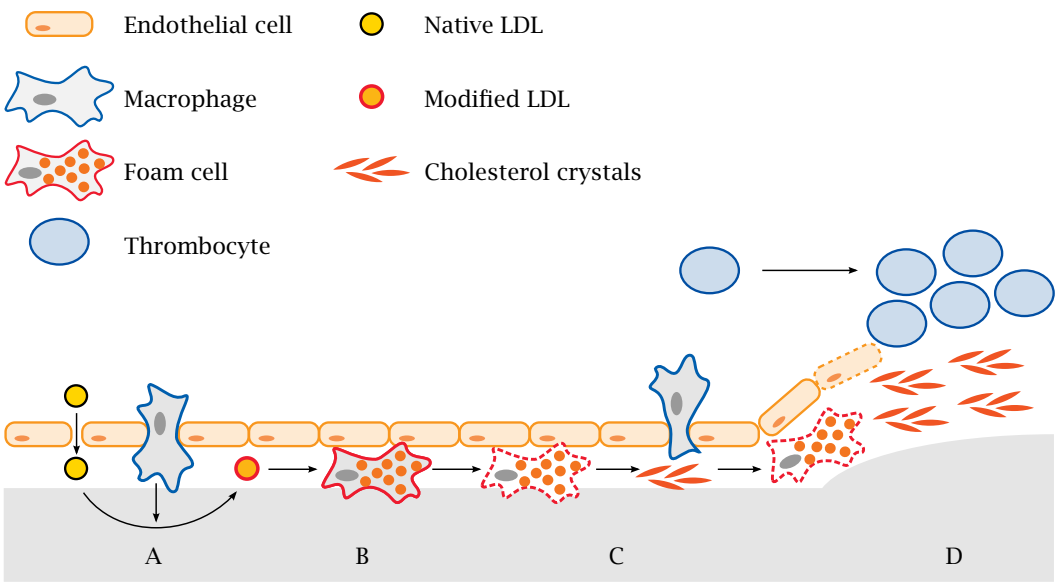
Detritus, fibrosis in advanced lesion

D



High-grade stenosis, thrombus

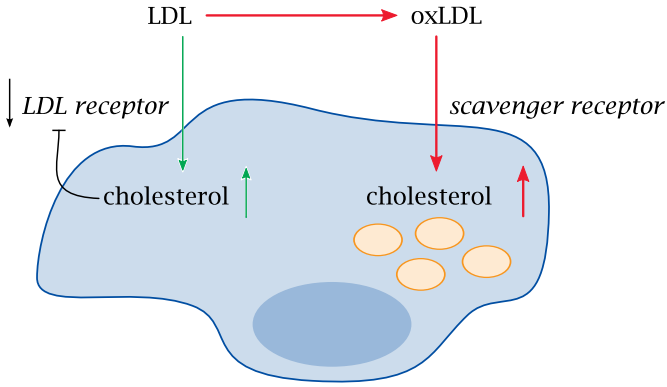
Development of an atherosclerotic lesion



Metabolic aspects of atherosclerosis

- cholesterol uptake, synthesis and degradation
- cholesterol transport in the circulation: LDL (low density lipoprotein) and HDL (high density lipoprotein)
- biochemical changes that turn physiological, benign LDL into an atherogenic agent

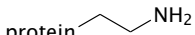
Two modes of uptake of cholesterol into macrophages



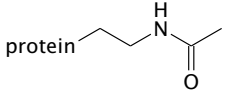
Modification of LDL is essential for excessive uptake by macrophages via the scavenger receptor

- LDL receptor is down-regulated once the cell is full up with cholesterol—no further LDL will be taken up
- Covalently modified LDL will be taken up by macrophages via *scavenger receptors*
- Various modifications have similar effects
- Modifications can affect both lipid and apolipoprotein components of LDL

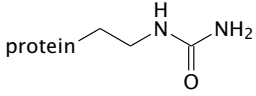
Experimental protein modifications that turn LDL into a ligand for the scavenger receptor



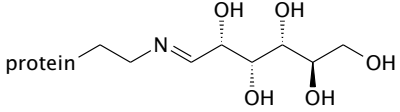
unmodified amine (native LDL)



acetylated amine



carbamylated amine



glucosylated amine

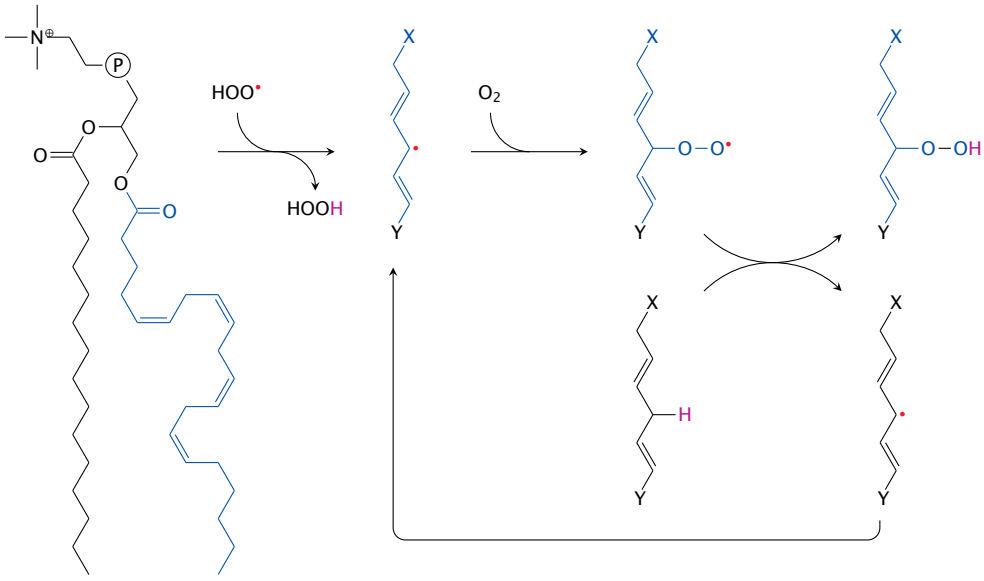
Which modifications of LDL are significant *in vivo*?

Modification	Possible causes
acetylation	easily achieved *in vitro*, but not plausible *in vivo*
carbamylation	promoted by urea, which is enhanced in kidney disease; also promoted by smoking
glucosylation	promoted by high blood glucose (diabetes)
partial proteolysis	proteases released from macrophages
oxidation of lipids and apolipoproteins	reactive oxygen species released from macrophages

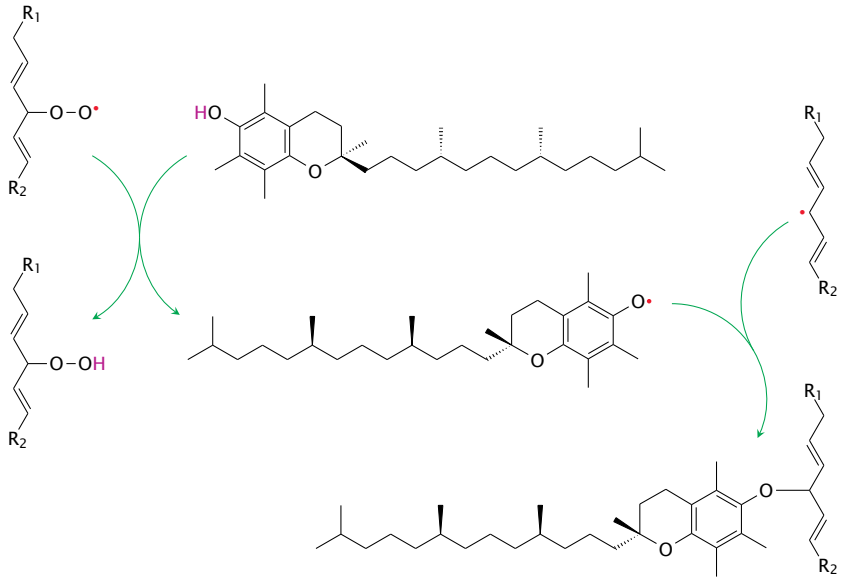
How does LDL become oxidized?

- Phagocytes produce reactive oxygen species
- Transition metals (Fe, Cu) exacerbate ROS activity
- Lipoxygenases convert fatty acids to radicals that can bind to LDL and induce lipid peroxidation

Self-sustained lipid peroxidation induced by peroxy radicals



α-Tocopherol intercepts lipid peroxidation



Experimental evidence implicating LDL oxidation in the pathogenesis of atherosclerosis

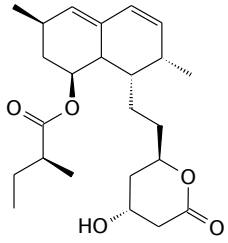
- Vitamin E reduces the severity of atherosclerosis in animal models—but *not* in clinical studies on humans
- Antibodies against oxidized LDL are found in blood; among these, IgG promotes atherosclerosis, whereas IgM inhibits it
- *Haptoglobin* alleles differ in the efficiency of hemoglobin clearance, which correlates inversely with susceptibility to atherosclerosis
- Production of HOCl by myeloperoxidase: chlorotyrosine residues detectable in oxLDL *ex vivo*—but myeloperoxidase k.o. mice have *increased* susceptibility to atherosclerosis

Lowering LDL cholesterol: therapeutic principles

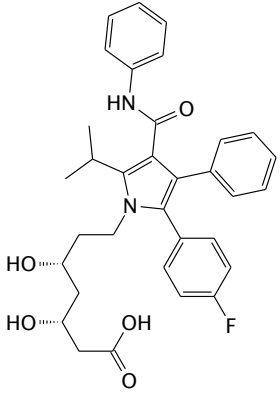
- inhibition of cholesterol synthesis
- inhibition of cholesterol uptake
- inhibition of cholesterol ester transfer protein
- inhibition of bile acid reuptake
- LDL apheresis

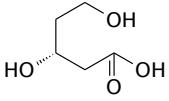
“Statins” inhibit HMG-CoA reductase

Mevastatin

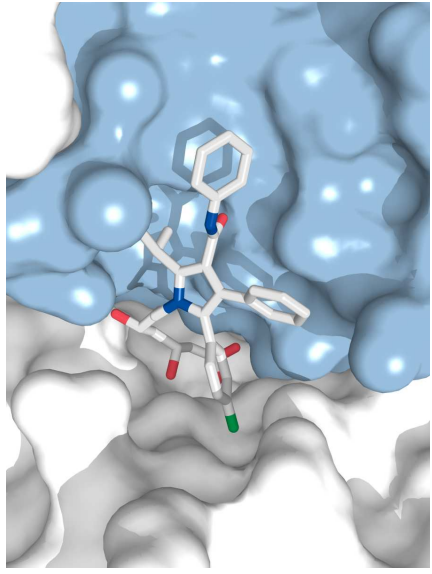


Atorvastatin

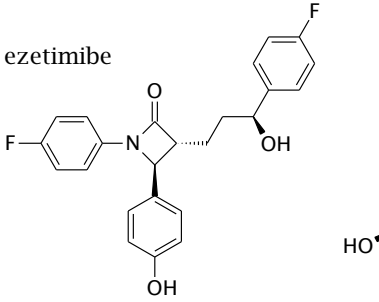


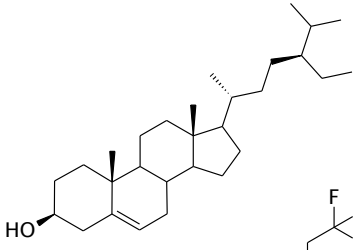


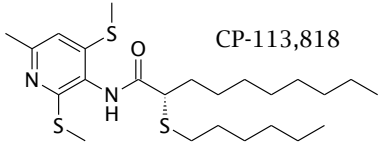
Mevalonate

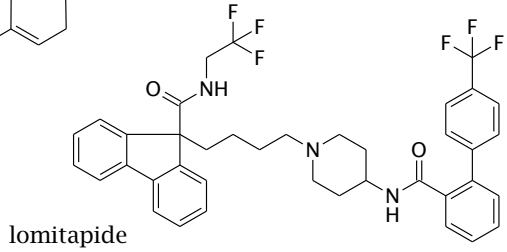


Inhibitors of intestinal cholesterol uptake



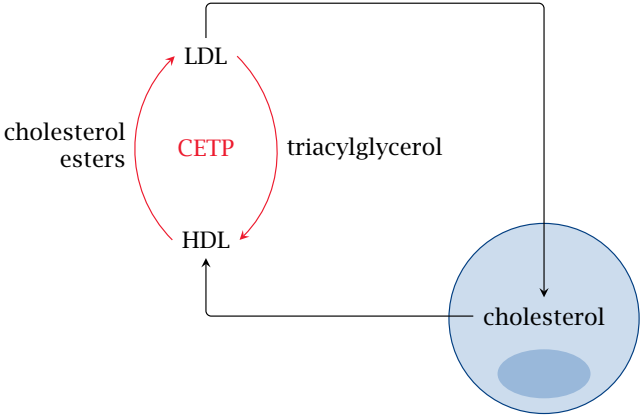


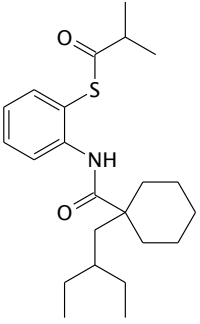




- intestinal uptake

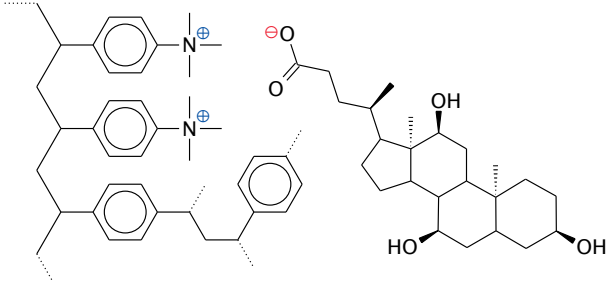
Cholesterol ester transfer protein (CETP) short-circuits cholesterol transport by lipoproteins





dalcetrapib

Cholestyramine particles absorb bile acids



cholestyramine cholic acid

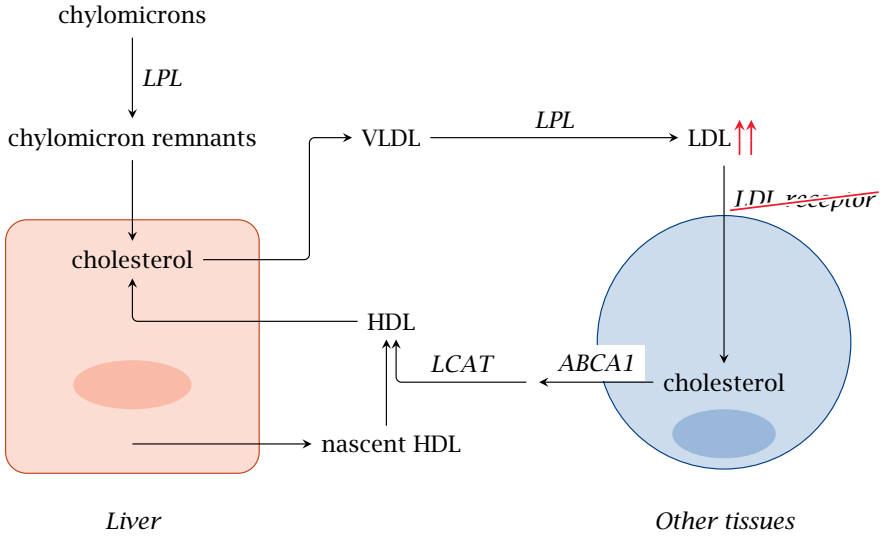
LDL apheresis

- Blood is diverted through an extra-corporeal filtration device
- cells are separated from plasma
- LDL is removed from plasma by affinity methods or size-based filtration
- The remaining plasma and cells are returned to the circulation
- The procedure is repeated in weekly or biweekly intervals

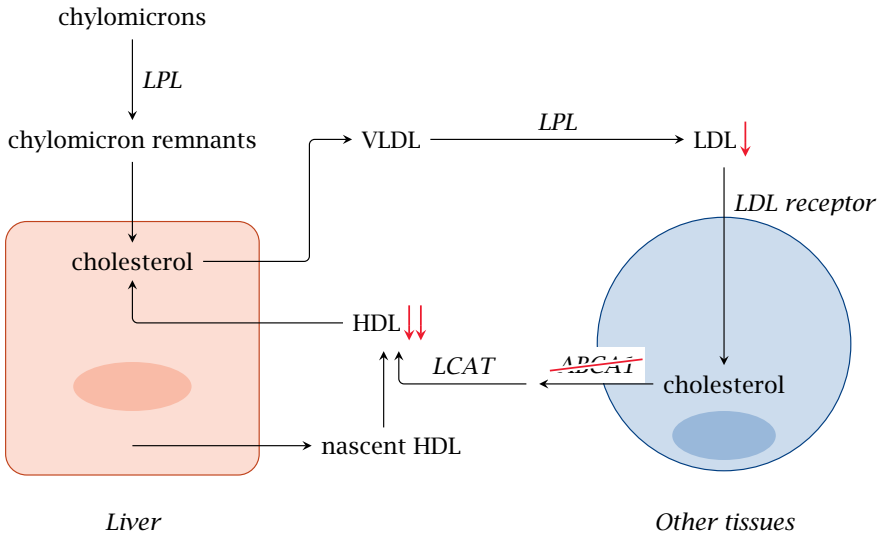
More . . .

- triparanol—an old drug, inhibits some CYP450 enzymes in the conversion from lanosterol to cholesterol; withdrawn due to toxicity
- bezafibrate—a PPARγ agonist
- nicotinic acid—activates hormone-sensitive lipase through a G protein coupled receptor named HM74A; 5 likely additional mechanisms
- probucol and succinobucol—supposedly antioxidants that prevent LDL oxidation, but also cause unrelated changes in other laboratory parameters
- guar gum and other carbohydrate fibers —absorb and prevent intestinal uptake of cholesterol and bile acids with variable efficiency
- thyroid hormone analogs—promote LDL utilization

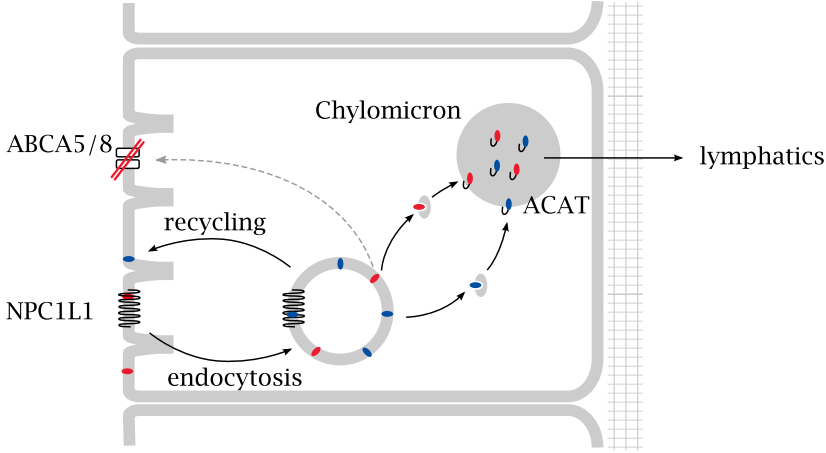
Familial hypercholesterolemia is due to a gene defect in the LDL receptor



Tangier disease: Disruption of cholesterol transfer to HDL



A defective plant sterol exporter causes sitosterolemia



Chapter 12

Amino acid metabolism

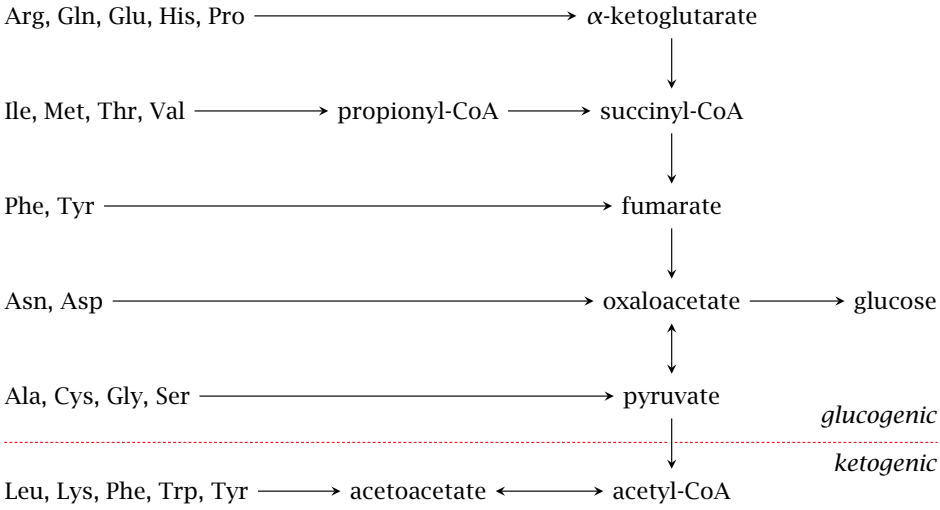
Metabolic uses of amino acids

- building blocks for protein synthesis
- precursors of nucleotides and heme
- source of energy
- neurotransmitters
- precursors of neurotransmitters and hormones

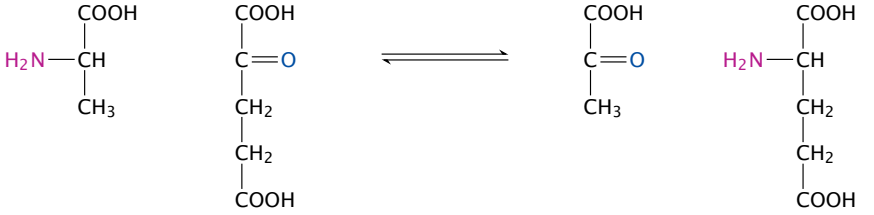
Outline of amino acid degradation

- The liver is the major site of degradation for most amino acids, but muscle and kidney dominate the degradation of specific ones
- Nitrogen is removed from the carbon skeleton and transferred to α-ketoglutarate, which yields glutamate
- The carbon skeletons are converted to intermediates of the mainstream carbon oxidation pathways via specific adapter pathways
- Surplus nitrogen is removed from glutamate, incorporated into urea, and excreted

Amino acid breakdown pathways join mainstream carbon utilization at different points of entry

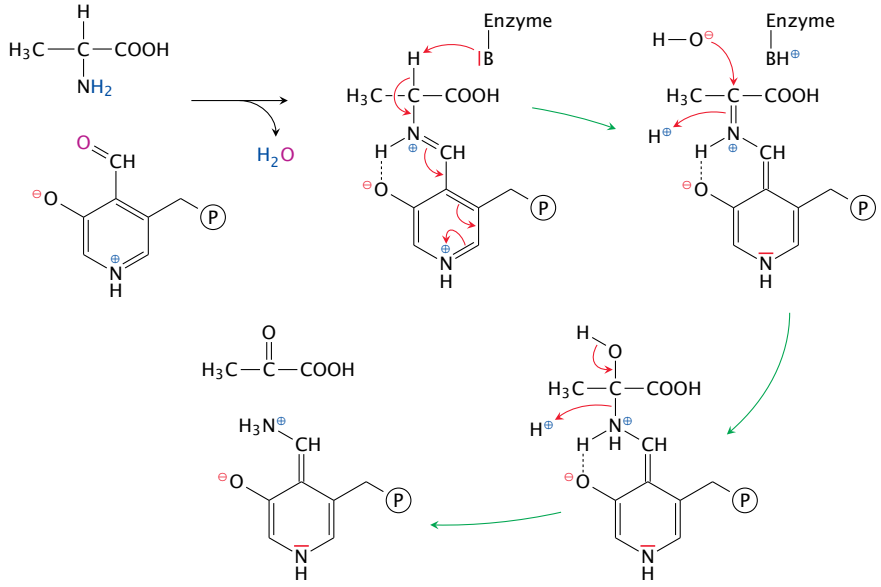


Transamination of amino acids

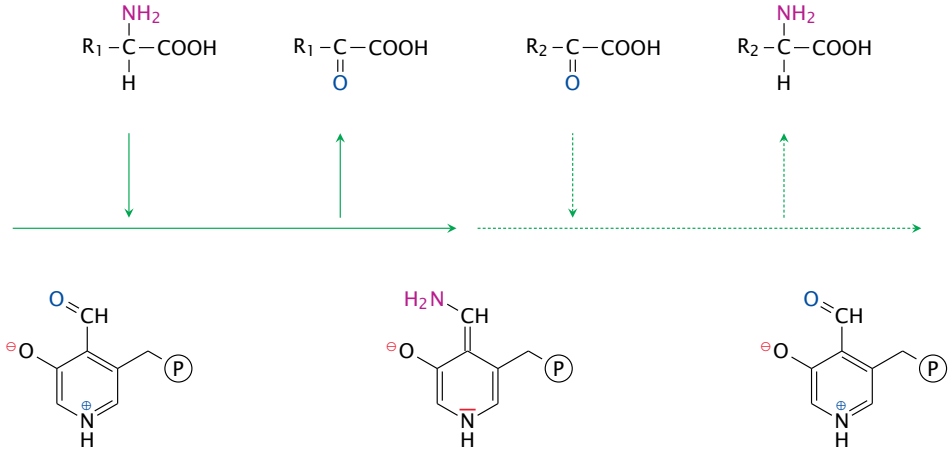


L-alanine α-ketoglutarate pyruvate L-glutamate

The reaction mechanism of transamination



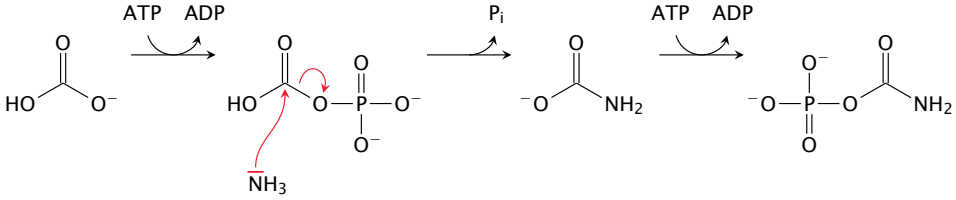
The *ping pong bi bi* mechanism of transamination



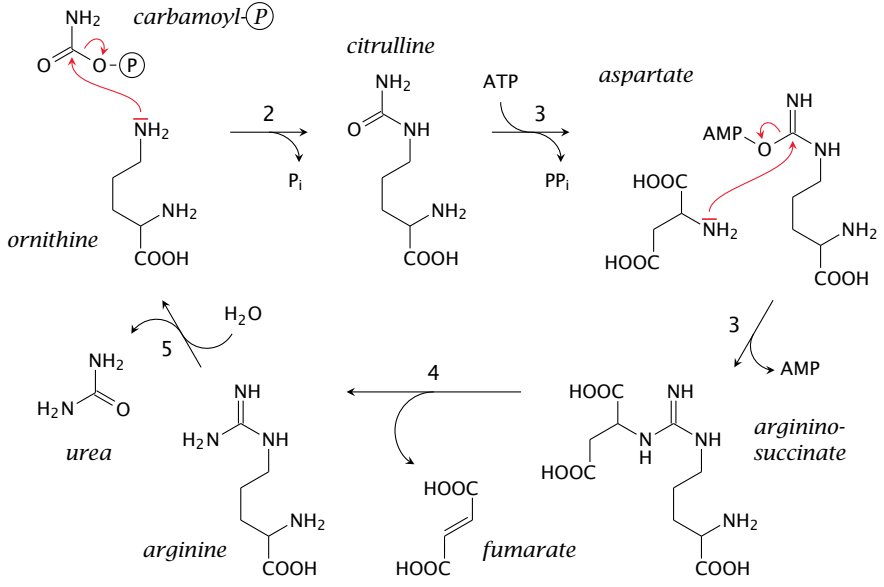
Nitrogen disposal and excretion

- Nitrogen accruing outside the liver is transported to the liver as glutamine or alanine
- In the liver, nitrogen is released as free ammonia
- Ammonia is incorporated into urea
- Urea is released from the liver into the bloodstream and excreted through the kidneys

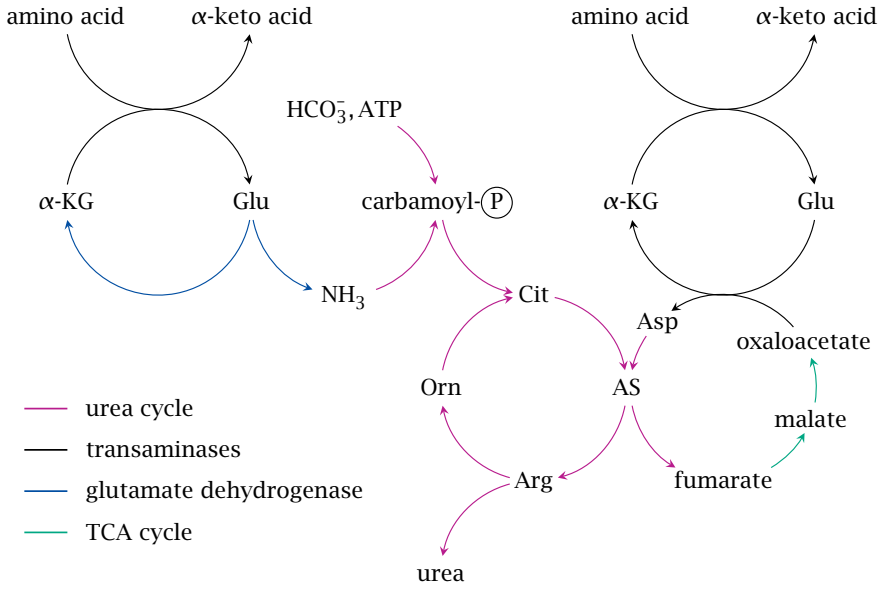
The urea cycle, part 1: carbamoylphosphate synthetase



The urea cycle, part 2: subsequent reactions

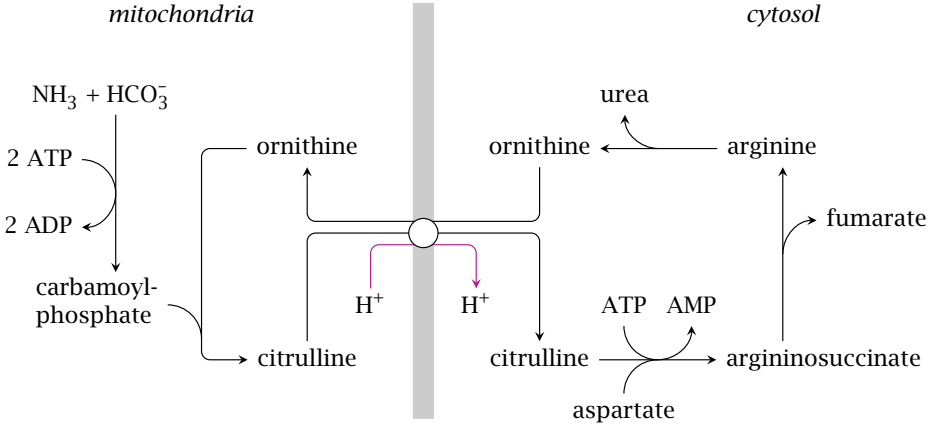


The urea cycle in context

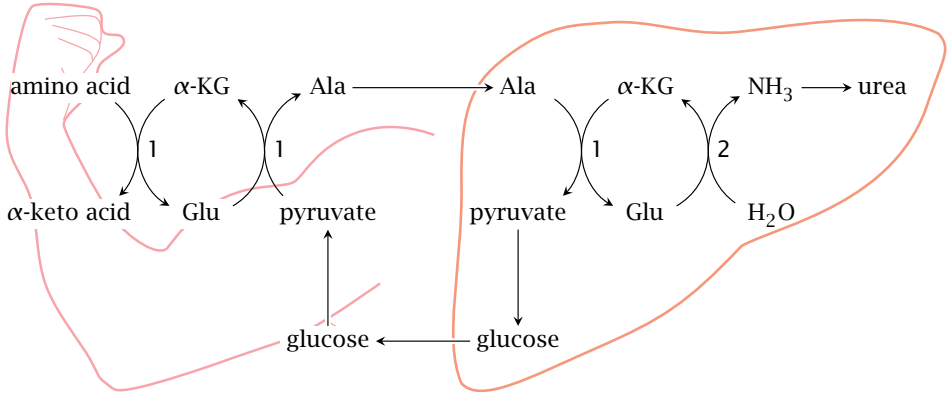


- malate aspartate shuttle

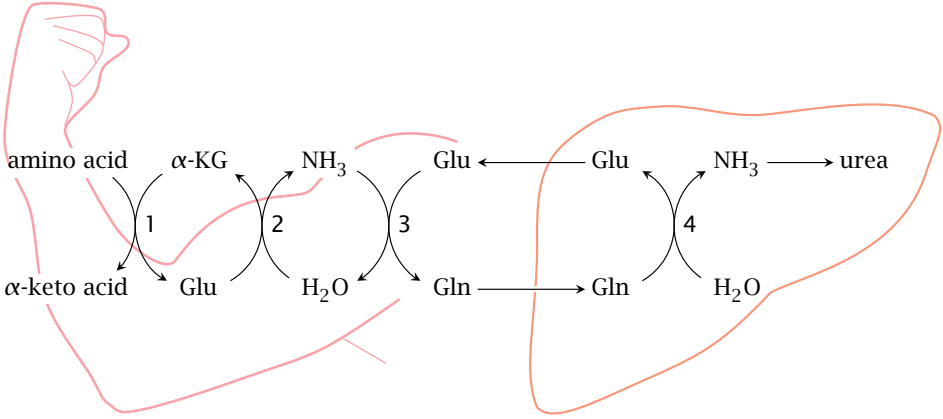
The urea cycle spans mitochondria and cytosol



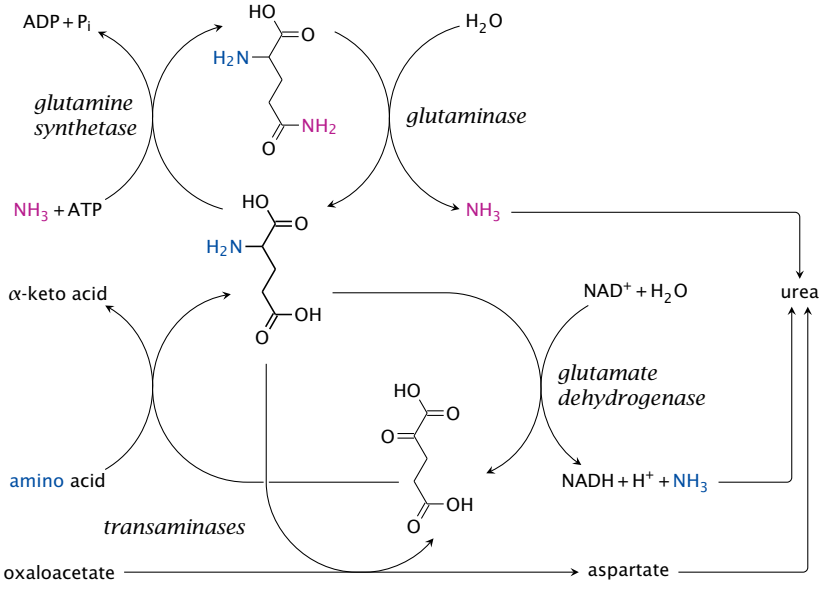
The glucose-alanine cycle



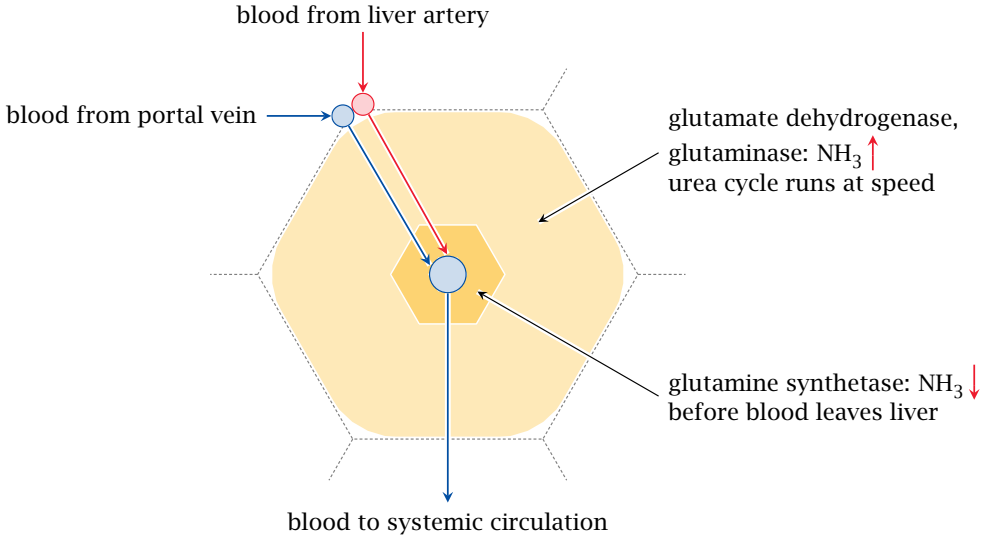
Nitrogen transport by glutamine



The central role of glutamate in nitrogen disposal

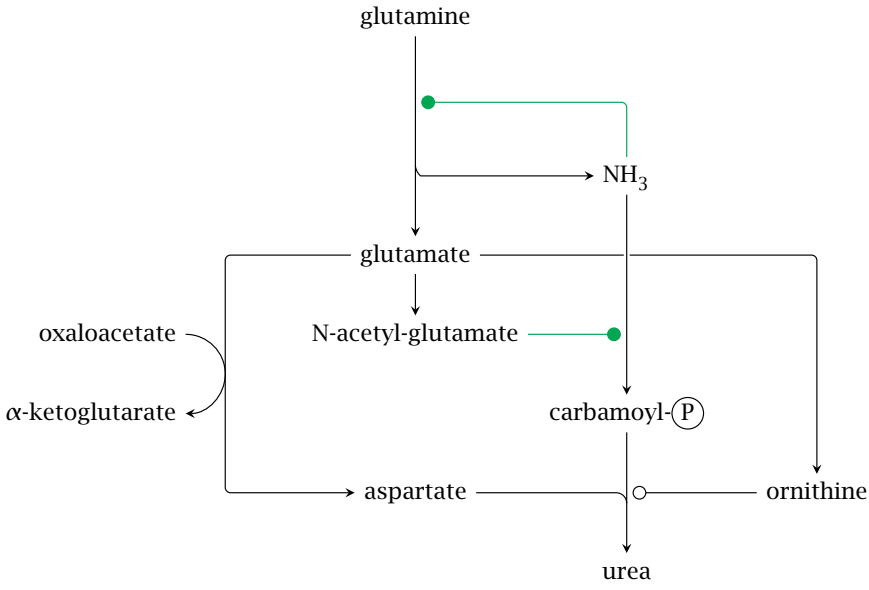


Control of ammonia levels in the liver lobule



- liver tissue

Regulation of the urea cycle

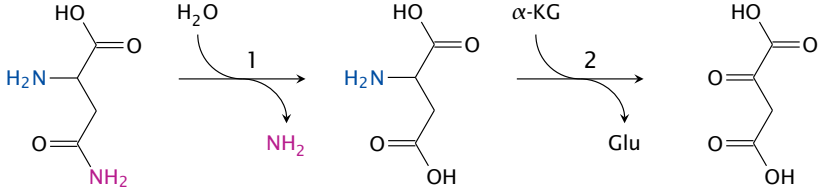


Hereditary enzyme defects in the urea cycle

- may affect any of the enzymes in the cycle
- urea cannot be synthesized, nitrogen disposal is disrupted
- ammonia accumulates, as do other metabolites depending on the deficient enzyme
- treatment
  - protein-limited diet
  - arginine substitution
  - alternate pathway therapy

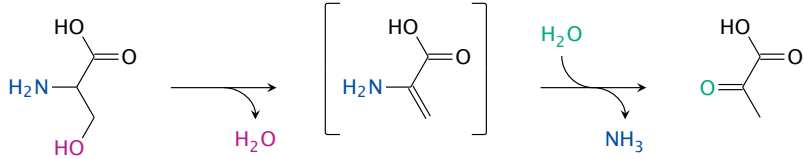
▸ glutamine conjugation

Asparagine degradation



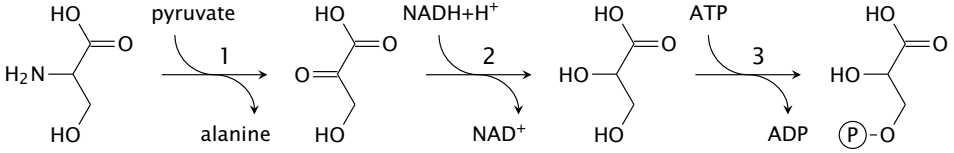
asparagine aspartate oxaloacetate

Serine dehydratase



serine aminoacrylate pyruvate

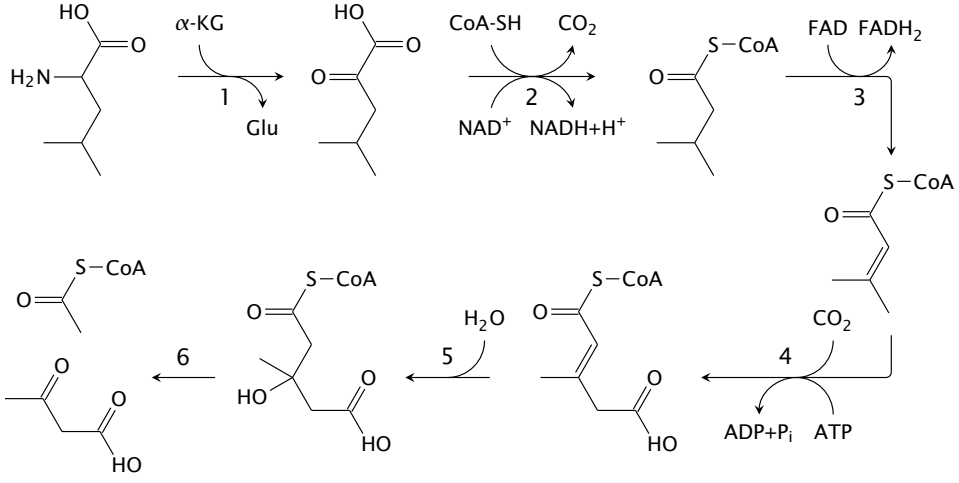
Serine-pyruvate transaminase



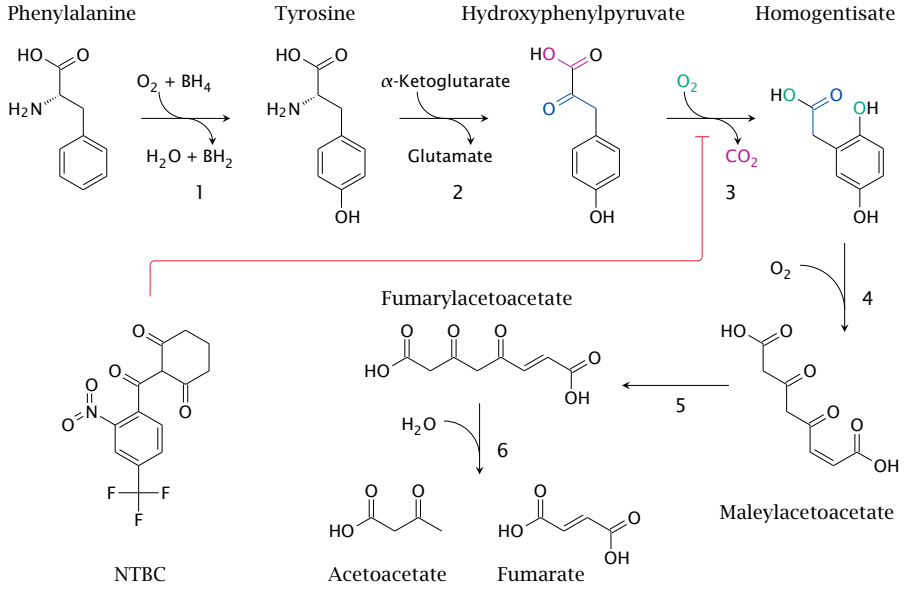
serine hydroxypyruvate glycerate 3-Ⓟ-glycerate

- dhfr

Degradation of leucine



Degradation of phenylalanine and tyrosine

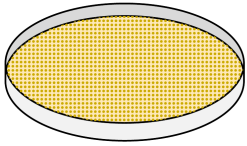


Phenylketonuria (PKU)

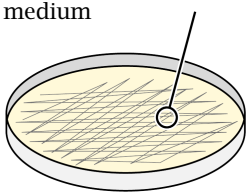
- homozygous defect of phenylalanine hydroxylase
- affects one in 10,000 newborns among Caucasians; frequency differs with race
- excess of phenylalanine causes symptoms only after birth; intrauterine development normal
- cognitive and neurological deficits, probably due to cerebral serotonin deficit
- treatment with phenylalanine-restricted diet
- some cases are due to reduced affinity of enzyme for cofactor THB, can be treated with high dosages of THB

The Guthrie test for diagnosing phenylketonuria

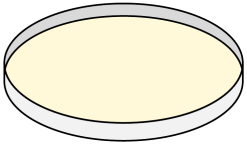
Grow *E. coli* Phe$^-$ on rich medium



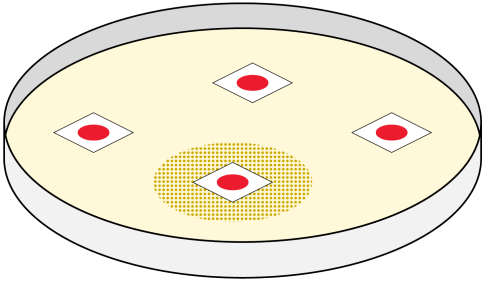
Spread on minimal medium



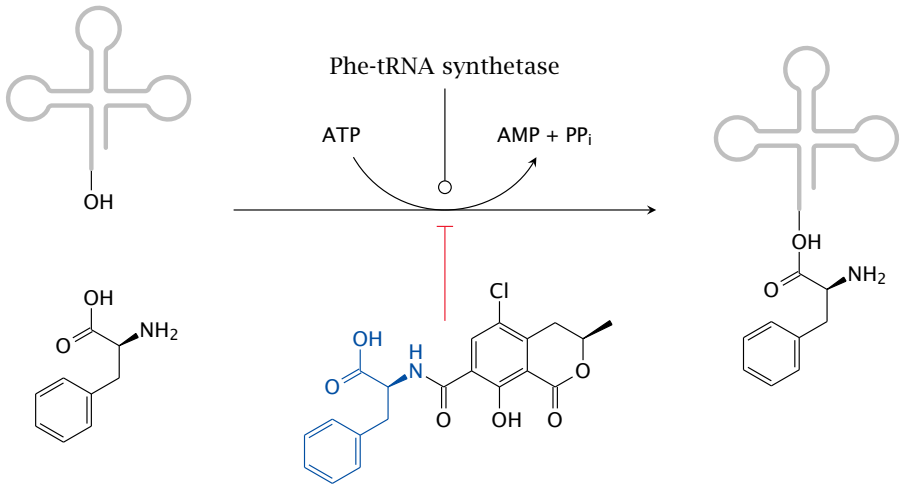
Cells persist, but do not grow



Place patients' blood samples onto inoculated agar—bacteria will grow around samples containing excess phenylalanine



Ochratoxin A inhibits phenylalanyl-tRNA synthetase



Tyrosinemia

- homozygous defect of fumarylacetoacetate hydrolase
- fumarylacetoacetate and preceding metabolites back up
- fumaryl- and maleylacetoacetate react with glutathione and other nucleophiles, causing liver toxicity
- the drug NTCB inhibits *p*-hydroxyphenylpyruvate dioxygenase, intercepting the degradative pathway upstream of the toxic metabolites
- dietary restriction of tyrosine required to prevent neurological deficit

▸ Phe degradation

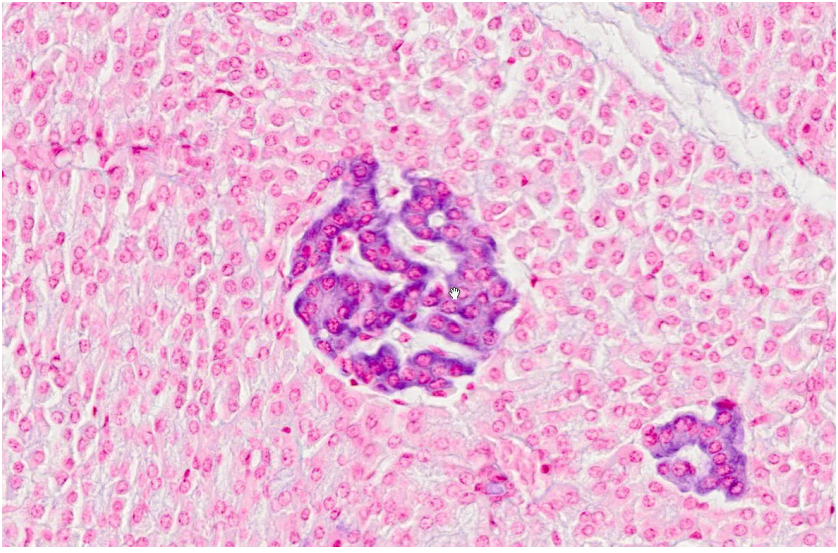
Chapter 13

Hormonal regulation of metabolism

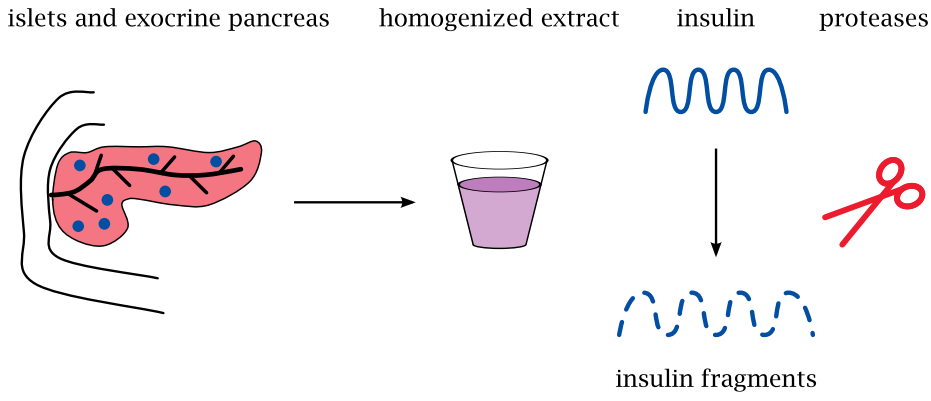
Hormones that affect energy metabolism

Hormone	Message
insulin	glucose and amino acids available, more substrates on the way
glucagon	glucose and amino acids in short supply, need to mobilize internal reserves
epinephrine	prepare for imminent sharp rise in substrate demand
glucocorticoids	prepare for extended period of high demand
thyroid hormones	increase basal metabolic rate

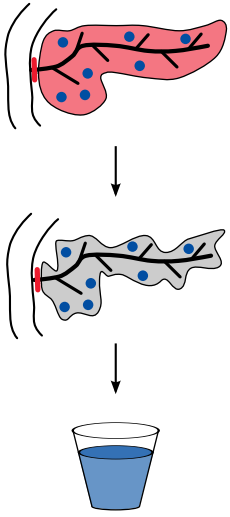
Langerhans' islets in the pancreas produce insulin and glucagon



A little bit of history: The purification of insulin—the problem



The purification of insulin—Banting's solution



apply ligature,
reseal abdomen

pancreatic juice backs
up, exocrine tissue
self-destroys

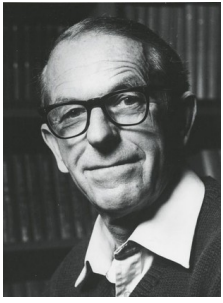
obtain protease-free
homogenized extract

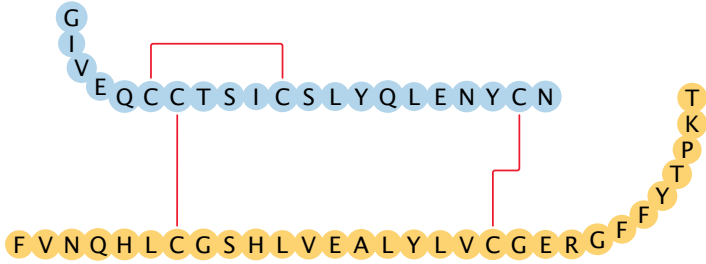
Historical side note: Norman Bethune, Banting's famous classmate

"Comrade Bethune's spirit, his utter devotion to others without any thought of self, was shown in his great sense of responsibility in his work and his great warmheartedness towards all comrades and the people. Every Communist must learn from him."

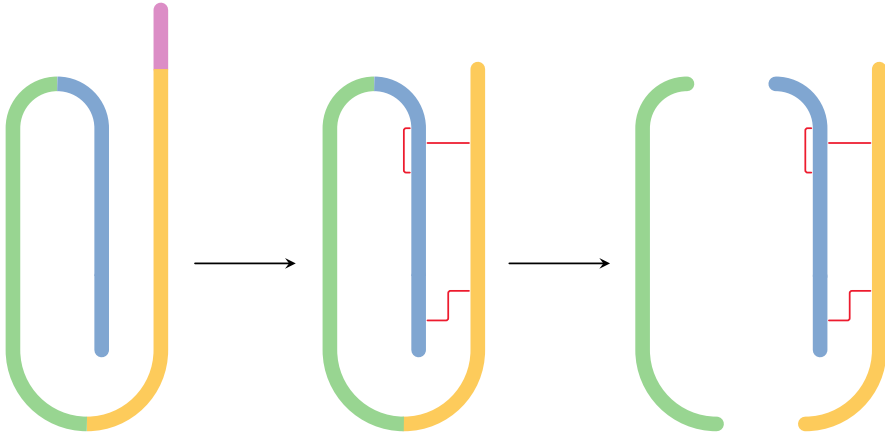
Mao Zedong, "In Memory of Norman Bethune"

Structure of insulin and its precursors (1)





Structure of insulin and its precursors (2)



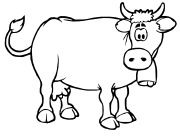
Sequences of human, swine, and bovine insulins

GIVEQCCTSICSLYQLENYCN

FVNQHLCGSHLVEALYLVCGERGFFYTPKT

GIVEQCCTSICSLYQLENYCN

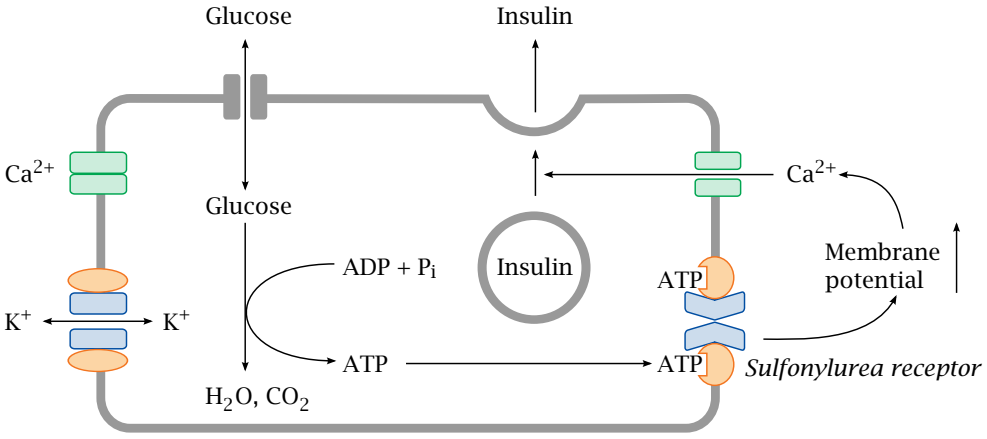
FVNQHLCGSHLVEALYLVCGERGFFYTPKA



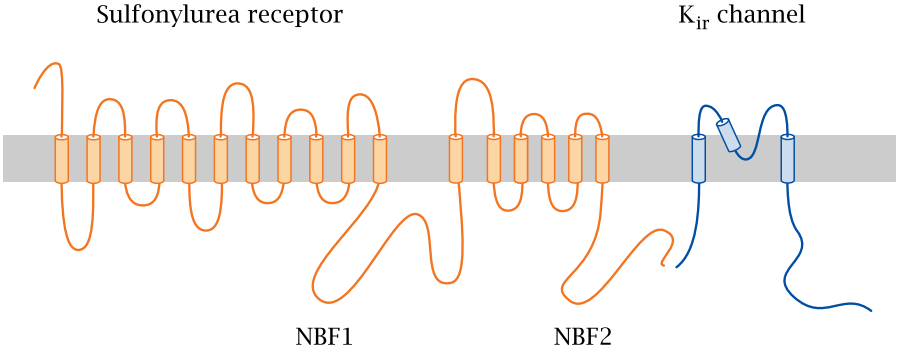
GIVEQCCASVCSLYQLENYCN

FVNQHLCGSHLVEALYLVCGERGFFYTPKA

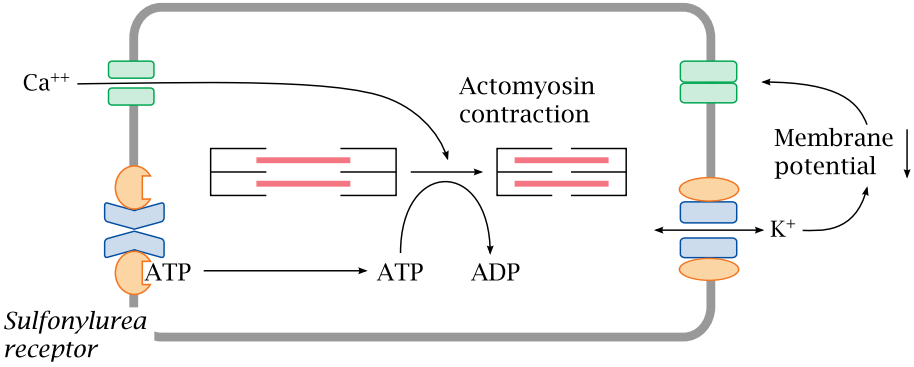
Insulin secretion in the β-cell is controlled by glucose and triggered by membrane depolarization



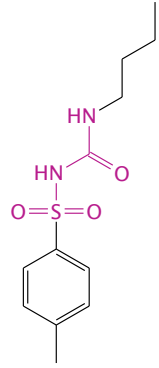
The sulfonylurea receptor controls an associated potassium channel



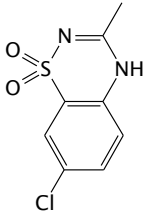
K_{ATP} channels also regulate the tone of smooth muscle cells



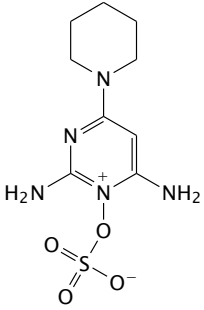
Tolbutamide promotes closing of the K_{ATP} channel



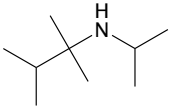
Tolbutamide



Diazoxide

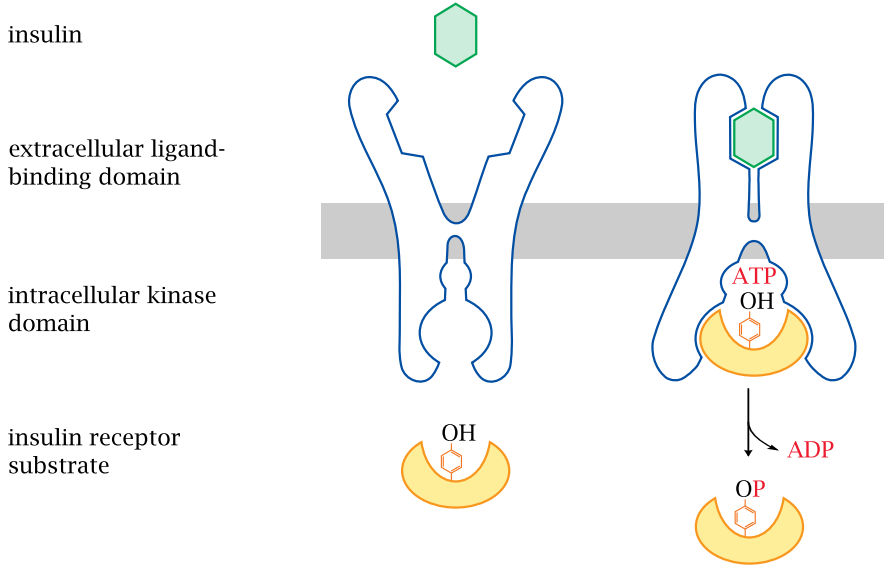


Minoxidil sulfate

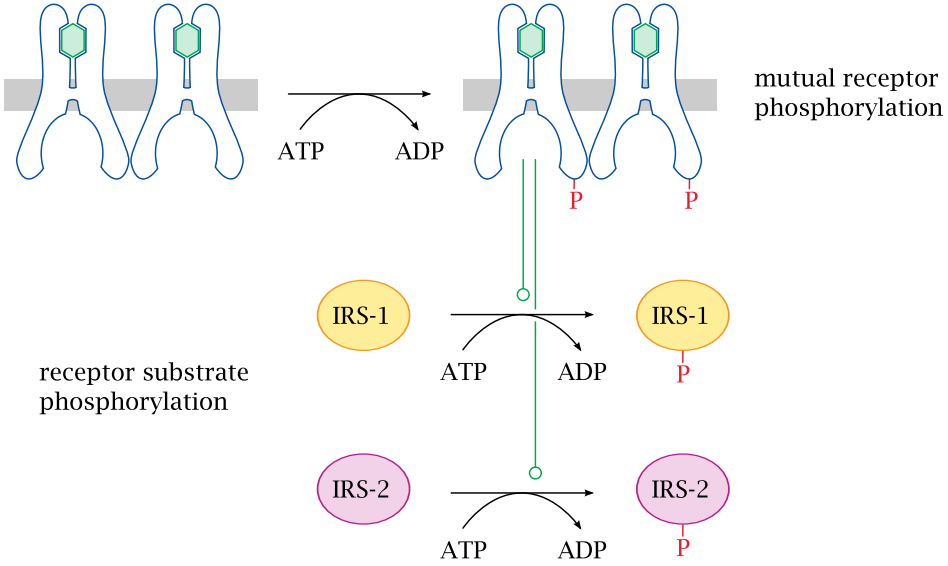


Iptakalim

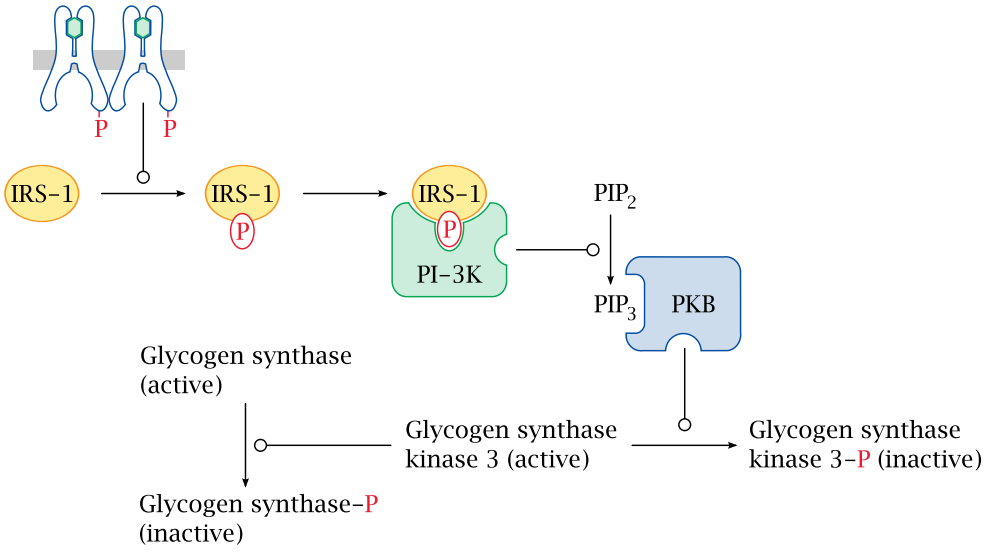
The insulin receptor is a receptor tyrosine kinase



Insulin receptor first phosphorylates itself and then a number of insulin receptor substrate proteins



Insulin effects on glycogen synthesis

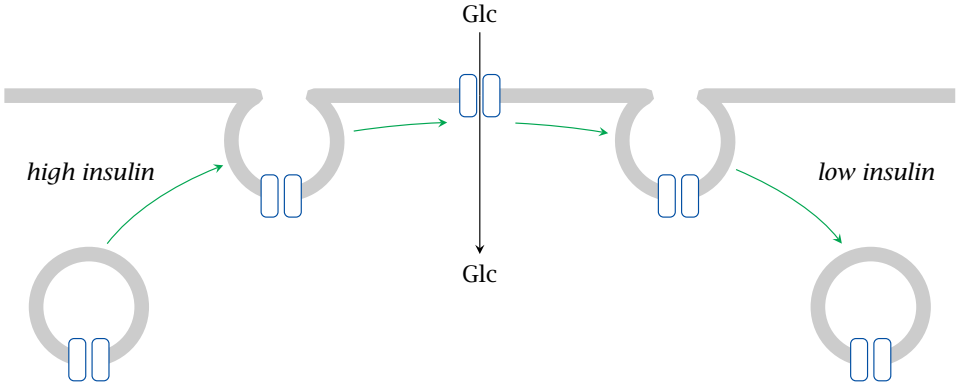


▸ PDE

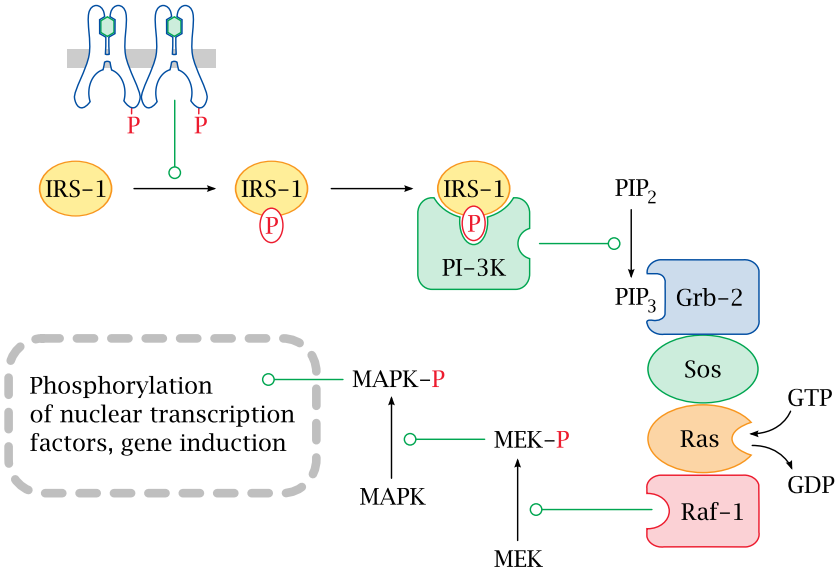
The role of insulin in glucose transport

	Active transport	Facilitated transport
insulin-independent	small intestine, kidney tubules	brain, β-cells, red blood cells, cornea and lens of the eye
insulin-dependent	never	muscle, fat, most other tissues

Insulin promotes glucose uptake by increasing the surface exposure of GLUT 4 transporters

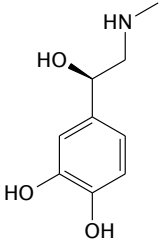


Transcriptional regulation by insulin

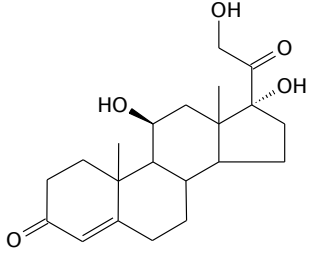


Other hormones

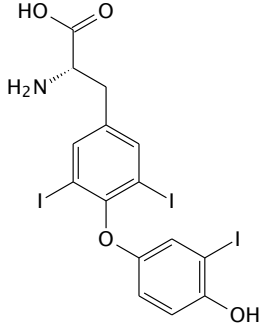
Glucagon



Epinephrine

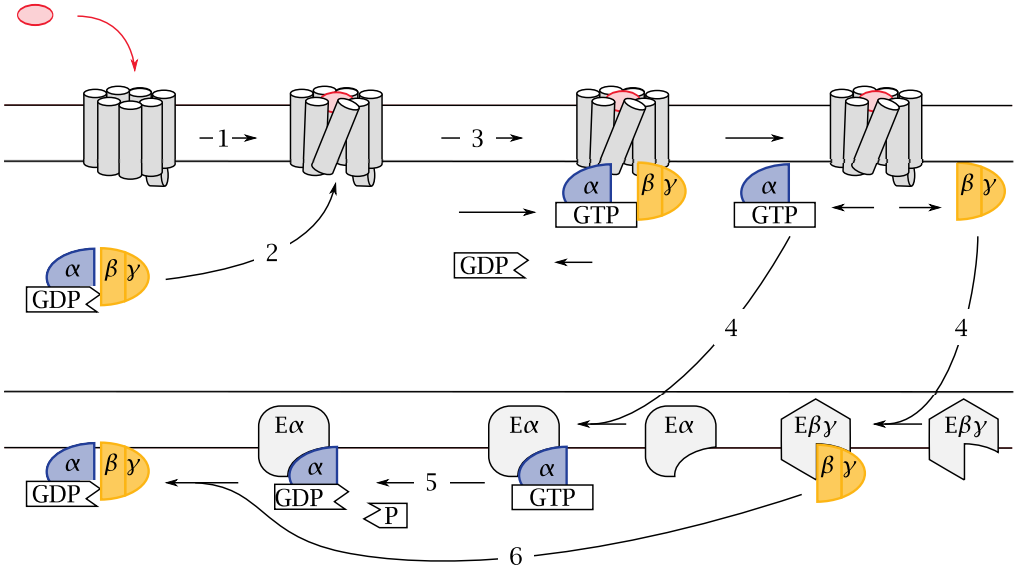


Cortisol

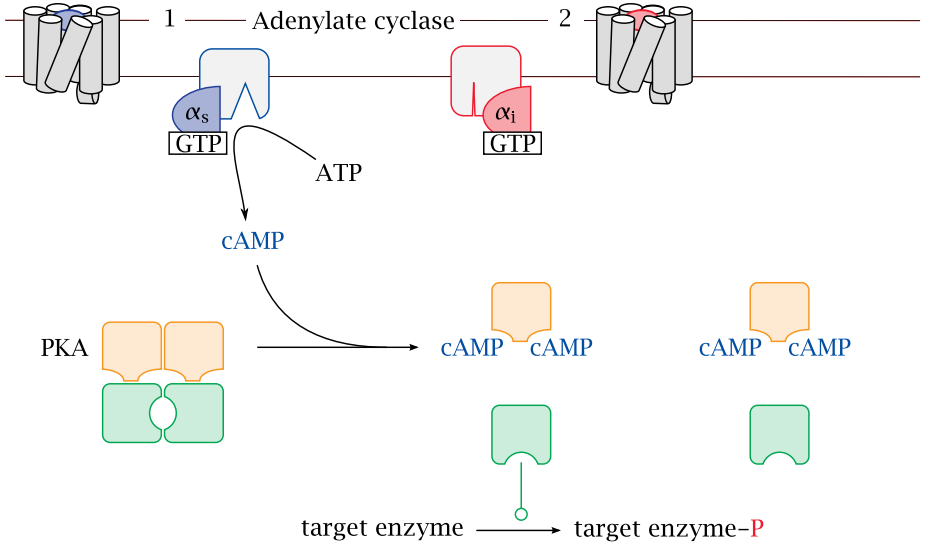


Triiodothyronine

Glucagon and epinephrine act via G-protein-coupled receptors



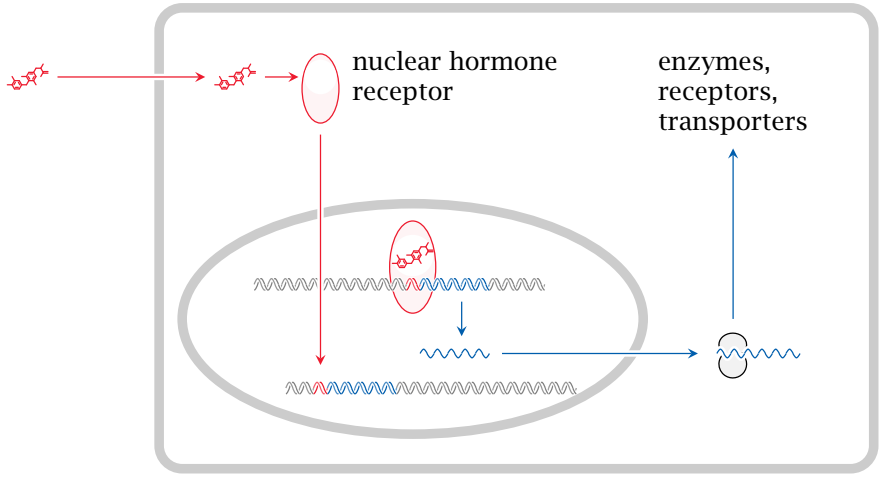
The glucagon and epinephrine receptors activate adenylate cyclase and protein kinase A



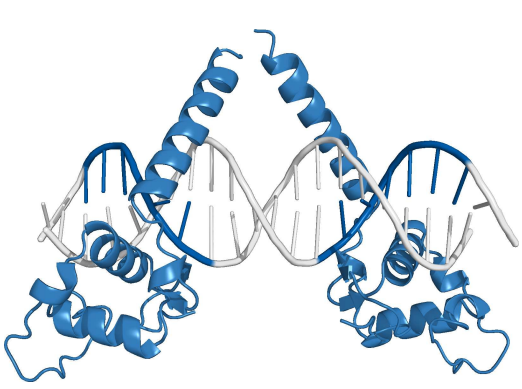
Metabolic effects of protein kinase A

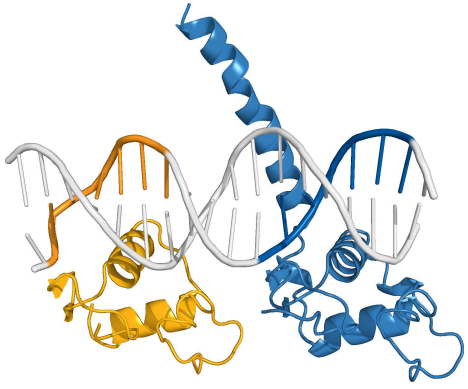
Target	Effect	Metabolic consequence
glycogen synthase	↓	glucose is not locked up in glycogen, remains available
phosphorylase kinase	↑	phosphorylase is activated, glucose is released from glycogen storage
PFK-2 / Fructose-2,6-bisphosphatase	↓ / ↑	Fructose-2,6-bisphosphate drops; glycolysis is inhibited, gluconeogenesis is activated
hormone-sensitive lipase	↑	fatty acids are mobilized for β-oxidation and ketogenesis

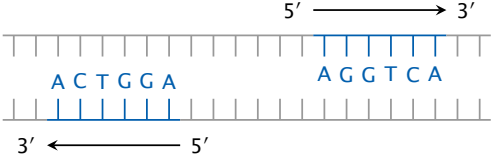
Glucocorticoids and thyroid hormones act on nuclear hormone receptors to activate transcription

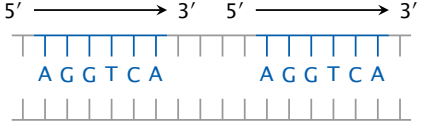


DNA binding by thyroid hormone receptors

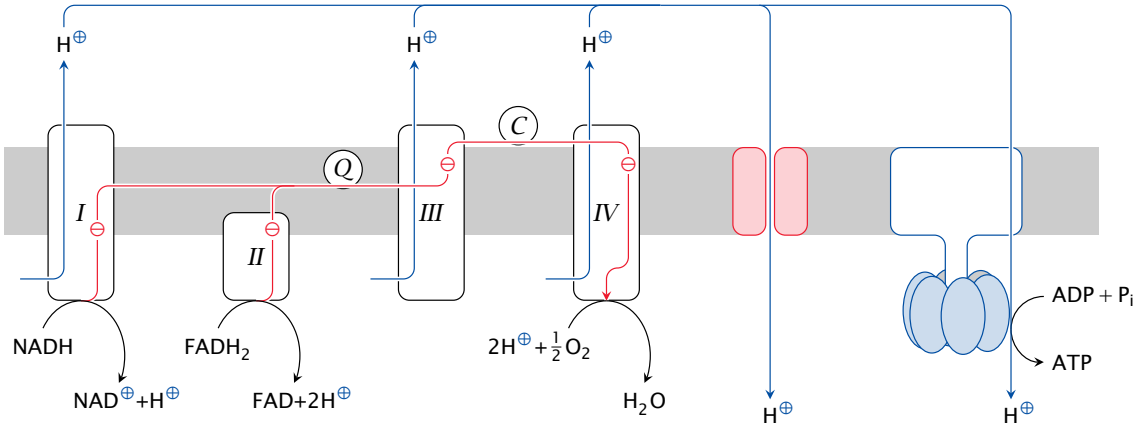








Thyroid hormones induce respiratory chain uncoupling proteins

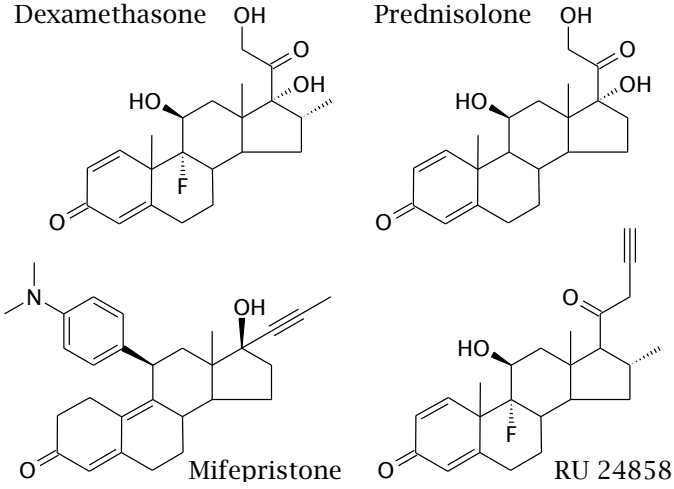


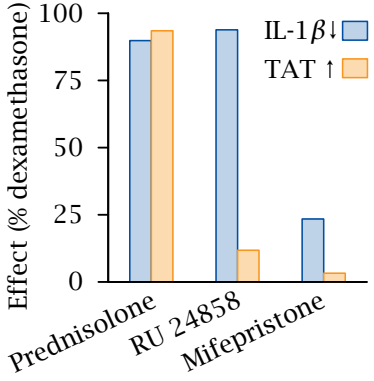
Metabolic effects of glucocorticoid hormones

- induction of enzymes for glycogen synthesis, glycogen breakdown, as well as gluconeogenesis
- induction of enzymes for protein breakdown, which supplies substrates for gluconeogenesis
- induction of adrenergic receptors

. . . overall, glucocorticoids increase blood glucose

Glucocorticoid receptor agonists and antagonists





Control of food intake by leptin

Chapter 14

Diabetes mellitus

Diabetes mellitus

What's in a name?

1. diabetes: "marching through"—urine is produced incessantly
2. mellitus: honey-sweet—as opposed to *diabetes insipidus* (insipid—without flavor)

What does the adjective tell us about a traditional method of diagnosis?

Forms and causes of diabetes mellitus

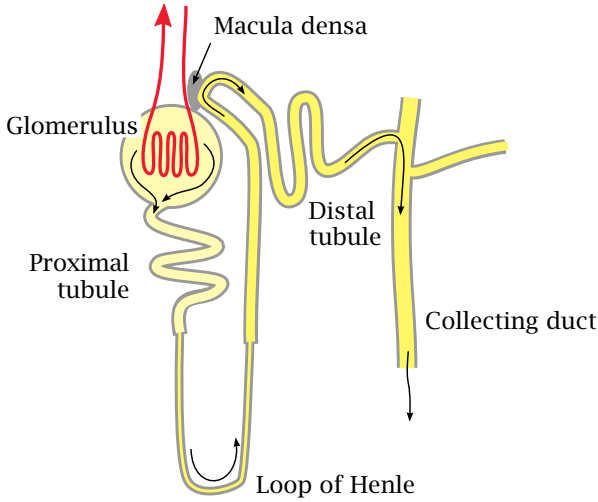
Form	Cause
type 1	lack of insulin due to destruction of β-cells in pancreas islets
type 2	lack of functional response to insulin
secondary	excess activity of hormones antagonistic to insulin

Overview of kidney function

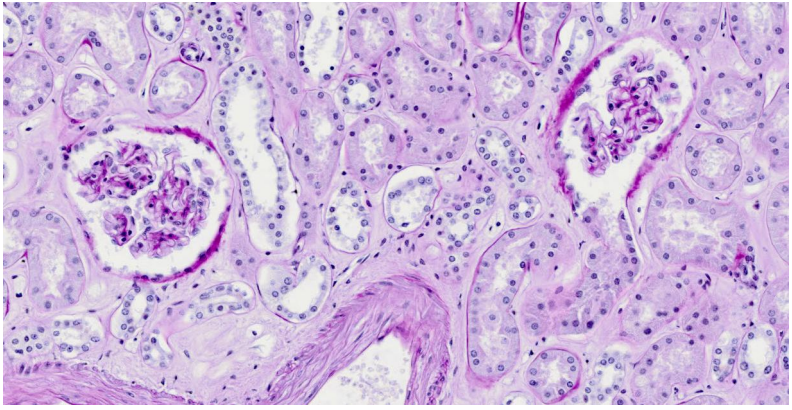
Urine is “distilled” from blood plasma in several stages:

1. ultrafiltration: 10-20% of the blood plasma volume that passes through the kidneys is squeezed across a molecular sieve; small solutes are filtrated, macromolecules are retained
2. solute reuptake: glucose, amino acids, salts etc. are recovered from the ultrafiltrate through active transport
3. water reuptake: driven by osmotic gradient
4. solute secretion: some substrates are actively secreted into the nascent urine

The nephron

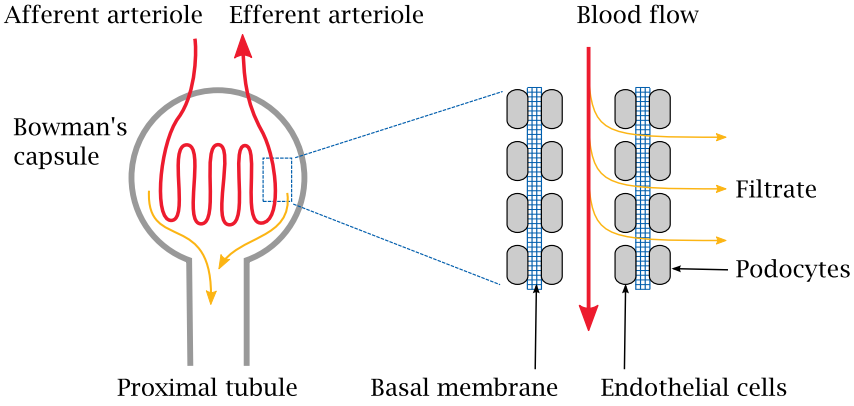

Macula densa
Glomerulus
Distal tubule
Proximal tubule
Collecting duct
Loop of Henle

Kidney tissue structure and function: Glomeruli and tubules

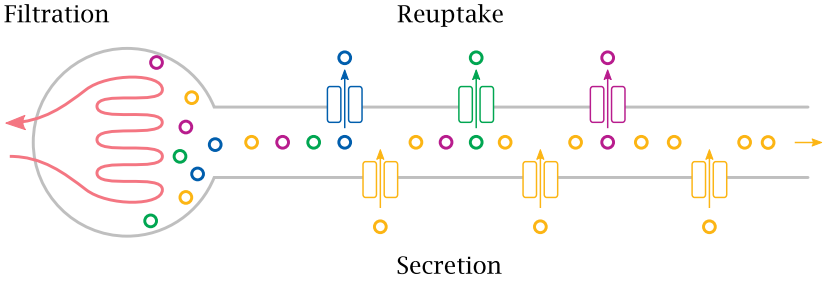


From pathorama.ch with permission

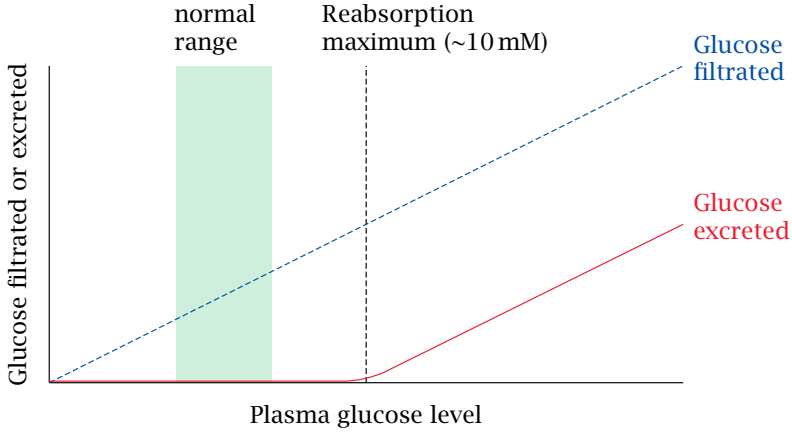
Primary filtration occurs in the glomerulus



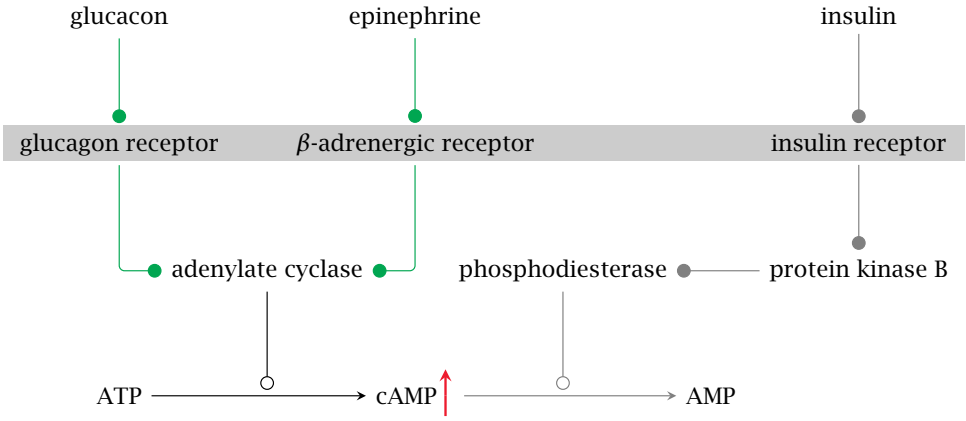
Reuptake and secretion occur in the tubular segments



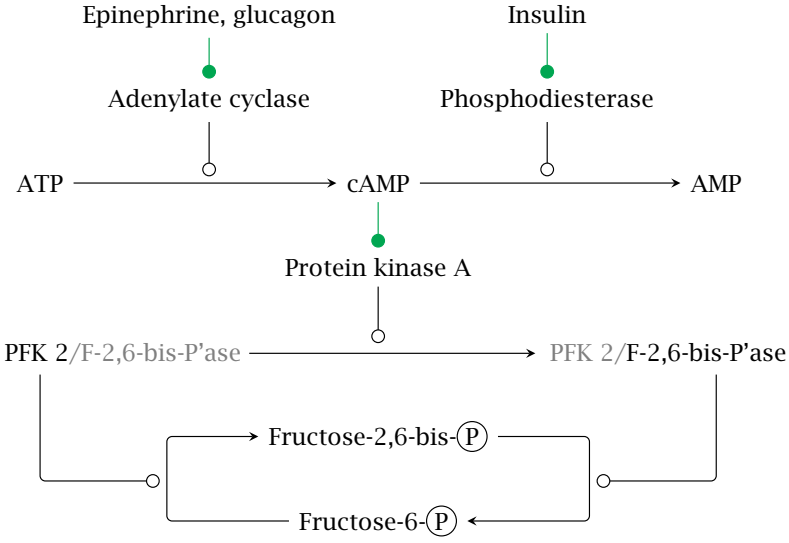
The capacity for glucose reuptake is saturated slightly above the physiological plasma concentration range



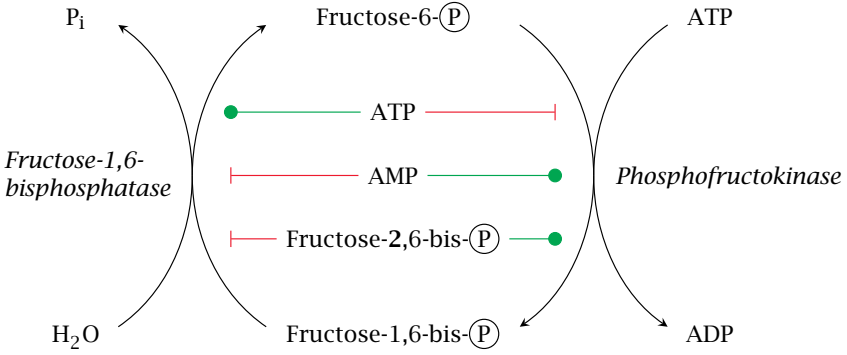
Lack of insulin drives up cAMP



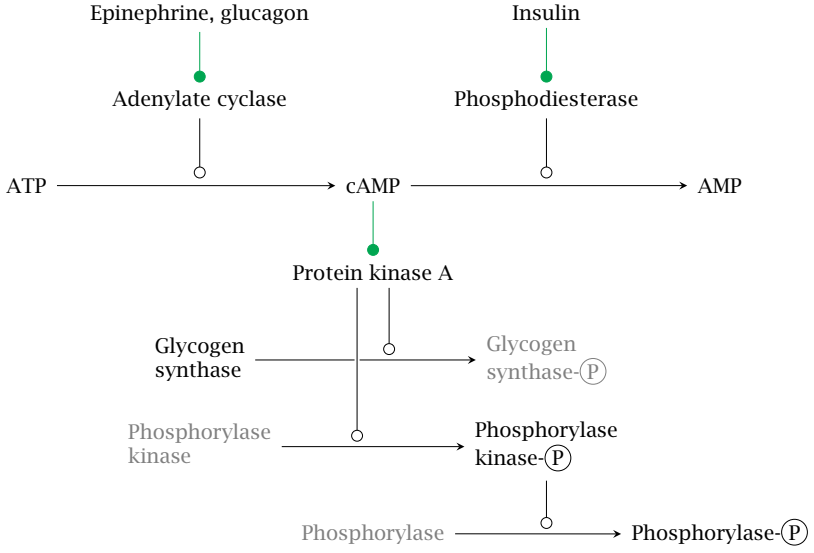
Lack of insulin promotes gluconeogenesis



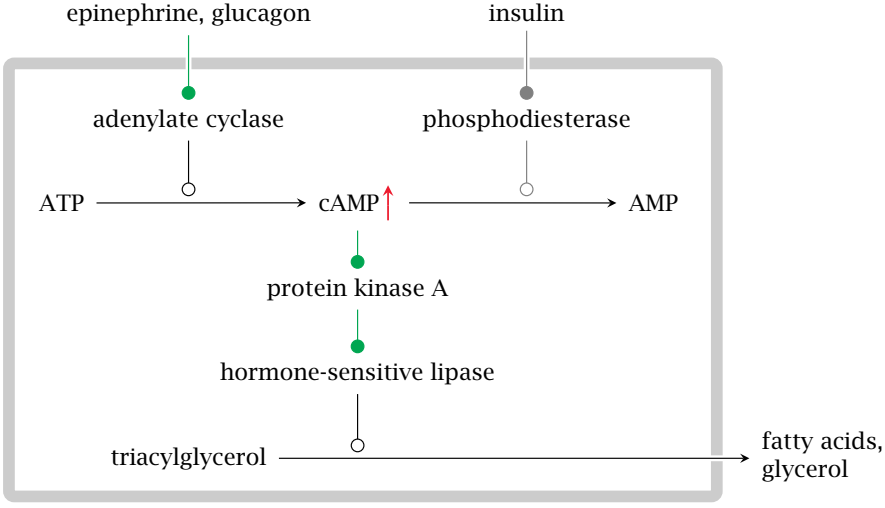
Lack of insulin promotes gluconeogenesis (2)



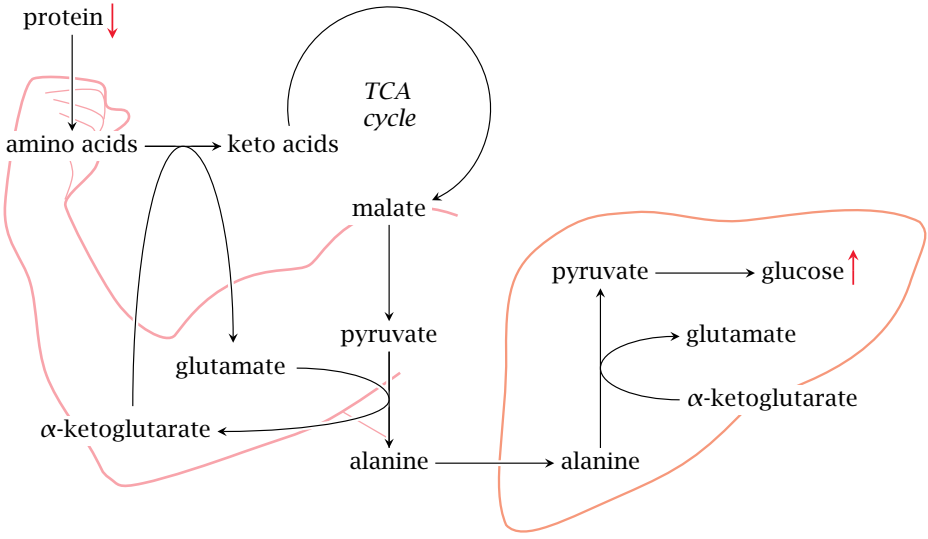
Lack of insulin induces breakdown and inhibits synthesis of glycogen



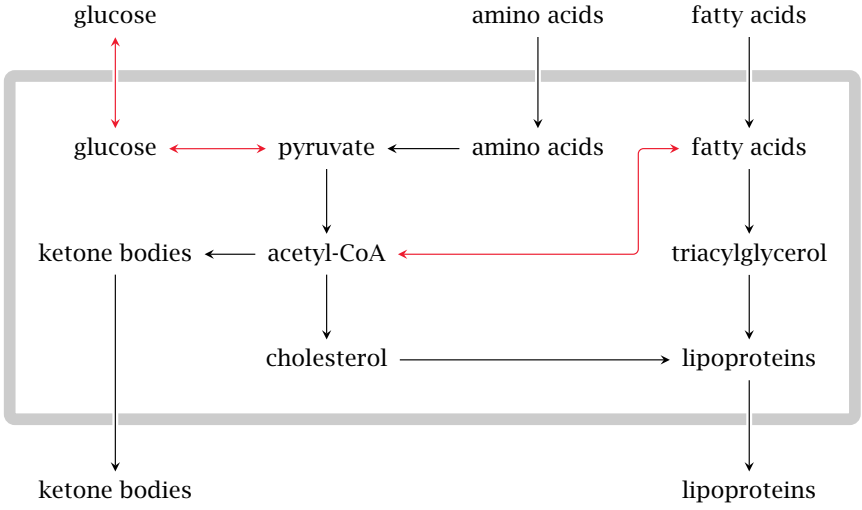
Lack of insulin induces triacylglycerol breakdown in fat tissue



Lack of insulin induces protein breakdown in muscle tissue



Substrate overload in the liver leads to ketogenesis and lipoprotein synthesis



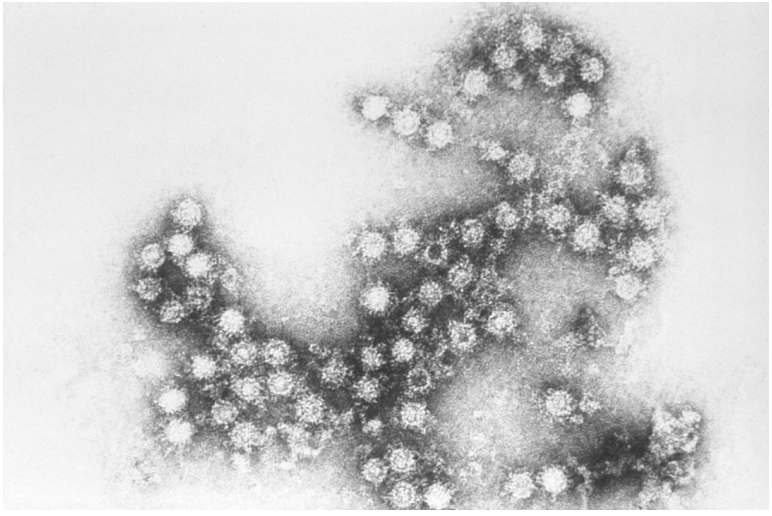
Laboratory findings in untreated or under-treated diabetes

Observation	Cause
increased blood glucose	excessive gluconeogenesis, lack of utilization
glucose excreted in urine	capacity for renal reuptake exceeded
acidosis (low blood pH)	high plasma levels of ketone bodies
increased urea levels	accelerated muscle protein breakdown
increased blood lipoproteins	increased synthesis and packaging of cholesterol and triacylglycerol in the liver

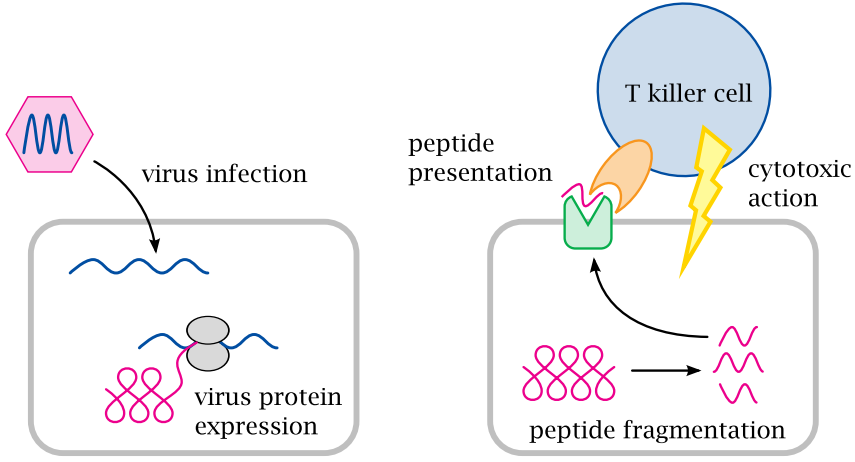
Typical symptoms and history in a new case of type 1 diabetes

Symptom	Cause
dehydration	osmotic diuresis due to glucose excretion
acetone smell	acetone forms from acetoacetate, is exhaled
coma	both acidosis and blood hyperosmolarity impair brain function
loss of body weight	dehydration, breakdown of proteins and fat
recent flu-like disease, possibly myocarditis	coxsackievirus infection

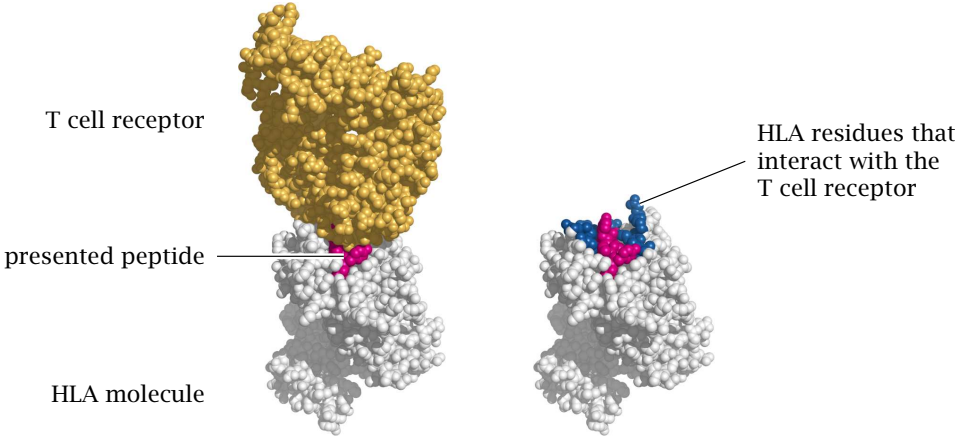
The role of coxsackieviruses in the pathogenesis of type 1 diabetes



Outline of T lymphocyte function in antiviral immune responses



Structure of a T cell receptor bound to its cognate peptide presented by an HLA molecule



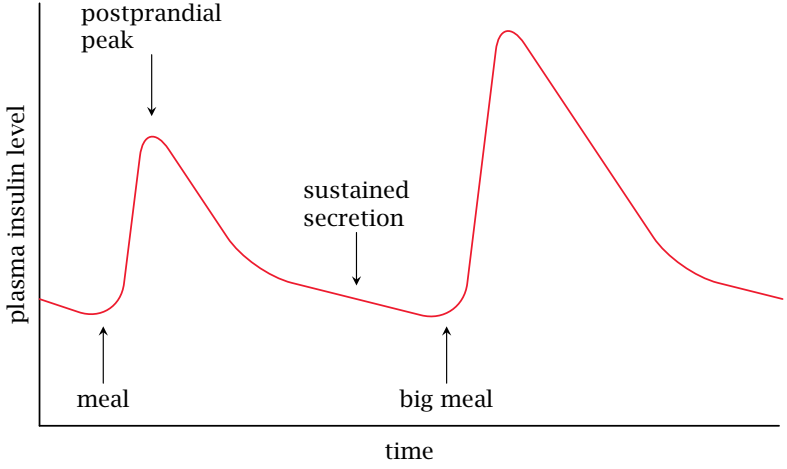
HLA alleles influence the risk of developing type 1 diabetes

HLA-DQ Haplotype	Relative risk	Absolute risk
A1: 0301-0302 / B1: 0501-0201	21	6%
B1: 0602	0.03	0.01%

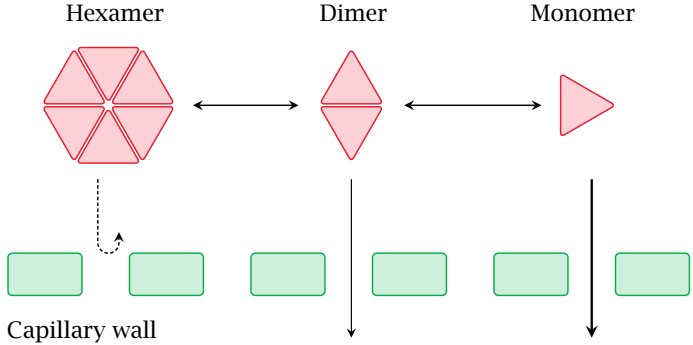
How to treat a fresh case of acute diabetes

1. Severely sick, possibly comatose patient
   - infusion therapy for fluid replacement, pH and electrolyte adjustment
   - parenteral nutrition with proportional insulin substitution
   - frequent monitoring of lab parameters (glucose, salts, pH) to adjust therapy
2. Upon stabilization
   - reversal to oral nutrition
   - train patient to adhere to a stable, regular diet and inject themselves with insulin
   - teach patient to monitor blood glucose and to recognize symptoms of hyper- and hypoglycemia

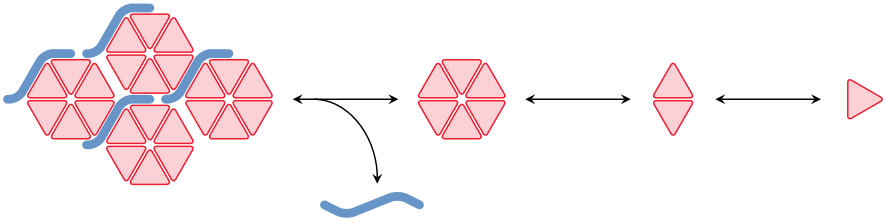
Kinetics of physiological insulin secretion



The reversible aggregation of insulin delays its diffusion from tissue into the circulation

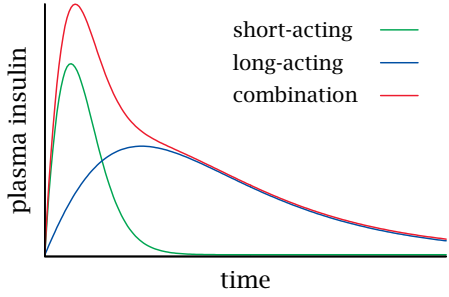


Delayed release of insulin from protamine complexes



protamine MARYRCCRSQSRSRYYRQRQRSRRRRRRSCQTRRRAMRCCRPRYRPRCRRH

Biphasic insulin preparations



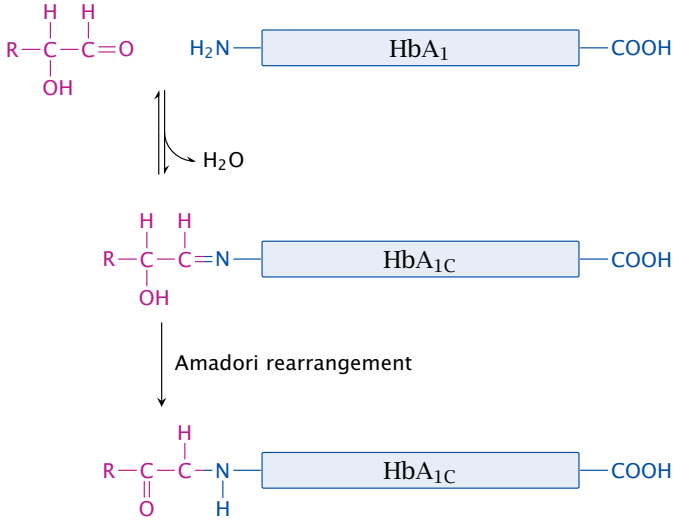
Short-term complications of insulin-requiring diabetes

Deviation	Symptoms
insulin too low	hyperglycemia, acidosis, . . . , coma
insulin too high	hypoglycemia, coma

Long-term complications of insulin-requiring diabetes

Biochemical deviation	Clinical manifestation
accumulation of sorbitol in the lens of the eye	cataract ‣ polyol pathway
increased conversion of glucose to lipids	increased blood fats, atherosclerosis
glucosylation of proteins? sorbitol accumulation?	damage to nerve fibres, kidneys, other organs

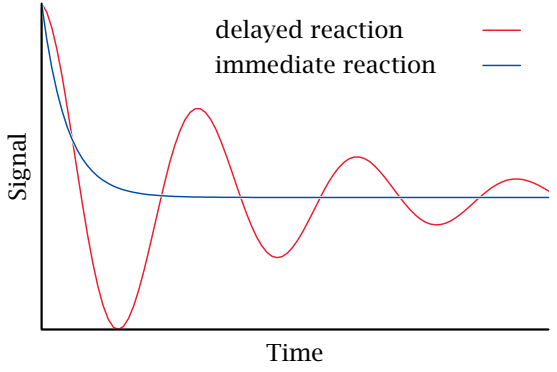
HbA_{1C} as a parameter of long-term glucose control



Intensive insulin therapy

- rationale: prevent long term complications through tight control of blood glucose
- means: frequent glucose sampling and injections, or continuous insulin application with pump, such that the rate of insulin infusion is controlled by the current glucose level
- challenge: avoid hypoglycemia through insulin overdose—we need to minimize the delay between insulin application and effect

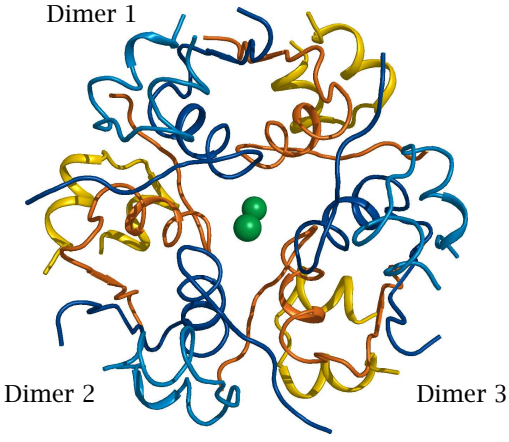
Nerdy intermission: delayed feedback causes signal oscillation

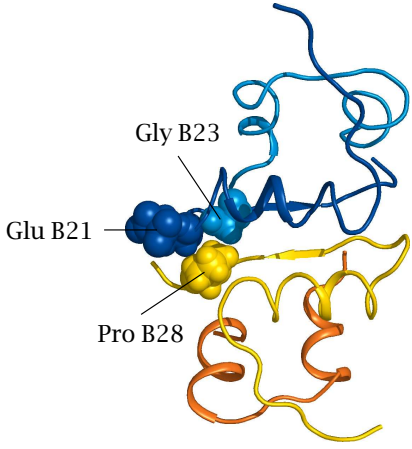


Mutant insulins optimized for rapid dissociation and uptake

- Insulin lispro: Proline B28 switched with lysine B29
- Insulin aspart: Proline B28 replaced with aspartate

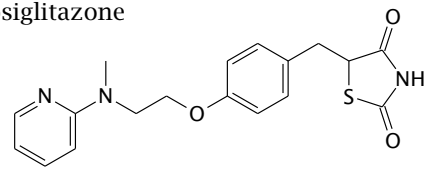
Structural basis for proline B28 mutations



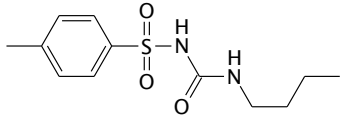


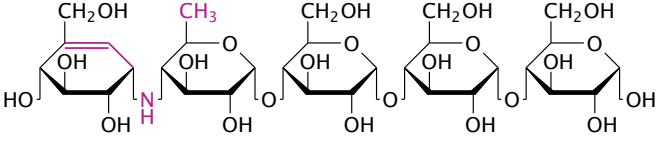
Oral antidiabetic drugs

Rosiglitazone

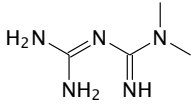


Tolbutamide





Acarbose

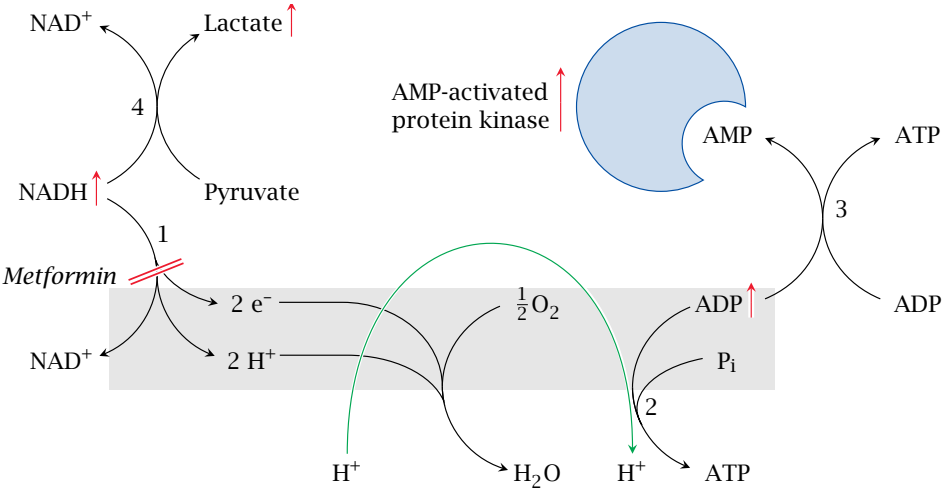


Metformin

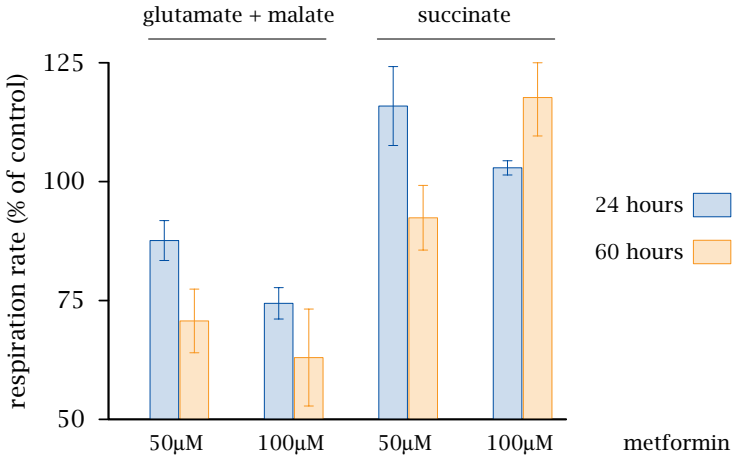
Action modes of oral antidiabetics

Drug	Action mechanism
tolbutamide	sulfonylurea receptor agonist
rosiglitazone	peroxisome proliferator-activated receptor γ agonist; inhibition of mitochondrial pyruvate transport
acarbose	inhibition of the brush border enzymes sucrase and maltase—reduced or delayed glucose uptake
tolrestat	aldose reductase inhibitor (withdrawn)
metformin	NADH dehydrogenase inhibition ?

Hypothetical mode of action of metformin



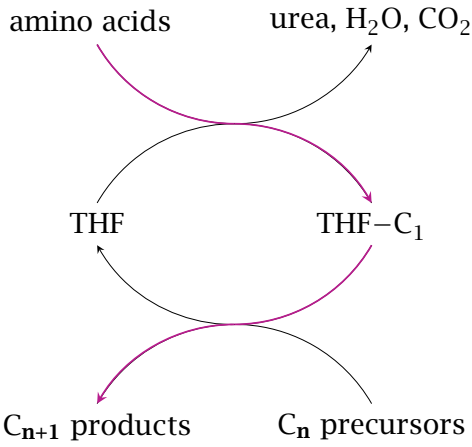
Inhibition of complex I of the respiratory chain by metformin



Chapter 15

Biosynthetic pathways using tetrahydrofolate and vitamin B_{12}

The role of tetrahydrofolate in biosynthetic reactions

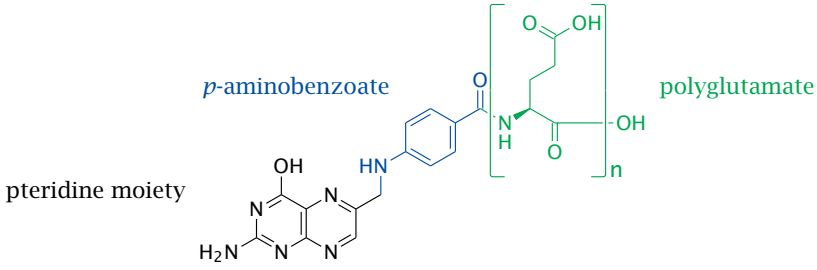


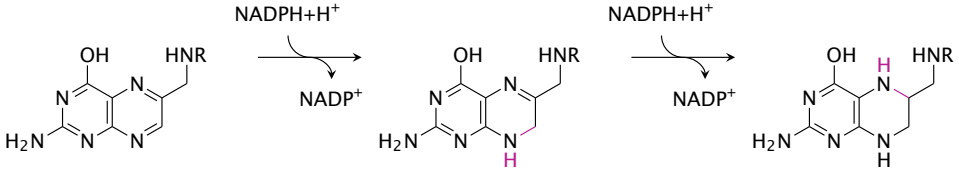
carbon sources

C_1-tetrahydrofolate pool

biosynthetic pathways

Folic acid is reduced by dihydrofolate reductase (DHFR)





Sources and destinations of C_1 units transferred by tetrahydrofolic acid

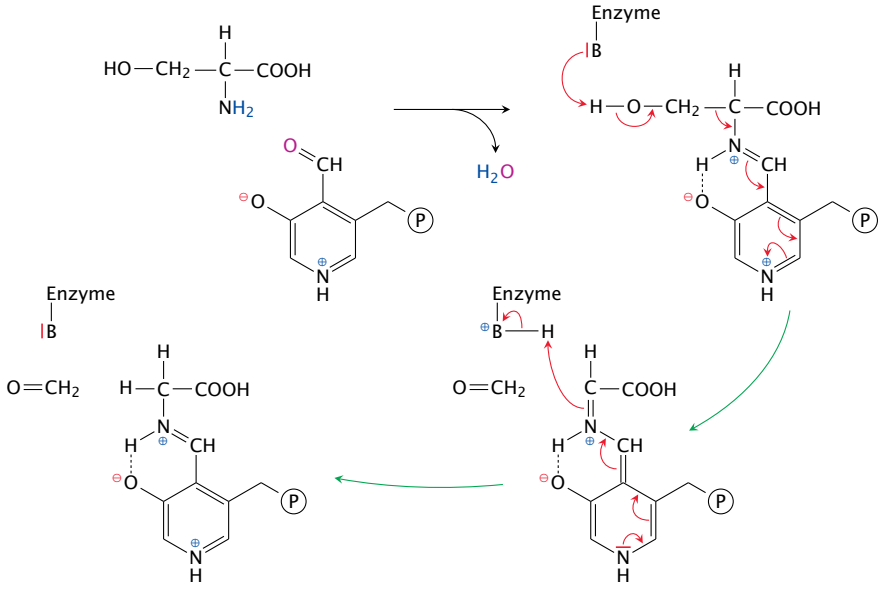
Sources:

1. *serine, glycine*
2. histidine, tryptophan

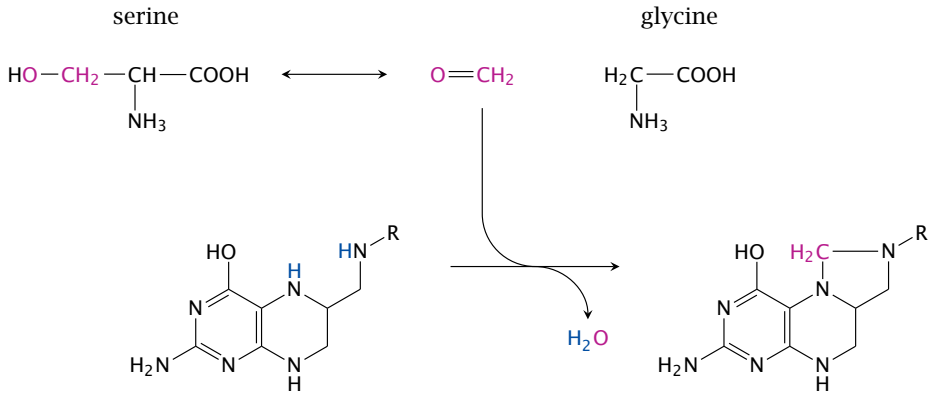
Biosynthetic destinations:

1. purine bases
2. thymine
3. *S*-adenosylmethionine $\longrightarrow$ choline phospholipids, creatine, epinephrine, DNA methylation

The serine hydroxymethyltransferase reaction: release of CH_2O



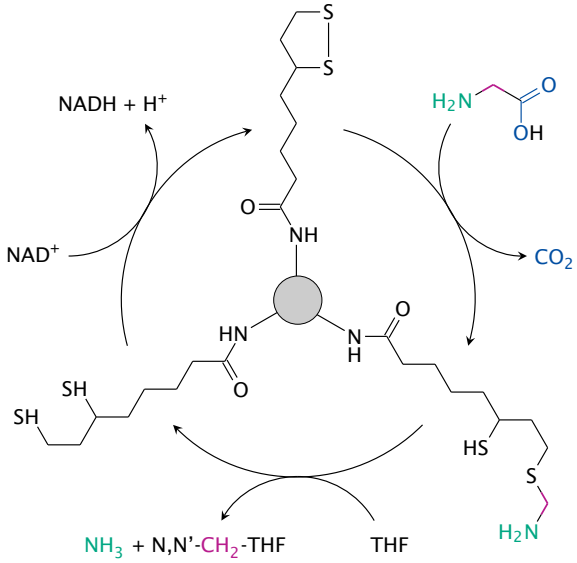
Capture of formaldehyde by THF yields N,N′-methylene-THF



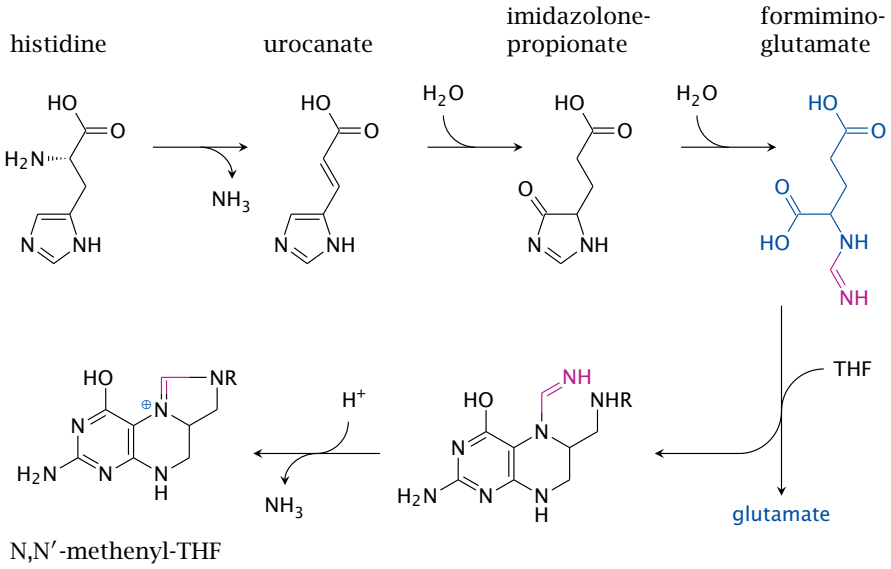
tetrahydrofolate

N,N-methylene-tetrahydrofolate

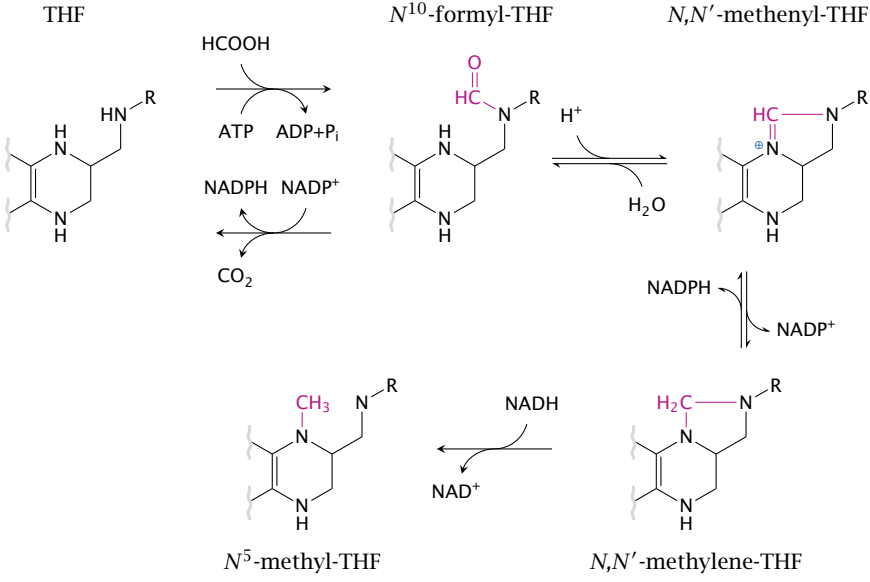
N,N′-methylene-THF production by the glycine cleavage system



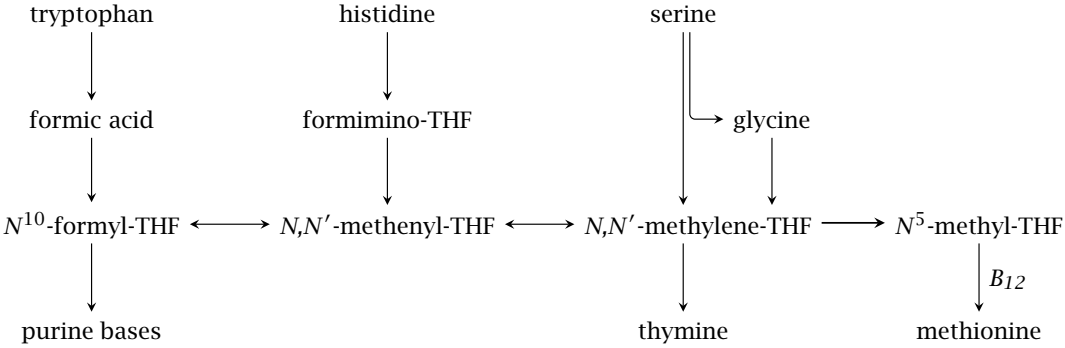
Histidine degradation produces *N,N′*-methenyl-THF



Redox transitions between various forms of C_1-THF

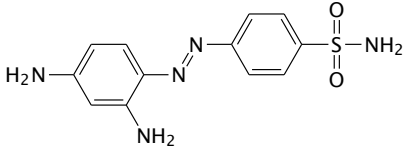


Overview of flux through the C_1-THF pool

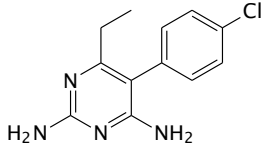


Folate antimetabolites as antibacterial and antiprotozoal agents

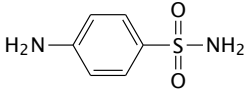
Sulfamidochrysoidine (Prontosil)



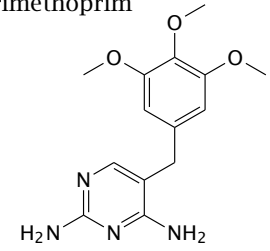
Pyrimethamine



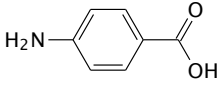
Sulfanilamide



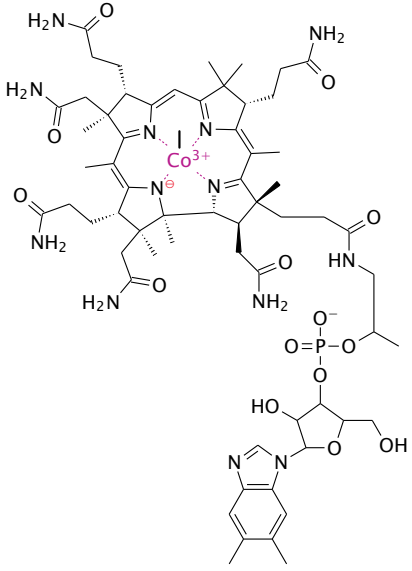
Trimethoprim

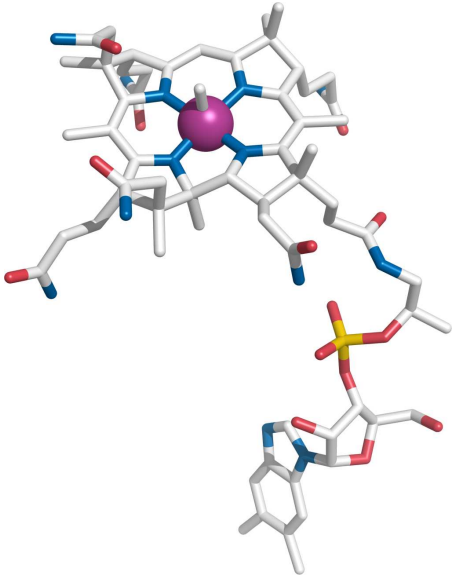


p-Aminobenzoic acid

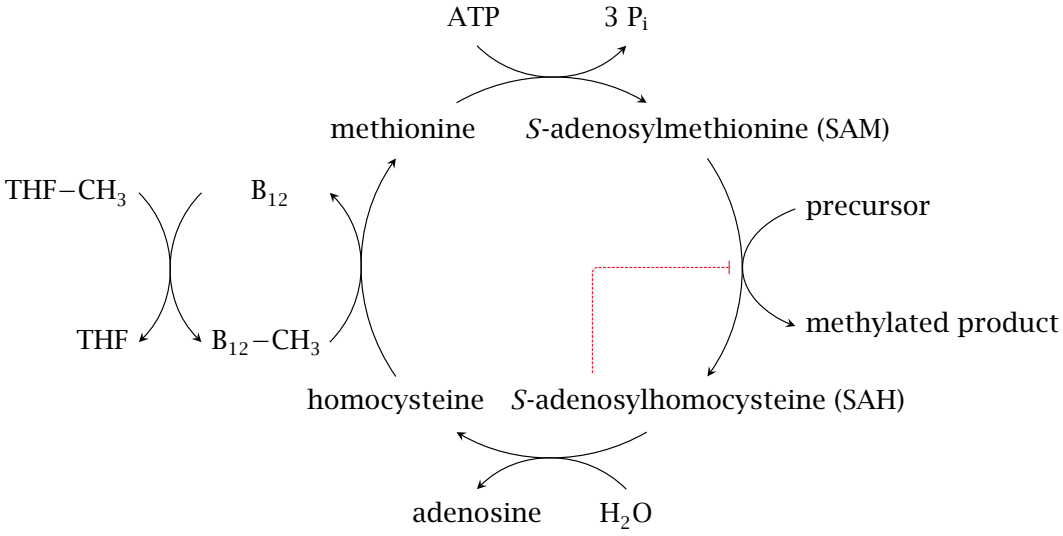


Structure of methylcobalamin

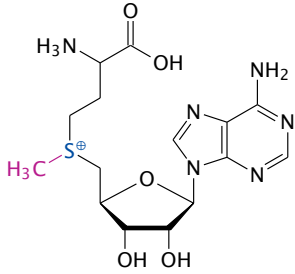




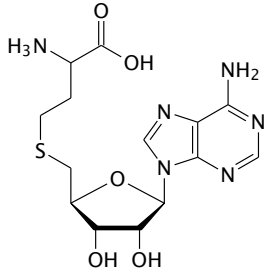
The *S*-adenosylmethionine (SAM) cycle requires vitamin B_{12}



Structures of S-adenosylmethionine and S-adenosylhomocysteine



SAM

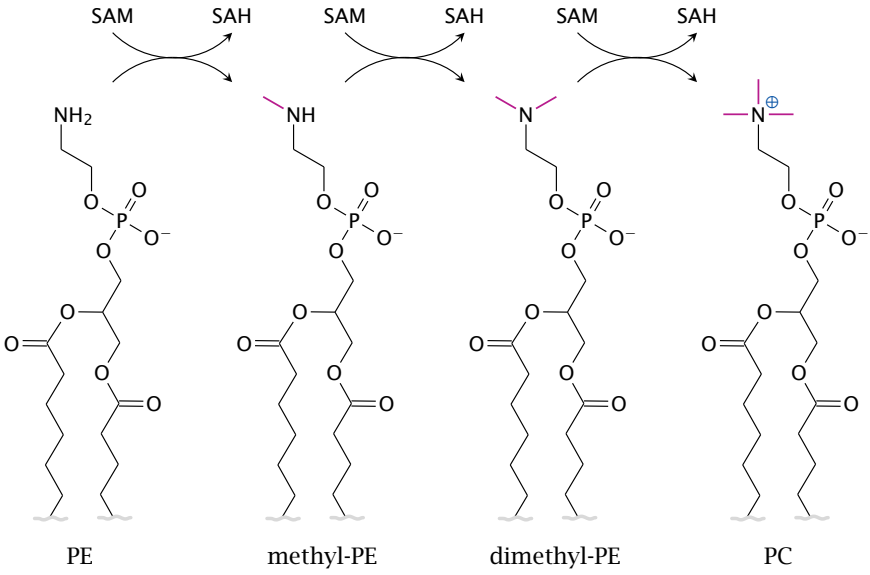


SAH

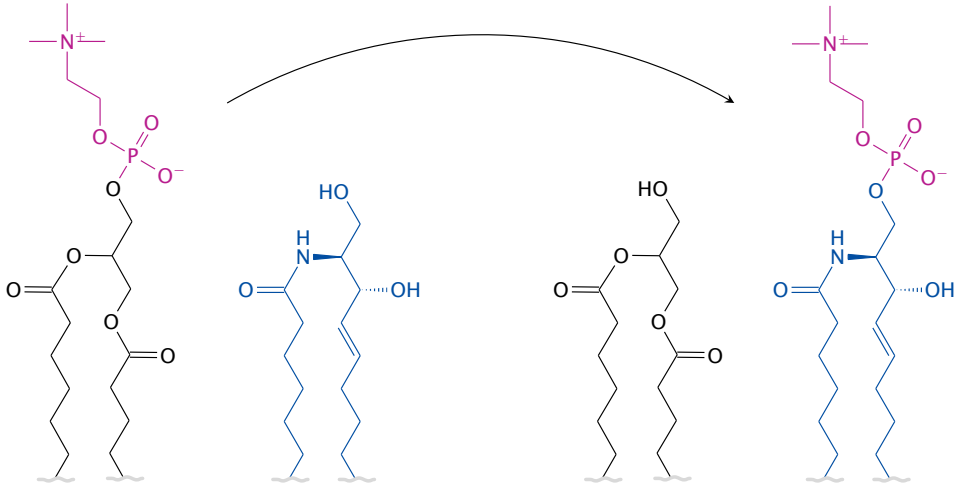
SAM-dependent methylation reactions

1. methylation of phosphatidylethanolamine (PE) to phosphatidylcholine (PC)
2. guanidinoacetate $\longrightarrow$ creatine
3. norepinephrine $\longrightarrow$ epinephrine
4. acetylserotonin $\longrightarrow$ melatonin
5. DNA methylation
6. methylation of drugs (e.g. mercaptopurine)

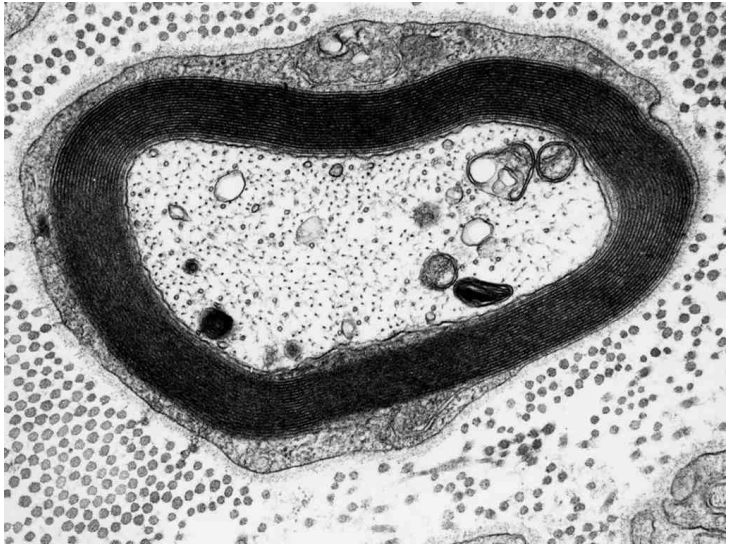
Phosphatidylethanolamine methylation



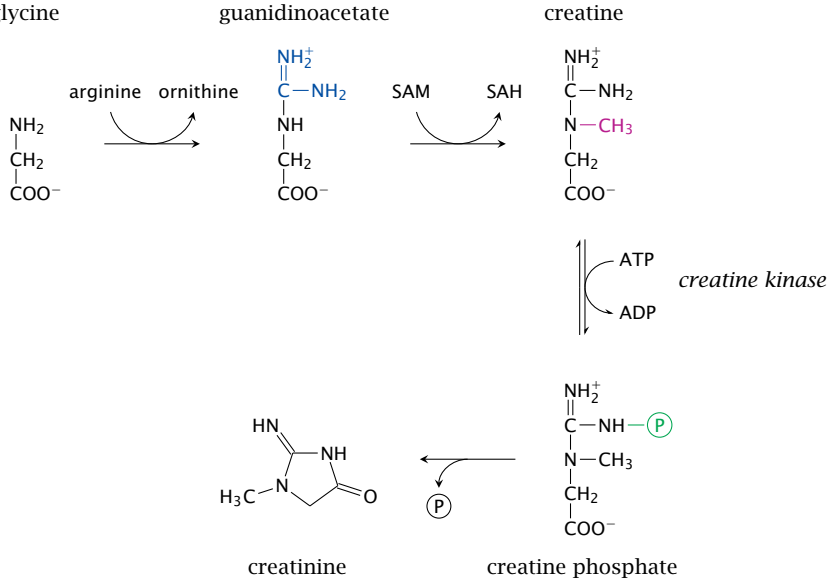
Sphingomyelin acquires its phosphocholine headgroup from PC



Major nerve fibers are myelinated



Creatine metabolism



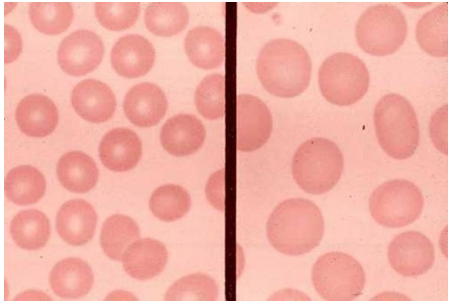
Uptake, transport and storage of folic acid

- contained in vegetables (Latin *folium* = leaf)
- synthesized by bacterial flora in the large intestine
- active transport mediates intestinal uptake and renal reuptake, as well as accumulation in the liver
- 50% of all folate is stored in the liver

Causes of folate deficiency

- malnutrition
- inflammatory bowel diseases
- surgical bowel resection (short intestine syndrome)
- cytochrome P450-inducing drugs
- excessive alcohol consumption—contentious

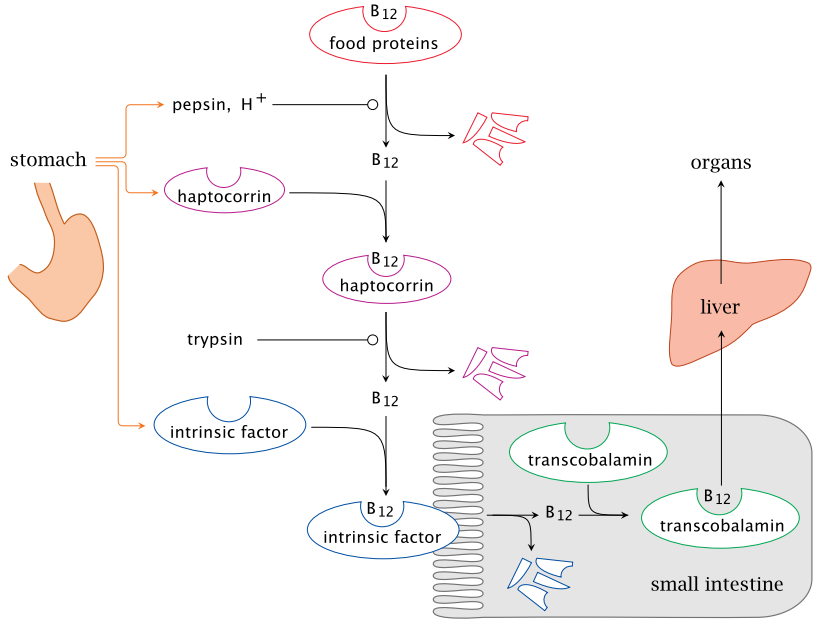
Folate deficiency causes macrocytic anemia



normal red cells macrocytic red cells

- C1-pool

Intestinal uptake of vitamin B_{12}

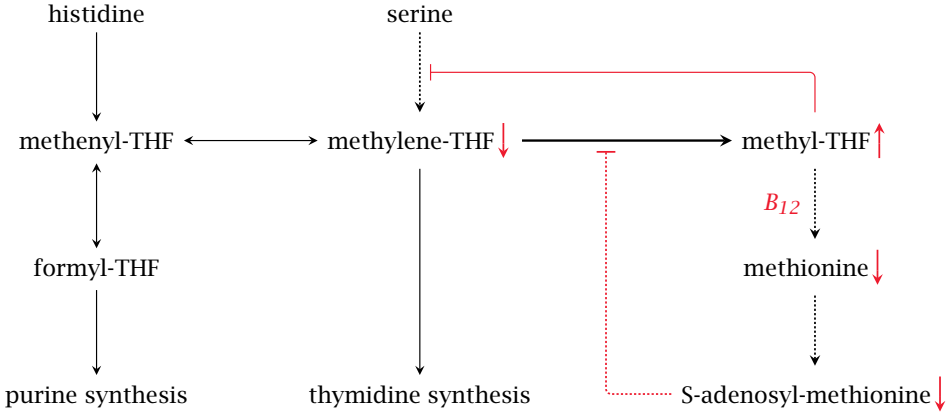


Various causes of B_{12} deficiency

Disease	Pathogenetic mechanism
autoimmune gastritis	destruction of the gastric parietal cells that produce gastric acid, haptocorrin, and intrinsic factor
pancreatic insufficiency	failure to digest haptocorrin
inflammatory bowel disease	disrupted uptake of B_{12} bound to intrinsic factor
receptor deficiencies	disrupted binding and cellular uptake of intrinsic factor or transcobalamin

▸ C1 pool

Vitamin B_{12} deficiency causes disruption of folate-dependent metabolism: the methyl trap 'hypothesis'



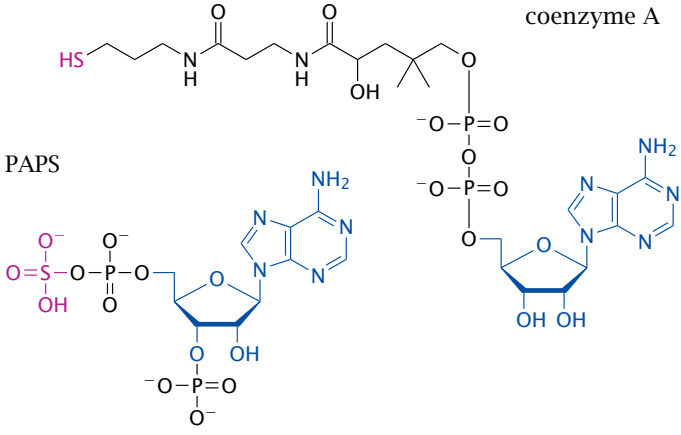
Chapter 16

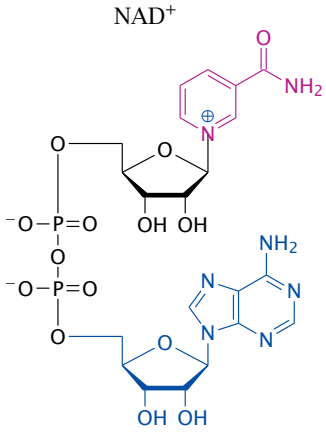
Nucleotide metabolism

Functions of nucleotides in biochemistry

- Building blocks of nucleic acids
- Cosubstrates and coenzymes
- Signaling

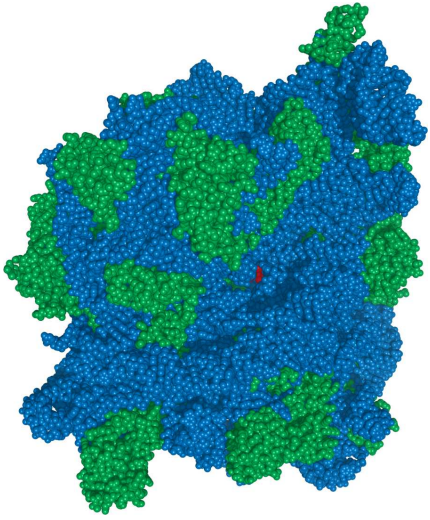
Structures of PAPS, acetyl-CoA, and NAD





▸ cobalamin structure

The RNA world hypothesis



RNA enzyme

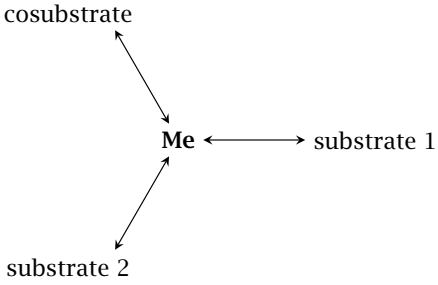
RNA enzyme
with peptide cofactor

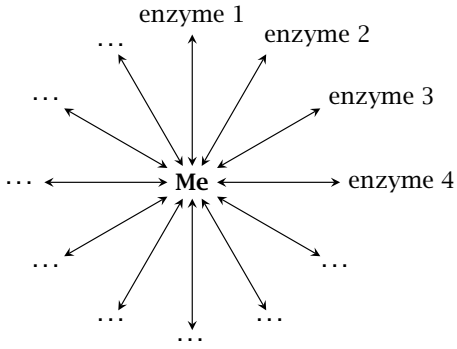
Hybrid enzyme

Pure protein enzyme

Why have cosubstrates become fossilized, whereas enzymes have not?

An enzyme's world . . .



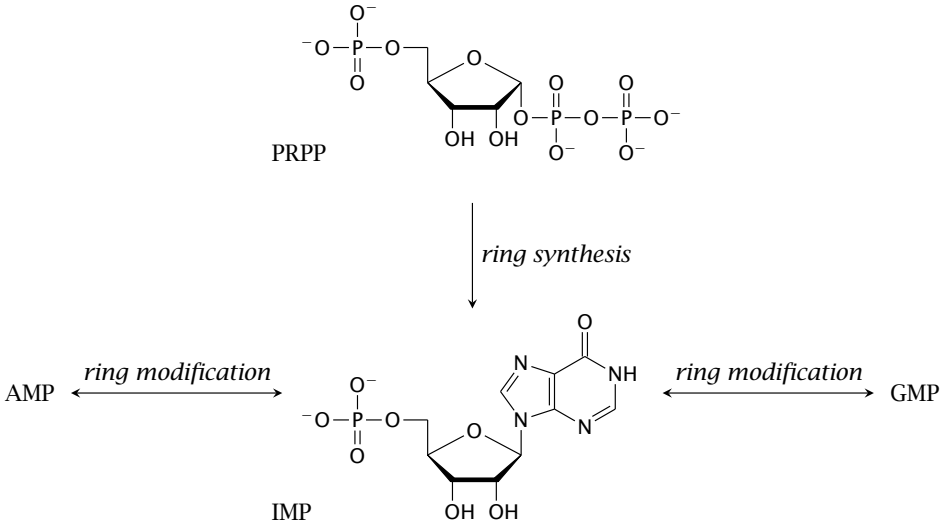


. . . and a cosubstrate's world

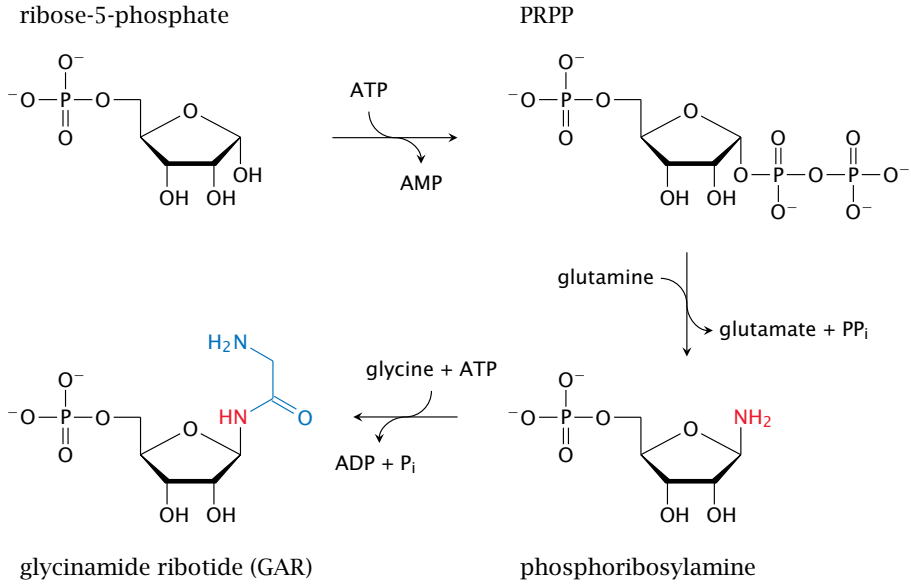
Metabolic routes and pathways of nucleotides

- *De novo* synthesis
- Intestinal uptake of nucleosides
- Endogenous turnover (partial degradation/salvage)
- Degradation and excretion

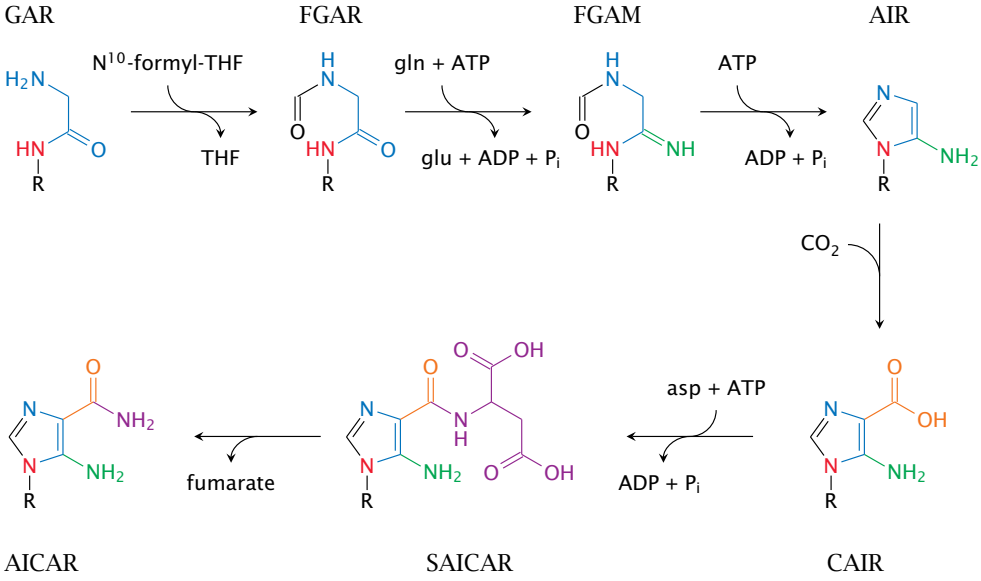
Overview of purine synthesis



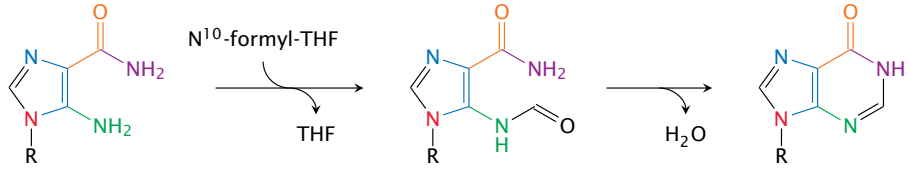
IMP synthesis (1)



IMP synthesis (2)

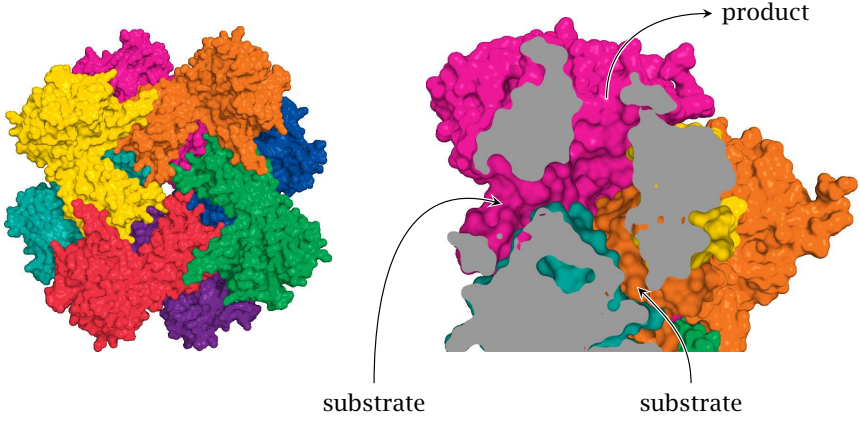


IMP synthesis (3)

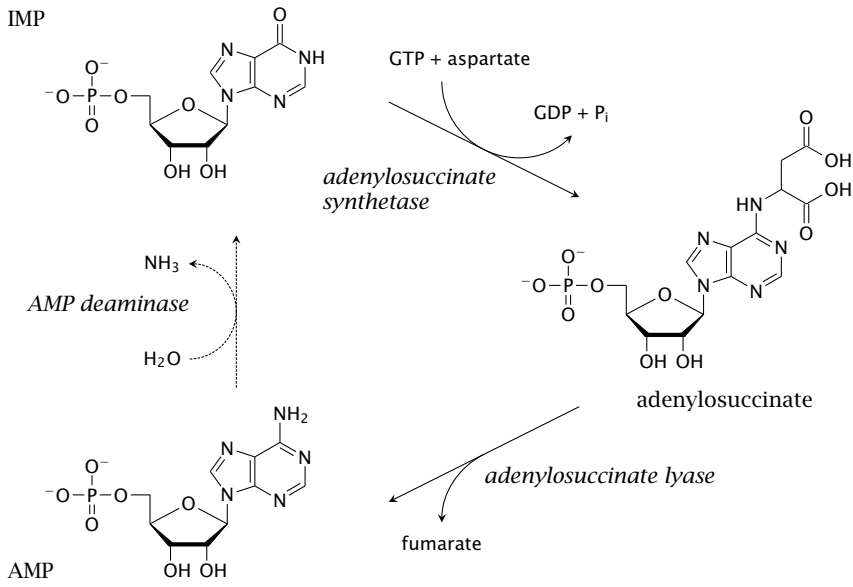


AICAR FAICAR IMP

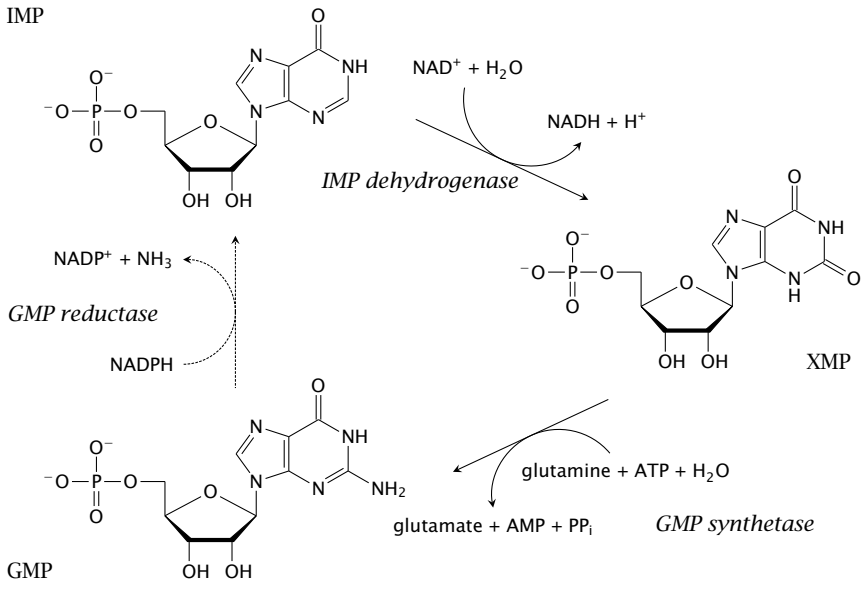
A bifunctional enzyme combines AIR carboxylase and SAICAR synthetase activities



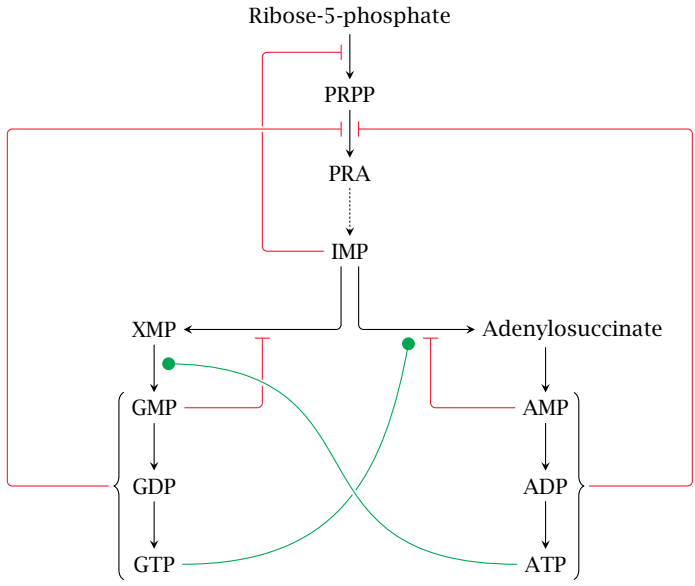
Synthesis of AMP from IMP



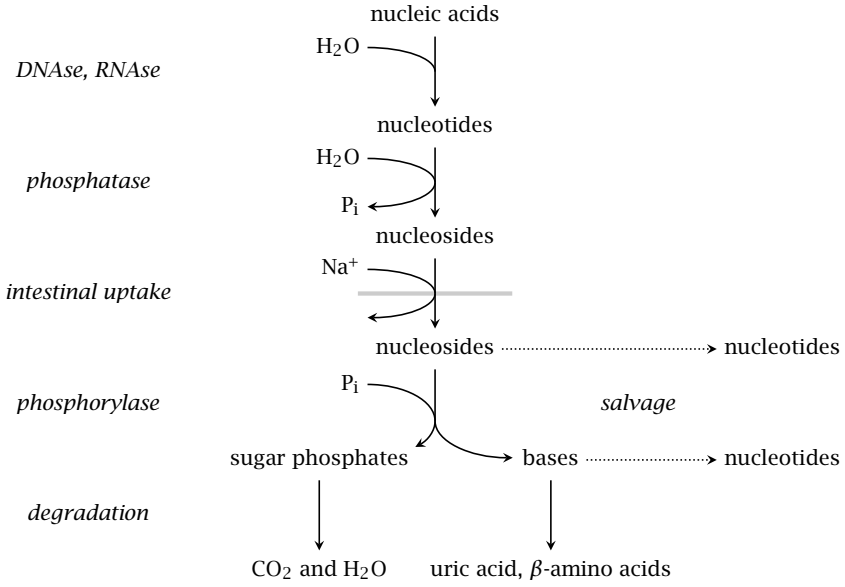
Synthesis of GMP from IMP



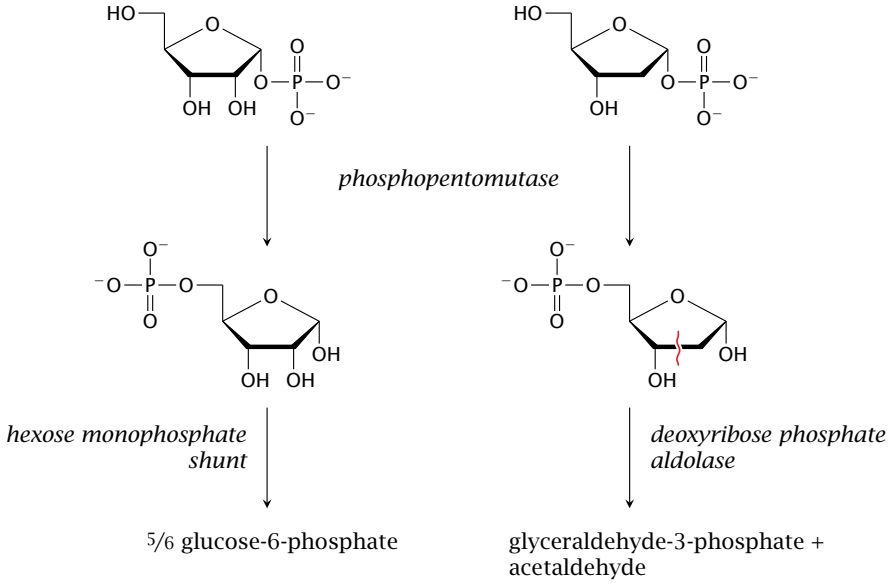
Feedback regulation in purine synthesis



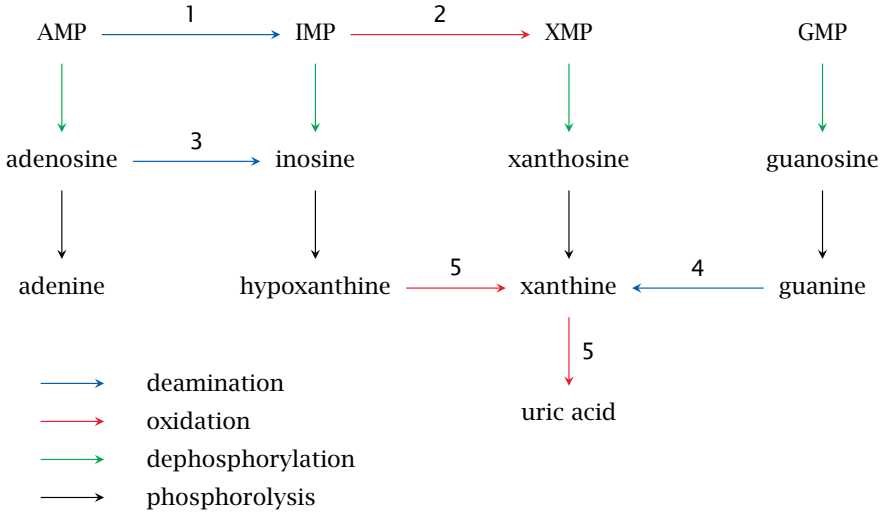
Overview of digestion and utilization of nucleic acids



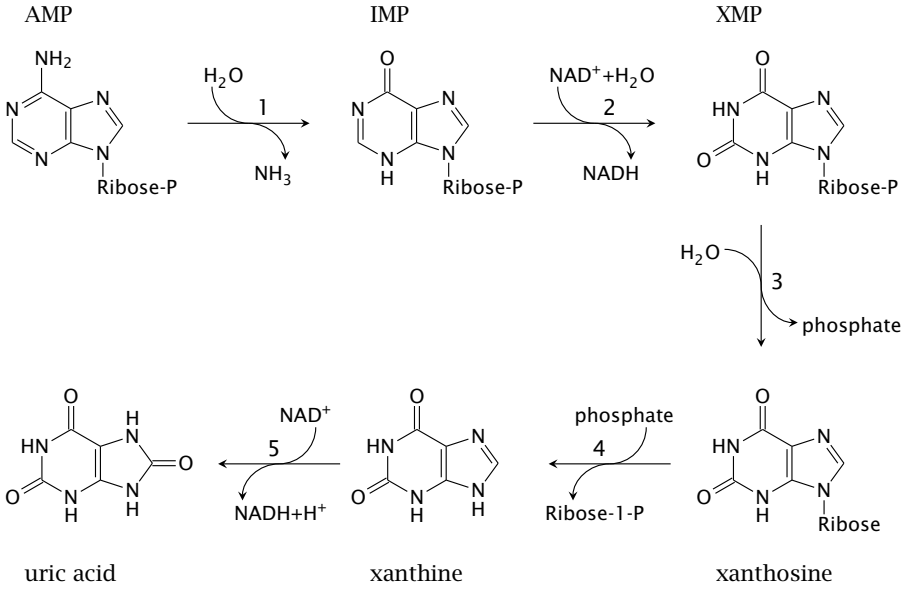
Utilization of ribose and deoxyribose



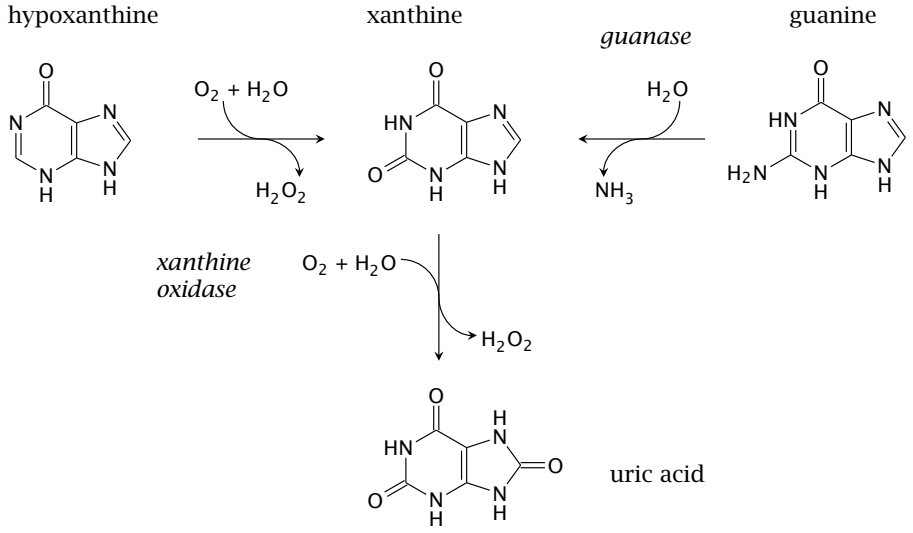
Degradation of endogenous purine nucleotides (overview)



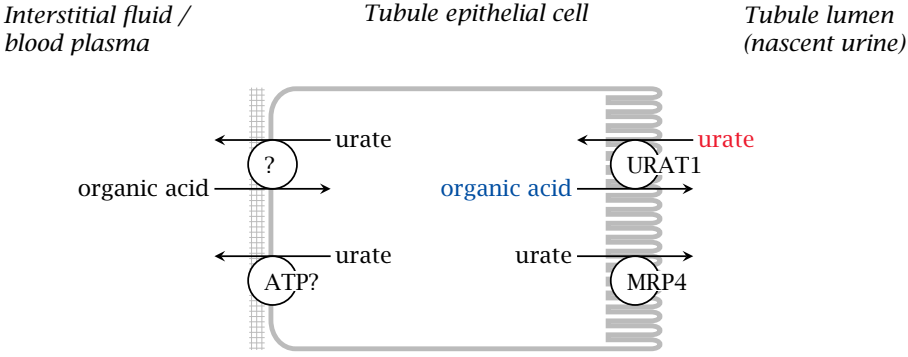
Adenine nucleotide degradation



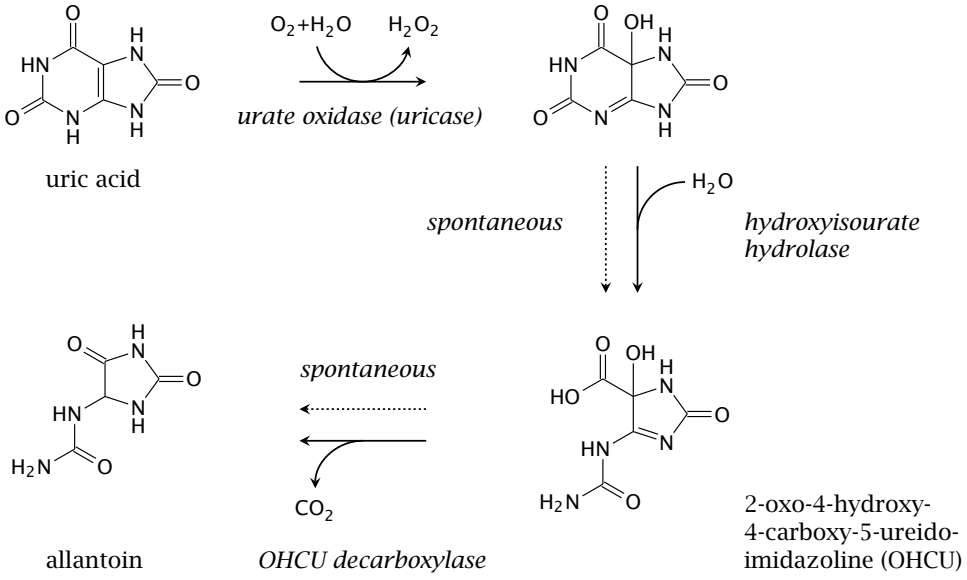
The guanase and xanthine dehydrogenase/oxidase reactions



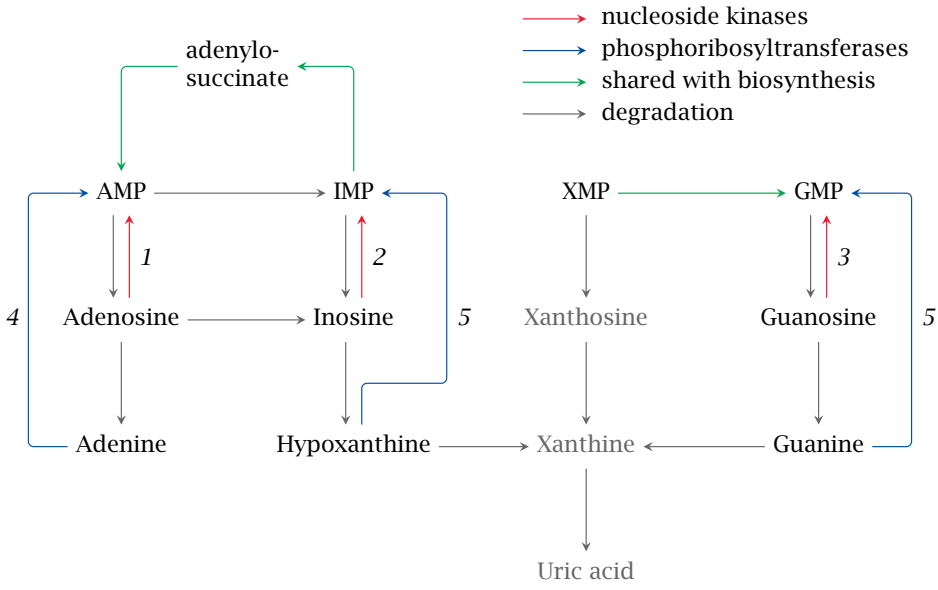
Renal urate elimination: tubular reuptake and secretion



Non-primates break down uric acid to allantoin



Overview of purine salvage reactions



Enzyme defects in purine degradation and salvage

Enzyme	Biochemical effects	Clinical symptoms
adenosine deaminase	accumulation of dA and dATP	severe combined immunodeficiency (SCID)
HGPRT	defective purine salvage, increased *de novo* synthesis and degradation	gout; impeded cerebral development and self-mutilation (Lesch-Nyhan syndrome)

- ADA deficiency

Gout

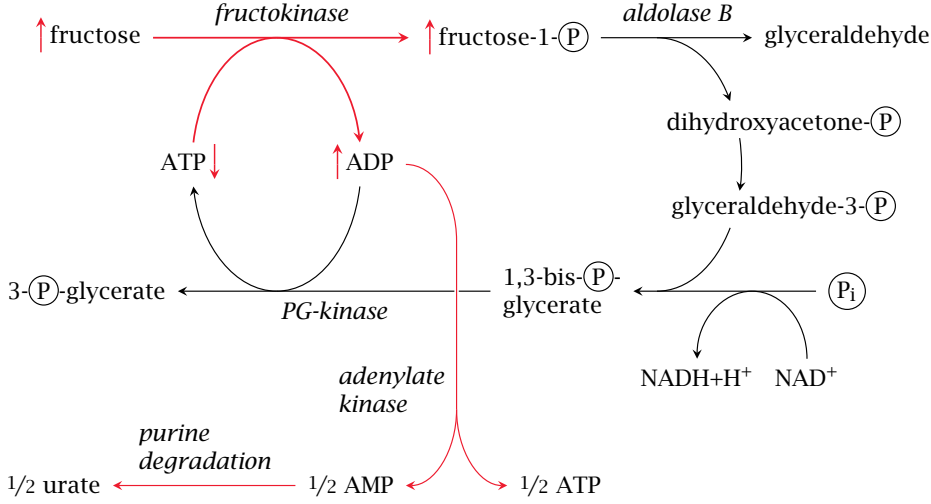
- Genetic or dietary factors cause chronically increased urate production or retention
- Urate has limited solubility and may form crystalline deposits, preferentially in joints and soft tissue
- Urate crystals activate inflammation and lead to arthritis that is painful and, in the long run, destructive

Diets and drugs that may promote gout

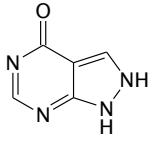
- too much food, too rich in purines
- excessive fructose or sucrose
- alcoholic beverages—but not all kinds: beer yes, wine no
- anorexia nervosa (!)
- drugs that interfere with uric acid secretion: pyrazinamide, salicylic acid
- drugs that contain purines: dideoxyadenosine

▸ renal urate elimination

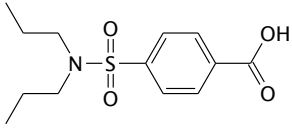
Gout: the fructose connection



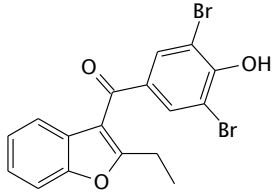
Drugs that affect purine degradation and elimination



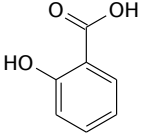
allopurinol



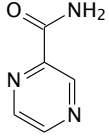
probenecid



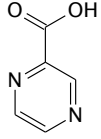
benzbromarone



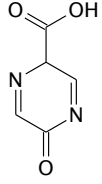
salicylic acid



pyrazinamide



pyrazinoate



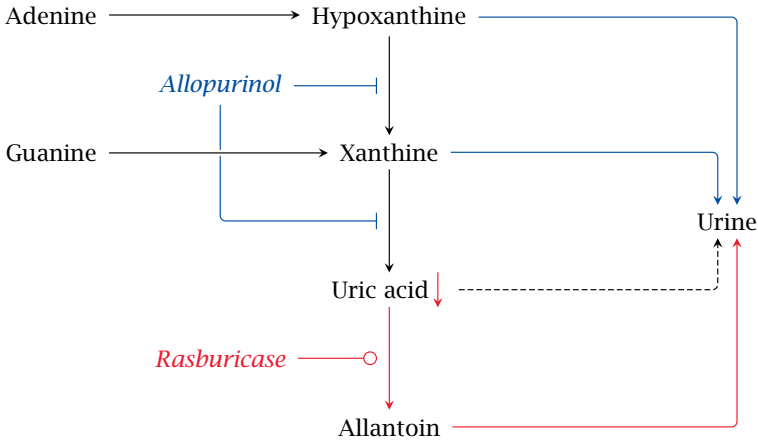
5-hydroxy-pyrazinoate

- renal urate elimination

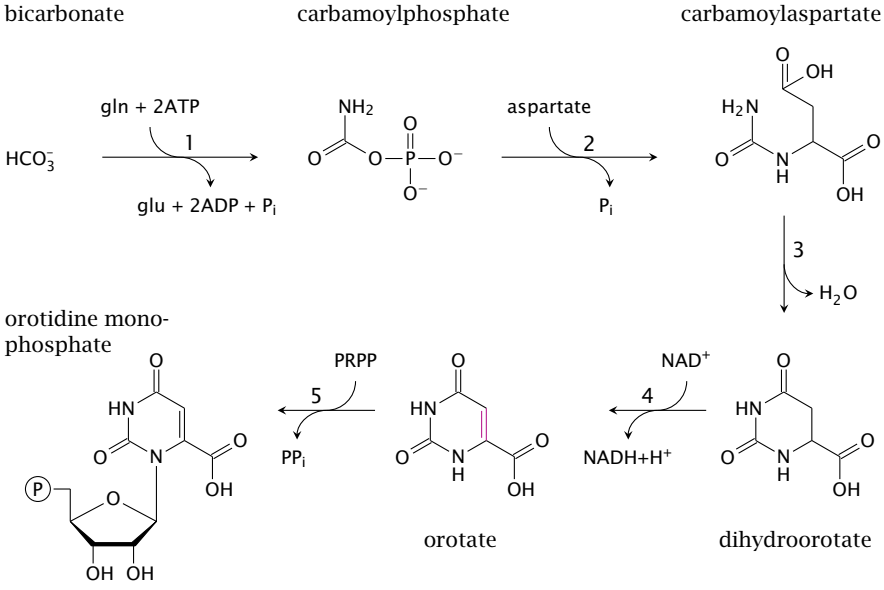
Acute urate nephropathy in tumor lysis syndrome

- Occurs during chemotherapy of malignancies, particularly with lymphomas and leukemias
- Chemotherapy causes acute decay of large numbers of tumor cells
- Degradation of nucleic acids from decaying cells produces large amounts of uric acid
- Uric acid in nascent urine exceeds solubility and precipitates, clogging up and damaging the kidney tubules
- Clinically manifest as acute kidney failure with high fatality rate

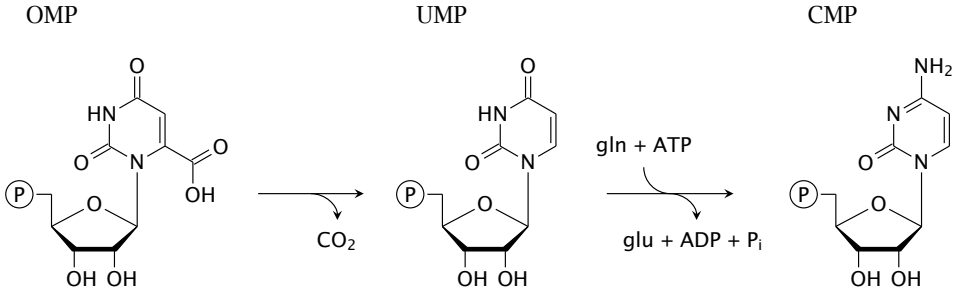
Rasburicase, a better preventive treatment for urate nephropathy



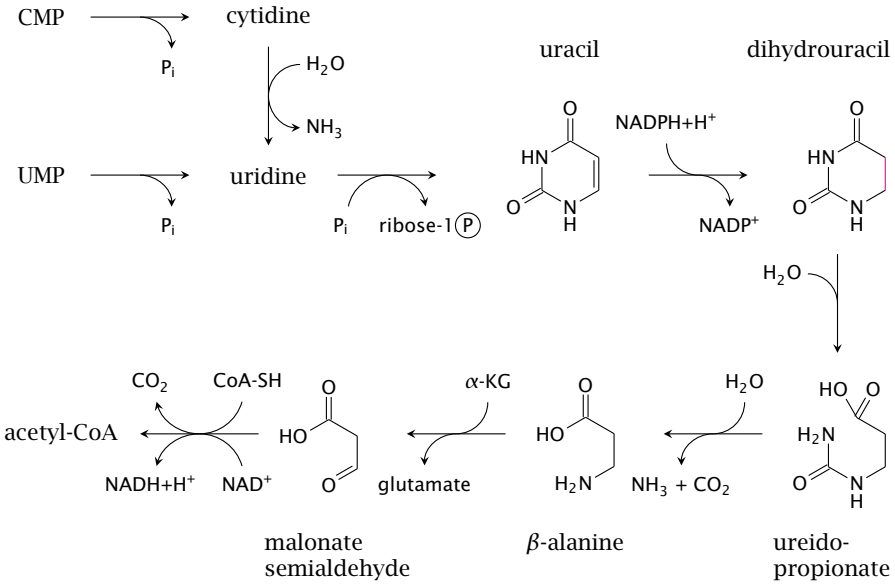
Synthesis of pyrimidines (1)



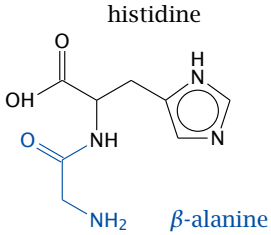
Synthesis of pyrimidines (2)

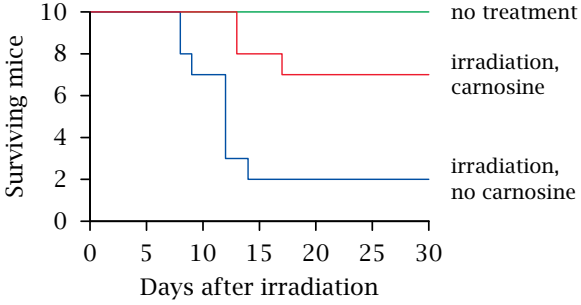


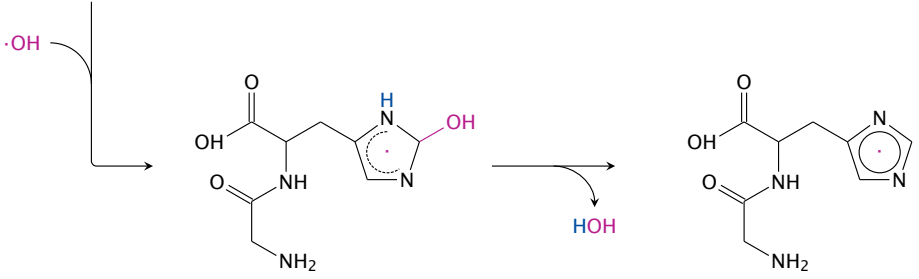
Degradation of pyrimidines



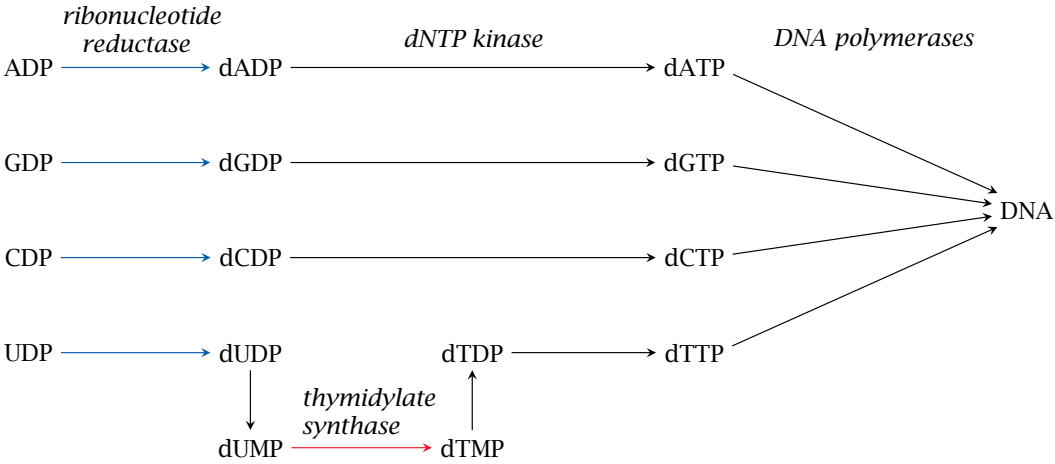
β-Alanine may be used to synthesize carnosine



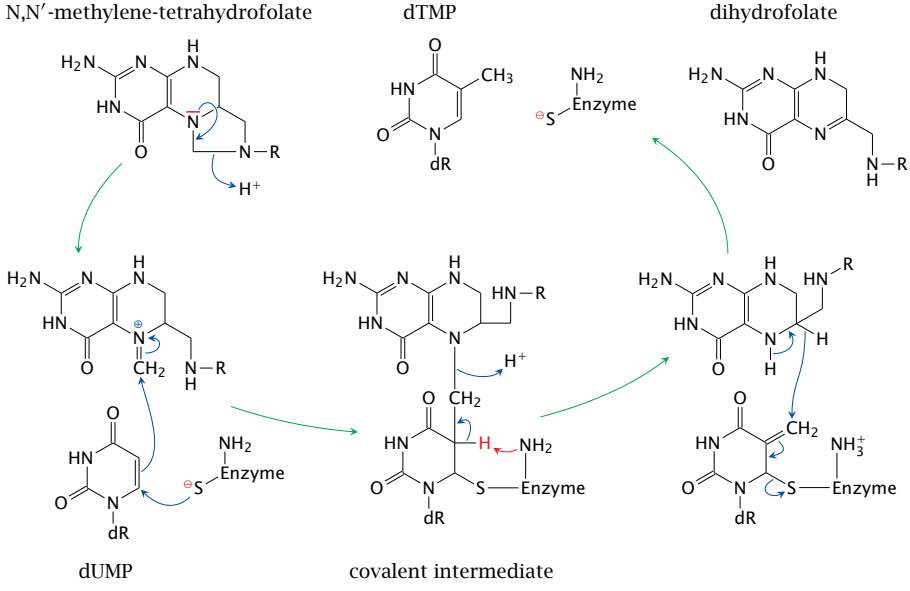




Synthesis of deoxyribonucleotides



The thymidylate synthase reaction

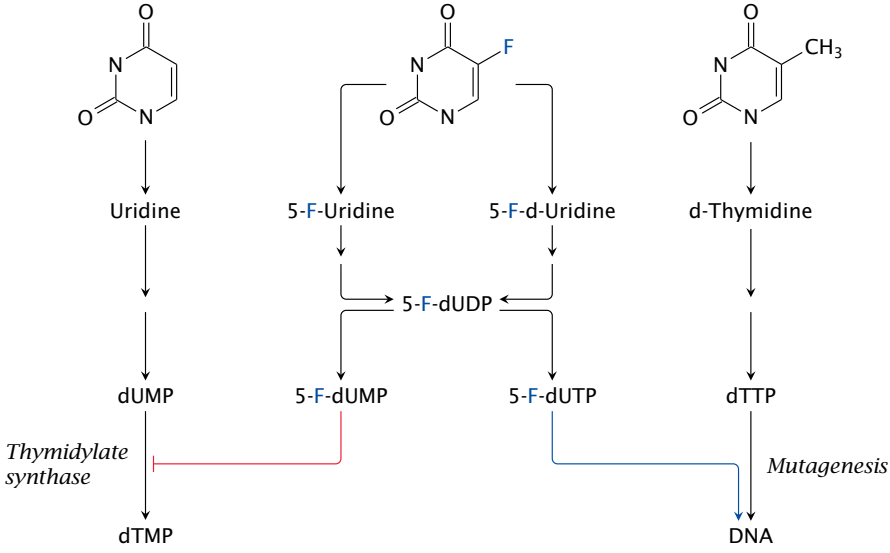


▸ DHFR

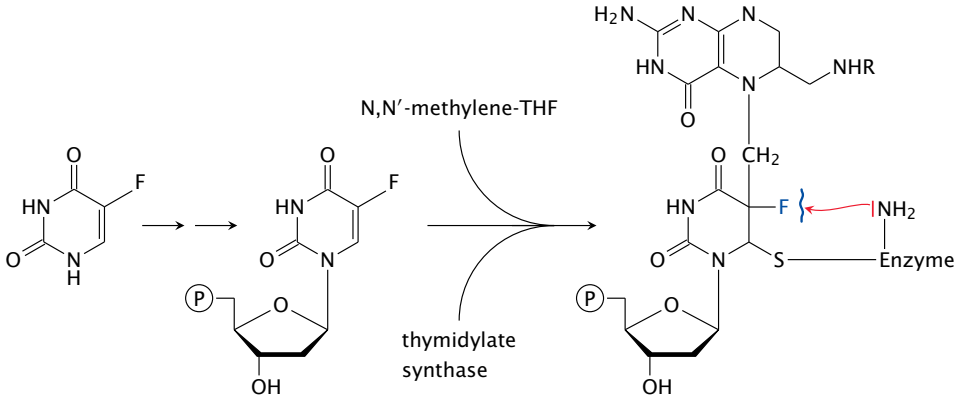
Nucleotide antimetabolites as anticancer and antiviral drugs

Therapeutic principle	Examples
direct inhibition of DNA/RNA polymerization	dideoxyadenosine, cytosine arabinoside, acyclovir
inhibition of nucleotide synthesis	mercaptopurine, fluorouracil, methotrexate
Incorporation of mutagenic analogues into DNA	idoxuridine

Dual action mode of 5-fluorouracil

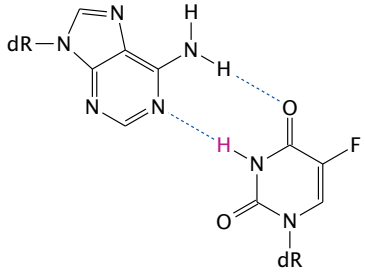


Inhibition of thymidylate synthase by 5-fluorouracil



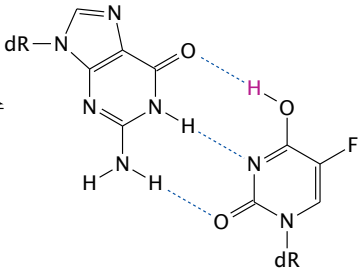
Mutagenesis through mispairing of the 5-FU iminol tautomer

Adenine



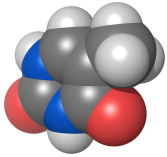
5-FU, amide form

Guanine

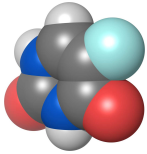


5-FU, iminol form

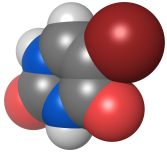
Thymine and various halogen analogues



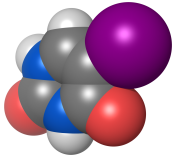
thymine



fluorouracil



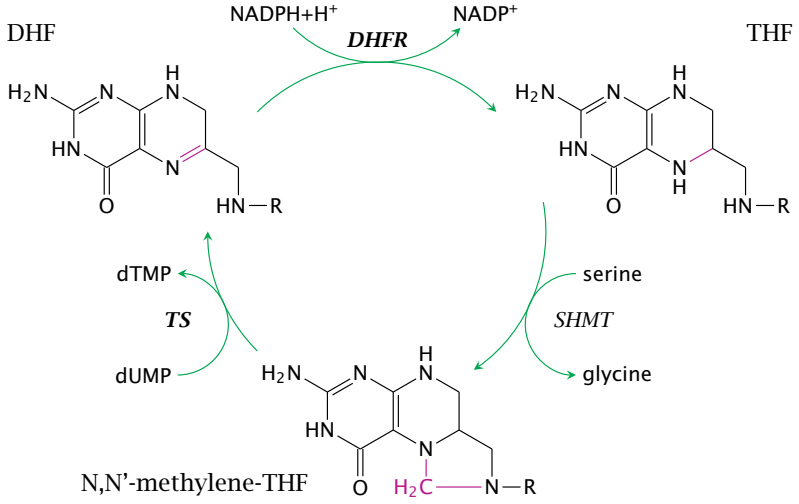
bromouracil



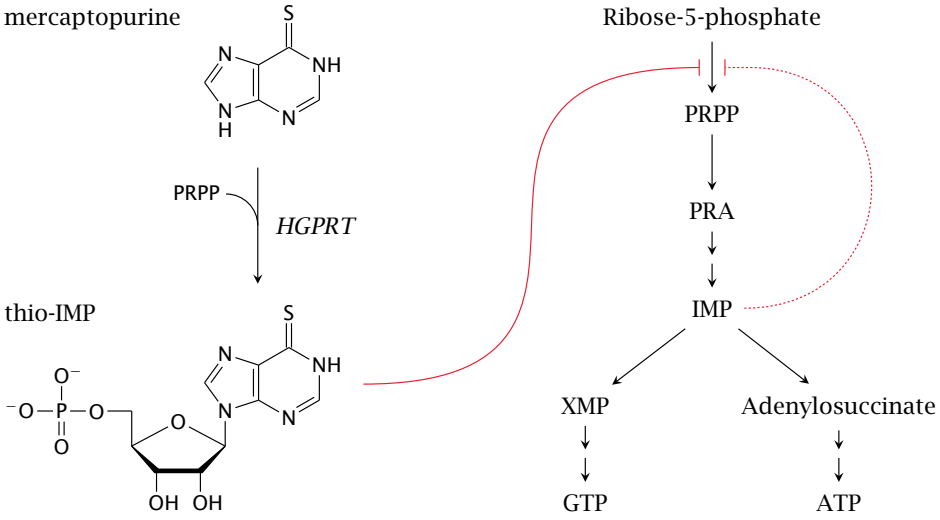
iodouracil

- Utilization of dietary nucleic acids

Indirect inhibition of thymidine synthesis by methotrexate

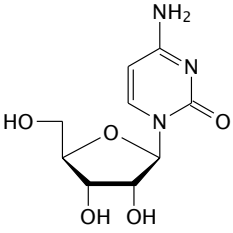


Mercaptopurine inhibits purine synthesis

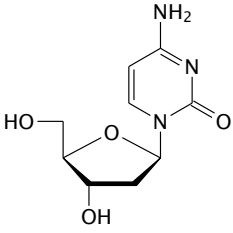


- purine salvage

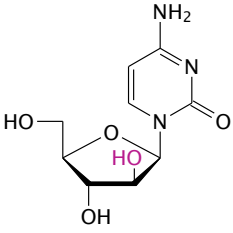
Structure of cytosine arabinoside (araC) and gemcitabine



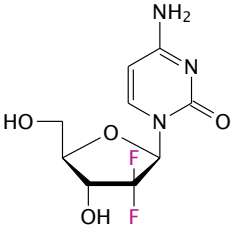
Cytidine



Deoxycytidine

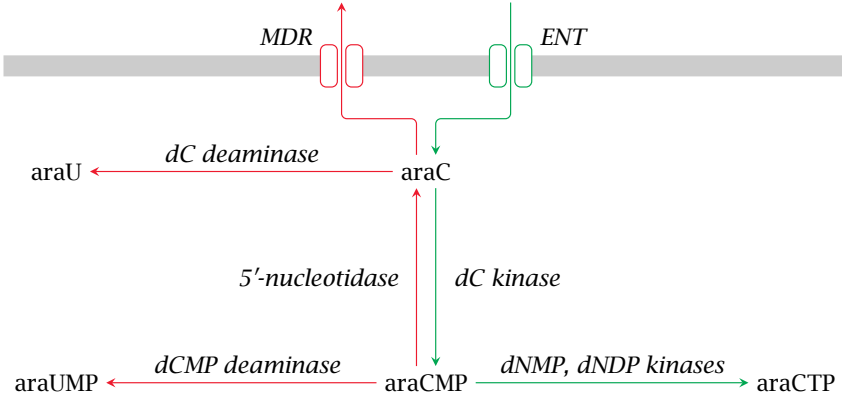


Cytosine arabinoside

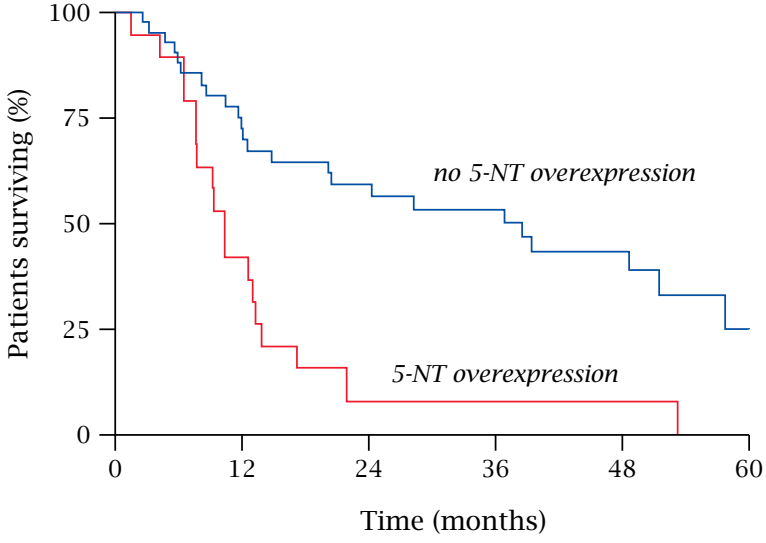


Gemcitabine

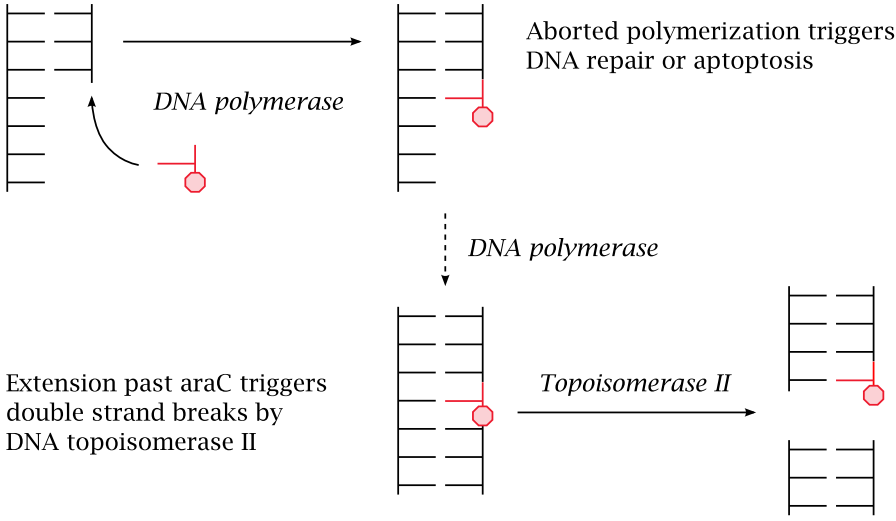
Metabolic activation and inactivation of araC



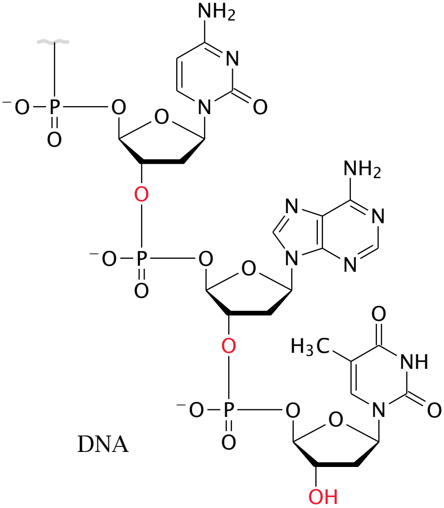
Overexpression of 5′-nucleotidase in leukemic cells shortens survival



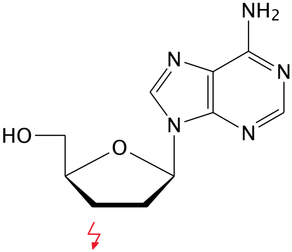
Action mode of araCTP



Dideoxyadenosine inhibits retroviral DNA polymerase



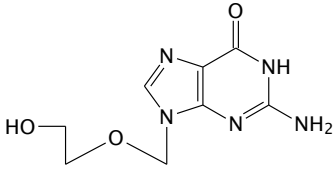
DNA



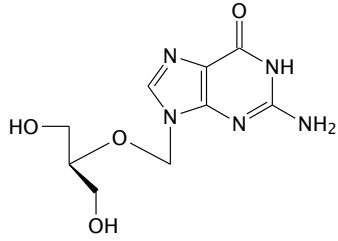
2,3-dideoxy-adenosine (ddA)

Aciclovir and ganciclovir

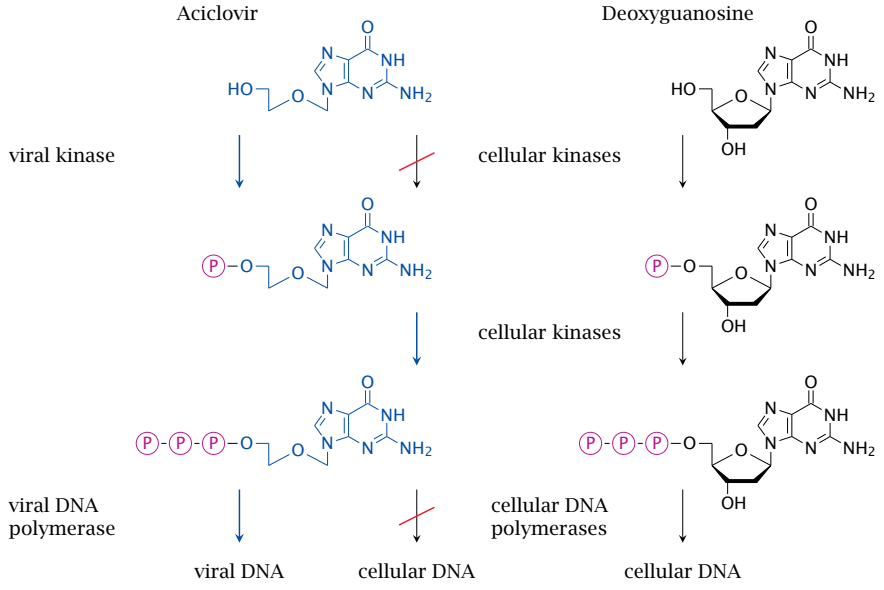
aciclovir



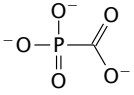
ganciclovir



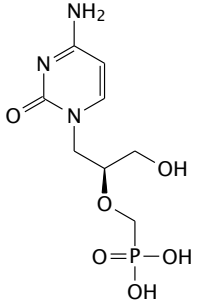
Aciclovir: mode of action on herpes virus



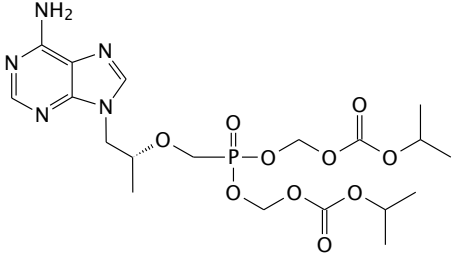
Some more inhibitors of viral nucleic acid synthesis



Foscarnet



Cidofovir

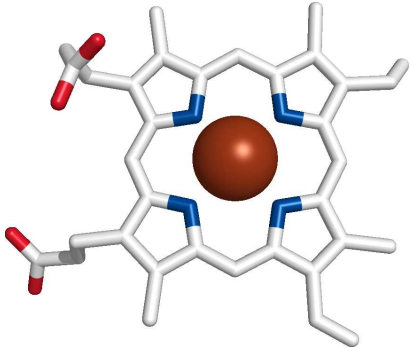


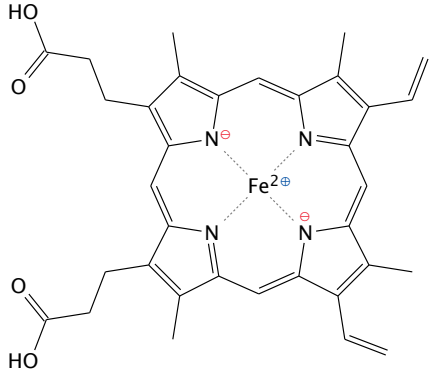
Tenofovir disoproxil

Chapter 17

Iron and heme metabolism

Iron and heme metabolism





Functions of heme

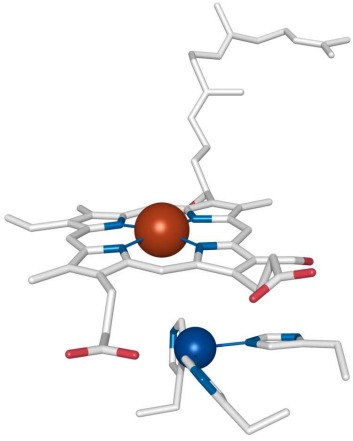
Redox chemistry

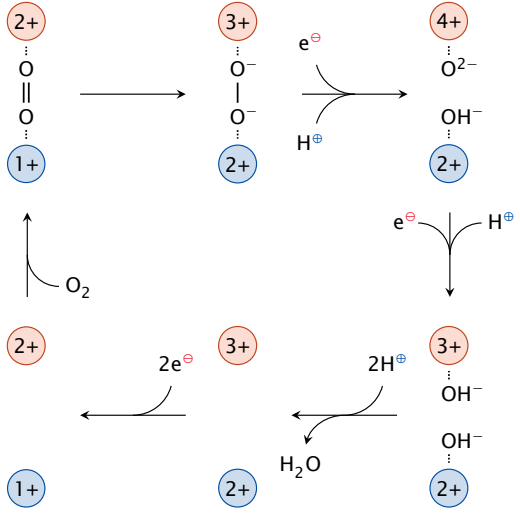
- electron transport: cytochromes in the respiratory chain
- enzyme catalysis: cytochrome P450, cyclooxygenase, others

Reversible binding of gases

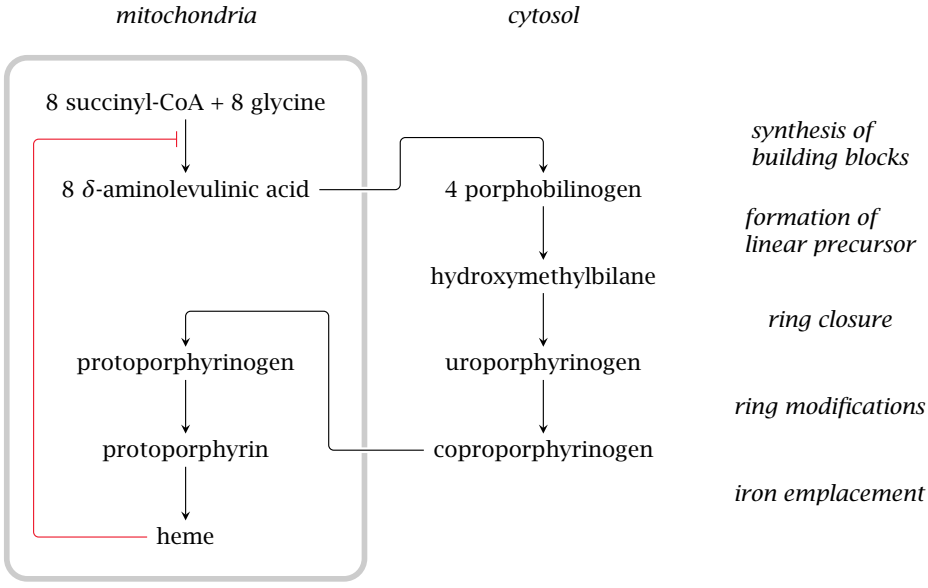
- O_2: hemoglobin and myoglobin (80–90% of all heme)
- NO: guanylate cyclase

Heme in cytochrome C oxidase

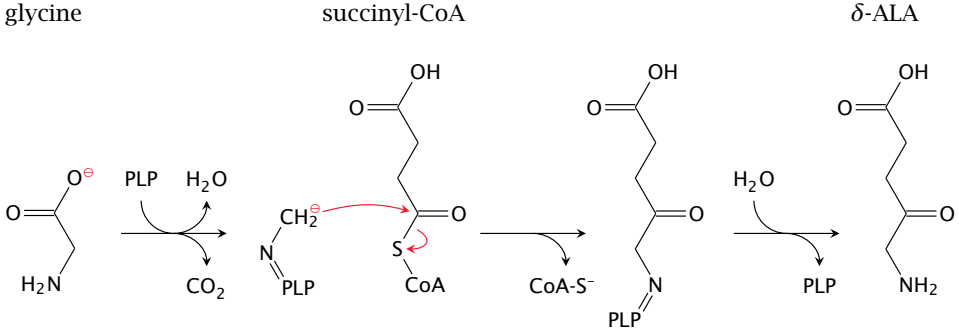




Heme synthesis (overview)

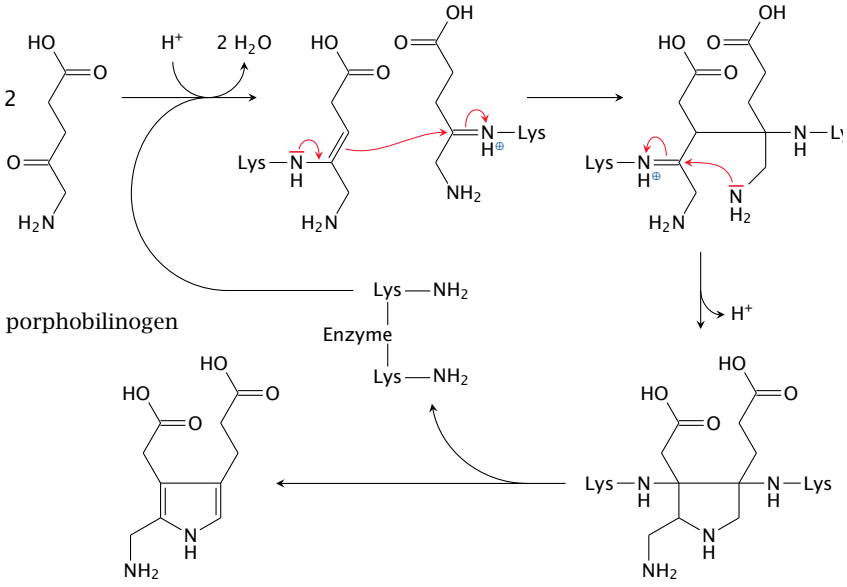


The δ-aminolevulinate synthase reaction

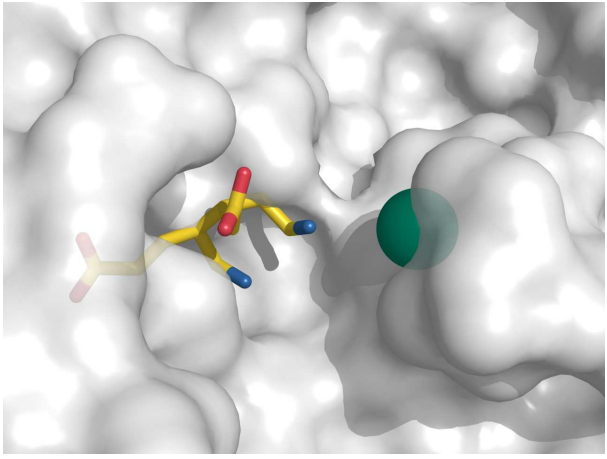


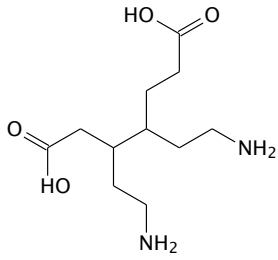
- SHMT

The porphobilinogen synthase reaction

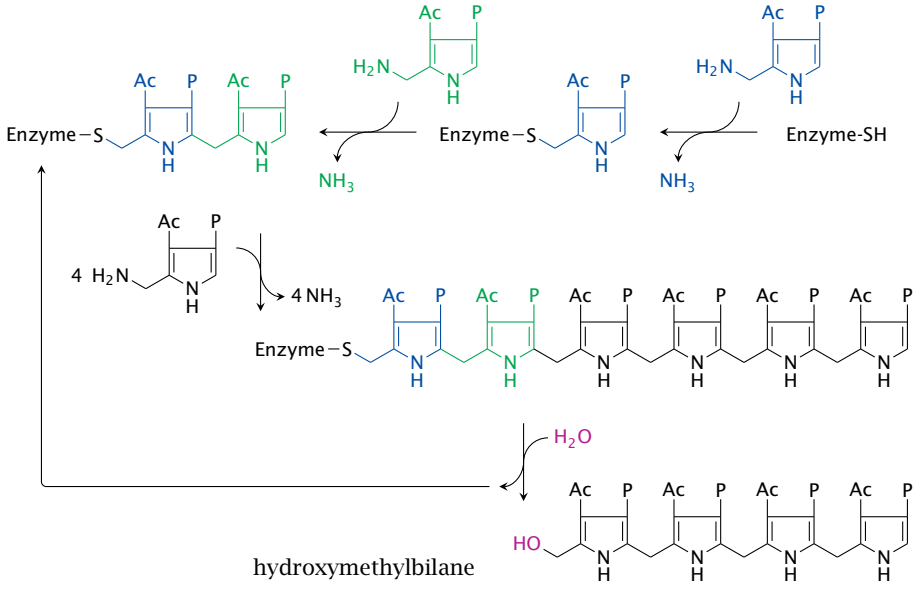


A substrate analogue next to zinc inside the active site of porphobilinogen synthase

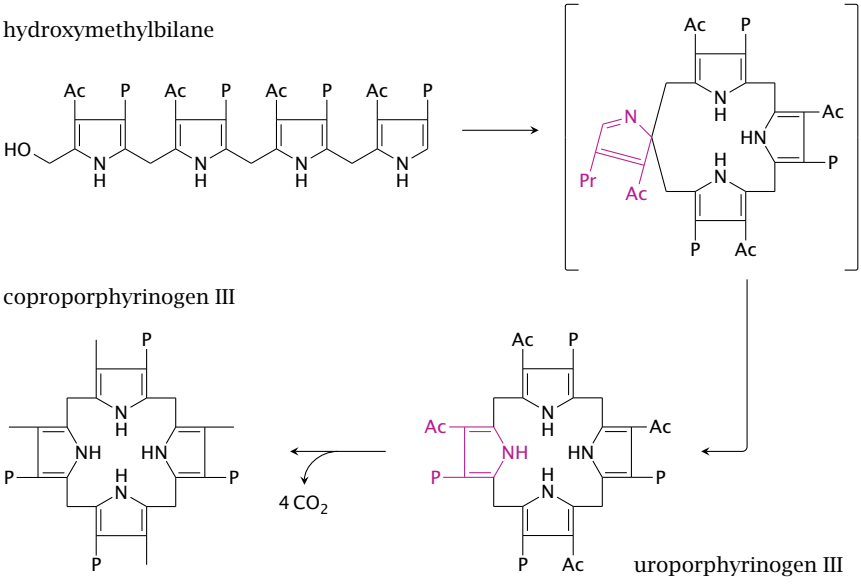




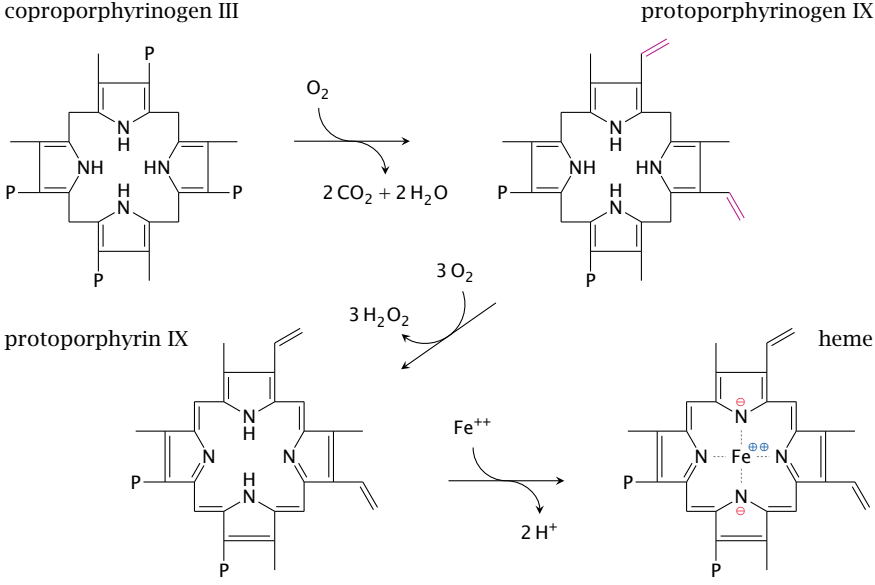
Porphobilinogen deaminase synthesizes hydroxymethylbilane



Synthesis of uro- and coproporphyrinogen III



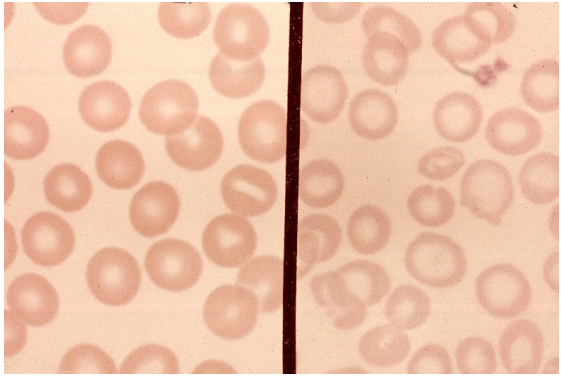
Final steps in heme synthesis



Disruptions of heme synthesis

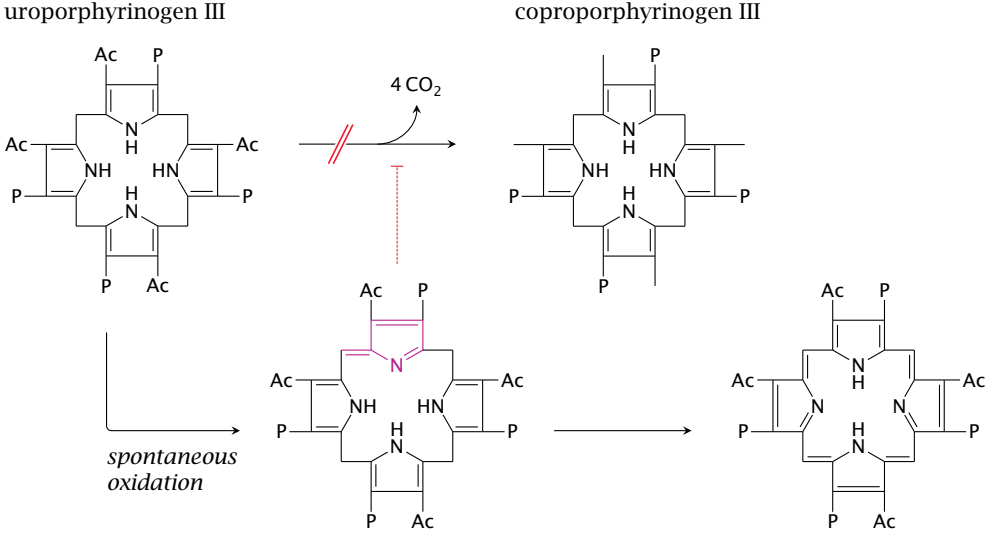
- iron depletion
- hereditary enzyme defects (porphyrias)
- vitamin B_6 deficiency—inhibition of aminolevulinate synthase
- lead poisoning—inhibition of porphobilinogen synthase

Disruption of heme synthesis causes microcytic, hypochromic anemia

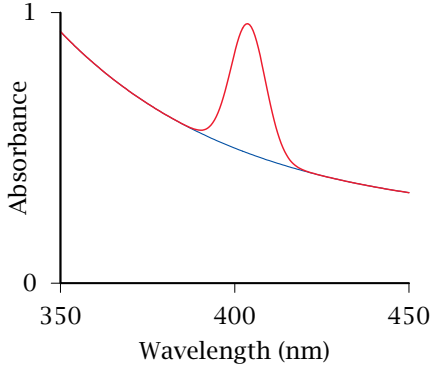


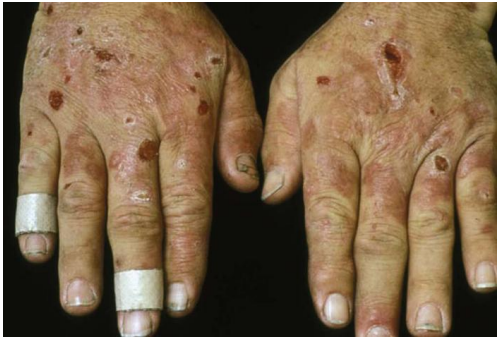
Normal red blood cells Microcytic anemia

Porphyria cutanea tarda (PCT) is caused by uroporphyrinogen decarboxylase deficiency



Laboratory and clinical findings in PCT

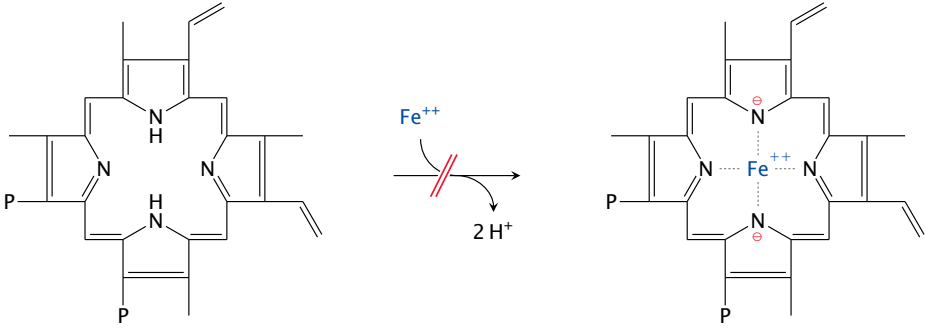




Causation of porphyria cutanea tarda

- hereditary—rare, autosomal dominant; enzyme defect is manifest in all tissues
- sporadic—exogenous, or related to a genetic defect in iron uptake regulation
  - caused by alcohol, halogenated hydrocarbons, other toxic substances
  - enzyme activity lacking in the liver but not erythrocytes and other tissues—enzyme is functional but inhibited by interfering metabolites
- iron overload seems important in both hereditary and sporadic forms

A defect of ferrochelatase causes erythropoietic protoporphyria



protoporphyrin IX

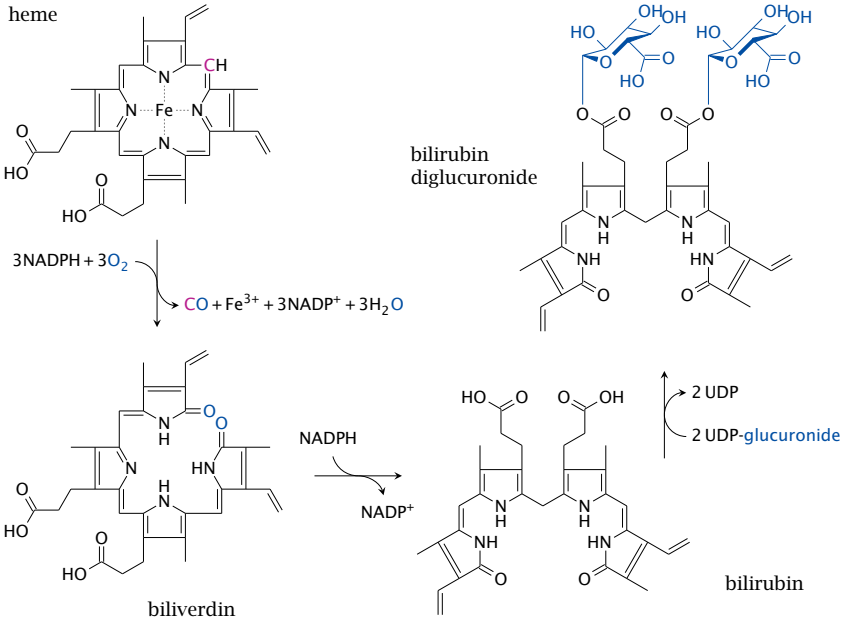
heme

Acute intermittent porphyria (AIP)

- deficiency of porphobilinogen deaminase, autosomal dominant
- excessive synthesis of δ-ALA in liver
- surplus porphobilinogen in urine—urine is colored red
- δ-ALA inhibits the $GABA_A$ receptor, causing
  - psychiatric symptoms ('organic psychosis')—too often misdiagnosed and mistreated
  - abdominal pain (neuropathic)
- episodes can be induced by drugs

‣ heme synthesis ‣ CYP induction

Heme degradation



- bile acid cycling

Jaundice

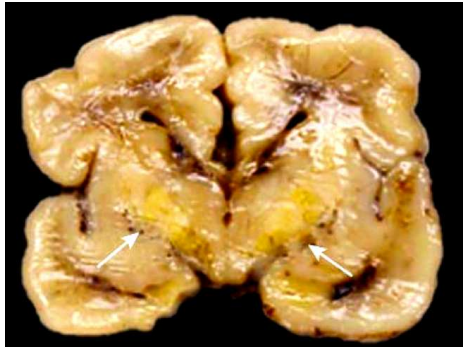
Accumulation of bilirubin in the body. Causes:

- increased production: hemolytic anemia (premature decay of red blood cells)
- decreased conjugation: enzyme defect, liver disease
- decreased excretion of conjugated heme: deficiency of ABCC2 transporter (Dubin-Johnson syndrome)
- mechanically blocked excretion: bile duct blocked by bile stone or tumor

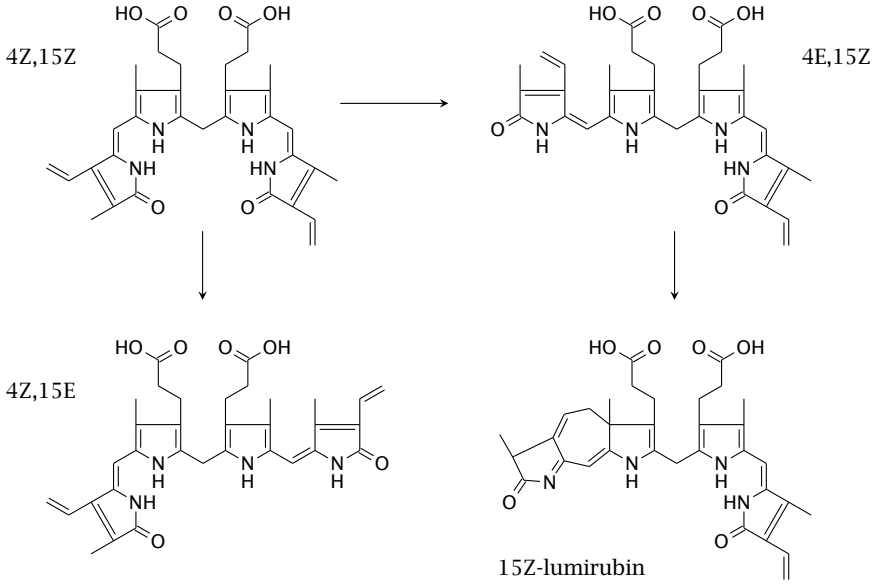
Enzyme defects in bilirubin conjugation by UDP-glucuronosyltransferase

- transient, usually mild: neonatal jaundice
- genetic, mild: Gilbert syndrome—asymptomatic jaundice
- genetic, severe, rare: Crigler-Najjar syndrome

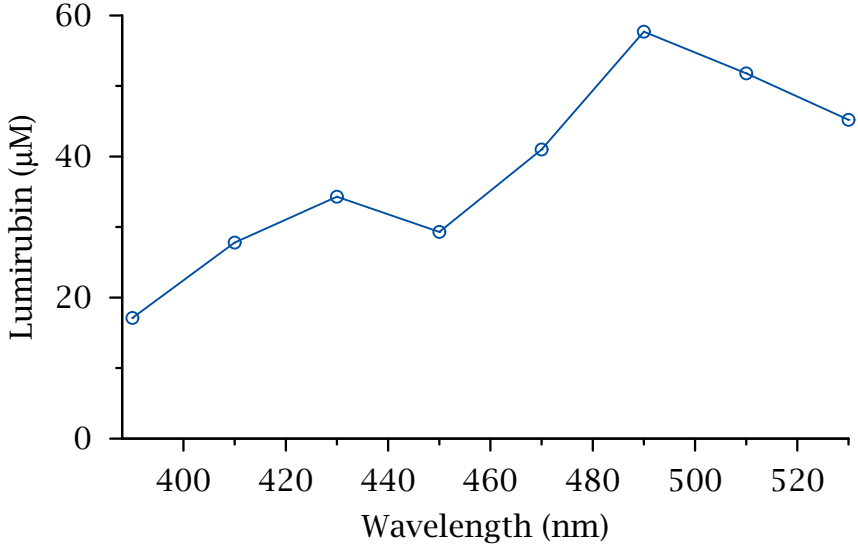
Bilirubin encephalopathy (“kernicterus”)



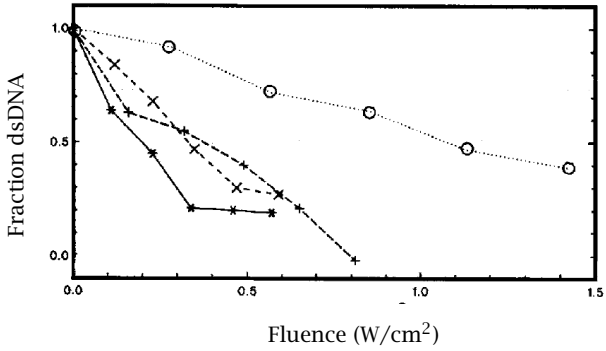
Photoisomerization products of bilirubin



Wavelength dependence of lumirubin formation



DNA strand breaks induced by bilirubin photo-activation (determined with Hoechst 32258)



∗ 410 nm

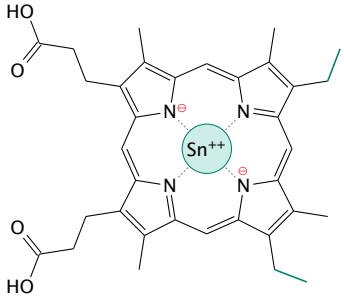
× 450 nm

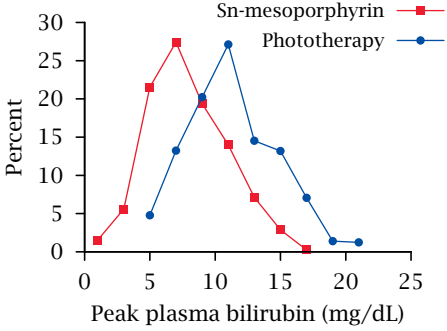
\+ 490 nm

o 530 nm

Christensen et al., Acta Paediatr 83:7-12 (1994)

Sn-mesoporphyrin, an inhibitor of heme oxygenase

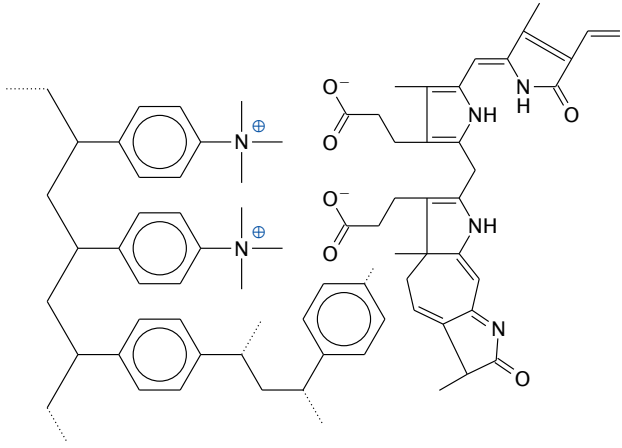




from Pediatrics 108:25–30

Long-term phototherapy of Crigler-Najjar syndrome

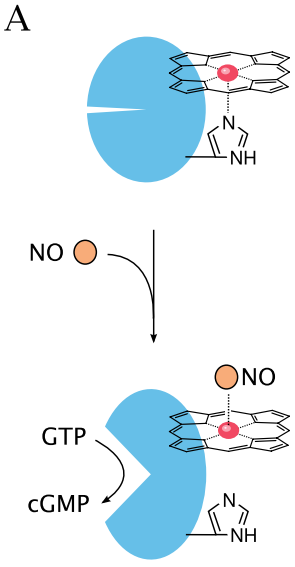
Cholestyramine particles absorb lumirubin

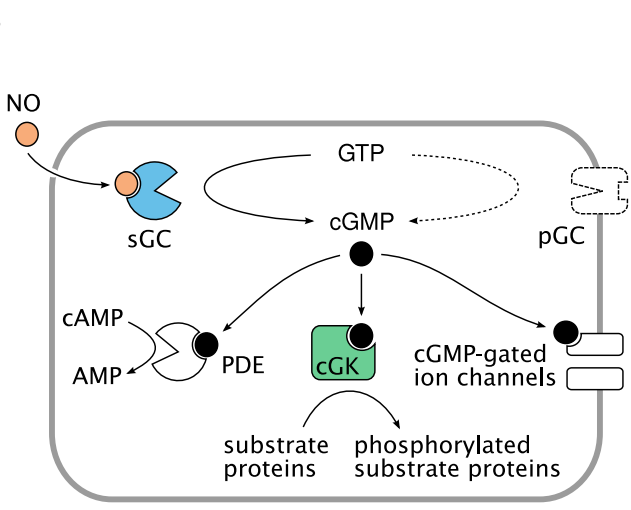


cholestyramine lumirubin

- cholestyramine/bile acids

Is CO a signaling molecule, like NO?



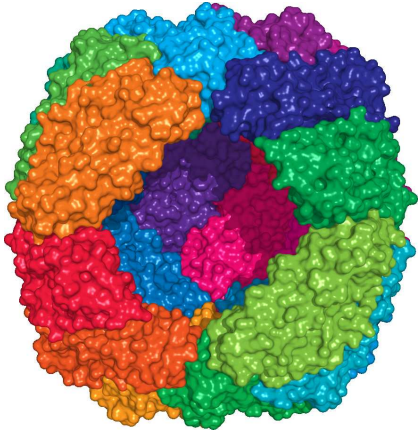


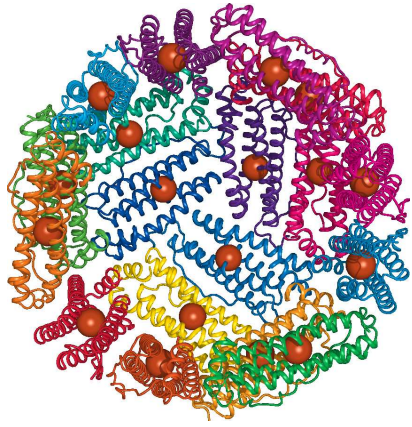
- heme degradation

Iron uptake, transport and storage

- uptake in the small intestine: Fe^{2+}—free or bound to heme
- transient storage as ferritin inside the intestinal epithelia
- transport in the blood: Fe^{3+}—bound to transferrin with very high affinity
- cellular uptake: endocytosis of transferrin, release of iron in acidic endosome
- storage: intracellular ferritin particles
- depletion: scaled-off cells, blood loss, breast milk

Structure of ferritin

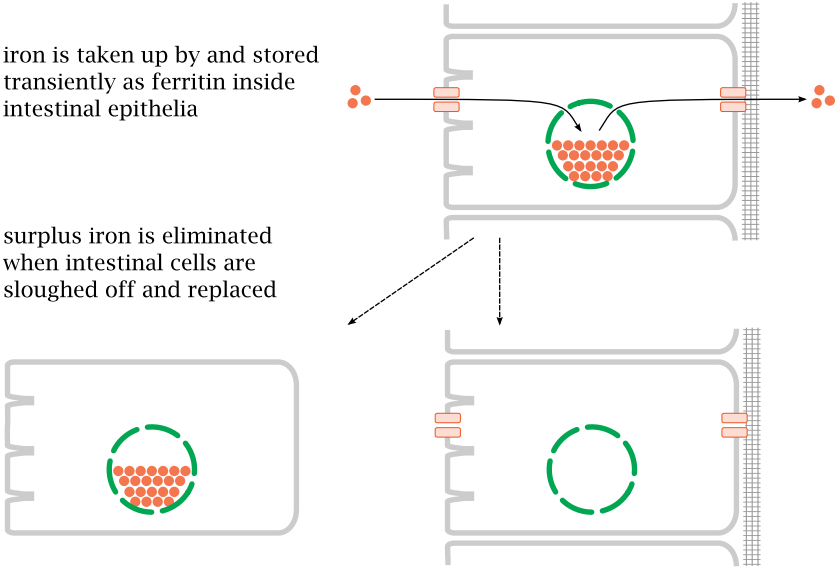




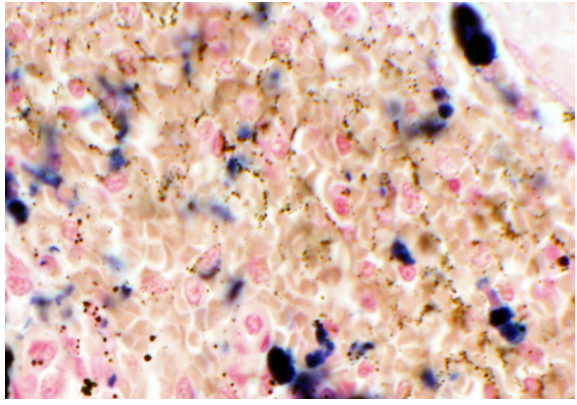
Ferritin in the small intestine regulates iron uptake

iron is taken up by and stored transiently as ferritin inside intestinal epithelia

surplus iron is eliminated when intestinal cells are sloughed off and replaced



Hemosiderin in liver tissue



Chapter 18

Metabolism of reactive species

The nature of “reactive species”

- Many RS are radicals ($OH^{\bullet}$, $O_2^{\bullet -}$), but some aren’t (H_2O_2, HOCl, singlet oxygen)
- RS are classified according to elemental composition, with some overlap—e.g. $^{\bullet}NO$ is both a ‘reactive oxygen species’ (ROS) and a ‘reactive nitrogen species’ (RNS)
- While most RS do contain oxygen and can thus be subsumed as ROS, there are exceptions such as thiyl radicals ($R-S^{\bullet}$) and chloramines ($R-HN-Cl$)

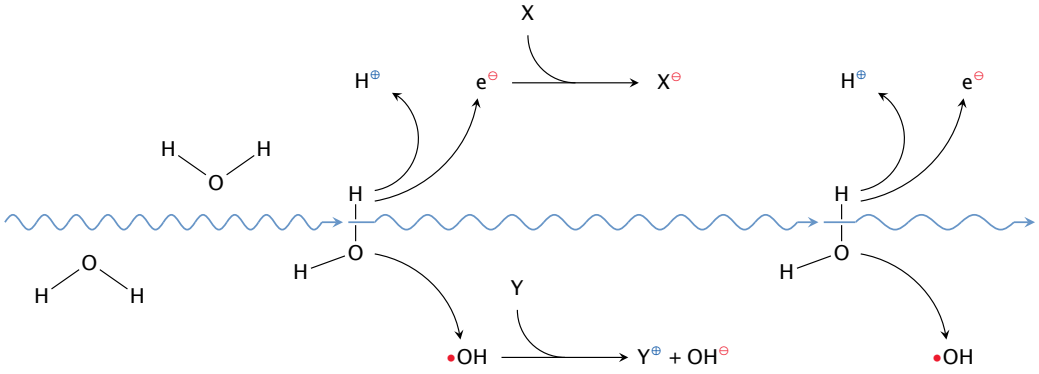
Reactive species in the human body: examples

Reactive species	Origin	Function or effect
$O_2^{\bullet -}$	respiratory chain	byproduct
$^{\bullet}OH$	ionizing radiation; Fenton reaction	DNA damage, lipid peroxidation (cell membranes, LDL)
H_2O_2	phagocytes	killing of microbes
	thyroid peroxidase	reaction intermediate
	superoxide dismutase	detoxification intermediate
HOONO	phagocytes	killing of microbes
singlet oxygen	photosensitization in porphyrias	skin damage
N-acetyl-*p*-quinone-imine (NAPQI)	metabolite of acetaminophen	drug toxicity
$R-S^{\bullet}$	secondary radical	detoxification intermediate

Do reactive species really matter in a class on metabolism?

- Reactive species are intermediates or byproducts of metabolic reactions
- Reactive species create ‘cross-talk’ between pathways—e.g., uric acid may scavenge reactive species produced in the respiratory chain
- Reactive species participate in the development of atherosclerosis and other metabolic diseases
- Metabolites and enzymes that scavenge radicals are highly abundant—e.g. glutathione (7-8 mM in liver cells), peroxiredoxins (1% of cellular protein)

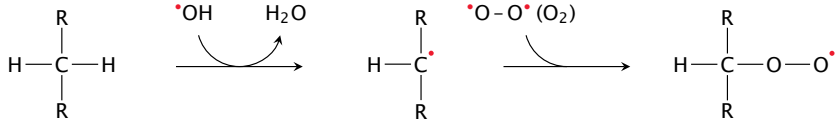
Reactive species and ionizing radiation

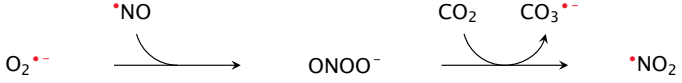


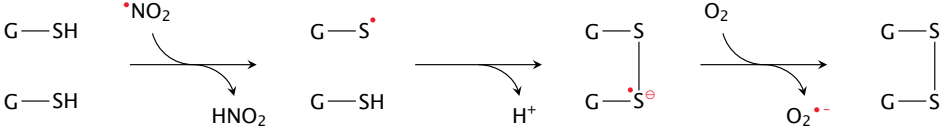
How toxic are $^{\bullet}$OH radicals?

- The LD_{50} of γ-radiation is 5 Gray (Gy) = 5 J/kg
- The main effect of γ-rays is to break up H_2O into H^+, e^-, and $^{\bullet}$OH
- The bond dissociation energy for the first bond in water is 500 kJ/mol, and that for ionizing the resulting hydrogen atom is 218 kJ/mol
- $\Rightarrow$ **at most 7 μmol/kg** of $^{\bullet}$OH is produced by one LD_{50} of γ-rays

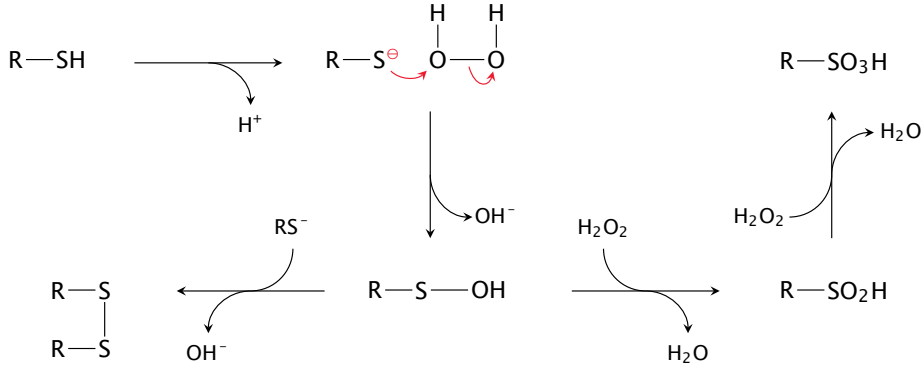
Reactions of radicals with each other and with non-radicals



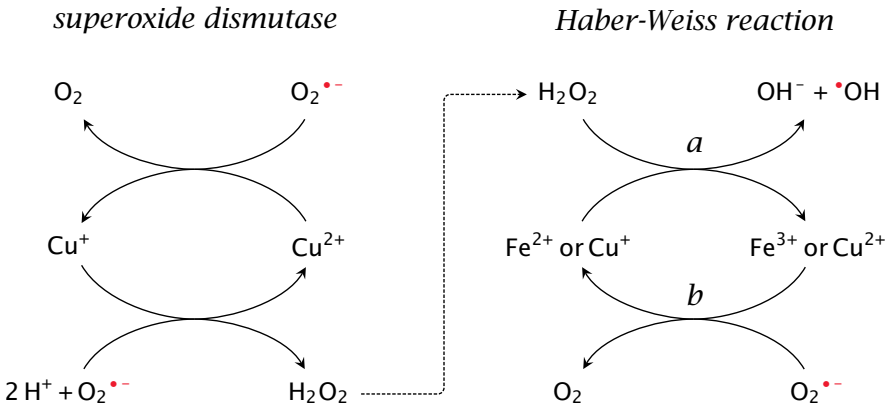




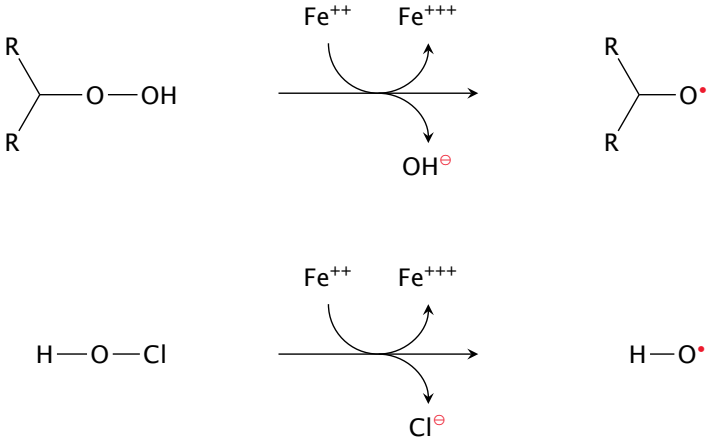
The reaction of H_2O_2 with thiol groups



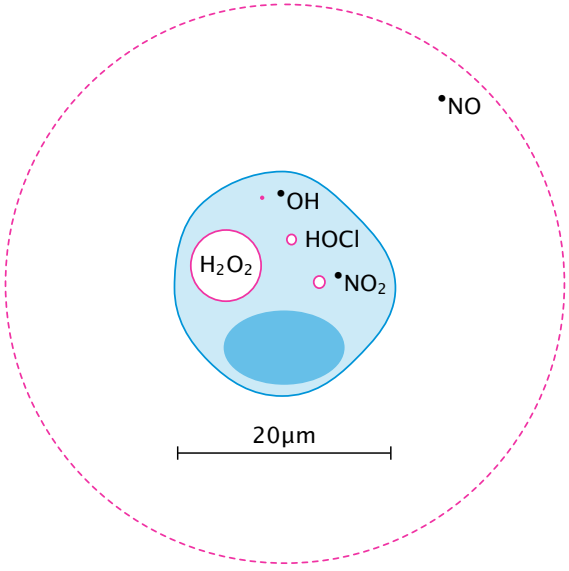
Radical reactions with transition metals



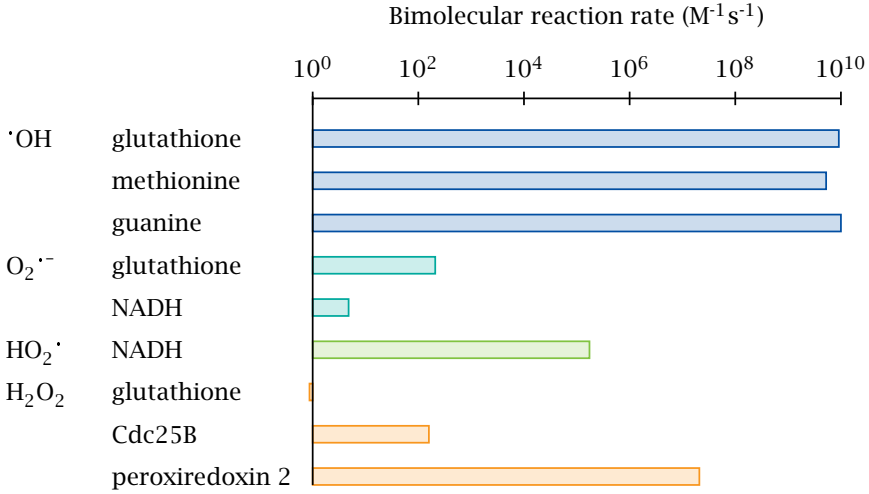
Fenton-like radical formation by transition metals



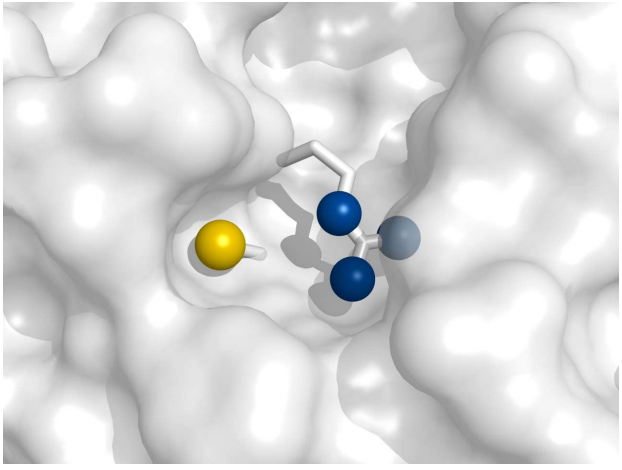
Diffusion distances of selected reactive species



Example bimolecular reaction rate constants



The active site of the protein tyrosine phosphatase Cdc25B

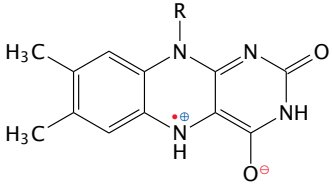


Standard redox potentials of selected radicals

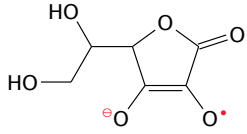
Oxidised form	$\longrightarrow$	Reduced form	$\Delta E^{0'}$ (V)
$^{\bullet}OH + H^{+}$	$+e^{-}$	H_2O	2.31
$R-O^{\bullet} + H^{+}$	$+e^{-}$	$R-OH$	1.60
$HO-O^{\bullet} + H^{+}$	$+e^{-}$	H_2O_2	1.06
$R-O-O^{\bullet} + H^{+}$	$+e^{-}$	$R-O-OH$	1.00
$R-S^{\bullet}$	$+e^{-}$	$R-S^{-}$	0.92
$H_2C=CH-CH^{\bullet}-CH=CH_2 + H^{+}$	$+e^{-}$	$H_2C=CH-CH_2-CH=CH_2$	0.60
ascorbyl$^{\bullet -}$ + H^{+}	$+e^{-}$	ascorbate^{-}	0.28
Fe^{+++}	$+e^{-}$	Fe^{++}	0.11
dehydroascorbate	$+e^{-}$	ascorbyl$^{\bullet -}$	−0.17
O_2	$+e^{-}$	$O_2^{\bullet -}$	−0.33
$R-SS-R$	$+e^{-}$	$R-SS^{\bullet -}-R$	−1.50
(water)	$+e^{-}$	e^{-} (solvated)	−2.87

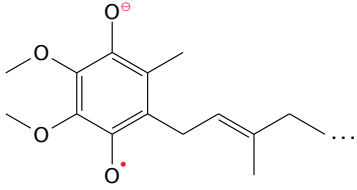
Some radicals are stabilized by resonance

FMNH

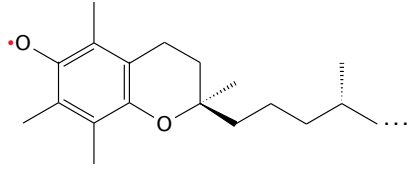


ascorbyl



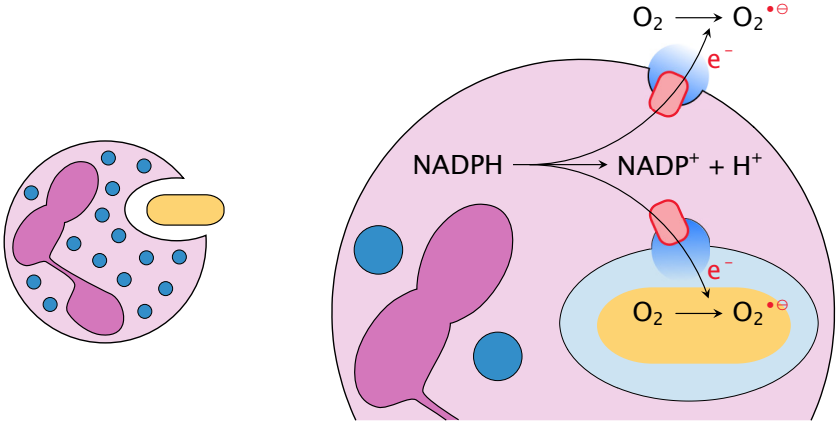


ubisemiquinone

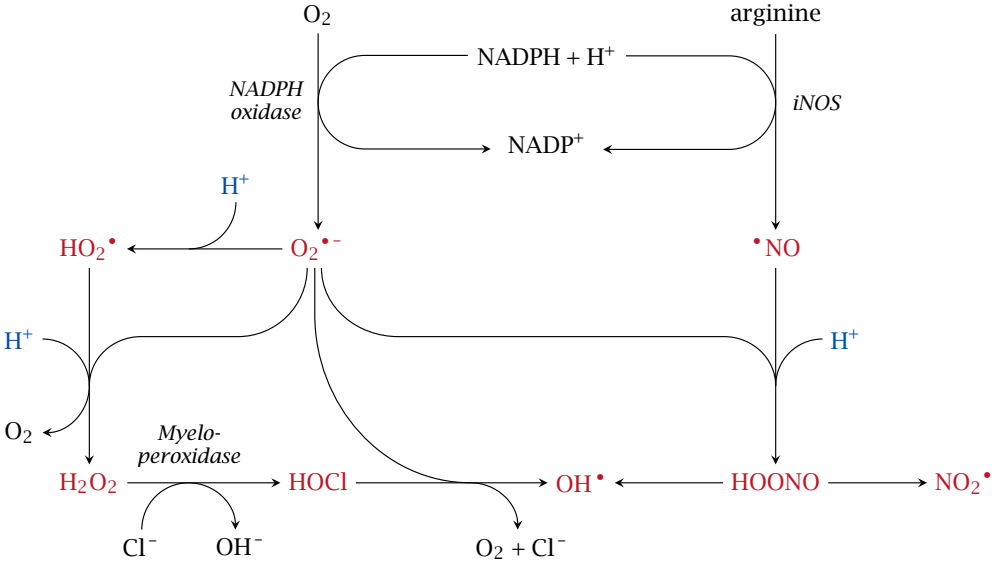


α-tocopherol

NADPH oxidase initiates ROS formation in phagocytes



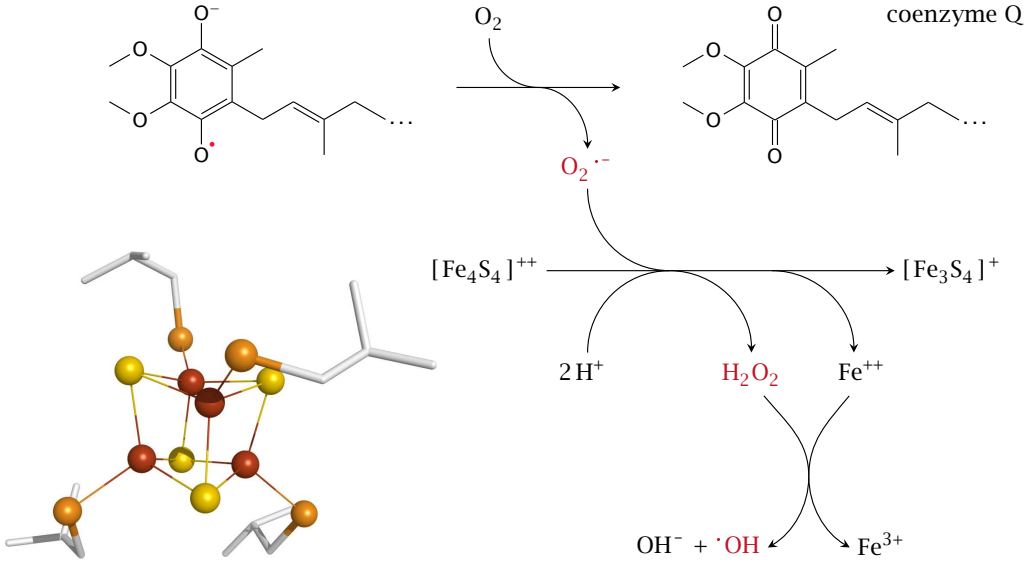
$O_2^{\bullet -}$ gives rise to other reactive oxygen species



Lessons from ROS generation in phagocytes

- ROS are produced in large amounts for killing microbes, even though they will also damage host cells
- ROS generation starts with reducing power, and often (as in this case) with enzymatic reactions
- Once primary RS have been generated—here, $O_2^{\bullet -}$ and $^{\bullet}NO$—they tend to spontaneously generate secondary ones
- pH matters—the weakly acidic endosomal pH seems optimized for generating peroxynitrite and $HO_2^{\bullet}$

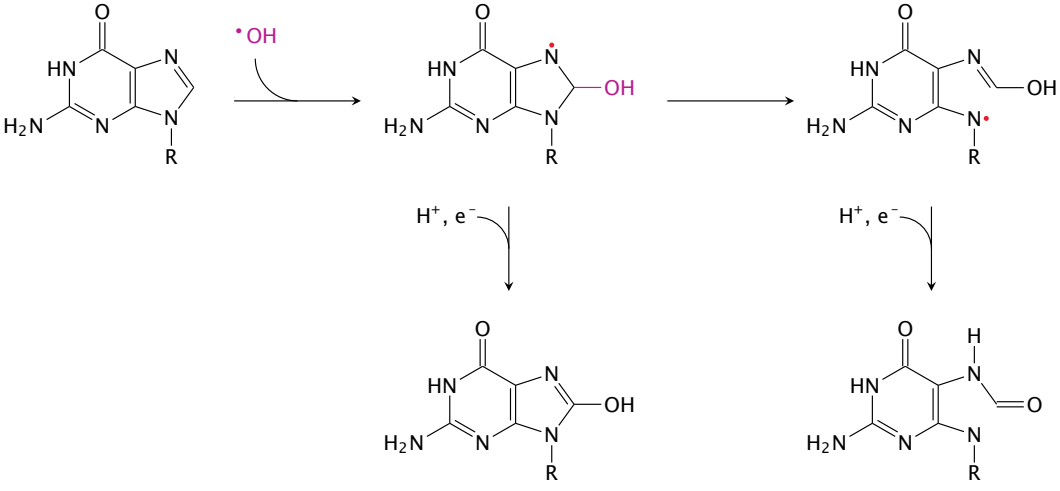
Production of reactive oxygen species in mitochondrial respiration



Mitochondrial energy state and ROS formation

- When ATP consumption is low, proton and electron transport chain back up
- Backed-up electrons will leak and produce more $O_2^{\bullet -}$
- $O_2^{\bullet -}$ activates uncoupling proteins, which will lower the proton-motive force and the ATP yield, but increase electron transport
- Increased $O_2^{\bullet -}$ formation has been observed in pancreatic β-cells in type 2 diabetes

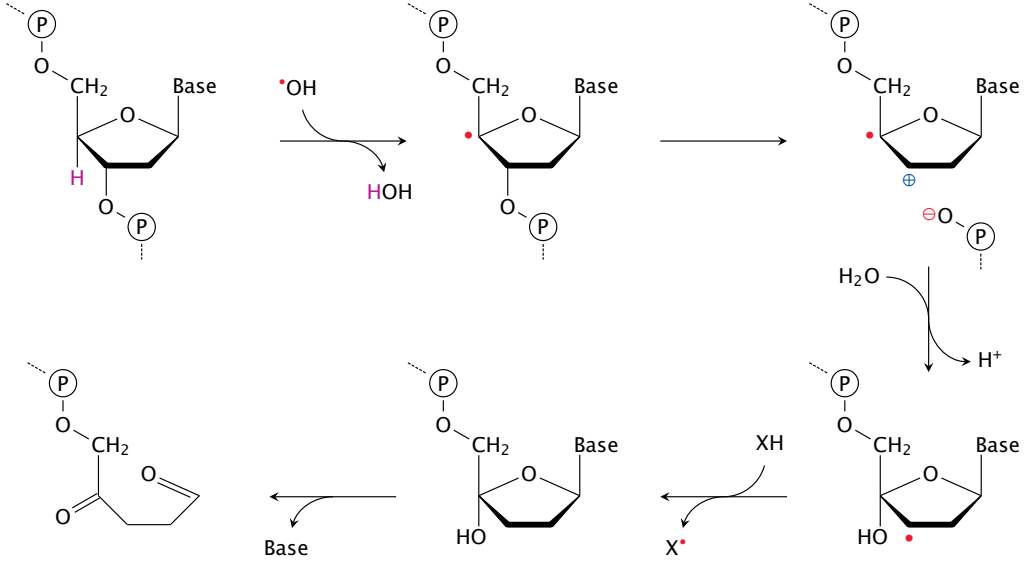
Hydroxyl radicals can modify DNA bases



8-hydroxyguanine

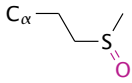
'FAPy-guanine'

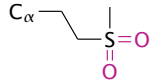
Hydroxyl radicals can break DNA strands



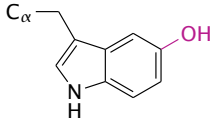
Protein modification by reactive oxygen species

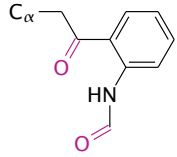
methionine



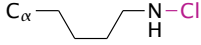


tryptophan

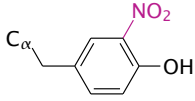


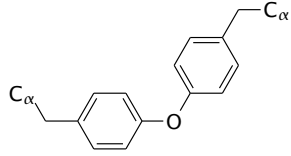


lysine



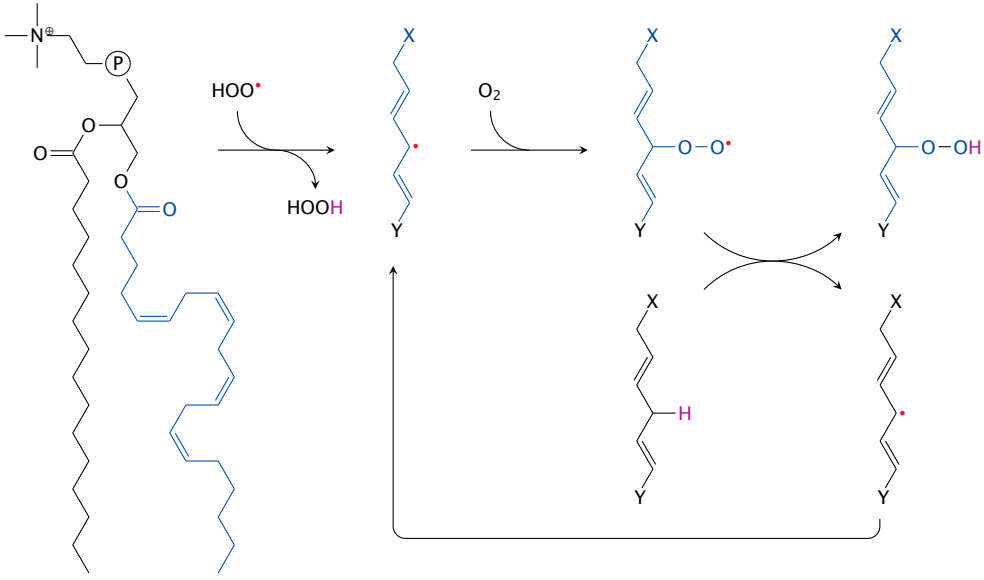
tyrosine





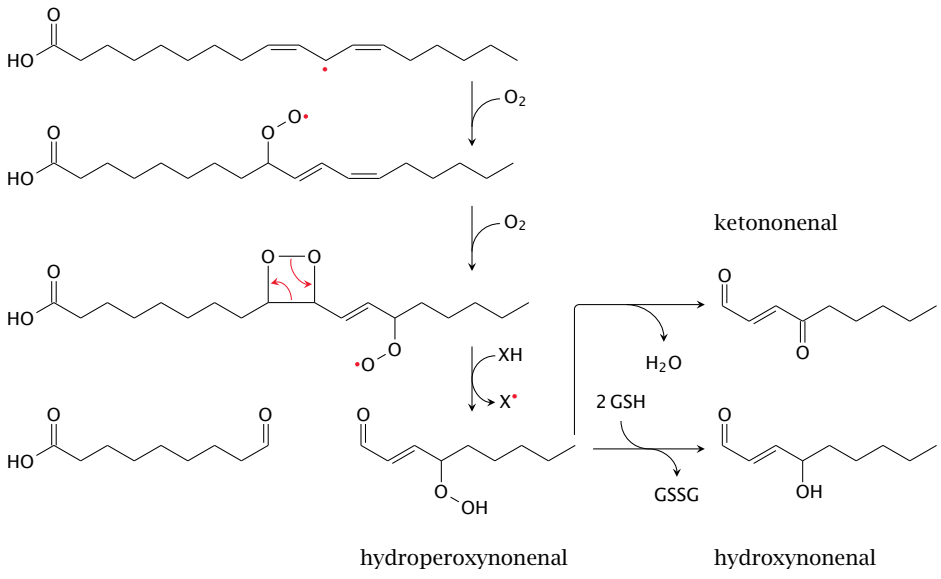
▸ thiol oxidation

Self-sustained lipid peroxidation induced by peroxyl radicals

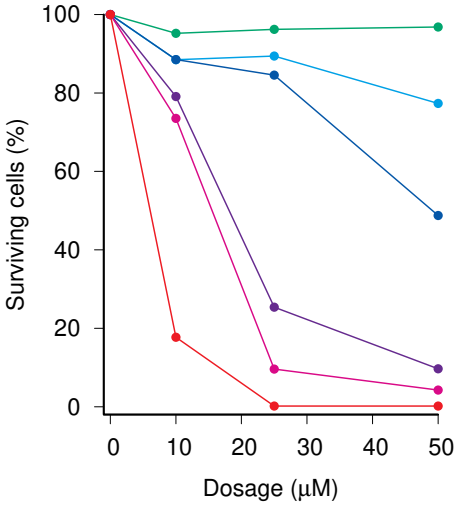


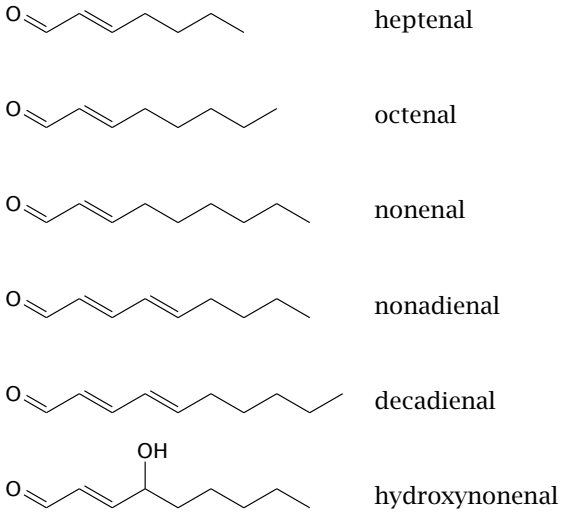
- Redox potentials

Toxic products of lipid peroxidation: hydroxynonenal

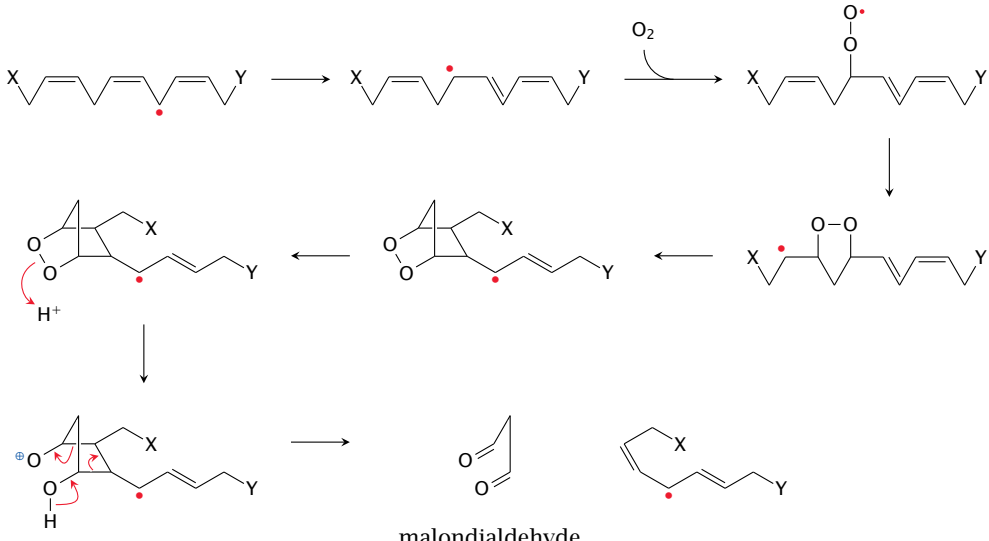


Hydroxynonenal cytotoxicity in cell culture

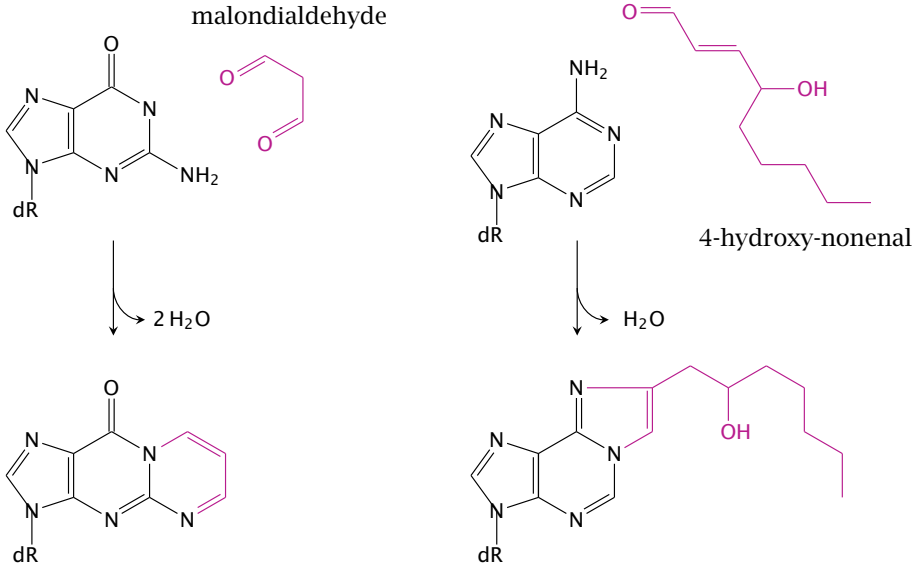




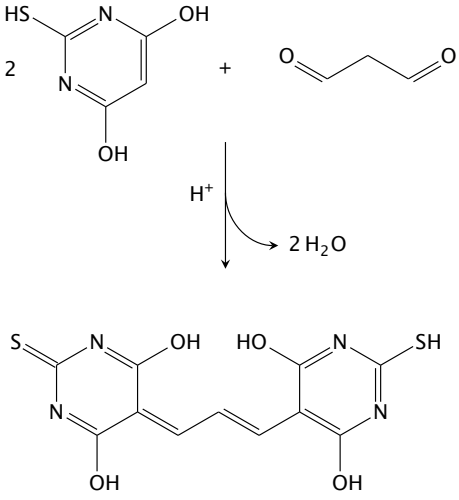
Toxic products of lipid peroxidation: malondialdehyde

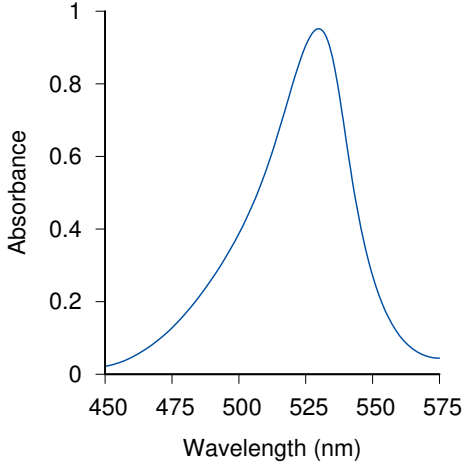


Formation of nucleobase adducts by hydroxynonenal and malondialdehyde

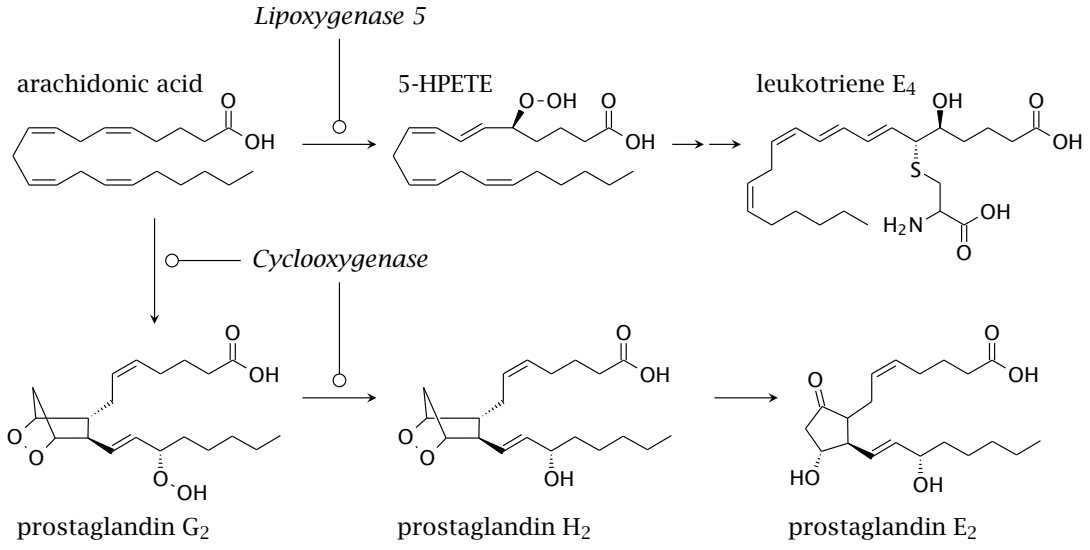


Detection of malondialdehyde with thiobarbituric acid

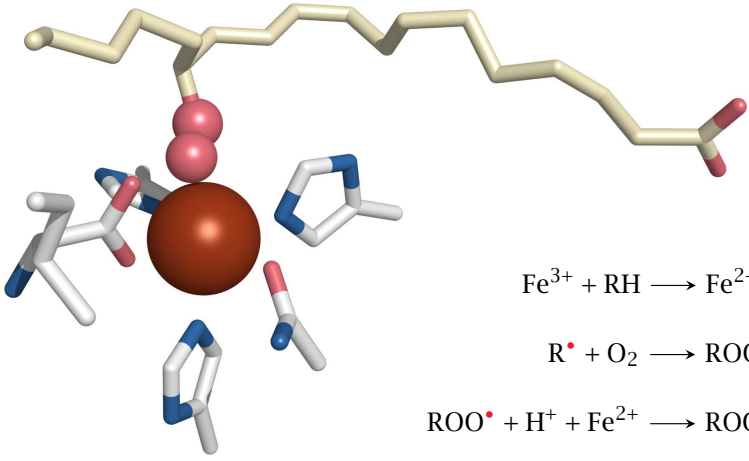




Formation of inflammatory mediators by enzymatic lipid peroxidation



Lipoxygenases use iron to abstract $H^{\bullet}$ from the substrate



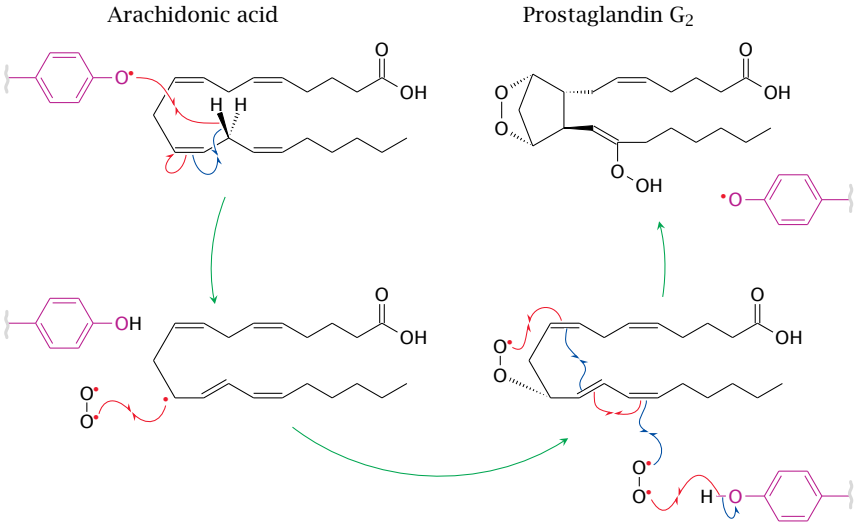
$$Fe^{3+} + RH \longrightarrow Fe^{2+} + H^{+} + R^{\bullet}$$

$$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$$

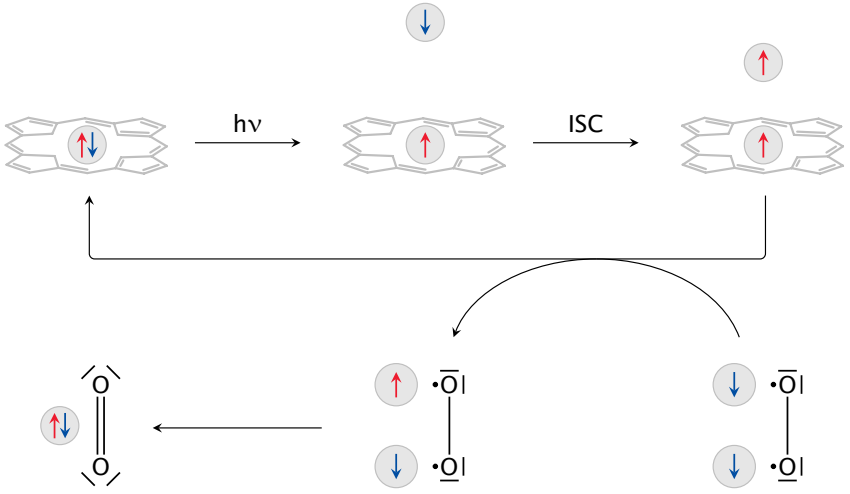
$$ROO^{\bullet} + H^{+} + Fe^{2+} \longrightarrow ROOH + Fe^{3+}$$

- redox potentials

A tyrosyl radical initiates the cyclooxygenase reaction



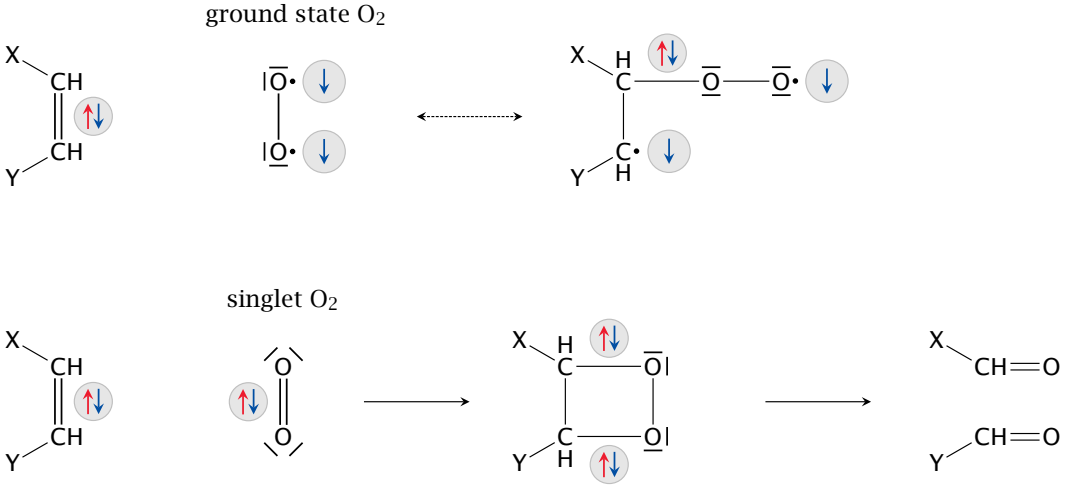
Photoactivated generation of singlet oxygen by porphyrins



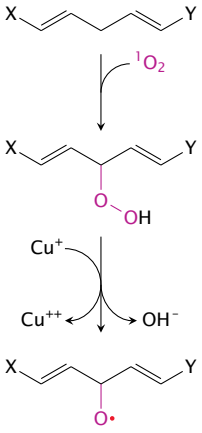
singlet O_2 ($^1\Delta_g$) singlet O_2 ($^1\Sigma_g^+$) triplet O_2 ($^3\Sigma_g^-$)

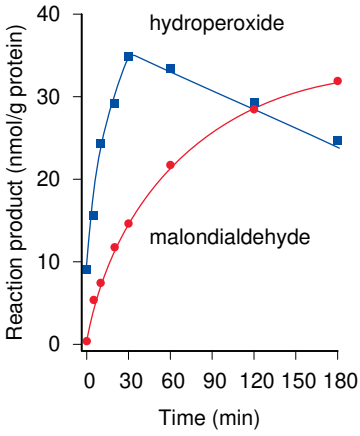
▸ PCT

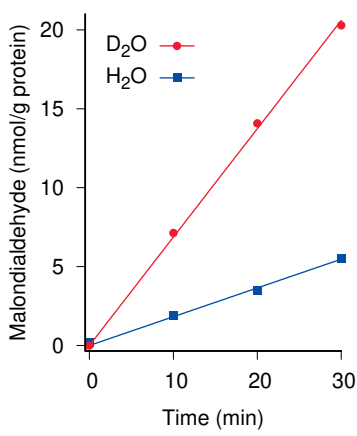
Singlet oxygen reacts readily with non-radicals



Singlet oxygen and transition metals in photoactivated lipid peroxidation



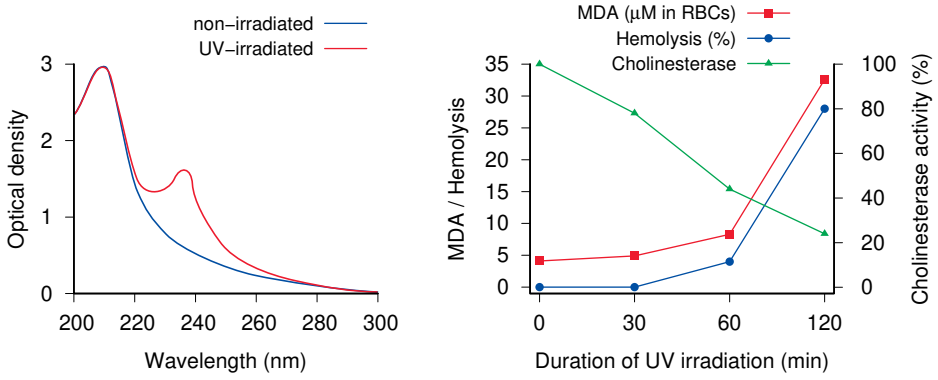




Data from Ding and Chan, 1984 (PMID 6717256)

‣ lipid peroxidation

UV-induced lipid peroxidation and membrane damage in erythropoietic protoporphyria



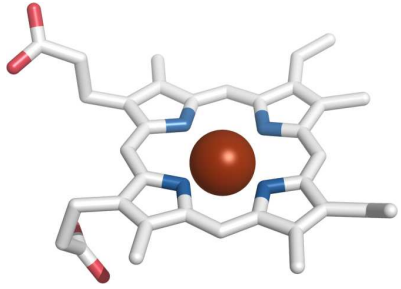
▸ EPP ▸ MDA

Data from Goldstein and Harber, 1972 (PMID 5014616)

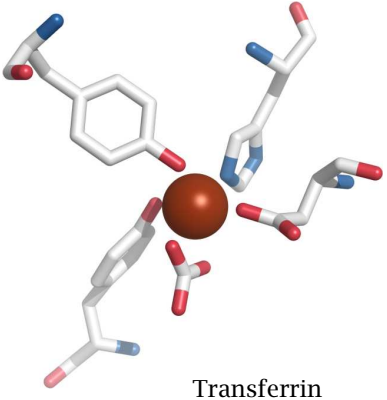
Protective mechanisms and molecules

- Metal sequestration (Fe, Cu)
- Enzymes
  - Superoxide dismutase
  - Catalase
  - Glutathione peroxidase family
  - Peroxiredoxins, glutaredoxins, thioredoxins
- Small molecules
  - Endogenous: glutathione, uric acid, bilirubin, coenzyme Q
  - Exogenous: ascorbic acid, vitamin E

Iron chelation by heme and by transferrin

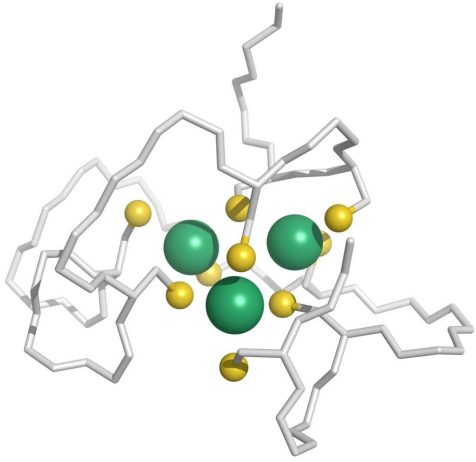


Heme

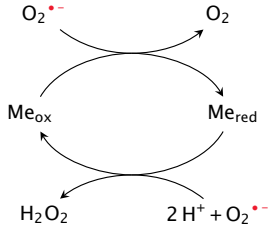


Transferrin

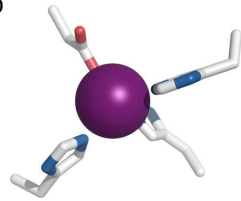
Metallothioneins sequester copper and other heavy metals

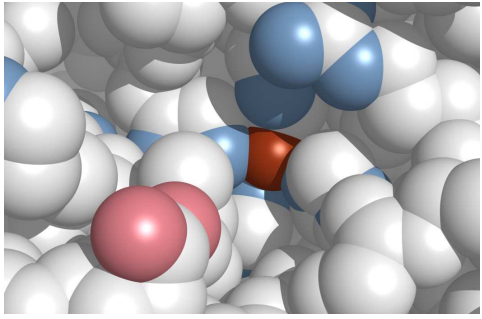


Superoxide dismutases contain transition metals

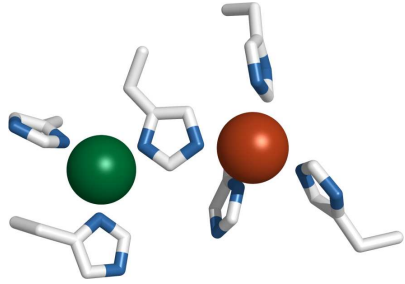


Mn - SOD



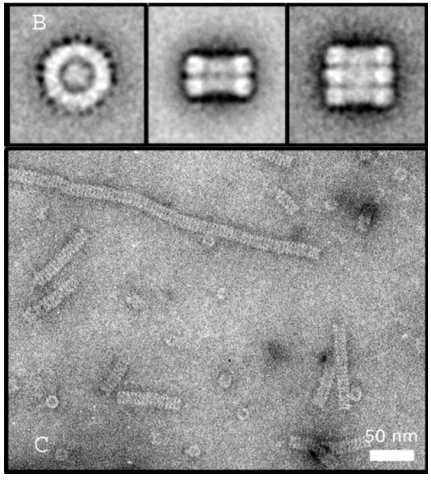


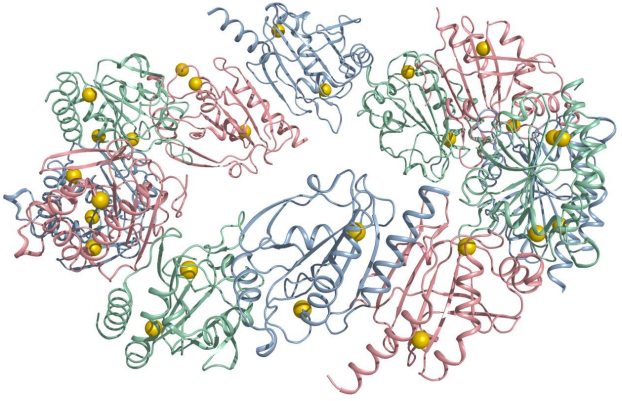
Cu, Zn - SOD



- Haber-Weiss

Structure of mitochondrial peroxiredoxin 3

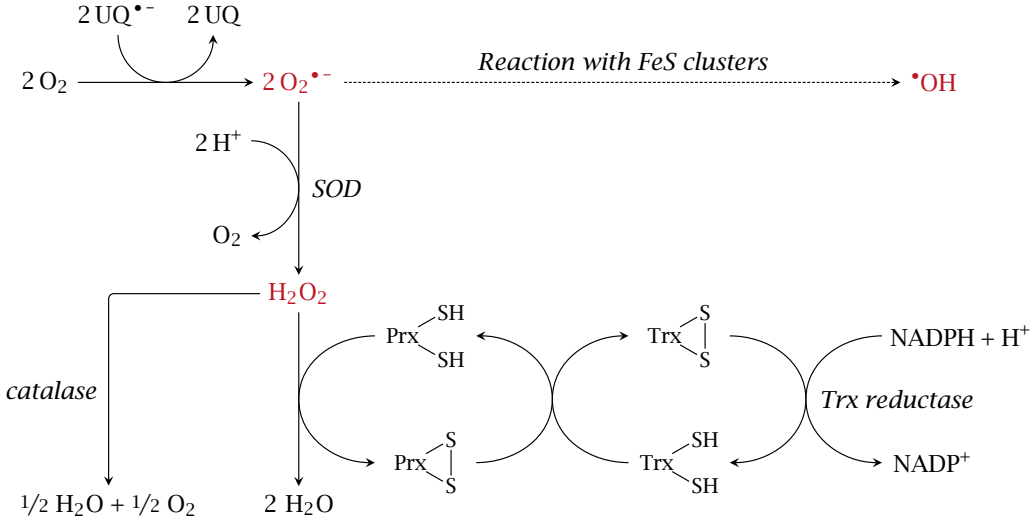




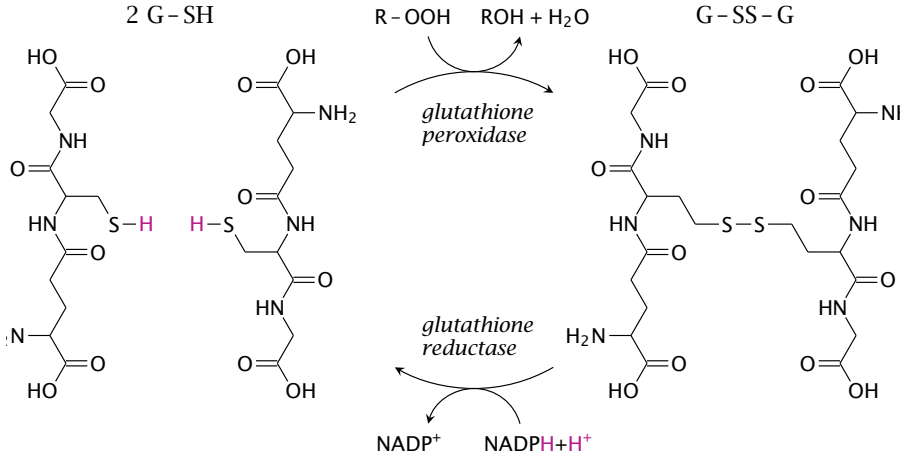
Other enzymes that carry out thiol/disulfide chemistry

Enzymes	Properties and functions
Glutathione peroxidases	contain selenocysteine in the active site; reduce organic peroxides
Thioredoxins	reduce protein disulfides, including peroxiredoxins
Thioredoxin reductase	reduces thioredoxin reductase using NADPH
Glutaredoxins	reduce protein/GSH mixed disulfides (P – SS – G) and dehydroascorbic acid
Thiol-disulfide isomerases	reside inside the ER; facilitate protein folding by resolving aberrant protein disulfides

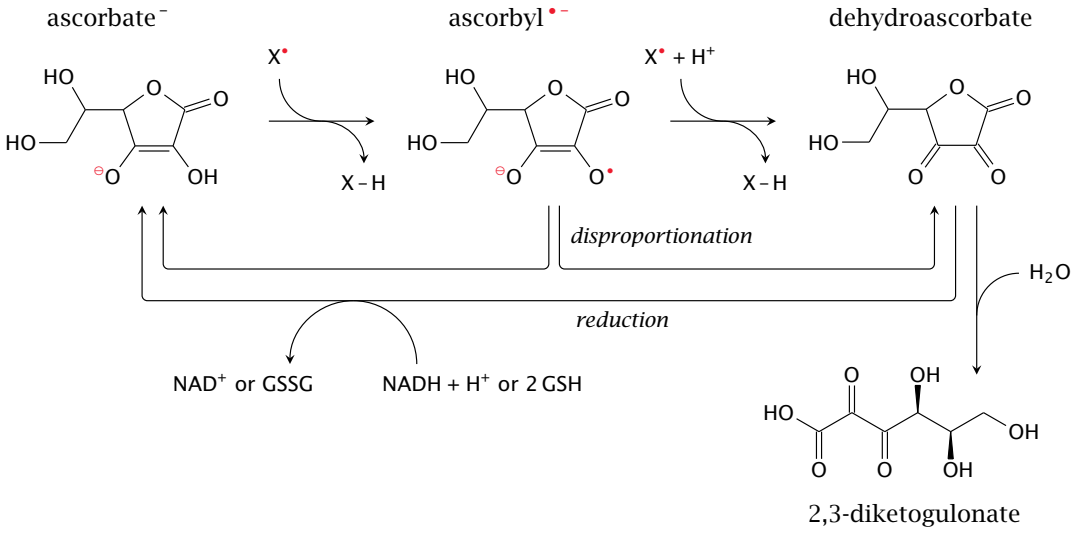
Detoxification of mitochondrial superoxide



Scavenging of organic peroxides by glutathione peroxidase



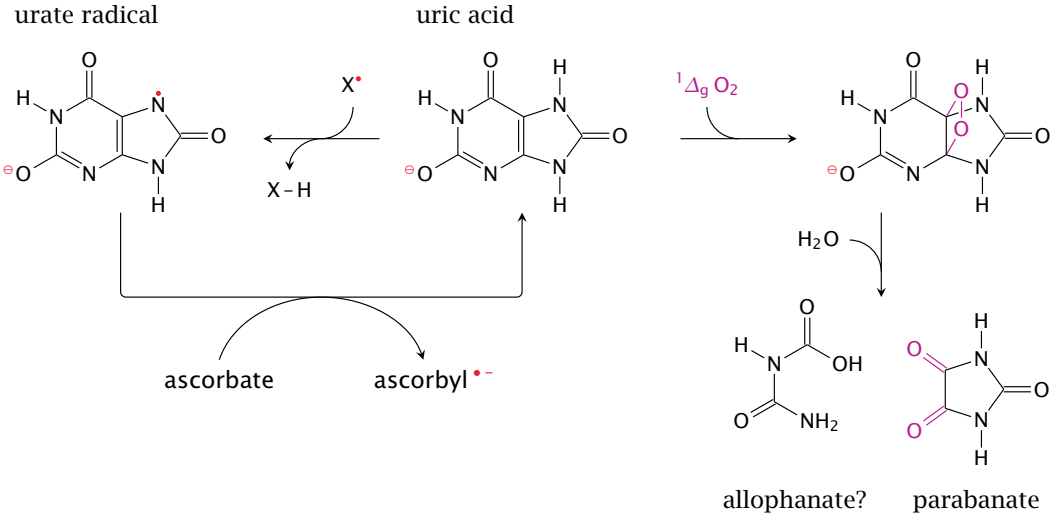
Ascorbic acid (vitamin C) is a major radical scavenger



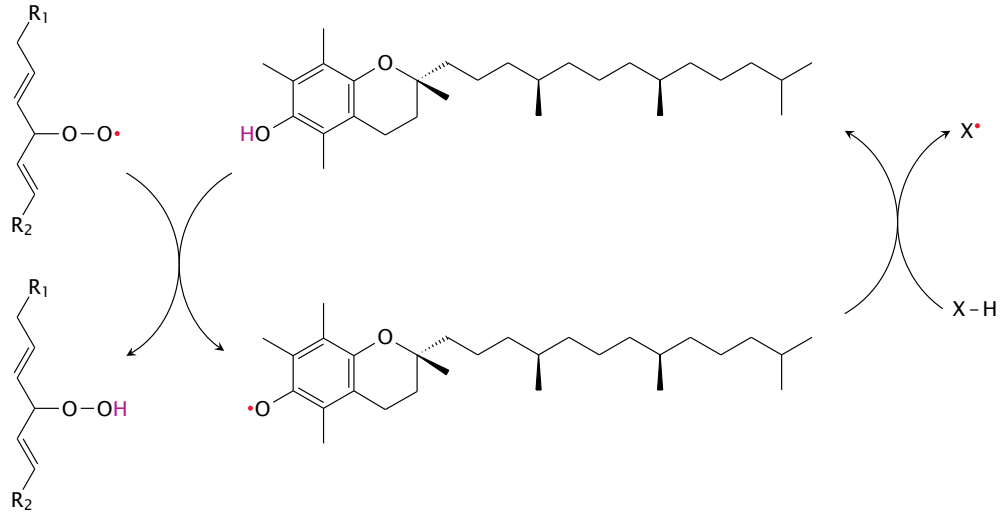
The energetics of ascorbyl disproportionation

Oxidised form		Reduced form	$\Delta E^{0'}$ (V)
ascorbyl$^{\bullet -}$	$-e^-$	dehydroascorbate	0.174
ascorbyl$^{\bullet -}$ + H^+	$+e^-$	ascorbate$^-$	0.282
2 ascorbyl$^{\bullet -}$ + H^+	$\longrightarrow$	ascorbate$^-$ + dehydroascorbate	0.454

Uric acid as a radical scavenger and antioxidant



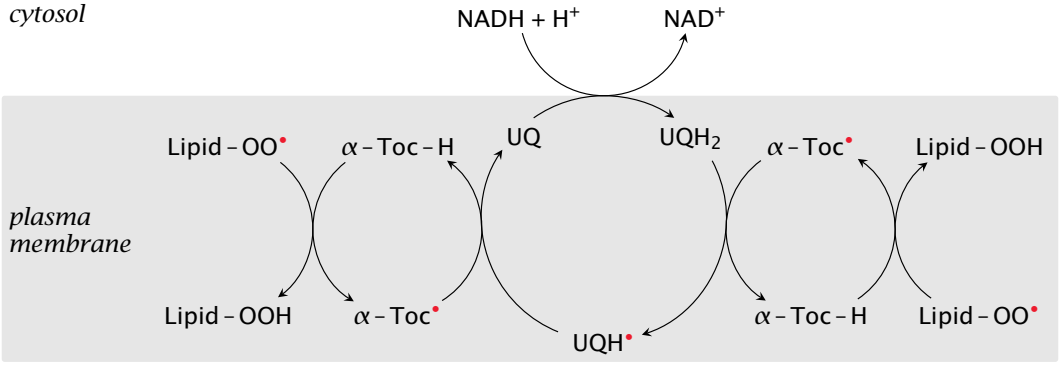
α-Tocopherol intercepts lipid peroxidation



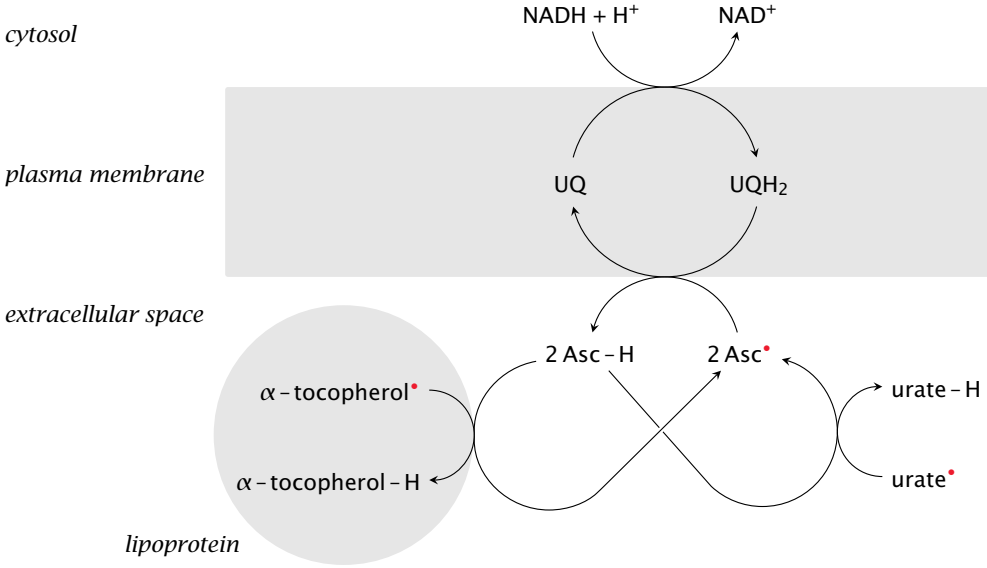
Extracellular antioxidants

- Small molecules: ascorbate, urate, glutathione
- Albumin
- Peroxiredoxin 4
- Selenoprotein P

Regeneration of α-tocopherol by ubiquinol



Regeneration of extracellular ascorbate and urate



Chapter 19

Metabolism of drugs and xenobiotics

Metabolism of drugs and xenobiotics

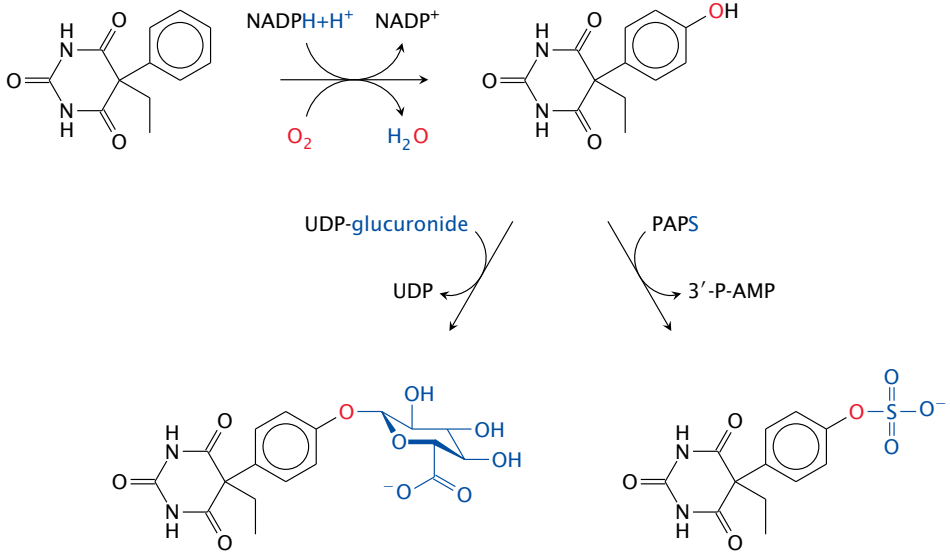
Functional significance:

- inactivation and facilitated elimination of drugs and xenobiotics
- activation of *prodrugs*
- formation of *active metabolites* with similar or novel activity
- detoxification of toxic xenobiotics
- *toxification* of non-toxic xenobiotics

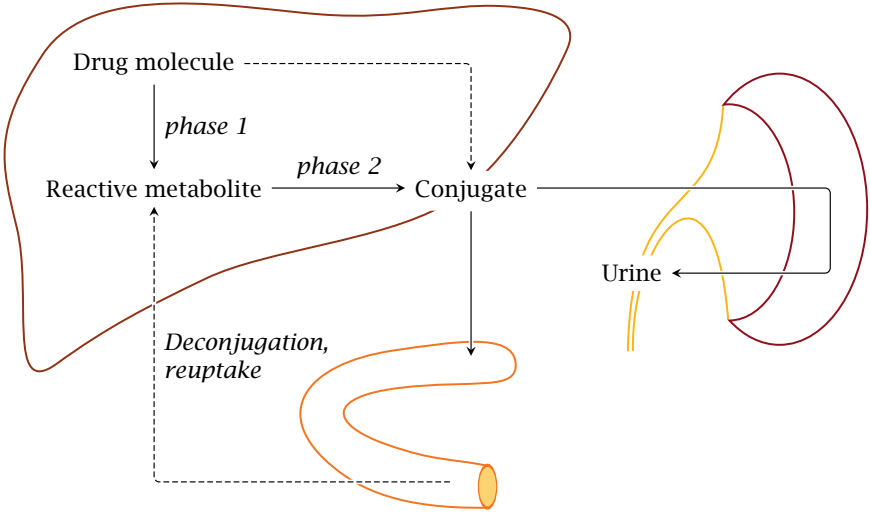
Enzyme specificity in drug metabolism

- key problem: a limited number of enzymes must cope with an unlimited number of substrates
- many drug-metabolizing enzymes have fairly broad specificities
- enzyme specificities overlap—many drugs give rise to multiple metabolites

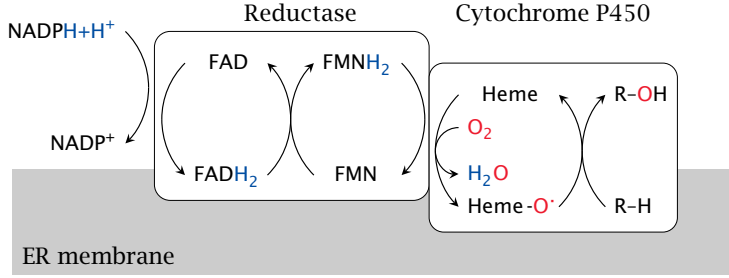
Example: metabolism of phenobarbital



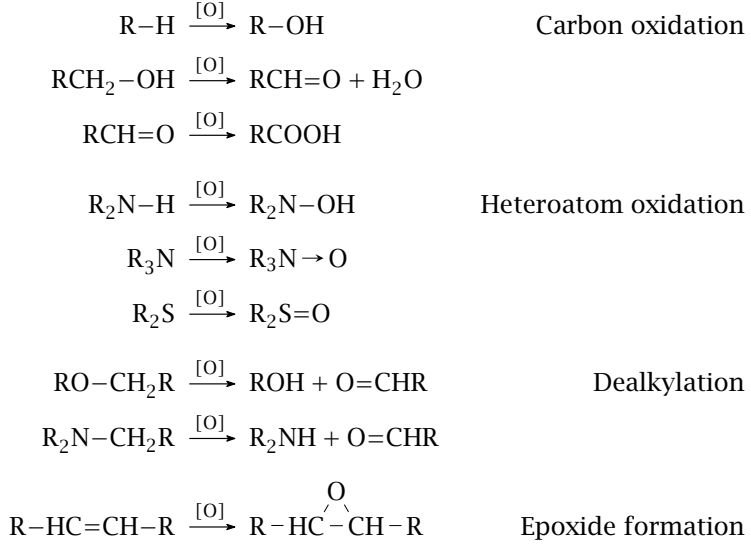
Drug metabolism facilitates drug elimination



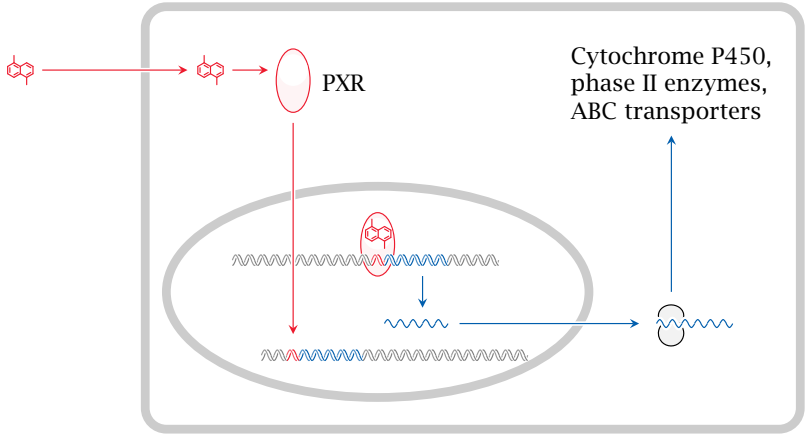
Mode of action of cytochrome P450 enzymes



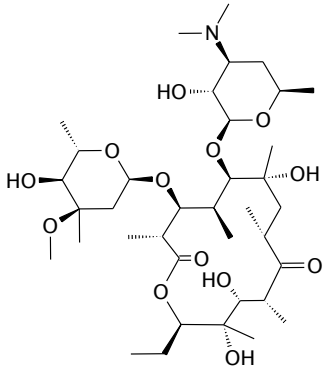
Reactions catalyzed by cytochrome P450

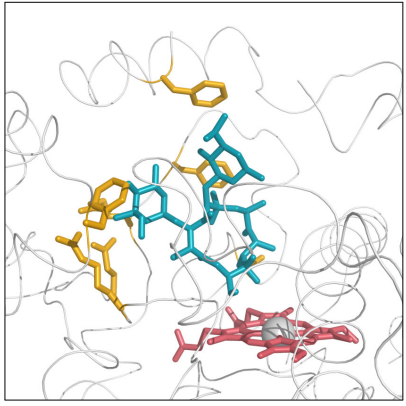


Transcriptional induction of CYP450 3A4

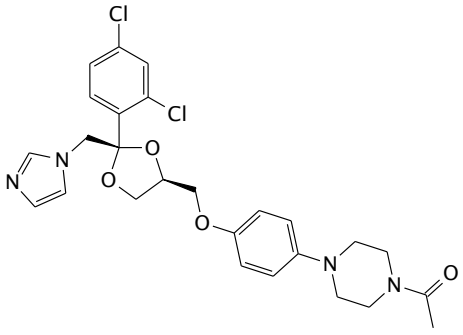


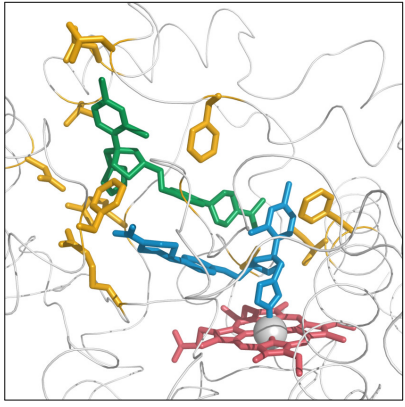
Structure of erythromycin bound to cytochrome P450 3A4



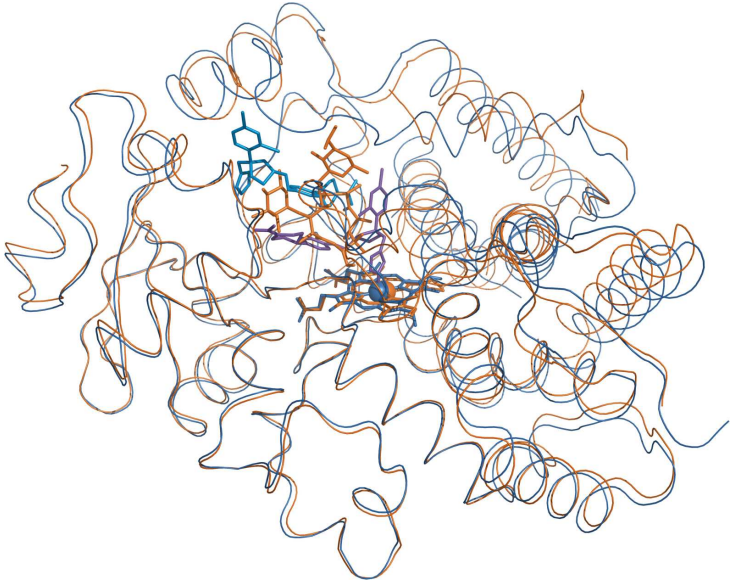


Ketoconazole bound to cytochrome P450 3A4

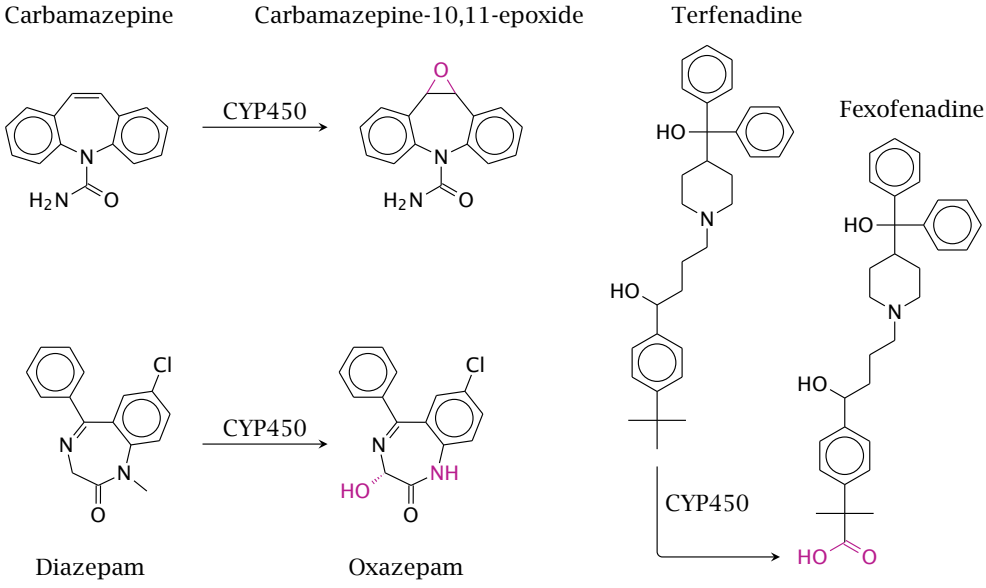




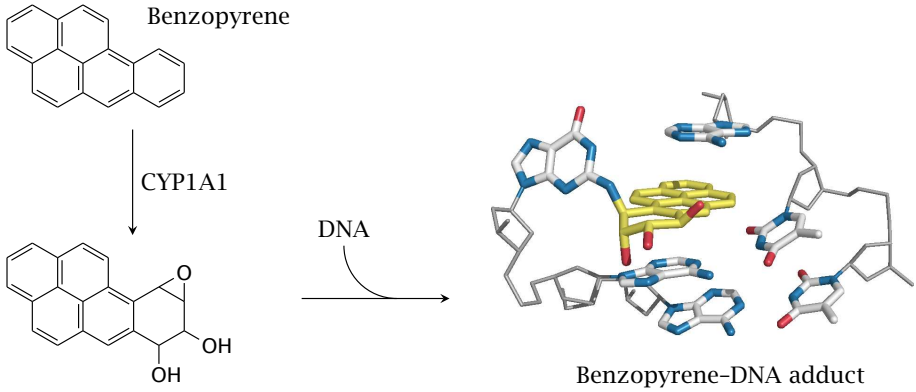
Superposition of the erythromycin- and the ketoconazole-bound structures



Examples of active metabolites formed by CYP450 enzymes



Benzopyrene as an example of harmful metabolism of xenobiotics

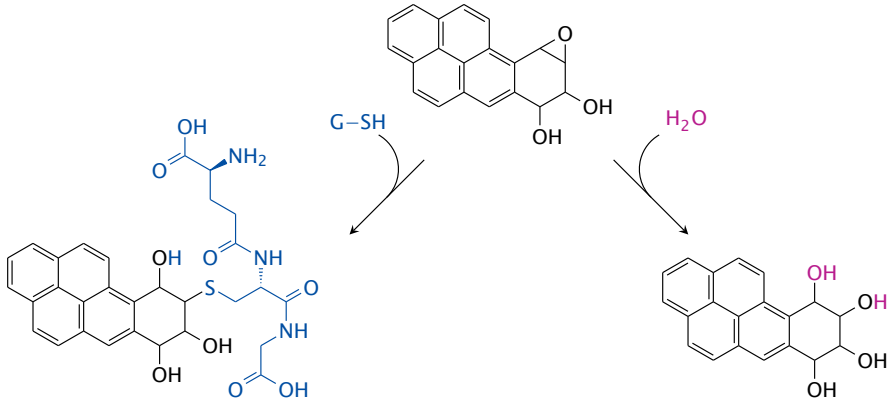


Benzopyrene–DNA adduct

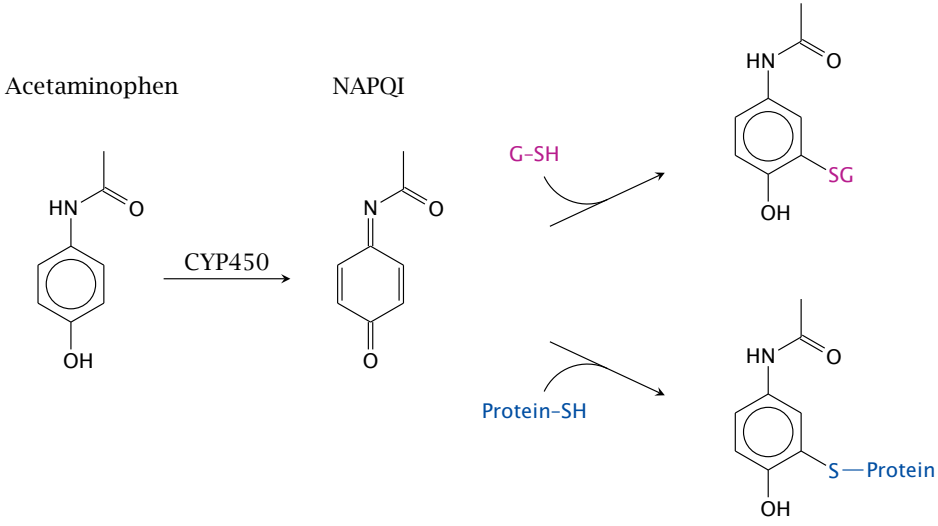
Summary of phase II reactions

Enzymes	Cosubstrates	Functional groups
UDP-glucuronosyl-transferases	UDP-glucuronide	–OH, $-NH_2$
sulfotransferases	PAPS	–OH, $-NH_2$
glutathione-*S*-transferases	glutathione	epoxy groups, double bonds
acetyltransferases	acetyl-CoA	–OH, $-NH_2$
methyltransferases	SAM	–OH, $-NH_2$, –SH
epoxide hydrolase	H_2O	epoxide groups
aminoacyltransferases	amino acids	–COOH

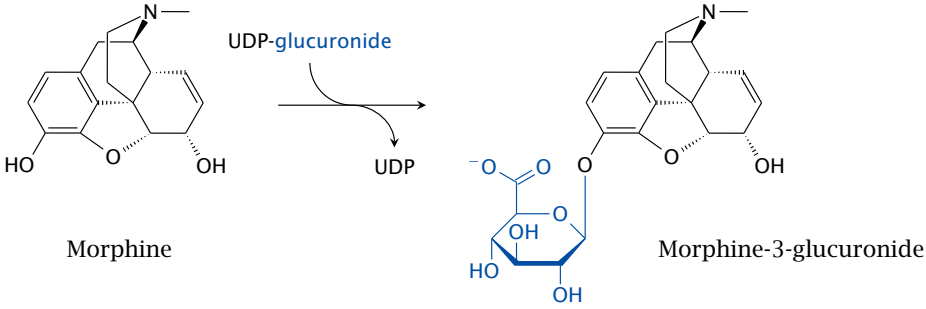
Detoxification of benzopyrene epoxide derivatives by epoxide hydrolase or glutathione-*S*-transferase



Metabolism of acetaminophen



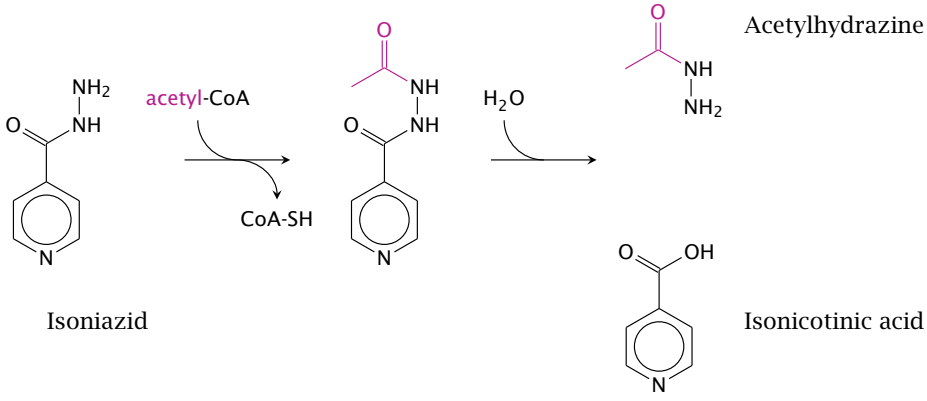
Morphine skips phase I and is conjugated directly



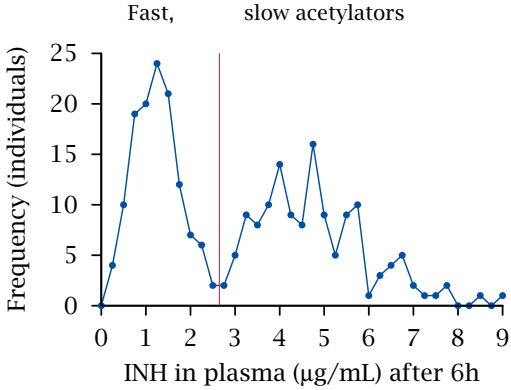
Morphine

Morphine-3-glucuronide

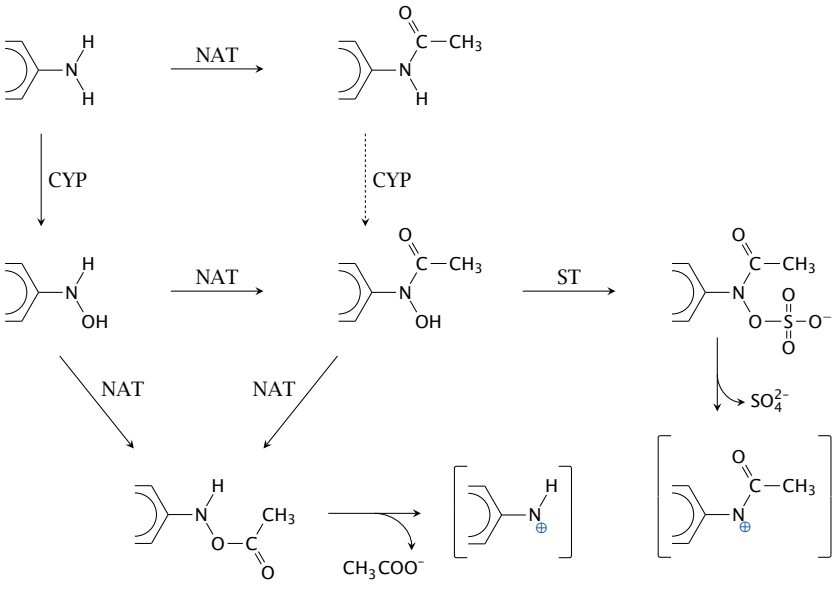
Acetylation of INH by N-acetyltransferase 2 (NAT 2)



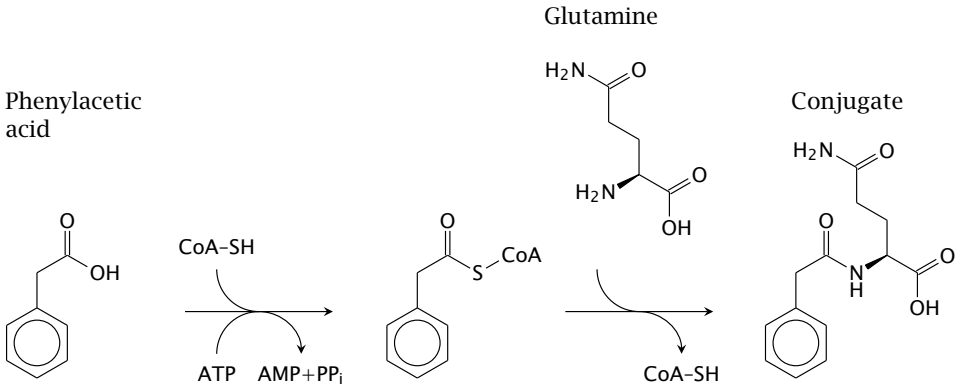
Bimodal distribution of INH acetylation speed



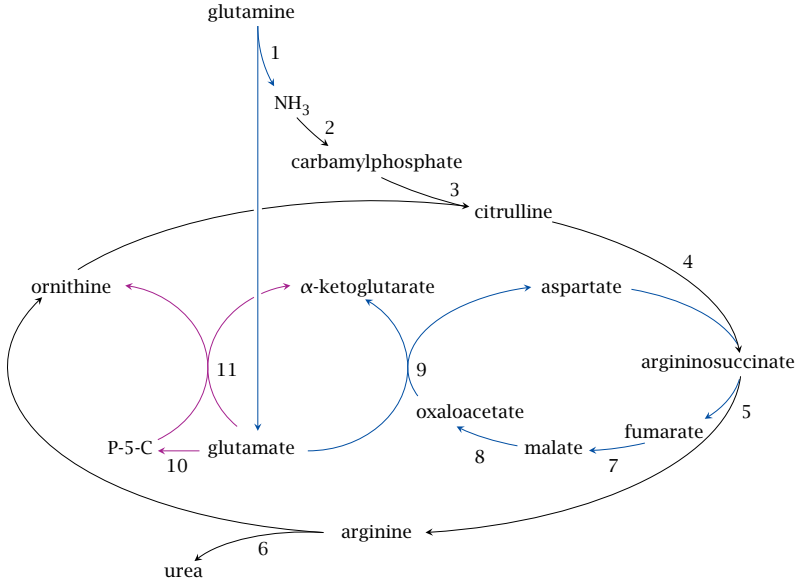
Metabolic activation of arylamine carcinogens



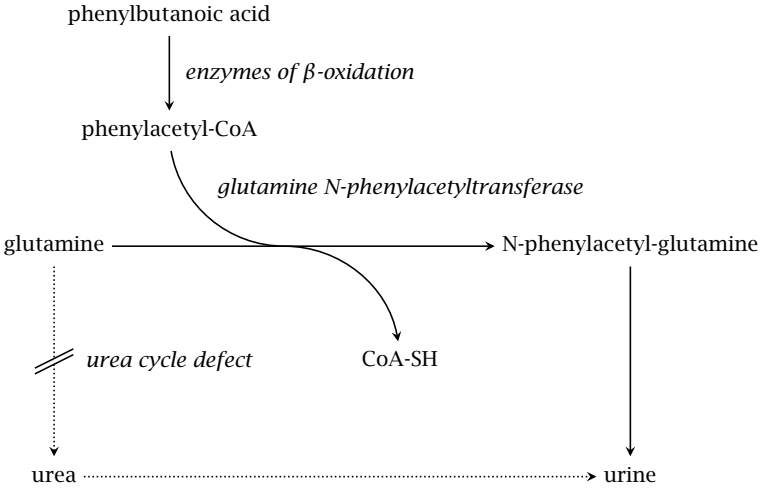
Amino acid conjugation: Glutamine conjugation of phenylacetate



Alternate pathway therapy of urea cycle defects (1)



Alternate pathway therapy of urea cycle defects (2)



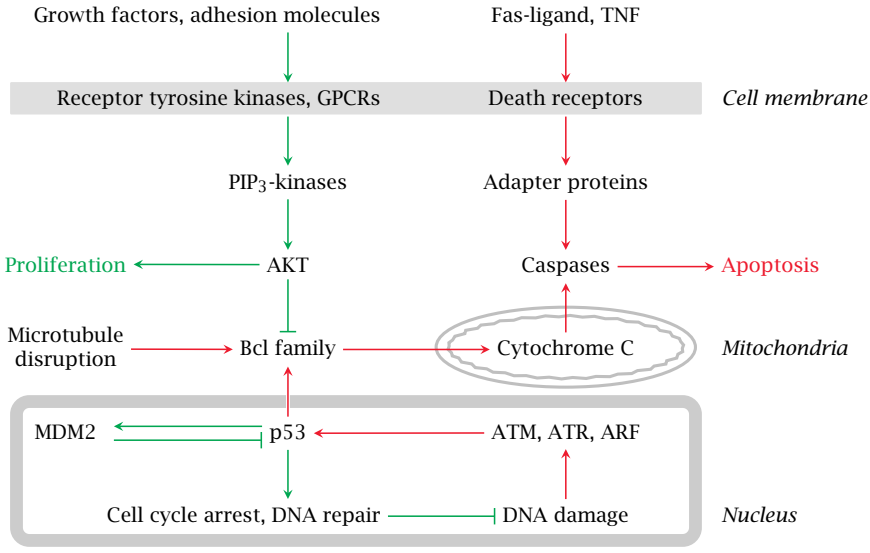
Reductive drug metabolism

Multiple enzymes:

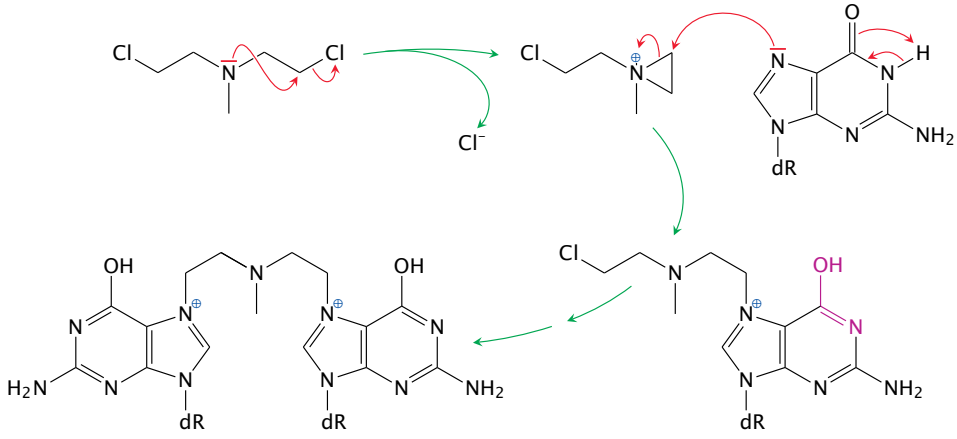
- methemoglobin reductase (diaphorase)
- cytochrome P450 reductase
- thioredoxin
- bacterial metabolism
- ...

- regeneration of tocopherol by ubiquinol

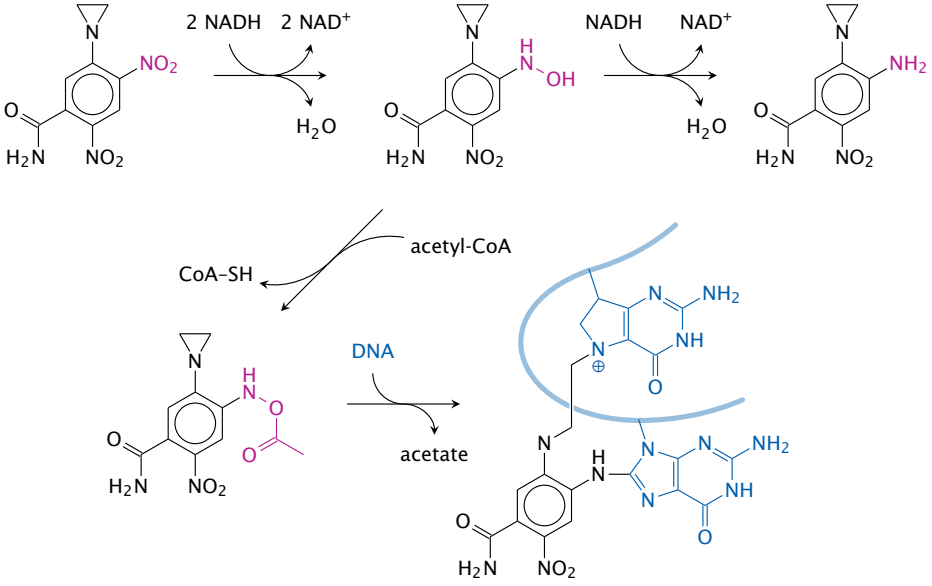
DNA damage triggers programmed cell death



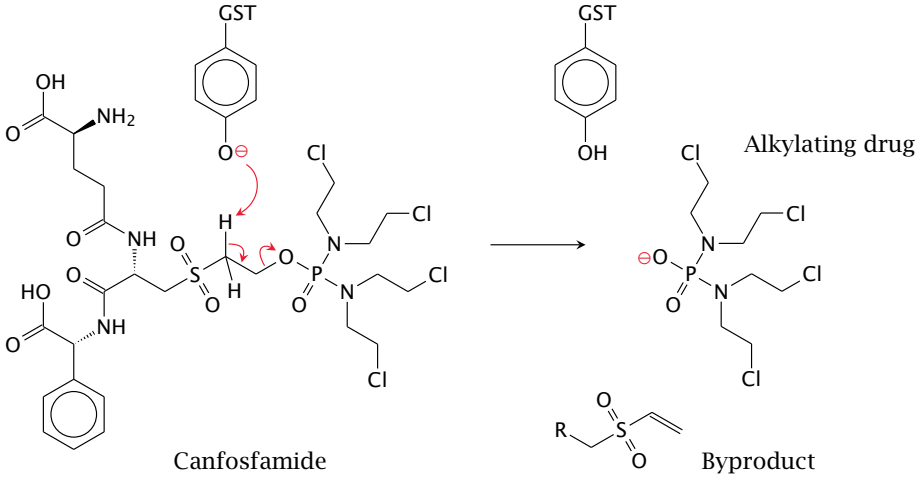
Mechlorethamine, a DNA-alkylating drug



CB 1954, an experimental antitumor drug that is activated by nitro group reduction and acetylation



Canfosfamide, an antitumor drug that targets alkylant-resistant tumor cells



Chapter 20

Enzyme and gene therapy of enzyme defects

Therapy of enzyme defects: general considerations

- How many organs are affected by the enzyme defect: One organ, a few, or all organs?
- How severe is the defect?
- Can the defect be adequately controlled by conventional treatment?

Conventional therapeutic strategies

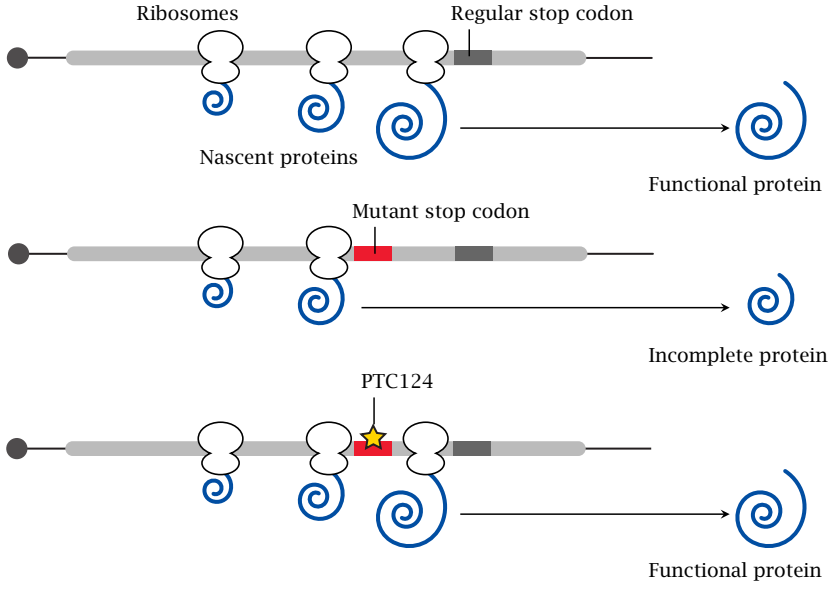
- diets
- drugs
- organ transplants

Therapeutic strategies based on molecular biology

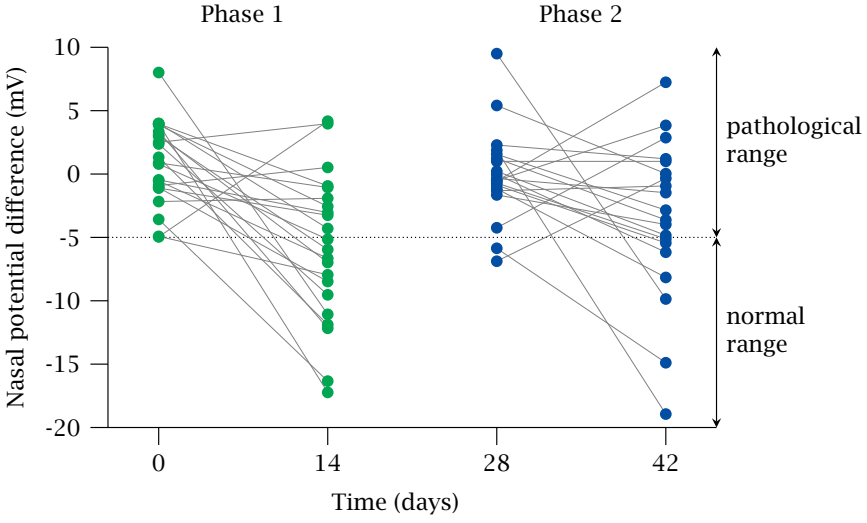
Correction of ...

- DNA: gene therapy
- mRNA: suppression of mutant stop codons with drugs
- protein: enzyme substitution

Translational antitermination with PTC124 (ataluren)



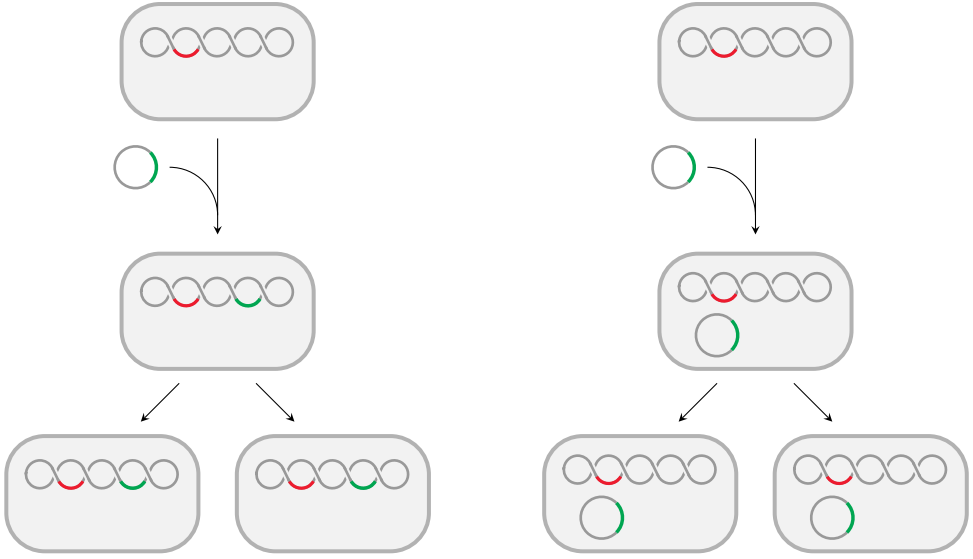
Ataluren in cystic fibrosis



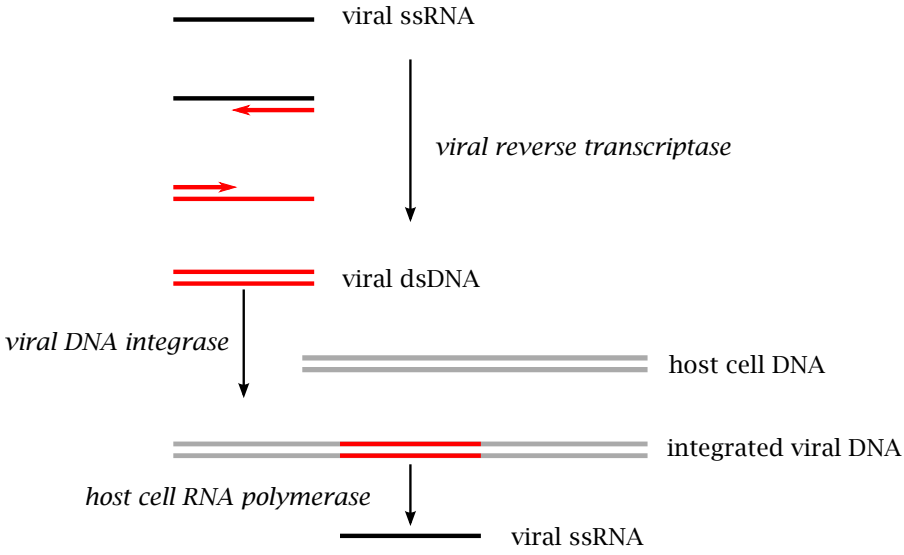
Technical considerations for gene therapy

1. gene transfer *in vivo* versus *in vitro*
2. transfer method: viral vectors *vs* naked DNA
3. location of transferred gene: chromosomal versus episomal
4. expression of transferred genes: transient versus permanent
5. immune reactions to vector (particularly where repeated application is required)

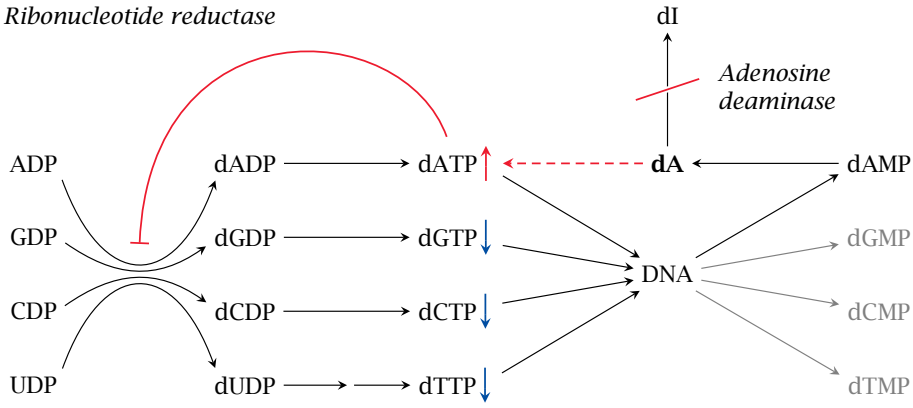
Chromosomal integration vs. episomal propagation of transferred genes



The life cycle of a retrovirus



An example: Adenosine deaminase deficiency

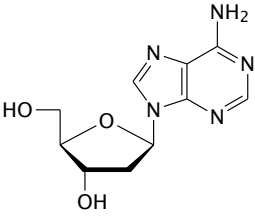


- purine degradation

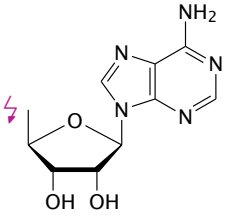
Conventional therapy of ADA deficiency: Allogenic bone marrow transplant

- currently the standard treatment
- side effects and complications can be severe
- requires compatible donor

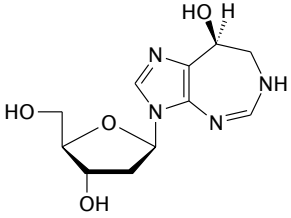
ADA deficiency: an in vitro model of drug treatment



2-deoxy-adenosine



5-deoxy-adenosine



pentostatin

Researching ADA enzyme therapy: first attempt

Adenosine Deaminase Enzyme Therapy Prevents and Reverses the Heightened Cavernosal Relaxation in Priapism

The Journal of Sexual Medicine (2010), 7:3011-3022

Researching ADA enzyme therapy: second attempt

Enzyme replacement therapy for adenosine deaminase deficiency and severe combined immunodeficiency

New Engl J Med (1976) 295:1337-43

- strategy: application of frozen irradiated red blood cells (!)
- therapy improved immune status and helped patient survive for 17 months (while waiting for blood marrow transplant)

Gene therapy of ADA deficiency

Still at the stage of clinical studies, not mainstream. A recent study was performed as follows:

- Non-myeloablative conditioning
- $CD34^+$ bone marrow cells (stem cells) were isolated from the blood, transduced in vitro with a retroviral vector carrying a functional ADA gene, and reintroduced into the body
- ADA expression achieved in lymphocytes: ~5% in bone marrow, ~75% in periphery
- All patients survived at time point of compilation of study (2–8 years after treatment), but some required additional enzyme treatment

New Engl J Med (2009) 360:447-58

Pompe disease

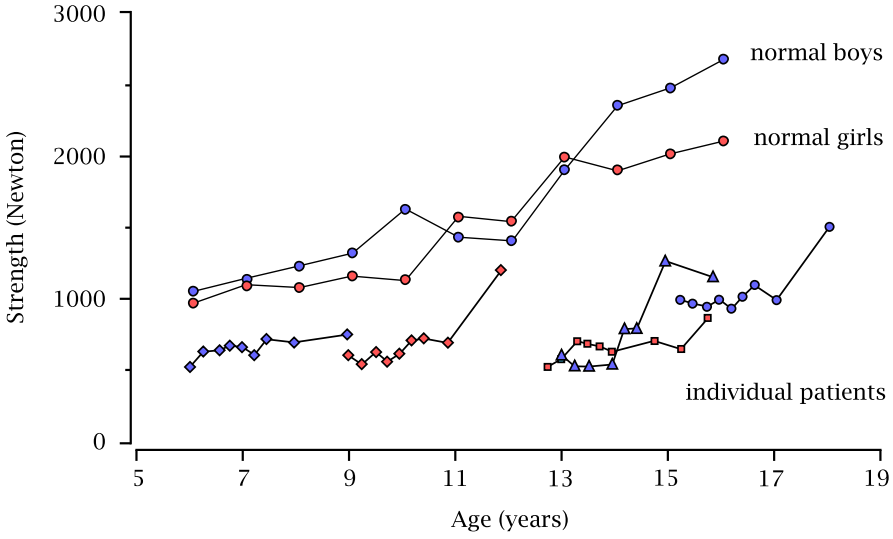
- defect of acid maltase, a lysosomal enzyme that breaks down glycogen particles
- lysosomal glycogen accumulates
- various forms: complete absence of enzyme (manifestation in infants) vs. residual activity (manifestation in older children or adolescents)
- affects mainly the skeletal muscle; glycogen accumulation leads to muscle tissue degeneration
- muscle strength progressively degrades, to the point that patients are no longer able to breathe

Enzyme therapy of Pompe disease

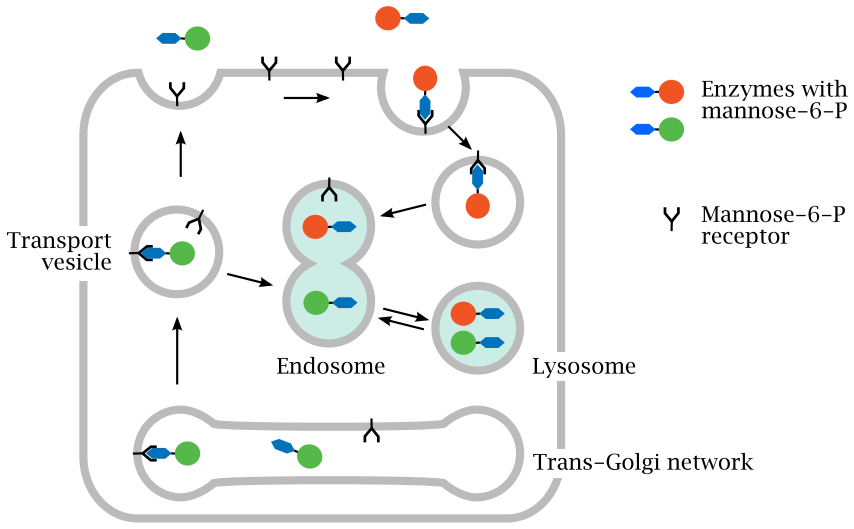
from Neuromuscular Disorders (2010) 20:775–782

- recombinant enzyme expressed in rabbit mammary glands, isolated from rabbit milk
- target group: juvenile patients (not infants)
- dosage: 20 $^{mg}/_{kg}$ every two weeks
- clinical outcome: improvement of muscle strength, but not to normal level
- no severe immune reactions

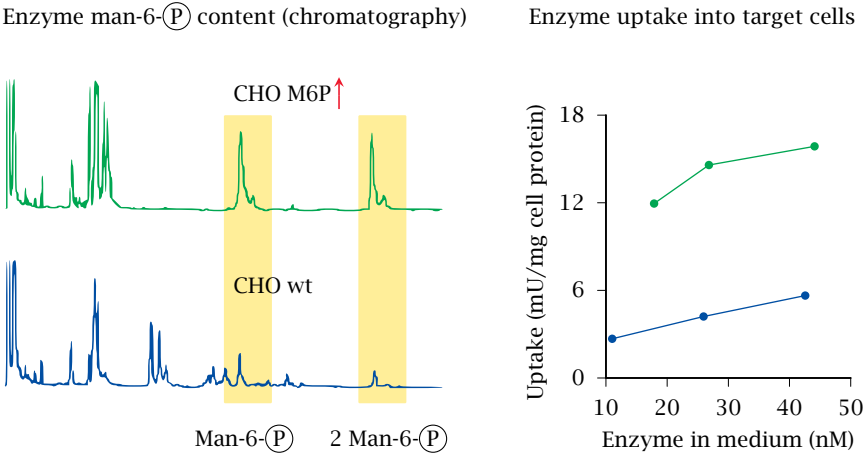
Clinical outcome of enzyme therapy: Muscle strength



The mannose-6-phosphate receptor targets proteins to the lysosome

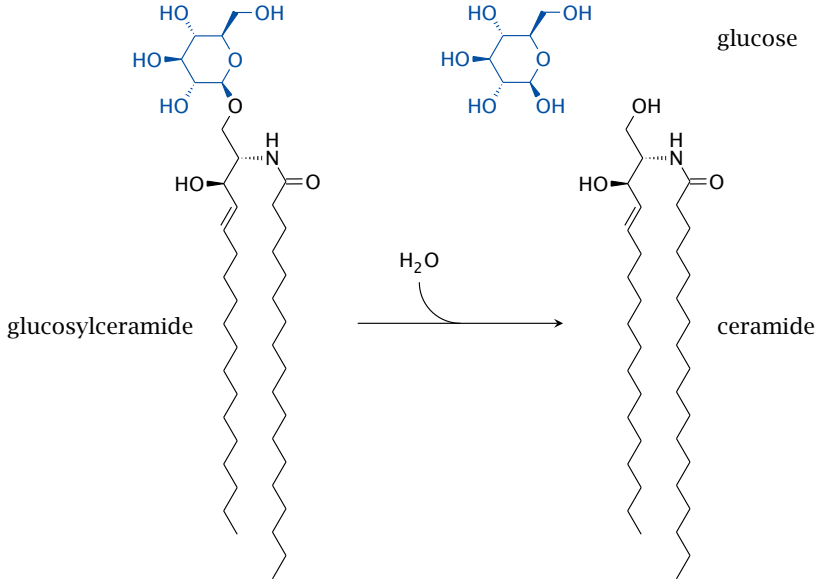


Optimization of acid maltase glycosylation

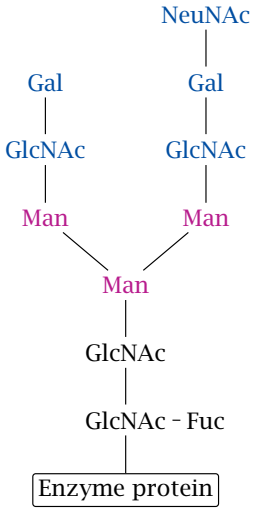


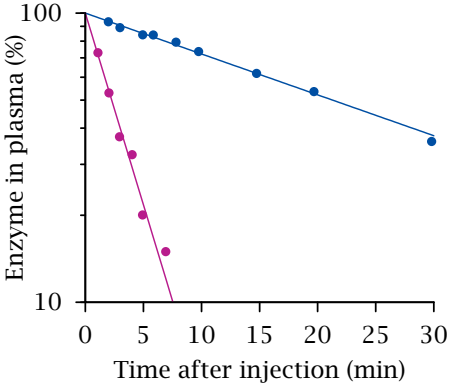
McVie-Wylie et al., Mol Genet Metab (2008) 94:448-455

The biochemical defect in Gaucher disease

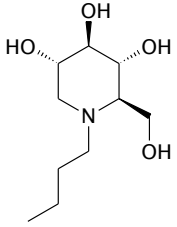


Partial deglycosylation of glucocerebrosidase accelerates uptake into macrophages





Drug treatment of Gaucher disease



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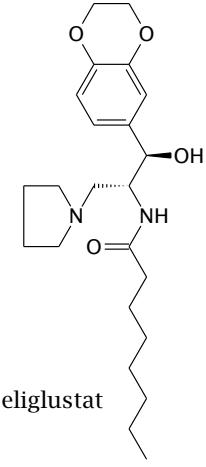

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