CELLULAR BASIS OF MEDICINE

Course 2 – Cell Metabolism and Specialisation

METABOLIC PATHWAYS

- PICTURE COMPLETION -

workbook

Vladimíra Kvasnicová

Dear students,

This workbook contains *blank schemes* of the main metabolic pathways you learn about in the course *Cell Metabolism and Specialisation*.

You can use it in any way you find useful and over time fill the schemes in with information you consider important.

But the best way how to work with this workbook is using the tasks on the facing pages and fill the schemes in step-by-step according to these tasks. The tasks were *not chosen randomly* but to guide you through the most important facts you should know about each of the metabolic pathways (main intermediates, enzymes, relationships). In a way they constitute a *written guide to the basic metabolism*.

In preparing this study material, feedback from colleagues involved in biochemistry teaching has been very helpful to me. My thanks are mainly to Pavla Balínová and Josef Fontana for review and valuable comments, to Jan Trnka for his support, help and especially for inspiration to create such a workbook. I would like also thank to all the other teachers who have worked with the workbook in their seminars in previous years. Some pictures from the 1st edition (2017) have been redrawn by student Joseba Andoni Fernández Pérez, whom I also thank very much for helping. The 3rd edition newly contained a list of the most important organic acids and dictionaries for vitamins and enzymes, to which I added one more exercise for the 4th edition. The 5th edition newly included a brief overview of the regulation of metabolic pathways and an appendix with a detailed solution of the assigned tasks. Two new tasks, on metabolism of cholesterol and lipoproteins, have been added into this 6th edition.

The solution to the tasks is provided in the appendix so that you can check your own answers. I do not recommend copying the answers to the blank maps from the study materials or from the appendix. If you first think about the questions yourself and fill in the blank maps from memory based on what you've already learned, this workbook will serve its purpose: it will help you understand in context what knowledge you should have about each metabolic pathway. And if you don't understand something enough to be able to solve the tasks in the workbook yourself, ask questions in the biochemistry seminars: your teachers will be happy to explain what will be needed.

I wish you, on behalf of all my colleagues from the biochemistry department, that this workbook will serve you as best as possible. We all hope this material will help you understand the principles of cellular metabolism.

If you have any questions arrange a consultation with your biochemistry teacher or use the e-mail vladimira.kvasnicova@lf3.cuni.cz. Use the e-mail also for any feedback or suggestions.

Vladimíra Kvasnicová

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1. ORGANIC ACIDS IN METABOLISM - tasks

a) first - *as a biochemical setting-up* – you can practise the structures and trivial (common) names of the substances you will meet very often during the study of metabolism: **write common names to each of the pictured substances**

b) have a look at the structural formulas ordered in one line and for these *having the same number of carbons* think about chemical reactions by which they can be interconverted (see their functional groups), and **write the type of each possible reaction into the picture**

c) mark the substances that are:

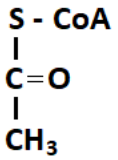
1) hydroxy derivatives of carboxylic acids
2) oxo derivatives (= keto derivatives) of carboxylic acids
3) amino acids
4) amides derived from dicarboxylic amino acids
5) acyls bound to coenzyme A (CoA)
6) organic compounds other than acids

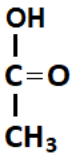
d) you may also **create small cards** (flash cards) for better learning: draw the structural formula of an organic acid on one side and write its common name on the other side; do biochemistry drills for some time - until you are able imagine each of the structure if you hear or read its name ;)

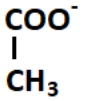
*NOTE: structural formulas of carboxylic acids and their derivatives are often drawn **vertically**, in an **ionized** form (this type of formula is preferred in biochemistry)*

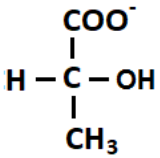
HINT:
Organic acids, important in metabolism, also include fatty acids and other proteinogenic amino acids: review their structural formulas and common names using the overview tables on the pages 57 – 60.

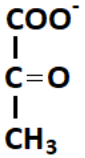
1. ORGANIC ACIDS IN METABOLISM - picture completion

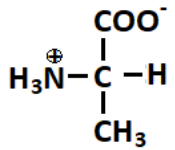


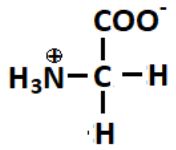


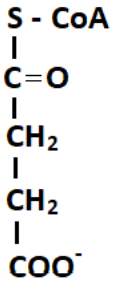


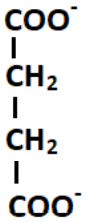


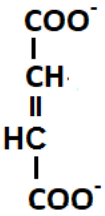


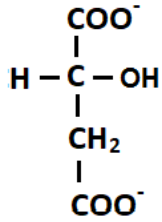


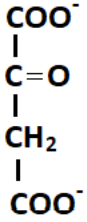


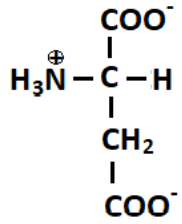


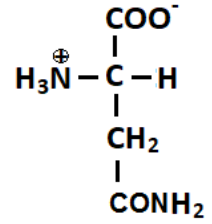


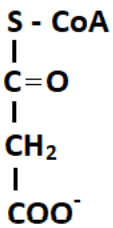


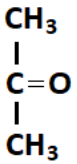


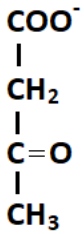


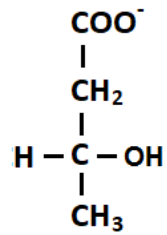


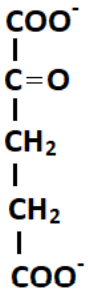


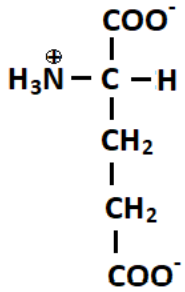


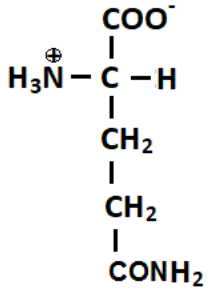












2. RESPIRATORY CHAIN - tasks

a) label parts of the picture: intermembrane space, mitochondrial matrix, inner mitochondrial membrane

b) add the coenzyme that is oxidized by Complex I, and list metabolic pathways producing this coenzyme

c) add the cofactor of Complex II; name this enzyme and state the metabolic pathway in which it is involved

d) state the alternative name of coenzyme Q (in both its oxidized and reduced form)

e) name 2 other enzymes of a cellular metabolism that transfer electrons directly to coenzyme Q and state the names of metabolic pathways, from which these enzymes carry electrons to the respiratory chain:

1)

2)

f) how many electrons can cytochrome c accept from Complex III? What is the mechanism of cytochrome c action?

g) add the name of Complex IV; what poison inhibits Complex IV?

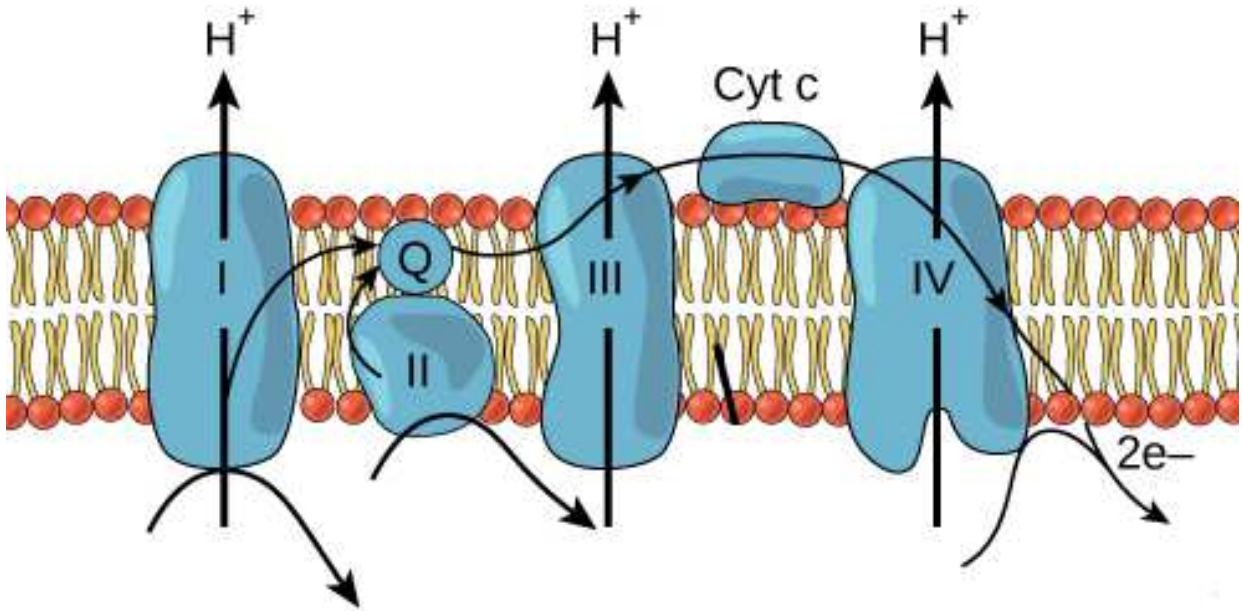
h) add substrates and the product of Complex IV (related to four transferred electrons)

i) complete the picture on the right side: draw ATP-synthase, ATP/ADP-translocase and an uncoupler protein (UCP)

j) describe the function (draw mechanisms of action) of each protein mentioned in the task i)

The picture was adopted from: https://www.boundless.com/biology/textbooks/boundless-biology-textbook/cellular-respiration-7/oxidative-phosphorylation-76/electron-transport-chain-362-11588/ (March 2016)

2. RESPIRATORY CHAIN - picture completion



3. KREBS CYCLE - tasks

a) mark acetyl-CoA in the picture and state its possible sources in a cell

b) find a sequence of three reactions describing the step by step oxidation of a saturated carbon skeleton up to a keto-derivative (saturated → unsaturated → hydroxy derivative → keto derivative), name all involved intermediates, add names (chemical types) of involved reactions, used cofactors and names of related enzymes (only those that are oxidoreductases)

c) find all reactions producing CO_2 and mark the one that is an analogous to the pyruvate dehydrogenase reaction; add names of all intermediates (substrates / products of these reactions) and cofactors used

d) mark all reactions of the Krebs cycle producing NADH; write below how this coenzyme is regenerated within mitochondrion:

e) find all intermediates that can be formed by a transamination of amino acids, give names of these amino acids

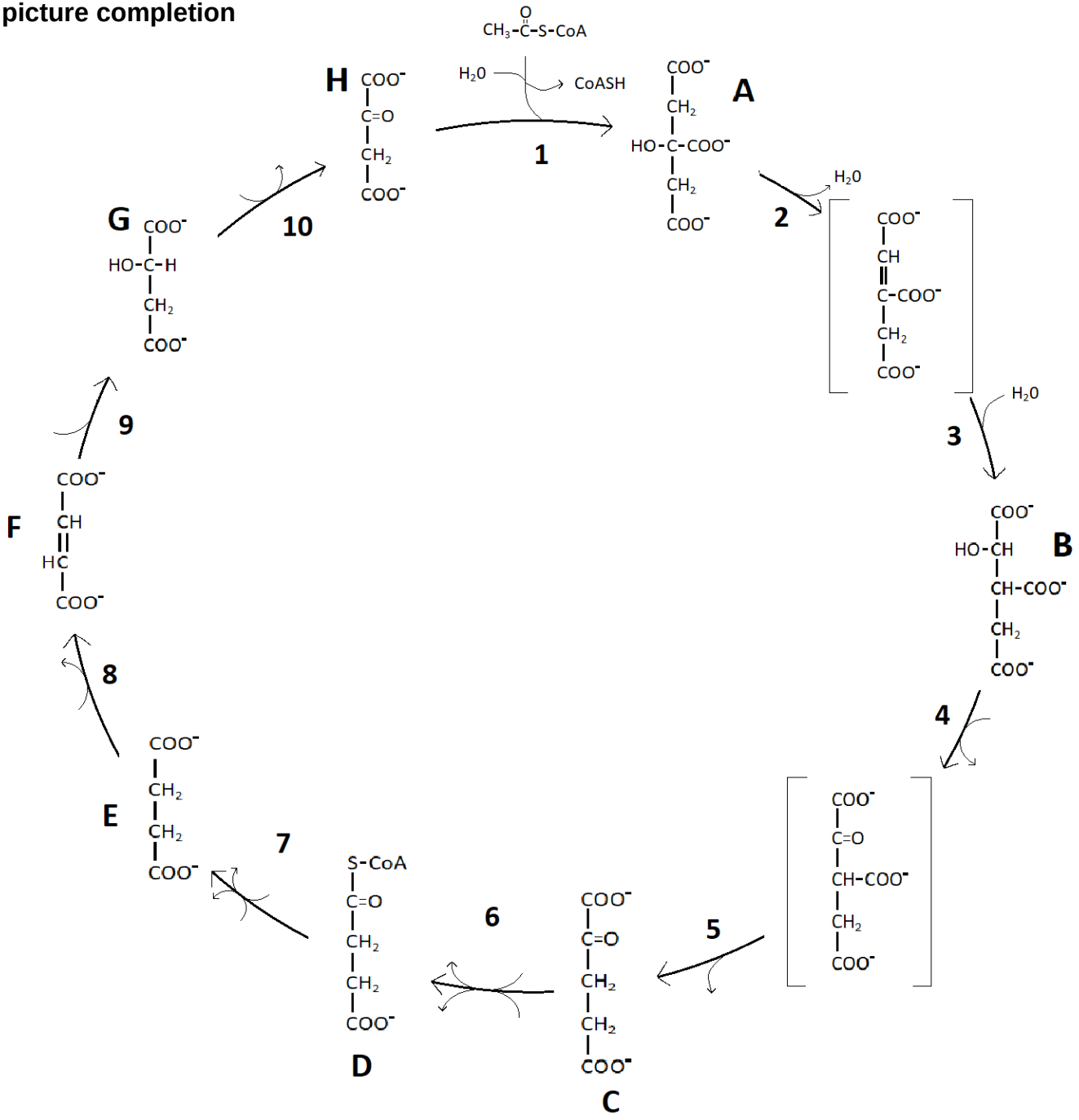
f) mark one intermediate that can be produced by an anaplerotic reaction from pyruvate and write this reaction

g) mark reactions catalyzed by regulatory enzymes of the Krebs cycle, name these enzymes and state below how they are regulated:

h) mark the *substrate level phosphorylation*; in some cells this reaction directly produces ATP, but in the liver rather GTP is produced: describe below how can GTP be used for ATP production?

i) mark the enzyme that is simultaneously involved in the electron transport chain, explain the connection

3. KREBS CYCLE - picture completion



4. REACTIONS OF PYRUVATE - tasks

a) find the reaction catalyzed by lactate dehydrogenase and add all the missing components of the reaction (including the enzyme´s cofactor and the name of the product); under which conditions does the reaction occur in cells and why is it important?

b) add all missing details to the reaction describing the fate of pyruvate under aerobic conditions (name and cofactors of the enzyme, names of all products of the reaction) and describe the fate of products of the reaction leading to the highest gain of ATP

c) one of the reactions is catalyzed by the enzyme ALT: state the whole name of the enzyme and add all missing details about the reaction (cofactor, the other reactant, names of products)

d) mark all reactions, through which pyruvate may enter the Krebs cycle

e) mark the reaction that enables the conversion of pyruvate to glucose; add the name of enzyme, its cofactor and all other components of the reaction (substrates and products)

f) mark all reactions that are somehow connected with the metabolism of amino acids and explain the connection

g) mark the reaction that does not occur in human cells and name the product of this reaction - this product can be hydrogenated by $NADH+H^+$ to ethanol; *(the two reactions describe the conversion of pyruvate to ethanol in yeasts during an anaerobic glucose metabolism)*

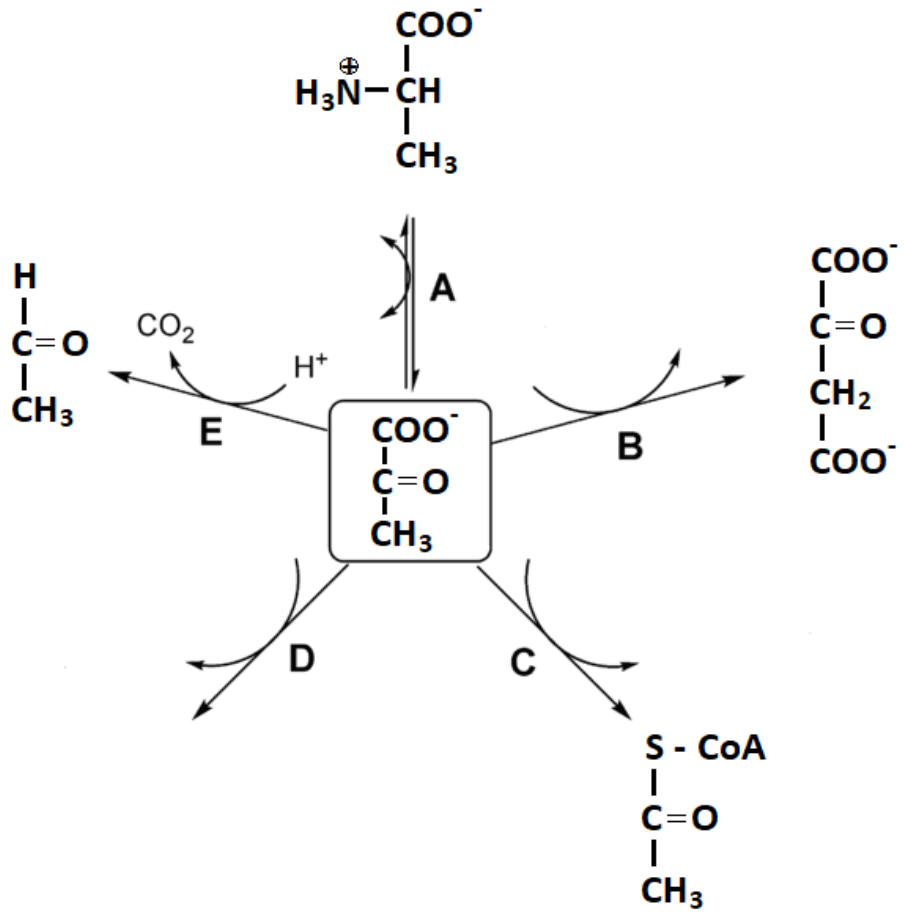
h) state the intracellular location of all reactions included in the picture (except the reaction from the task g) and mark all reactions which are irreversible under physiological conditions

i) state all possible other metabolic conversions of the product of reaction B

j) state all possible other metabolic conversions of the product of reaction C

k) state all possible nutrients, which can be converted to pyruvate in the cellular metabolism:

4. REACTIONS OF PYRUVATE - picture completion



5. GLYCOLYSIS - tasks

a) mark two reactions referred to as *substrate-level phosphorylations* and add the names of all reactants and products of these two reactions; explain the term “substrate level phosphorylation”:

b) mark the reaction producing NADH and add the names of a substrate and a product of the reaction; add the second substrate of the reaction (not pictured in the picture); how can be NADH regenerated back to NAD^+ ?

c) mark all reactions in which ATP acts as the second substrate; name enzymes catalyzing the reactions and add names of their substrates and products

d) mark the reaction catalyzed by aldolase and name products of the reaction

e) mark three reactions referred to as isomerizations; the reaction No. 10 also includes an isomerization (after releasing a phosphate group): what types of isomers are involved in the reaction?

f) starting from the reaction No. 6 all involved intermediates are formed in duplicate (not drawn) – explain why is it so:

g) mark the intermediate converted to 2,3-bisphosphoglycerate (2,3-BPG) in red blood cells and explain the role of this molecule; during its conversion to 3-phosphoglycerate inorganic phosphate is produced instead of ATP - why?

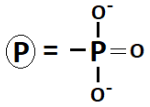
h) mark the reaction catalyzed by a different enzyme in the liver and pancreatic β-cells compared to most other cells of the body; what is the name of the enzyme? why is it referred to as the „glycemic sensor“ of cells?

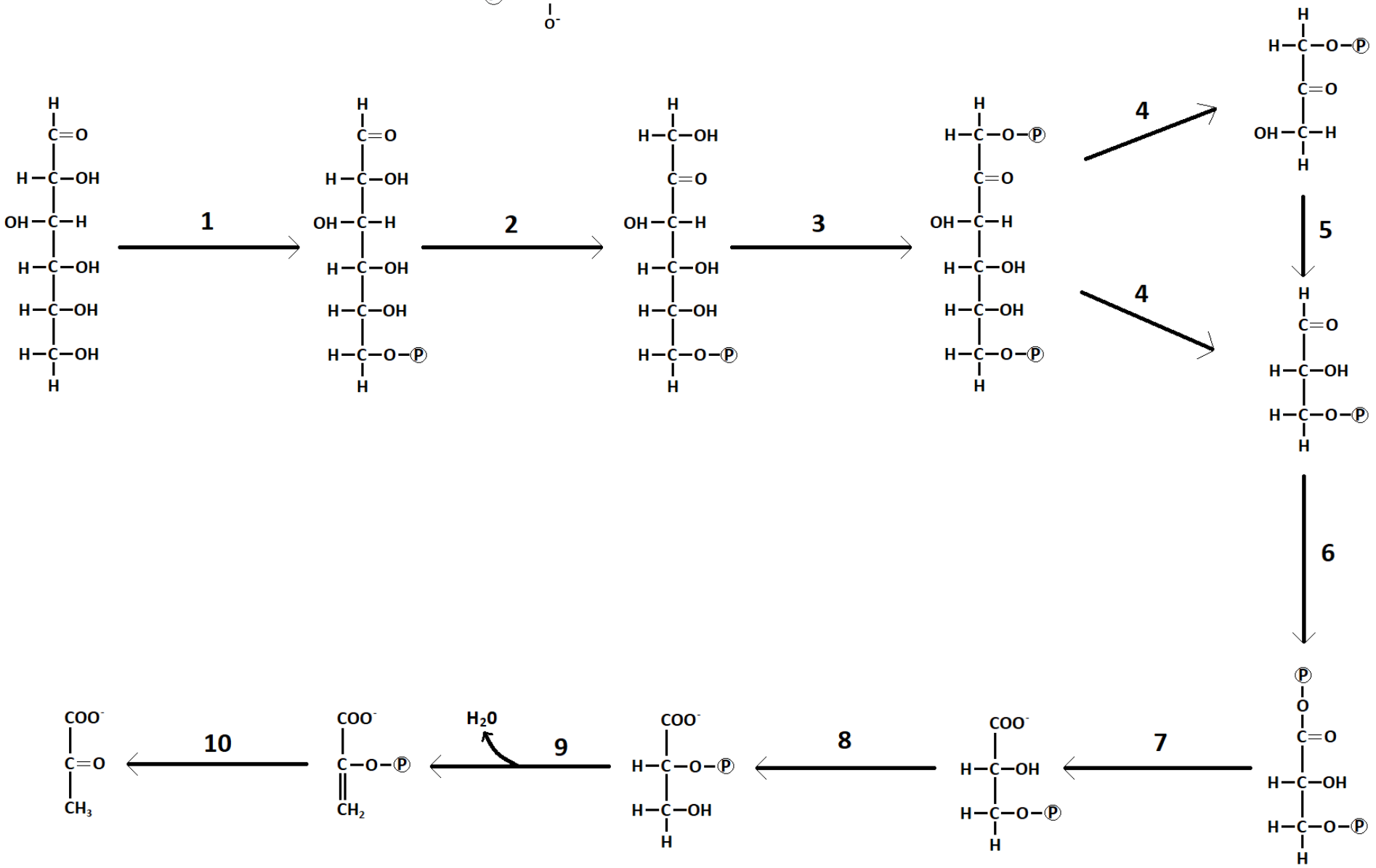
i) mark all intermediates of glycolysis which are also involved in the pentose phosphate pathway; explain the use of this interconnection (by the shared intermediates) of the two metabolic pathways

j) mark all “irreversible” reactions of glycolysis; why are the reactions virtually irreversible under physiological conditions?

k) mark and name all regulatory enzymes of glycolysis; state how and by what substances they are regulated

5. GLYCOLYSIS - picture completion

Ⓟ = 



6. GLUCONEOGENESIS - tasks

a) gluconeogenesis proceeds in almost all reactions as a “reverse glycolysis” (see the picture from bottom), the only differences are in reactions A, B and C: add names of enzymes catalyzing reactions B and C in gluconeogenesis; how do these reactions differ (in their mechanism) from reactions of glycolysis?

b) in which cellular compartment the reaction C of gluconeogenesis occurs? the enzyme catalyzing the reaction is not available in all cells - list cells in which the enzyme is present:

c) the reaction A of gluconeogenesis proceeds in two steps: add the steps to the picture, including structures and names of all reactants and products; add names and intracellular location of all involved enzymes

d) in the Cori cycle lactate is used as a substrate of gluconeogenesis: add the reaction to the picture (structural formula of lactate, names of the enzyme and coenzyme involved); what is the intracellular location of the enzyme?

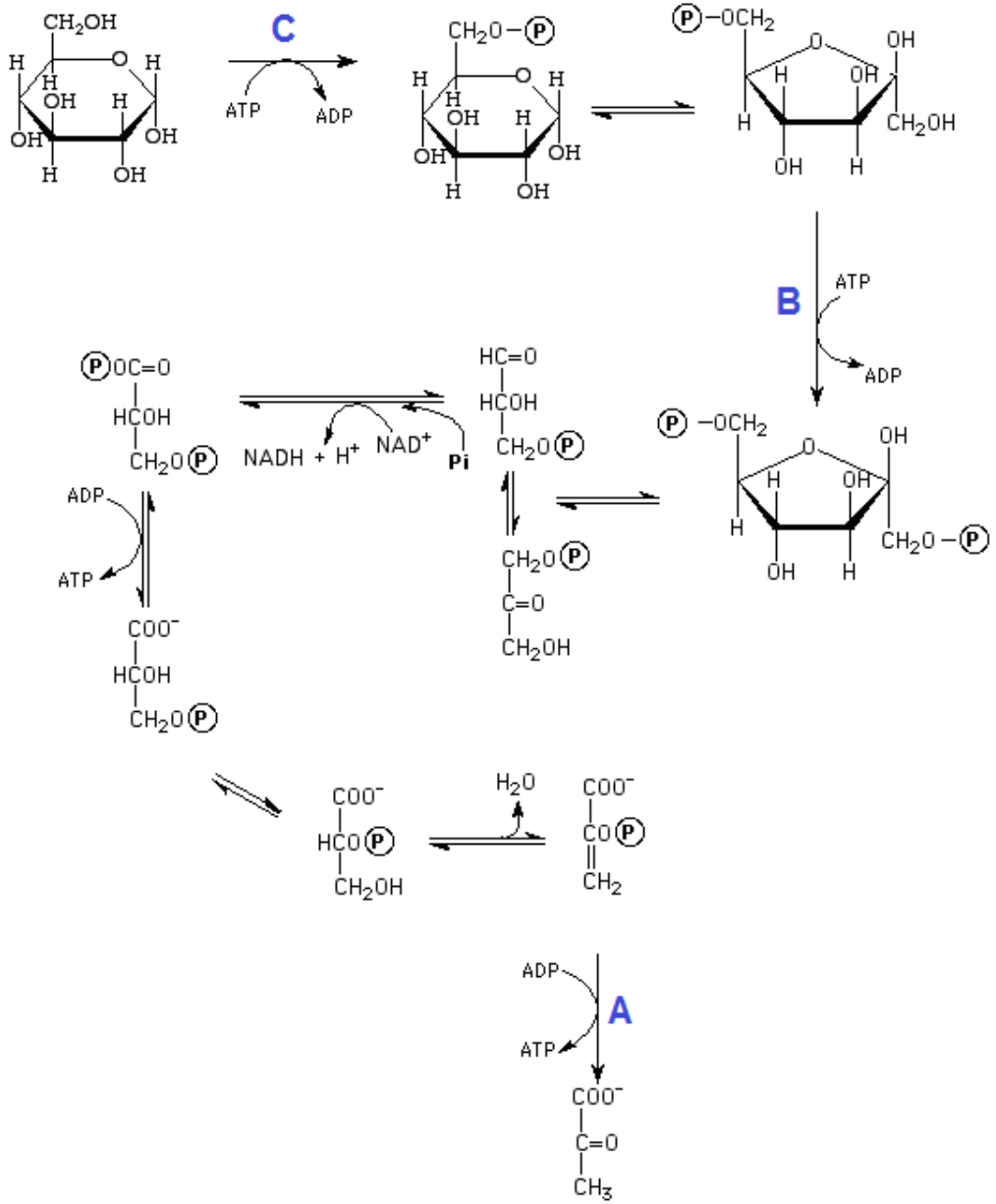
e) *during starvation* glycerol is one of important substrates of gluconeogenesis: what is the major source of this substrate in this situation? draw the connection (two reactions involved) between glycerol and gluconeogenesis: add its intracellular location, names of enzymes involved and their cofactors, and the name and formula of the intermediate

f) add the amino acids (names and formulas) connected with gluconeogenesis by their conversion to 1) pyruvate and 2) oxaloacetate; add the names of enzymes catalyzing these transamination reactions in the cytosol

g) Krebs cycle (KC) intermediates are important substrates of gluconeogenesis: draw the connection of KC with gluconeogenesis - which intermediate of KC leaves the mitochondrion, how is it transported through the inner mitochondrial membrane and how it enters gluconeogenesis in the cytosol?

h) reactions A, B, C are regulatory reactions of both gluconeogenesis and glycolysis but enzymes involved are different: describe the regulation of gluconeogenesis

6. GLUCONEOGENESIS - picture completion (from bottom toward top)



7. DEGRADATION OF GLYCOGEN - tasks

a) number the carbon atoms in the molecule A; what type of a glycosidic bond is found in the structure?

b) reaction no.1: fill in the name of the second substrate (a) and the name of the enzyme catalyzing this reaction; describe the regulation of the enzyme:

c) fill in the name of substance B, number its carbon atoms and explain how the position of the phosphate group is related to the type of bond being broken in the glycogen molecule

d) reaction 2: what kind of a chemical reaction is it? fill in the name of product C

e) reaction 3: fill in the names of products (D, d), the name of the enzyme and its cellular localization; is this enzyme present in all cells containing glycogen? In which EC class does this enzyme belong?

f) draw an arrow where glycogenolysis is connected to glycolysis when it occurs in the same cell; does this connection alter the amount of ATP gained from glycolysis?

g) in which two forms do substances „a“ and „d“ exist in a cell and what functions do they serve?

h) there are genetic disorders called glycogenoses connected with glycogen metabolism: explain what kind of a disorder glycogenoses are in general *(it is not necessary to describe individual types of the disorder)* and how glycogenoses affect cells / organs which store glycogen:

i) *glycogenosis type Ia* (Von Gierke's disease) affects only the liver, kidneys and the intestine, it manifests by severe fasting hypoglycemia: find in the picture the enzyme, which is deficient in this glycogenosis and explain the clinical connotations *(the enzyme occurs mainly in the mentioned organs)*

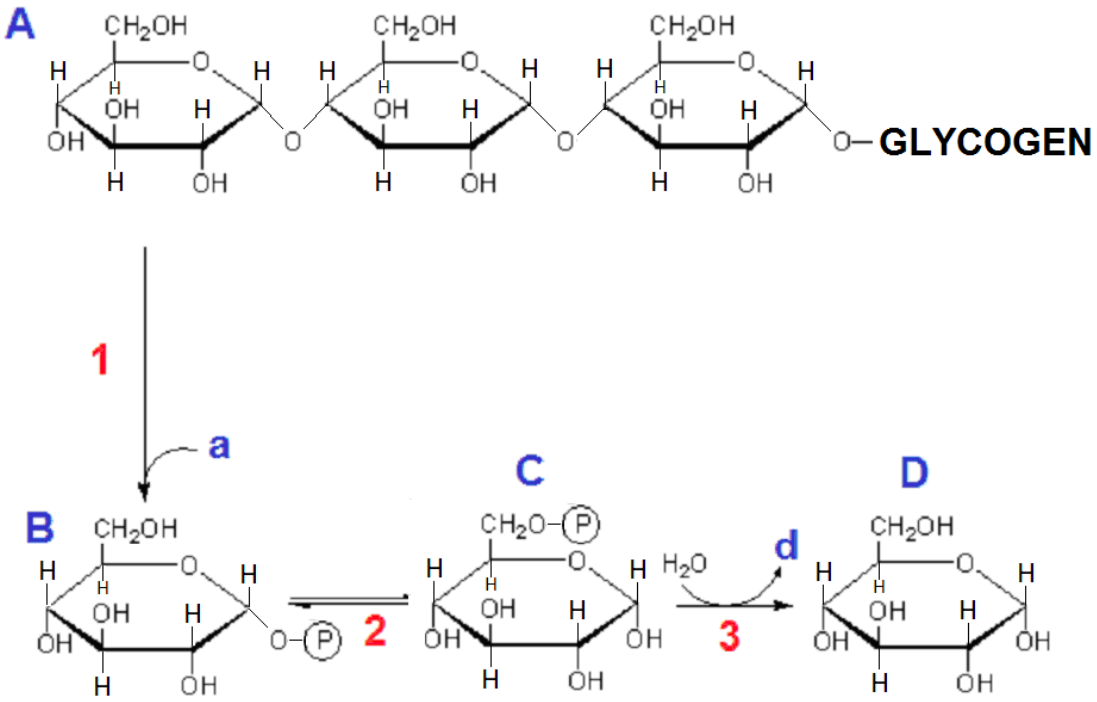
j) *glycogenosis type V* (McArdle disease) is caused by a deficit of the glycogenolysis enzyme, which is different in the muscle and in the liver (see different regulation of this enzyme) and manifests by transient weakness and muscle spasm during exercise accompanied by increased plasma myoglobin; explain the causes of these symptoms; which enzyme is deficient?

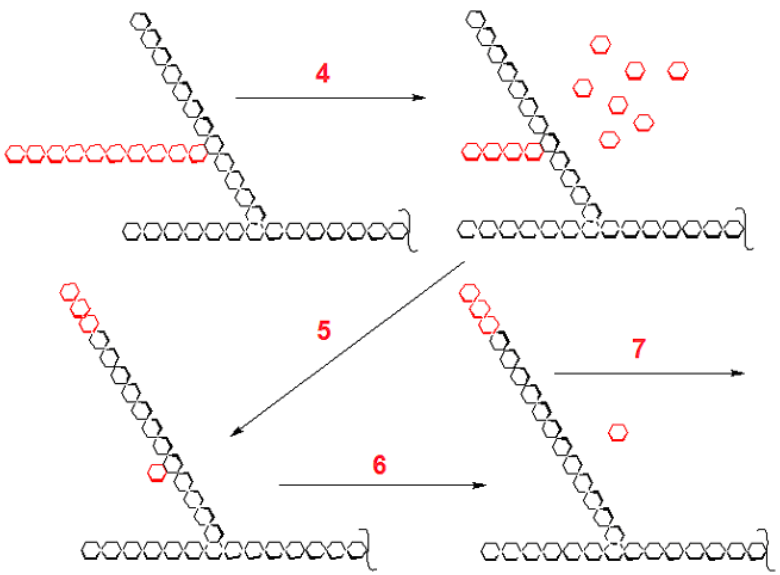
k) *glycogenosis type III* (Cori's disease) is caused by a deficiency of the de-branching enzyme, which has two enzyme activities in humans: transferase and hydrolase; label in the picture on the right side reactions catalysed by this enzyme, and distinguish the two reaction types catalysed by this enzyme.

l) fill in the name of the product of the hydrolase activity of the de-branching enzyme; how does this product differ from the products of glycogenolysis catalysed by glycogen phosphorylase?

m) fill in the picture on the right the missing names of enzymes other than the debranching enzyme

7. DEGRADATION OF GLYCOGEN - picture completion





8. SYNTHESIS OF GLYCOGEN - tasks

a) number the carbons in the molecule A

b) reaction no. 1: fill in the names of substrates (A, a), products (B, b) and the enzyme that catalyzes the reaction in the liver;
what is the name o the enzyme catalyzing this reaction in extrahepatic tissues and how does it differ from the liver enzyme?

c) what type of a reaction is the transformation of B to C? fill in the name of the substance C

d) reaction no. 3: fill in the name of substance c; what kind of a substance is it?

e) write the names of enzymes catalyzing reactions 3 and 3´; what is the importance of reaction 3´ for reaction 3?

f) reaction no. 4: fill in the name of substrate D and product d; write down the precise type of the chemical bond that formed in the extended glycogen molecule (note from which carbon of substrate D the substance d was removed)

g) reaction no. 4 is catalyzed by the regulation enzyme of glycogenesis: write its name and state how it is regulated

h) point out in the right-hand corner (schematic structure of glycogen), in which specific locations can reaction no. 4. occur, note the reducing and non-reducing ends; what does non-reducing / reducing mean?

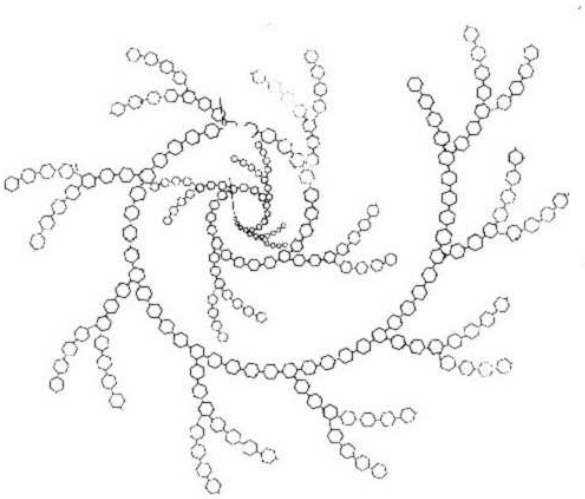
i) the de-novo synthesis of glycogen is initiated by an enzyme that glycosylates itself: which amino acids are glycosylated? state the name of the enzyme and label in the picture in the right-hand corner, where it is bound.

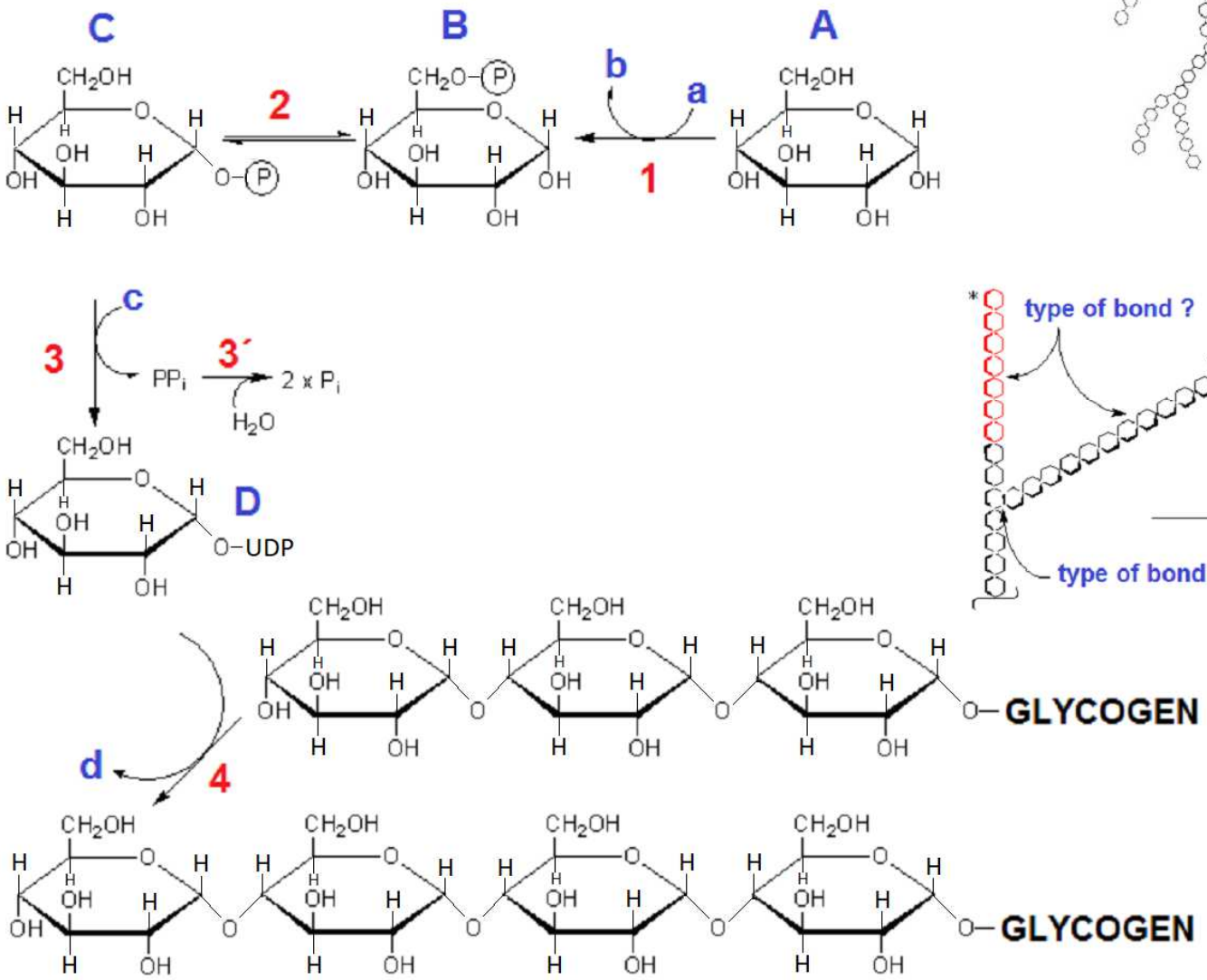
j) the formation of branches in the glycogen molecule occurs in the reaction no. 5: name the enzyme that catalyzes it

k) in the picture with reaction no. 5 fill in the precise name of the type of bonds in the glycogen molecule

l) does the asterisk denote a reducing or a non-reducing end? what is its importance for further growth of the glycogen molecule?

8. SYNTHESIS OF GLYCOGEN - picture completion





9. PENTOSE PHOSPHATE PATHWAY - part 1 - tasks

Draw into the picture the structural formulas of all given monosaccharide derivatives and state what type of a saccharide each of them is according to the number of carbons and the principal functional group.

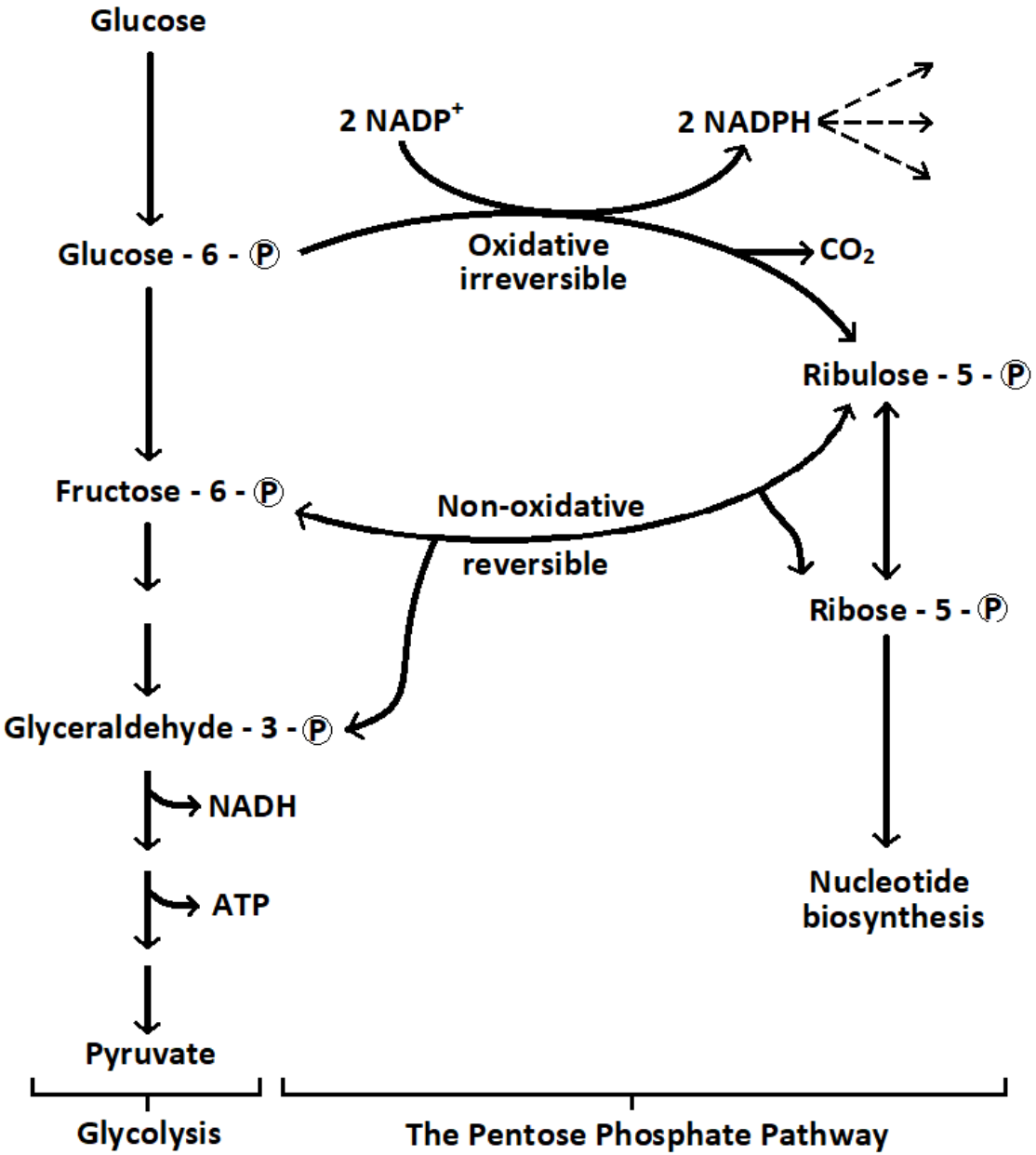
a) state the various uses of NADPH in cells

b) use a colour pencil and mark the „pentose cycle“ (= a sequence of reactions proceeding in a cycle), which is an alternative pathway of glucose oxidation up to CO_2 and produces NADPH but not ATP

c) use a colour pencil and mark the „pentose phosphate pathway“ producing ribose-5-phosphate (mark 2 possibilities of this pathway)

d) use a colour pencil and mark the „shunt of glycolysis“ used for production of NADPH together with NADH and ATP production in glycolysis itself (i.e. the use of glc-6-phosphate for production of both: NADPH and energy)

9. PENTOSE PHOSPHATE PATHWAY - part 1 - picture completion



9. PENTOSE PHOSPHATE PATHWAY - part 2 - tasks

oxidative phase:

e) add missing names (substrate / product / enzymes)

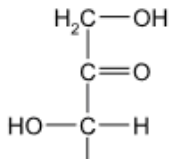
f) what is the cause of hemolytic anaemia in the case of a failure of these reactions?

non-oxidative phase:

g) add missing names

h) mark all products of the oxidative phase of the cycle

i) mark the ketoses containing the following structure:

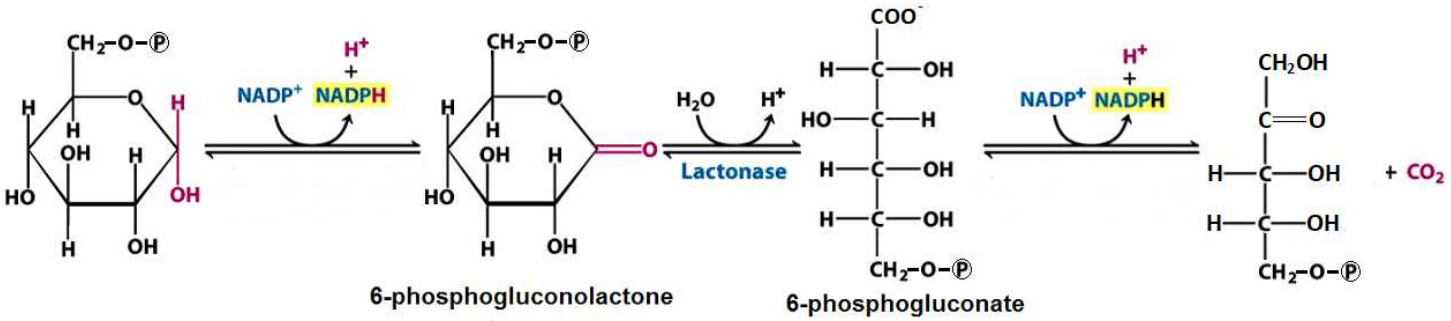


j) sugar-fragments are always transferred from a ketose, containing the above mentioned structure in its molecule, to an aldose: an aldose is formed from the ketose and a ketose forms from the aldose; add the size of fragments (number of carbons) transferred by **transketolase** and by **transaldolase**

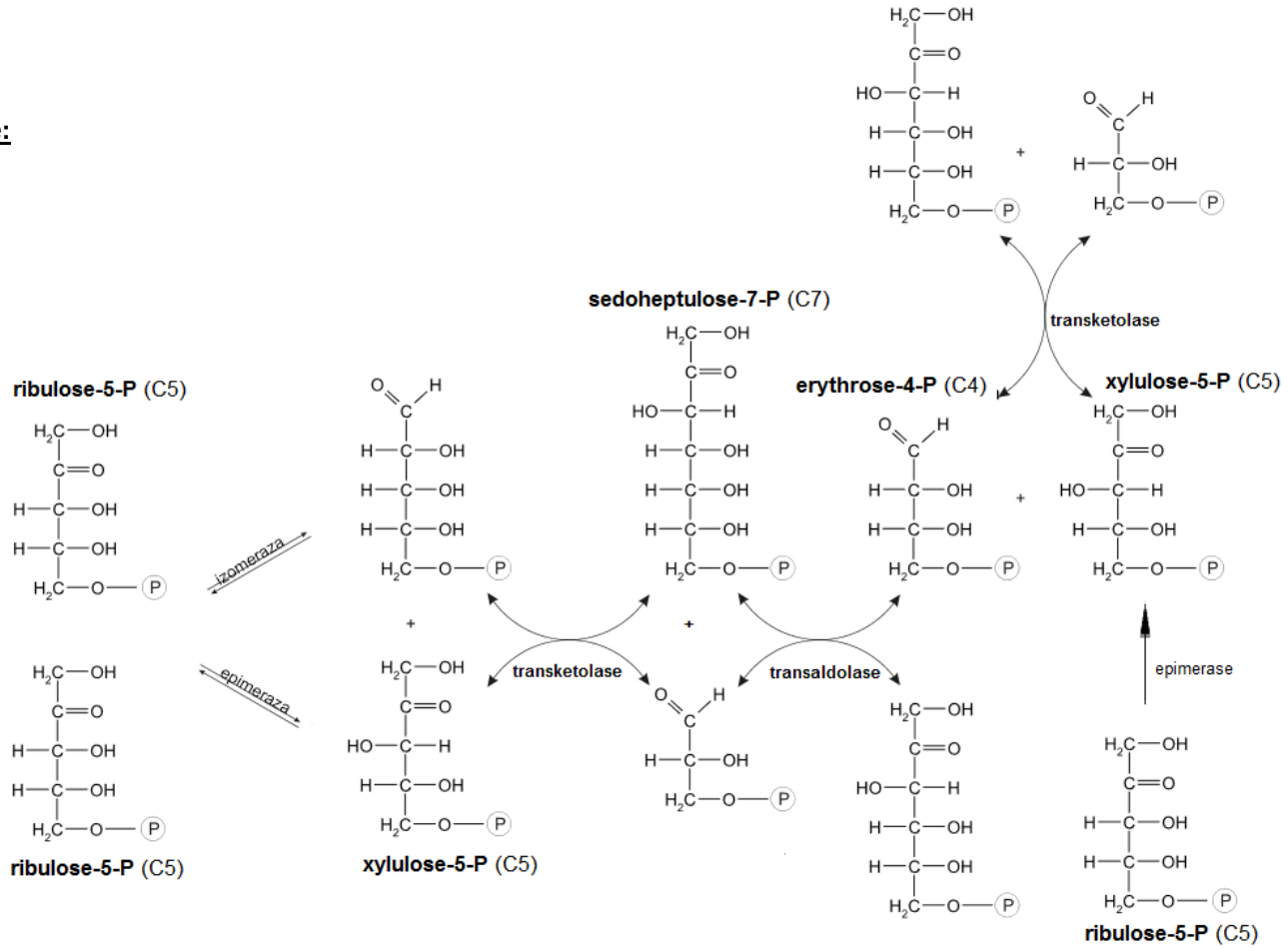
k) mark all final products of this sugar interconversion; what is their fate?

9. PENTOSE PHOSPHATE PATHWAY - part 2 - picture completion

oxidative phase:



non-oxidative phase:



10. METABOLISM OF FRUCTOSE - tasks

a) name all the structures and number all their carbons; find the relationship between the two reactions

b) first reaction: add the name of the enzyme catalyzing the reaction and its intracellular and organ location; what is the name of genetic disorder caused by a deficiency of the enzyme? what is its manifestation?

c) second reaction: add the name of the enzyme catalyzing the reaction and its intracellular and organ location; what is the name of genetic disorder caused by a deficiency of the enzyme? what is its manifestation?

d) second reaction: describe the interconnection of the first product (upper one) with glycolysis: draw the structural formula of a substance to which it is converted, state its name and the name of enzyme catalyzing this conversion

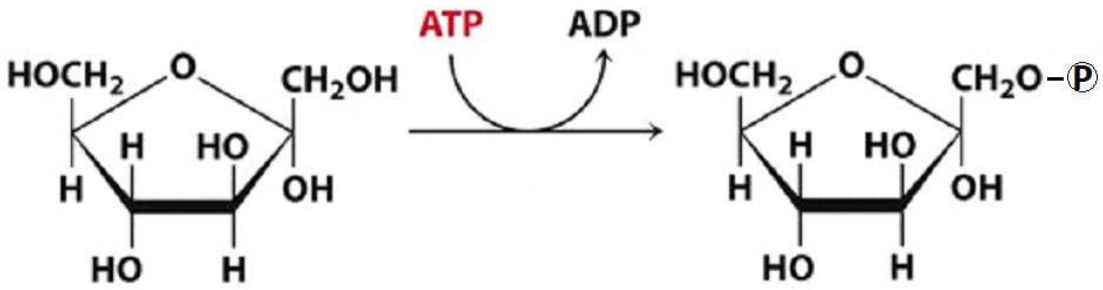
e) second reaction: second product of the reaction (lower one) can be converted either directly to an intermediate of glycolysis or to glycerol: draw both these reactions, including names of the products, enzymes and cofactors

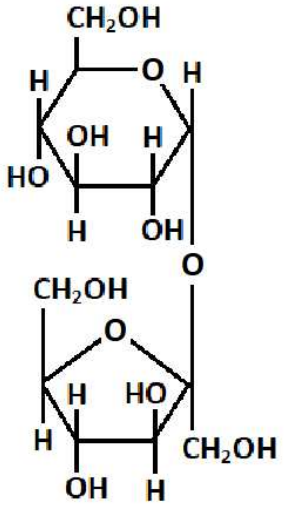
f) draw other conversions of glycerol leading to the connection with glycolysis (these reactions are the same as in the gluconeogenesis from glycerol): state formulas and names of all involved substances and enzymes

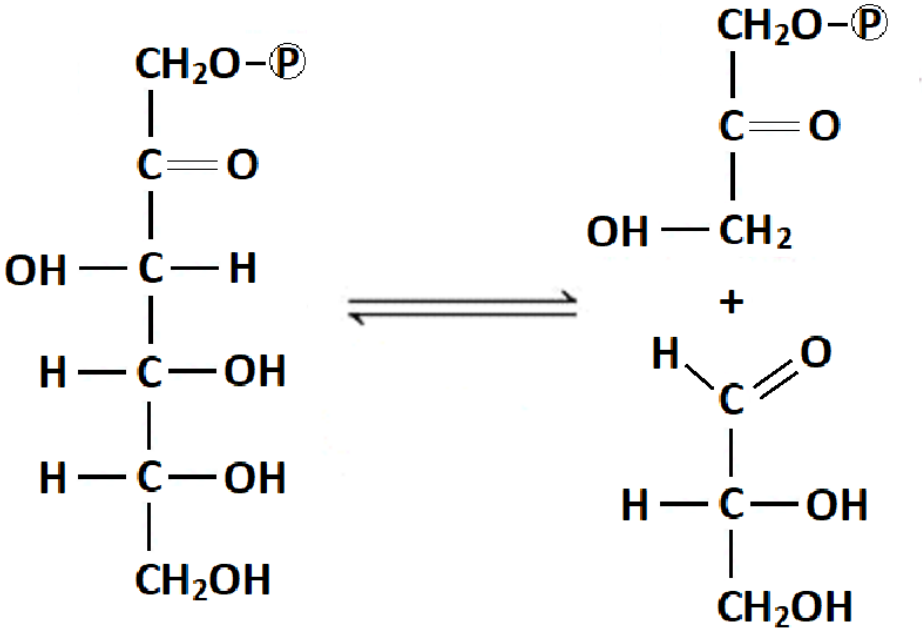
g) what are metabolic causes of a faster conversion of fructose to body fat in comparison with the glucose metabolism?
(draw the interconnection with TAG synthesis in the picture)

h) name the disaccharide (on the right up) which is a source of fructose in the diet; add names of its components and the name of enzyme hydrolyzing the disaccharide in the small intestine

10. METABOLISM OF FRUCTOSE - picture completion







11. METABOLISM OF GALACTOSE - tasks

a) add names to all structural formulas used in the picture

b) name the enzyme A and state its location in the body and within cells; what is the name of a genetic disorder caused by a deficiency of the enzyme? what is its manifestation?

c) name the enzyme B - what is the type of the reaction it catalyzes? add the reaction in which the substrate of the reaction B (bottom on the left) is produced: write the name and structural formula of substance from which it is formed and the name of the enzyme catalyzing this reaction in the liver

d) add the other substrate and the other product of the reaction C (they are not included in the picture); what is the other use of the substance pictured bottom on the right in cellular metabolism? (out of the galactose metabolism)

e) name the enzyme D and explain the role of this reaction in a human metabolism

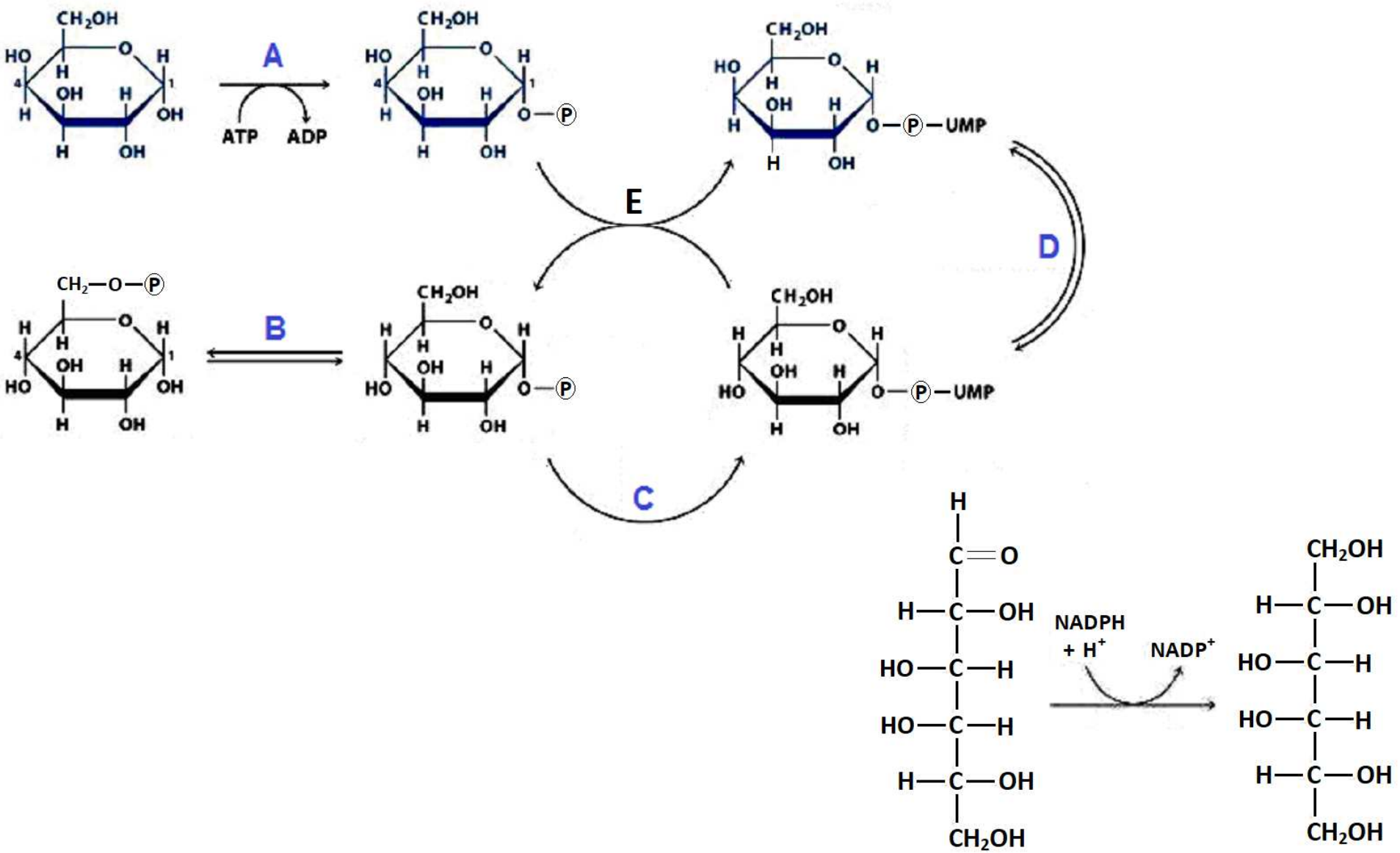
f) reaction E is catalyzed by uridyltransferase *(the exact name of the enzyme is UDP-glucose-hexose-1-phosphate uridylyltransferase)*; what is the name of genetic disorder caused by a deficiency of this enzyme? what is its manifestation?

g) indicate in colour the sequence of reactions converting free galactose to free glucose; add the name of the enzyme catalyzing the last reaction of this metabolic conversion (not pictured)

h) the picture in bottom right describes another possibility of galactose conversion in the body: add names of the substrate, product and enzyme catalyzing the reaction; describe clinical correlations (under what conditions the reaction proceeds and what are its pathological consequences?)

i) what saccharide is a source of galactose in human diet? in which food does it occur? what is the name of the enzyme necessary for its digestion in the small intestine? what is the name of a genetic disorder caused by a deficiency of this enzyme? what is its manifestation?

11. METABOLISM OF GALACTOSE - picture completion



12. BETA-OXIDATION OF FATTY ACIDS - part 1 - tasks

a) add the general name of substance „a“ ; what would be its concrete name if R = CH_3-$(CH_2)_{14}$- ?

b) name the enzyme A and add its cofactor (entering and leaving the reaction) and describe its interconnection with the electron transport chain (ETC);
what type of oxidation is the reaction A? (= the type of an organic reaction)

c) add the name of substance „b“ ; what type of the organic reaction is the reaction B? *mark β-carbon in each formula*

d) add the cofactor of enzyme C (entering and leaving the reaction) and describe its interconnection with the electron-transport chain;
what type of oxidation is the reaction C? (= the type of an organic reaction) *mark β-carbon in each formula*

e) name the enzyme D, add the name of substance „c“ and name, formula and fate of the substance „d“; what type of the organic reaction is the reaction D?

f) substance „a“ at the start and at the end of one β-oxidation cycle is not the same: by how many carbon atoms does it differ?

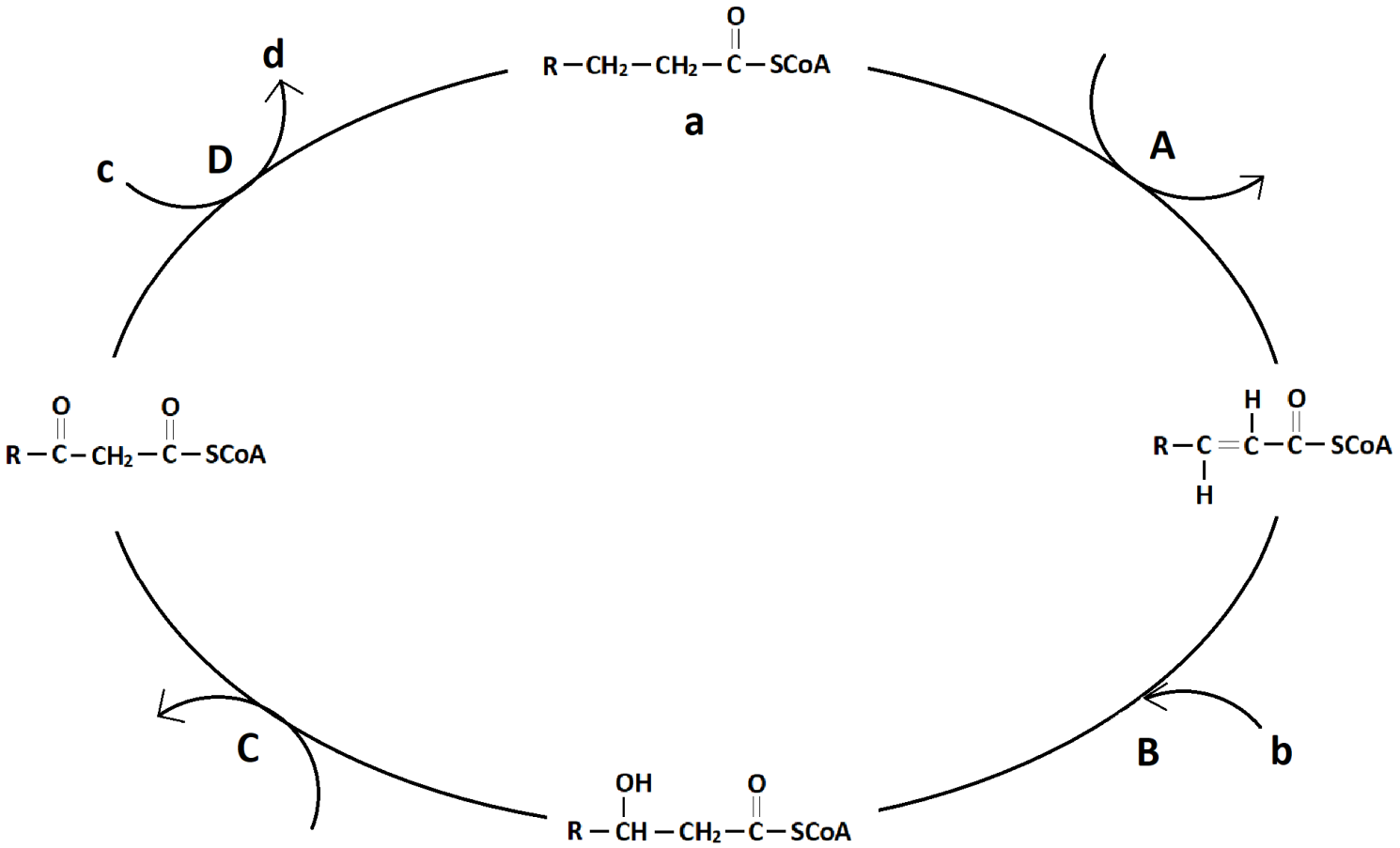
g) name the substance „a“ found at the end of the cycle if the substance entering the cycle was

1) stearoyl-CoA,

2) butyryl-CoA

h) state how many times the cycle in the picture must proceed to enable a complete degradation of stearoyl-CoA;
how many and what products will be formed altogether in the β-oxidation of stearoyl-CoA?

12. BETA-OXIDATION OF FATTY ACIDS - part 1 - picture completion



12. BETA-OXIDATION OF FATTY ACIDS - part 2 - tasks

i) what kind of fatty acid must enter β-oxidation to produce substance „a“? state its name:

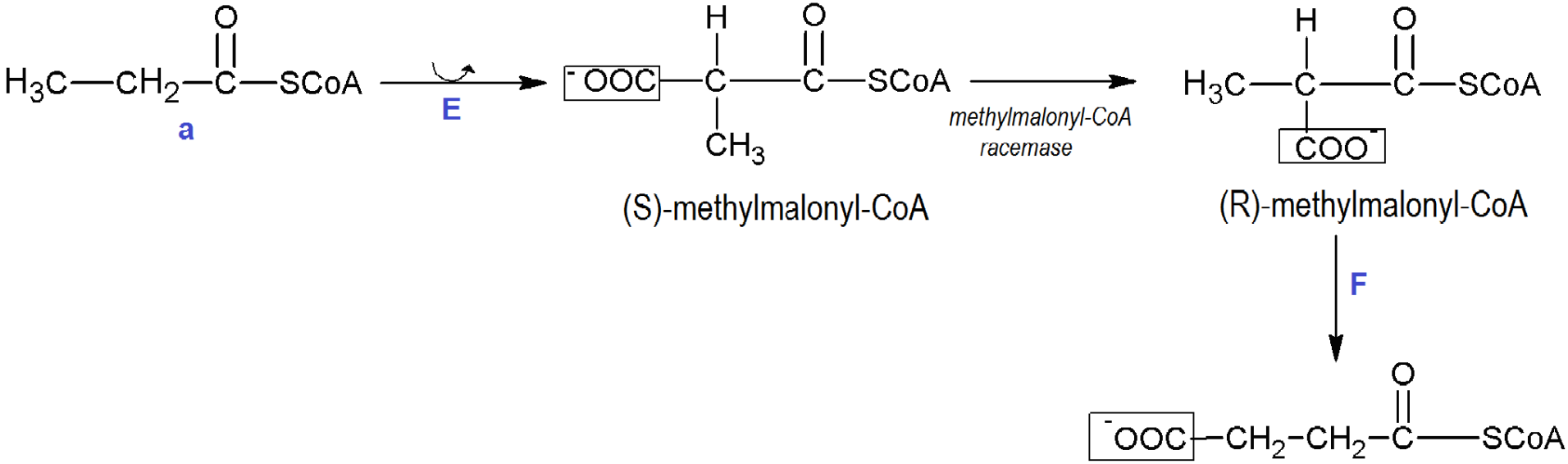
j) add the name of enzyme E, its cofactor and its other two substrates and two products (not included in the picture)

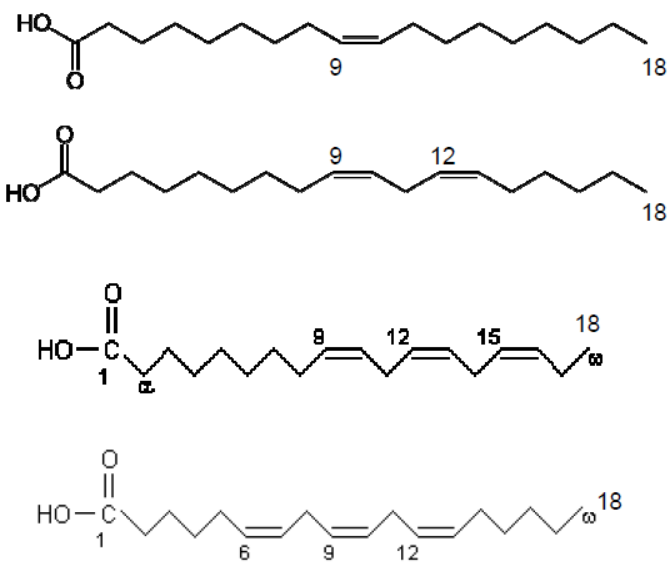
k) add the name of enzyme F, its cofactor and the name and the fate of its product

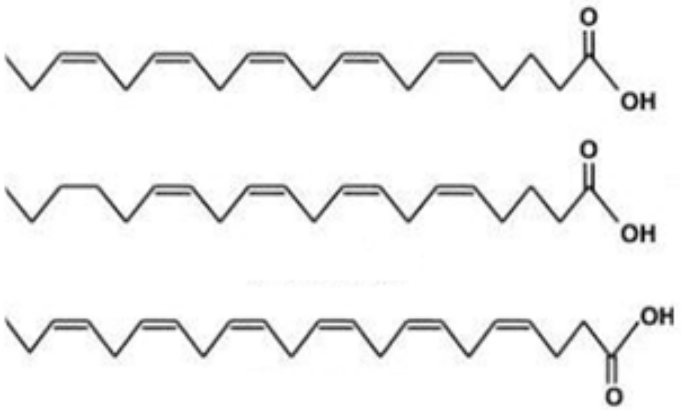
l) name the given unsaturated fatty acids and add also their nutritional classification „omega“ for each (ω-3 / ω-6 / ω-9 or n-3 / n-6 / n-9)

m) explain how the presence of double bonds affects the process of beta-oxidation of these fatty acids; will it affect the amount of ATP produced as well?

12. BETA-OXIDATION OF FATTY ACIDS - part 2 - picture completion







13. METABOLISM OF KETONE BODIES - part 1 - tasks

Synthesis:

a) reaction A: add names of its substrate, product and enzyme; what metabolic pathway is the most important source of the substrate used in this synthesis?

b) reaction B: add the structural formula and the name of its second substrate („b“) and the name of its second product (not pictured)

c) add the full name of the 6-carbon containing product of reaction B; this substance is formed by the same reactions (A+B) also in another metabolic pathway - in which one?

What is the intracellular location of enzymes A and B?

d) reaction C: add names of both products and the name of the enzyme catalyzing the reaction; interconnect the product „c“ with the reaction B by an arrow („c“=„b“)

e) reaction D: add the names of the enzyme, its cofactor („d“) and the reaction product

f) reaction E: add the name and formula of „e“ and explain how this reaction is interrelated with the term „ketone bodies“ used for the substrate and the product of the reaction D

g) add the name of the 3rd ketone body (the product of the reaction E); what is its fate?

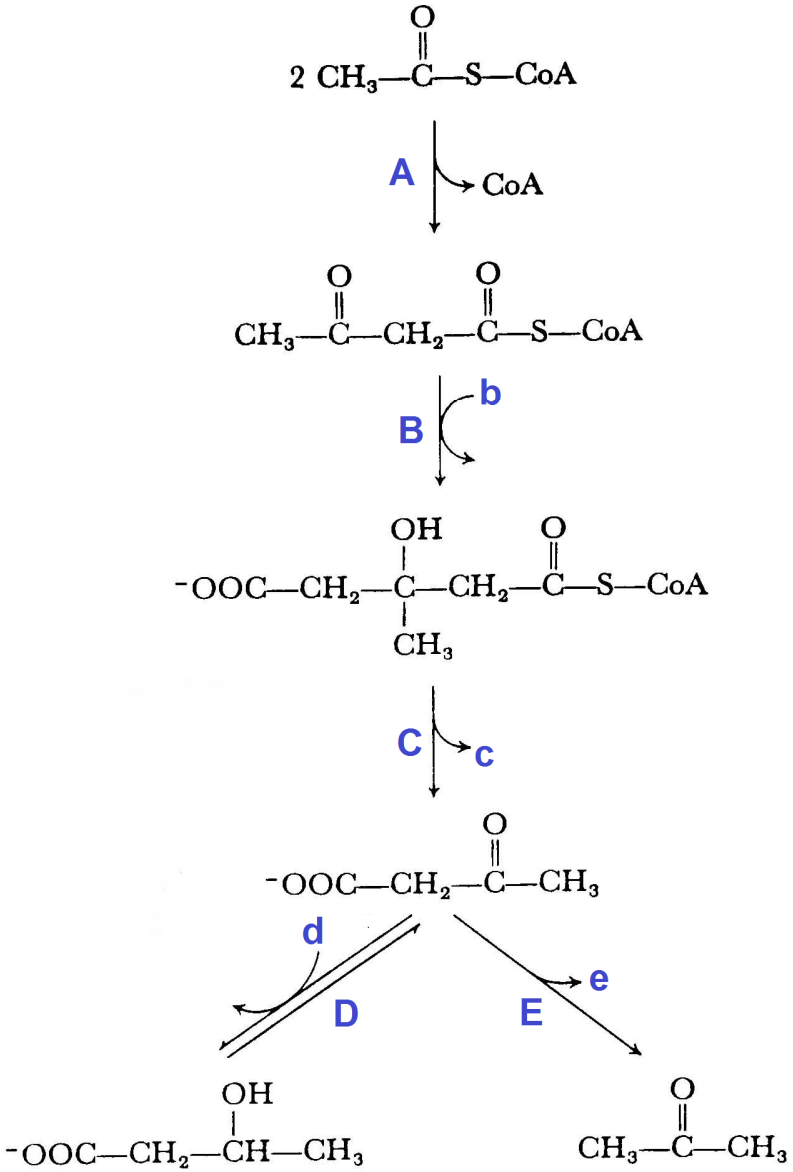
h) what is the location of ketogenesis?

1) in the body:

2) within cells:

13. METABOLISM OF KETONE BODIES - part 1 - picture completion

SYNTHESIS:



13. METABOLISM OF KETONE BODIES - part 2 - tasks

Degradation:

a) reaction D: it is catalyzed by the same enzyme as reaction D in the ketogenesis: add the names of the substrate, product, enzyme and its cofactor

b) reaction F (activating ketone bodies by an attachment of CoA): add names of substances „f“ and „g“; state the name of another metabolic pathway in which „f“ and „g“ are involved

c) add the name of enzyme F; this enzyme is missing in the liver - what are metabolic consequences?

d) reaction A is catalyzed by the same enzyme as the reaction A in the ketogenesis: add the names of its substrate, product and enzyme; what is the name of the substance „a“?

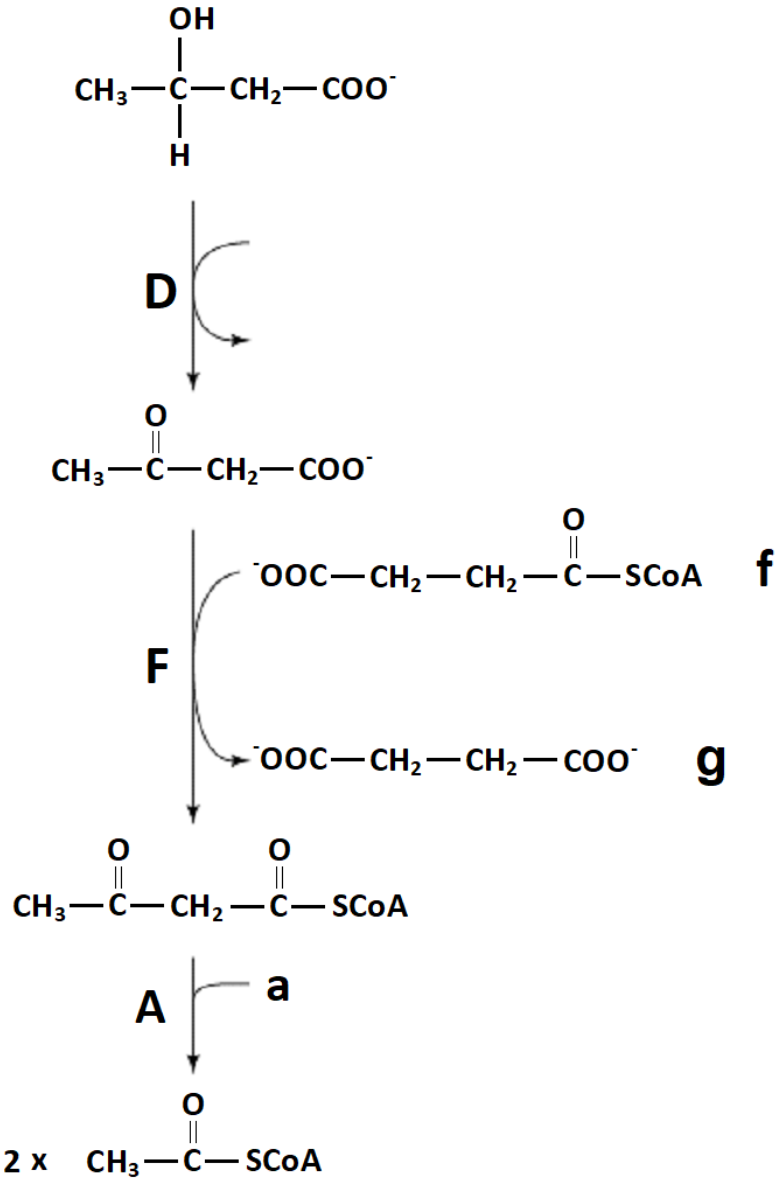
e) reaction A is also involved as the last reaction in another metabolic pathway - which one?

f) what is the fate and function of products of the reaction A? (function of ketone bodies degradation?)

g) state the intracellular location of the degradation of ketone bodies:

13. METABOLISM OF KETONE BODIES - part 2 - picture completion

DEGRADATION:



14. SYNTHESIS OF FATTY ACIDS - tasks

Fatty acid synthase contains two binding sites: peripheral one (see the label „enzyme“) and catalytic one („ACP“) in which all synthetic reactions proceed. The peripheral site binds already completed FA-chain only between individual cycles of the synthesis (the synthesized FA is put aside there for a while).

cycle 1 (left) / cycle 2 (right)

a) mark all acyls in the picture that are present at the peripheral binding site and add their names

b) malonyl-CoA serves as the source of C2 fragments during FA synthesis: mark all malonyls in the picture bound to ACP binding site

c) write the reaction in which malonyl-CoA is synthesized from acetyl-CoA (state all formulas, names, involved enzyme and its cofactors):

d) reaction A: in the structure of one of its substrate mark the source of CO_2 produced by this reaction; add the name of product and mark which part of its structure comes from acetyl transferred from the peripheral site

e) mark all alpha and beta carbons in all 4-carbon containing acyls in the picture

f) add cofactors of reactions B and D; which metabolic pathways produce these cofactors?

g) reaction D: add the name of its product; after transfer of this product to the peripheral site the second cycle of the synthesis will start (again on the ACP binding site)

h) in a structure of one of substrates mark the source of CO_2 produced by the reaction A-2

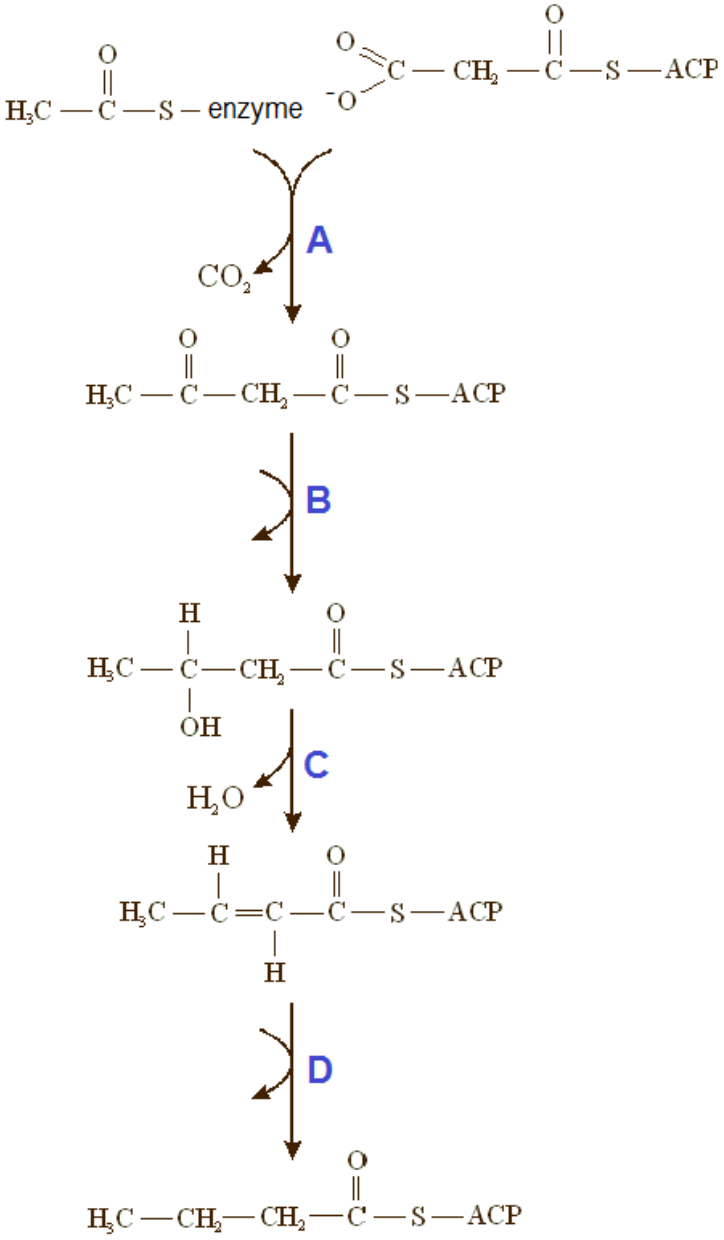
i) mark all alpha and beta carbons in all 6-carbon containing acyls in the picture and look how the step-by-step reduction of the beta-carbon proceeds in following reactions (B-2 → C-2 → D-2)

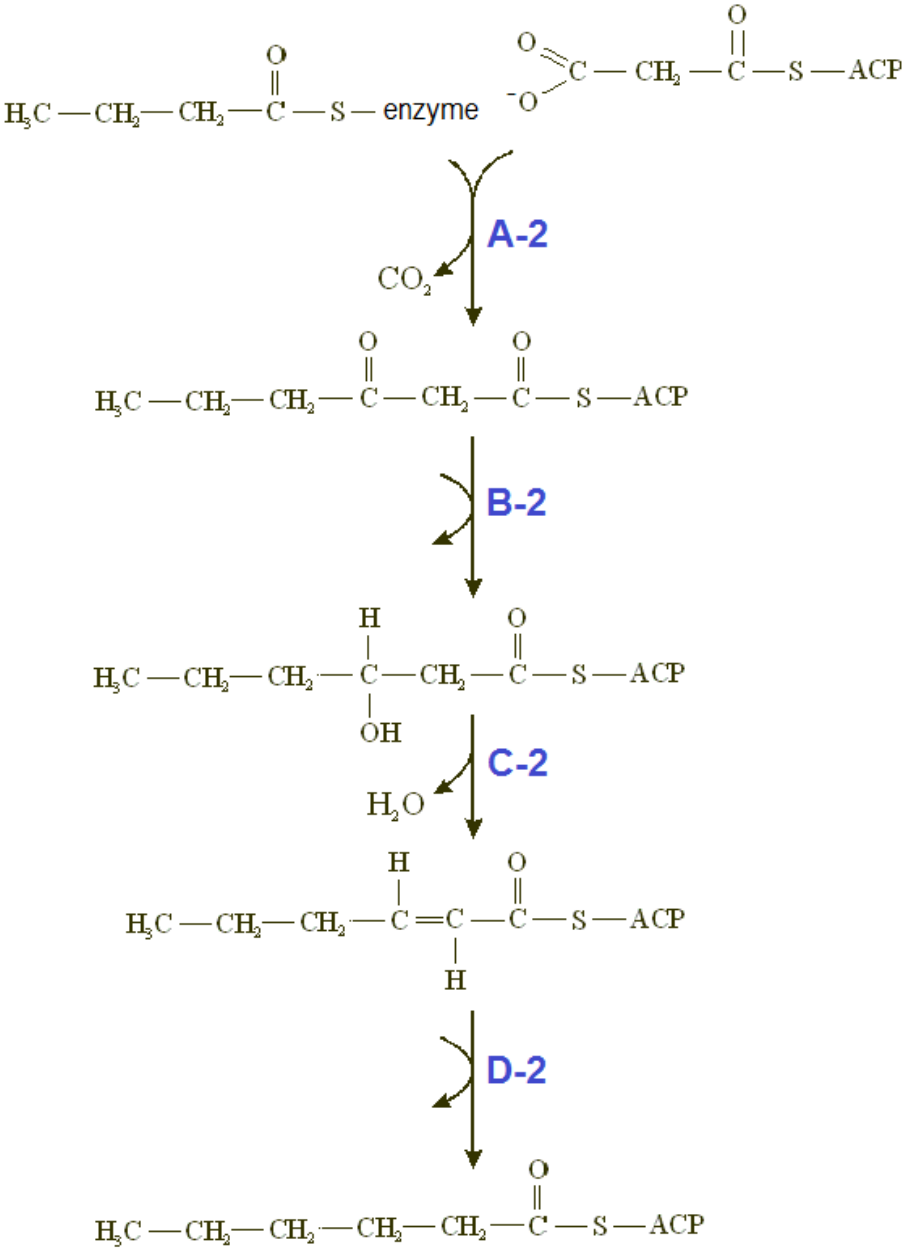
j) add cofactors of reactions B-2 and D-2; what is the fate of the oxidized forms of the cofactors produced by these reactions?

k) name the final product of the synthesis (bottom) and mark its omega-carbon: which substrate pictured at the beginning of the whole synthesis (in the reaction A) is the source of this omega-carbon?

l) complete the synthesis up to the final product palmitoyl-CoA: state the number of other malonyl-CoA molecules and enzyme cofactors needed; what can be other use of palmitoyl-CoA within cells?

14. SYNTHESIS OF FATTY ACIDS - picture completion (2 cycles)





15. METABOLISM OF TRIACYLGLYCEROLS - tasks

a) name substances A and B, and state how they are formed in the body

b) add names of enzymes and their cofactors next to the arrows (left) converting A and B to C; what is the location of these enzymes in the body?

c) add names of all substances and enzymes to all the reactions of the conversion C to E; what is other possible use (out of this metabolic pathway) of the intermediate D?

d) state the fate of the <u>final product</u> of the above reactions if it is synthesized in the

1) liver

2) adipose tissue

e) the picture on the right (up) describes a hydrolytic cleavage of TAG (lipolysis): mark and name all cleaved bonds

f) add names of enzymes catalyzing the lipolysis:

1) in the small intestine

2) in blood

3) within adipocytes

g) how are the enzymes from the question f) 2) and 3) regulated?

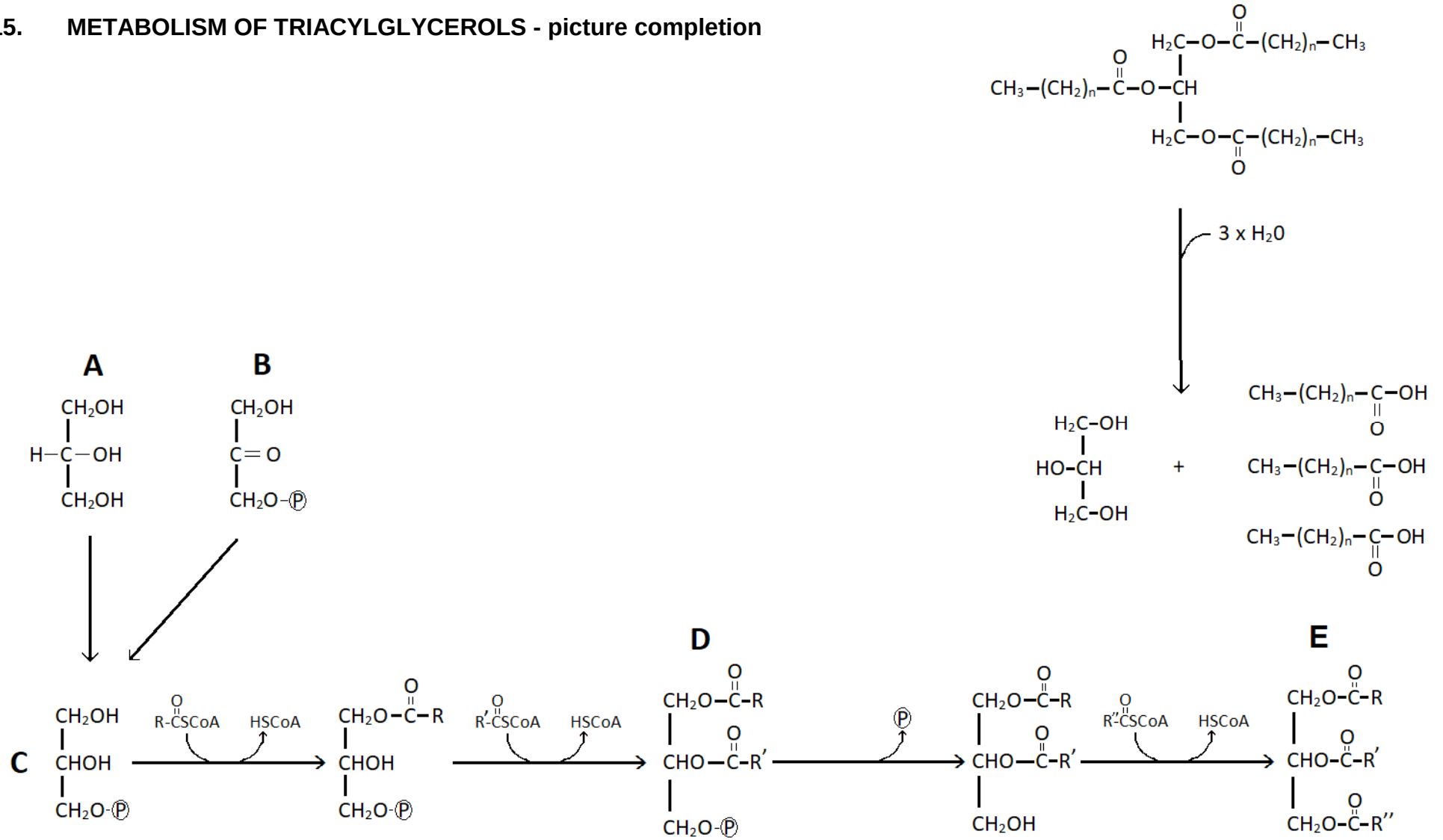
h) add names and the fate of the final products of the lipolysis if it proceeds:

1) in blood supplying muscles

2) in blood supplying adipocytes

3) within adipocytes

15. METABOLISM OF TRIACYLGLYCEROLS - picture completion



16. UREA CYCLE - tasks

a) find urea in the picture; look at its structure - what type of a chemical compound it is?

b) fill in the substrates of the reaction no. 2 into the box and write reactions producing these substrates

c) state the name of the enzyme catalyzing the reaction no. 2 and the name of its product

d) the enzyme no. 2 is activated by an allosteric activator produced by the reaction no. 1: add names of the substrates and the product of this reaction

e) find aspartic acid in the picture and add the reaction in which it can be formed (use structural formulas)

f) one of urea cycle intermediates is an essential proteinogenic amino acid: find it in the picture and state its name

g) state the name of enzyme no. 3 - it is a hydrolase

h) fill in names of all other intermediates of the urea cycle

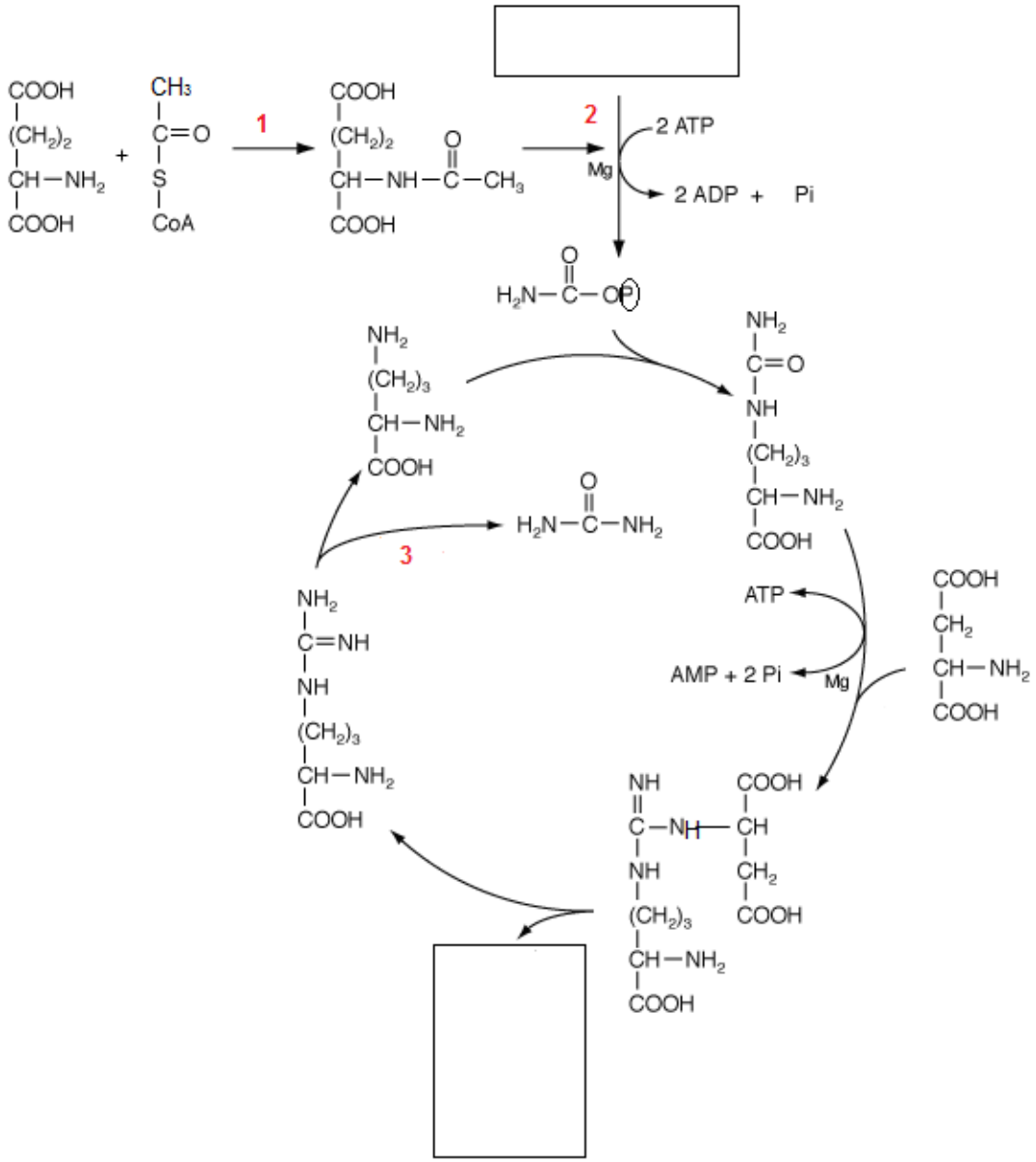
i) fill in the structural formula and the name of the other product leaving the cycle (see the box); what can be its fate?

j) use a colour pencil and mark all reactions of the urea cycle located in mitochondria; encircle substances that need to be transported between cytosol and a mitochondrion for the urea cycle purposes

k) what is the ATP requirement for the urea synthesis? (in number of phosphates cleaved from ATP)

l) find analogies between the urea and Krebs cycles; how do the cycles differ in an energy consumption / production?

16. UREA CYCLE - picture completion



17. TRANSAMINATION - tasks

a) the pair of substances (A) and (B) is involved in all transaminations mentioned below: name them

b) write the general name of the enzyme catalyzing transaminations; what is its cofactor?

c) draw structural formulas of the pair of substances (C1) and (D1) involved in the transamination catalyzed by the enzyme ALT; write the full name of the enzyme and names of the substances

d) draw structural formulas of the pair of substances (C2) and (D2) involved in the transamination catalyzed by the enzyme AST; write the full name of the enzyme and names of the substances

e) draw structural formulas of the pair of substances (C3) and (D3) if (C3) is valine: name the enzyme catalyzing this transamination

f) take notice of the enzyme names: they are based on the specific amino acid involved (you have added) or on its type if the enzyme specificity is broader (in case of the question e)

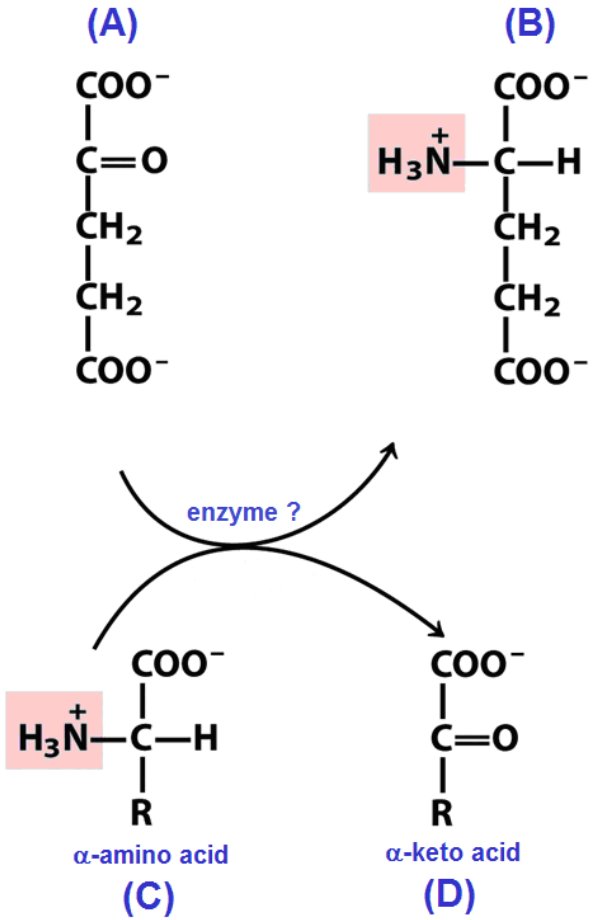
g) all the pictured transamination reactions are reversible: mark the reverse reactions to the picture

h) substance (B) can be converted to glutamine and glutamine can be converted to the substance (B): draw the structural formula of glutamine to the picture (up on the right) and add both the reactions (they are not transaminations!) including names of the enzymes and other substances involved

i) asparagine can be synthesized from one of the amino acids (you have added) by the similar but not the same reaction as glutamine from the substance (B): write the reaction; what is the difference?

j) substance (B) can be converted to the substance (A) also by another reaction (it is not transamination): add the reaction to the picture (up on the left), write the name of the enzyme and its coenzyme involved; what type of reaction it is? is it reversible?

17. TRANSAMINATION - picture completion



C1 D1 C2 D2 C3 D3

18. METABOLISM OF TYROSINE - tasks

a) find tyrosine in the picture; is it essential in the diet?

b) add the name of enzyme and its coenzyme to the reaction A → B; what is the name of inherited disorder caused by an insufficiency of this enzyme? what is the name of the substance that accumulates in blood under these conditions?

c) if the substance A accumulates in the body it is converted to the substance C that is excreted in urine: add the name of the substance C (its structure is somehow connected with the name of disorder from the question b)

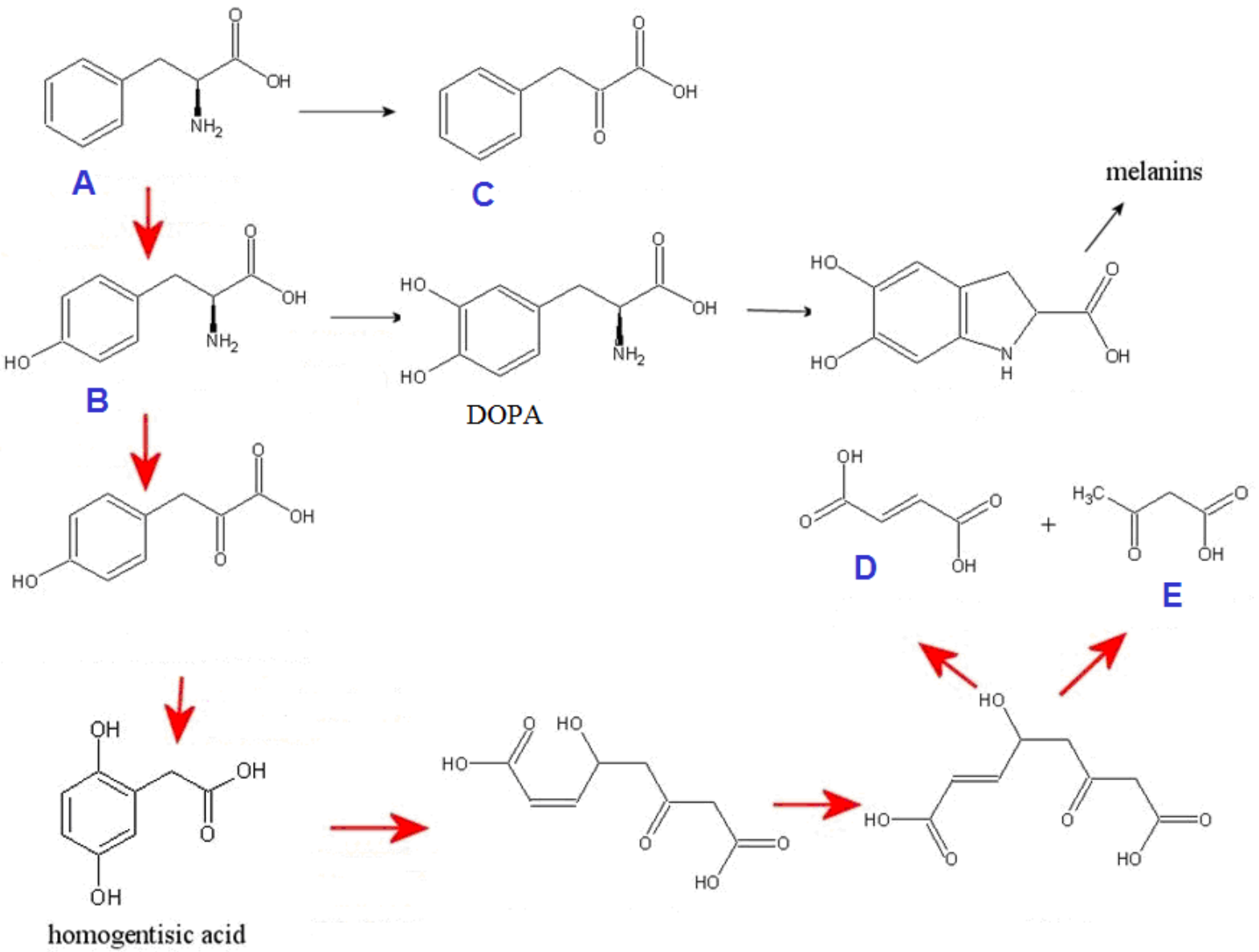
d) which signalling molecules are synthesized from the substance DOPA? (out of this pathway - not pictured)

e) what are melanins, also synthesized from DOPA? name the inherited disorder caused by a deficiency of the melanin synthesis:

f) one of intermediates formed during the degradation of the substance B is homogentisic acid: name the inherited disorder associated with the accumulation of this acid in the body

g) name substances D and E; state their subsequent metabolic use in the body:

18. METABOLISM OF TYROSINE - picture completion



19. METABOLISM OF METHIONINE - tasks

a) name substances A, B, C and D; which one is essential in the diet?

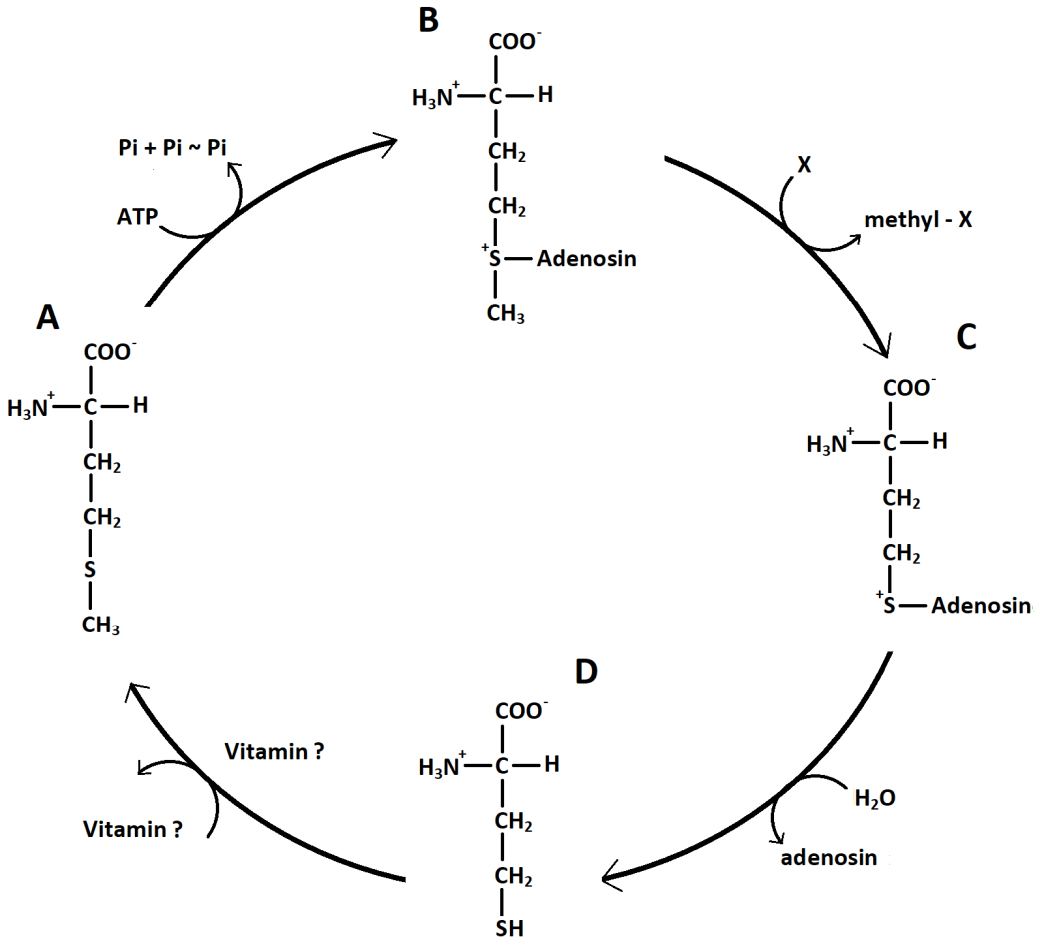
b) substance B: mark methyl group that is transferred to the compound X (see the reaction no. 2); add to the picture at least 3 examples of metabolic reactions involving this methyl-transfer (i.e. what substances need this methylation for their synthesis?)

c) add to the picture an alternative use of the substance D in cells: draw the synthetic pathway of a non-essential amino acid from the substance D (describe all reactions, including the names and formulas of all intermediates)

d) add to the picture the names of two vitamins necessary for the reaction no.4; which other vitamin is required for reactions you have added in the question c) ?

e) what disease is connected with an elevated blood concentration of the substance D? what can be the cause of the substance D accumulation in blood? what are the consequences?

19. METABOLISM OF METHIONINE - picture completion



20. METABOLISM OF BRANCHED CHAIN AMINO ACIDS - tasks

a) write names of substances (A), (B) and (C) to the picture; are all of them essential in the human diet?

b) reaction no. 1 of all the above mentioned substances is catalyzed by the same enzyme, its second substrate (not pictured) is α-ketoglutarate:
draw its structure formula to the picture and state the name of the enzyme and its cofactor; where in the body is this enzyme localized predominantly?
draw the second product of this reaction (formed from α-ketoglutarate) and state its name

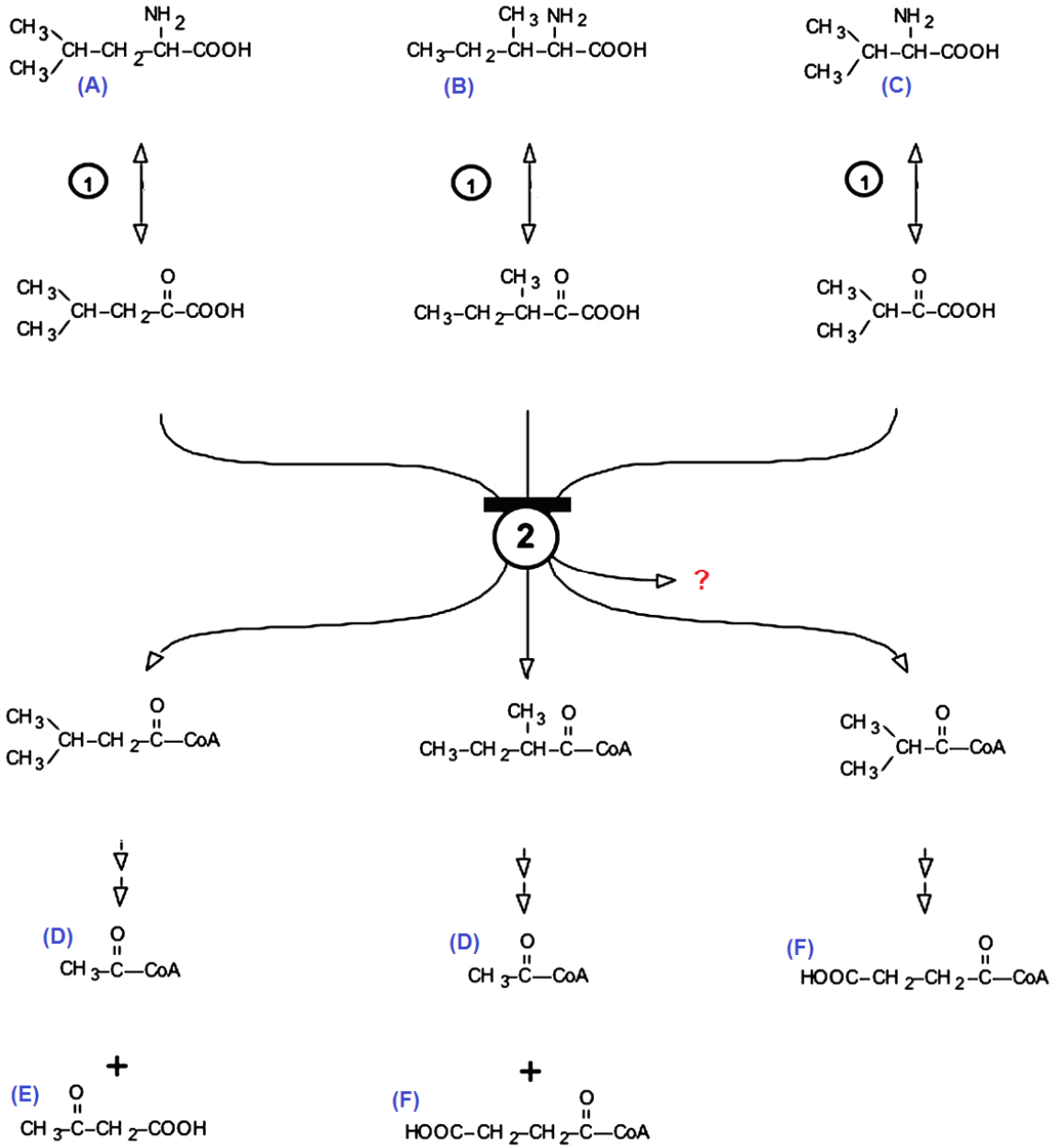
c) reaction no. 2: compare the formulas of its substrates and products - how do they differ? add the missing product of the reaction to the arrow marked by (?);
state what type of reaction it is and where it is localized within cells; write the name of the enzyme catalyzing the reaction and state all its cofactors;
which cofactor leaves the reaction as its other product (not pictured)?

d) molecules (D) and (E) are final degradation products of the substance (A), they are produced by cleavage of HMG-CoA (not pictured): add the names of the molecules and name the enzyme producing them from HMG-CoA; in which other metabolic pathway is this enzyme involved?
can be the products (D) and (E) converted to glucose? state how they are subsequently degraded up to CO_2 and water

e) substances (B) and (C) are first metabolized to propionyl-CoA (not pictured) that is subsequently converted to the product (F): write the reactions describing the conversion of propionyl-CoA to the product (F) including the enzyme cofactors needed for these reactions (= derivatives of two vitamins);
what is the name and the fate of the substance (F)? can it be converted to glucose?

f) newborn screening includes the test searching for a deficiency of the enzyme catalyzing the reaction no. 2: state the name of this inherited disorder;
what is its manifestation? what are its consequences?

20. METABOLISM OF BRANCHED CHAIN AMINO ACIDS - picture completion



21. SYNTHESIS OF CHOLESTEROL - tasks

a) reaction A: add the names of the substrate, product and enzyme; the same enzyme is involved in two other metabolic pathways - in which ones?

b) reaction B: add the name of the second substrate and the full name of the product; in which other metabolic pathway is the same reaction involved?

c) reaction C: add the names of the product, enzyme and its cofactor; in the formula of substrate mark and name the functional group which has been reduced by the reaction C to form a primary alcohol group (after removing HS-CoA, not pictured)

d) state mechanisms how the enzyme C is regulated:

e) reaction D: 2 ATP molecules are consumed for the phosphorylation: add names of reaction products

f) reaction E: another ATP is consumed during the decarboxylation: in the structure of the substrate mark the source of CO_2 (produced by the reaction) and add names of reaction products

g) reaction F describes the isomerization of the product of reaction E: add the name of the isomer

h) the isomers are referred to as the „active isoprene“, draw the structure of isoprene:

i) reactions G, H and I describe the polymerization of various „isoprenoid units“ - add their names

j) what is another use of products of the reactions G and H? (in another metabolic pathway)

k) which coenzyme of the electron transport chain is synthesized from these isoprenoid intermediates?

l) state the number of carbons found in the product of the reaction I; what type of „terpene“ is it?

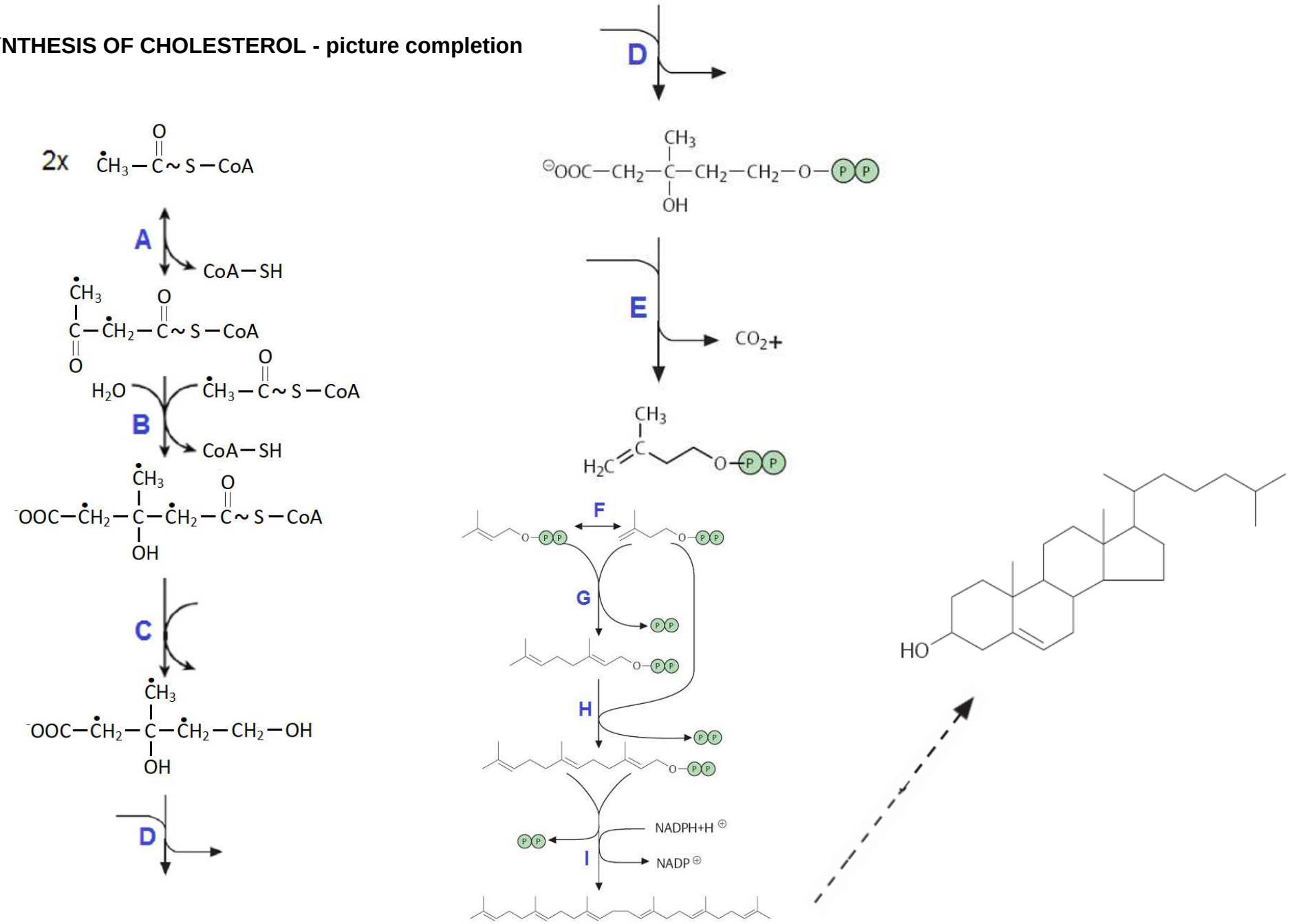
m) following reactions of the cholesterol synthesis (not pictured) consume, as well as the reaction I, molecules of NADPH: what is the intracellular source of them?

n) number all carbons in the structure of cholesterol in a proper order

o) mark the possition to which fatty acid binds during an esterification of cholesterol; where is free / esterified cholesterol localized within cells?

p) describe other use of cholesterol in a metabolism

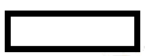
21. SYNTHESIS OF CHOLESTEROL - picture completion



22. METABOLISM OF LIPOPROTEINS - tasks

a) use the frame „INTESTINE“ to write down types of lipids that can be absorbed by enterocytes from the diet

b) write names of pictured lipoproteins <u>into **frames**</u>, and names of enzymes metabolizing them <u>into bubbles</u> and state the body location of these enzymes





(LPL = lipoprotein lipase, LCAT = lecithin-cholesterol-acyltransferase, CETP = cholesterol-ester-transfer-protein, HTGL = hepatic lipase)

c) is free cholesterol transported into the core or the surface part of lipoproteins? add free cholesterol into pictures of lipoproteins

d) mark the receptor called „LDL-receptor“; what is the name of an inherited disease caused by its lack? how does this disease manifest itself?
by what mechanism does the cell regulate the number of LDL-receptors in its membrane?

e) which apoprotein acts as a ligand of LDL-receptor?

f) ABCA1 transporter transfers excess cholesterol from the surface of cells to one of the lipoproteins: what is the subsequent fate of this cholesterol?

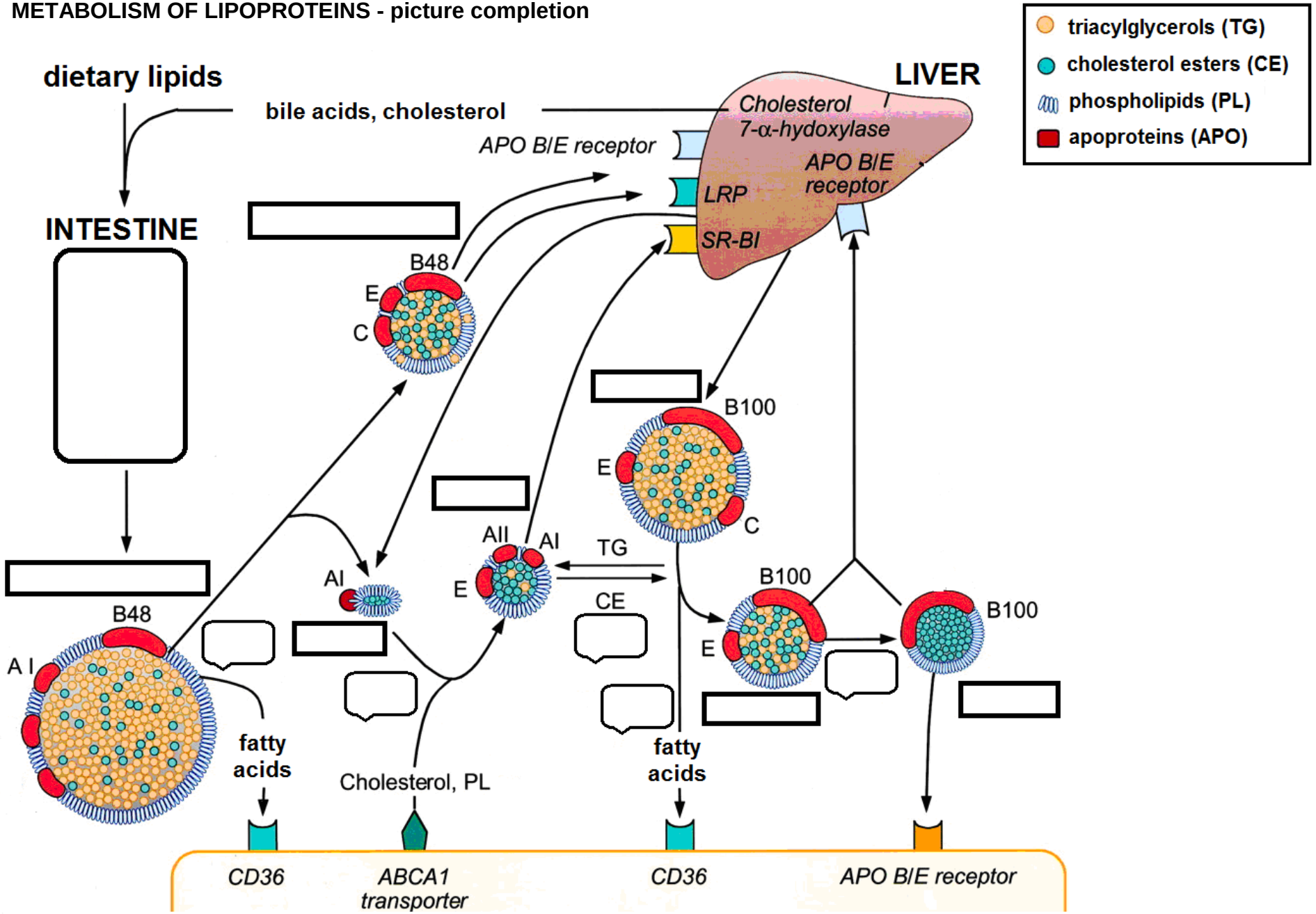
g) which apoprotein activates LCAT?

h) add the name of the apoprotein, activating lipoprotein lipase, into the picture of the chylomicron

i) what is the function of the enzyme cholesterol-7-α-hydroxylase in the liver? how is it connected with the digestion of lipids?

picture adopted from http://circ.ahajournals.org/content/110/13/1868/F2.large.jpg (April 2016)

22. METABOLISM OF LIPOPROTEINS - picture completion



23. METABOLISM OF HEME - part 1 - tasks

SYNTHESIS:

a) what is the intracellular localization of the reactions (1, 6, 7, 8) and where are remaining reactions localized?

b) reaction (1): state the names of reactants A and B, product C and the enzyme, that catalyzes the reaction; which vitamin is an essential precursor of the cofactor used by this enzyme? what is the locant for $-NH_2$ group of the substance C? *(as locants use both - a number and a Greek letter, and write the whole name of the substance C)*

c) how is the enzyme, catalyzing the reaction (1), regulated? A) in the bone marrow
B) in the liver

d) reaction (2): state the names of its product and the enzyme catalyzing the reaction; this enzyme is inhibited by lead (Pb) – what are the pathophysiological consequences of such poisoning?

e) reaction (3) and (4): one of products of the reaction (3) is a linear tetrapyrrole that is converted by the reaction (4) to a cyclic tetrapyrrole: what enzyme is essential for the formation of the right isomer (substance E)? state the name of the substance E

f) reaction (5): state the name of its product (substance F) and describe how it structurally differs from the substance E

g) name substances G and H and state what is the main difference in their structures (a physico-chemical property) – notice the type of the reaction (7)

h) reaction (8): state the name of the enzyme catalyzing the reaction; do you know some of its inhibitors?

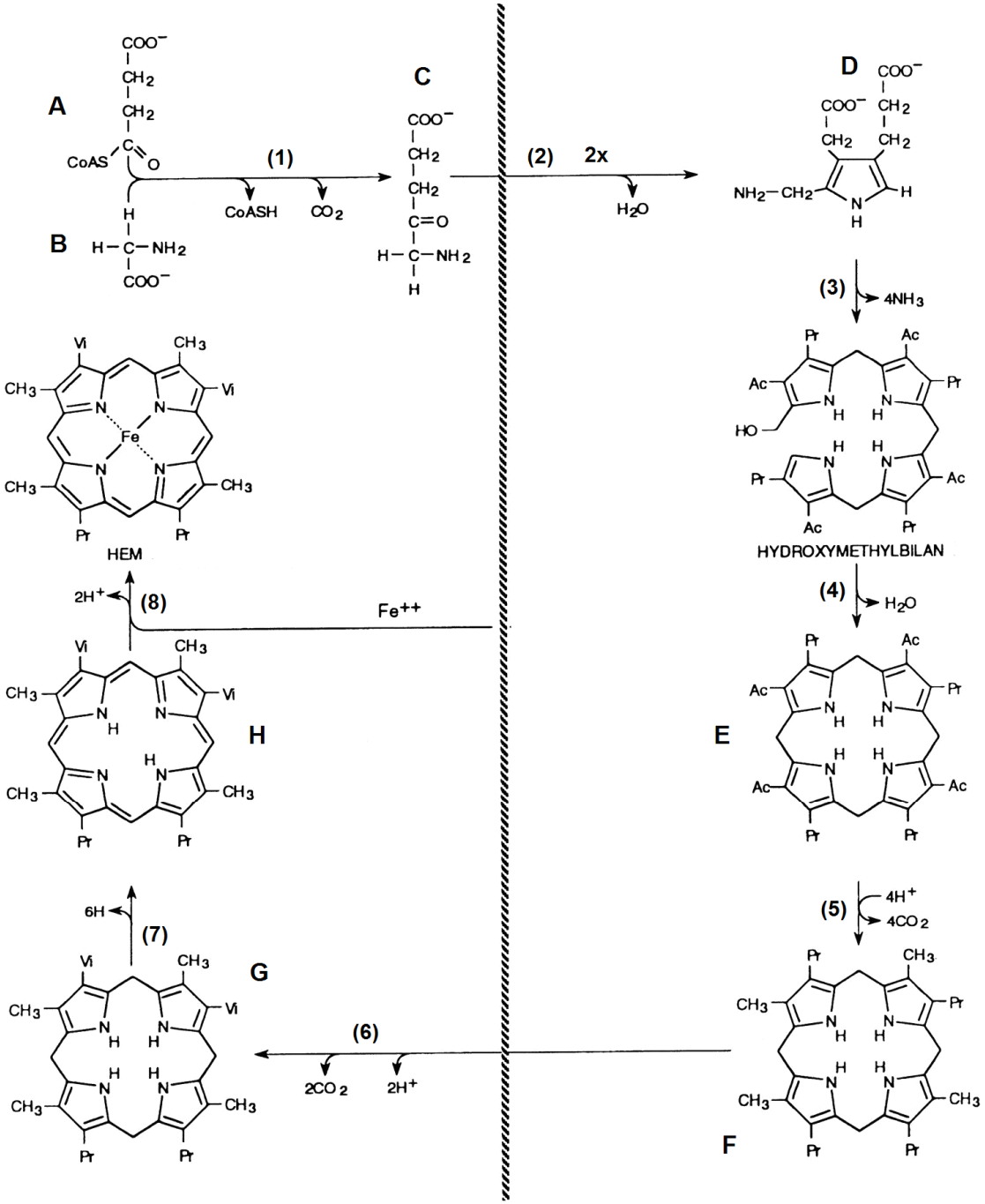
i) what is the name of hereditary disease caused by a deficiency of some of the enzymes (3) - (8)? what is its manifestation?

j) heme is one of porphyrins - what part of its structure results in its colour? under which conditions can other porphyrins be formed in the human body? what are the pathophysiological consequences?

picture adopted from http://www.bloodjournal.org/content/89/1/1?sso-checked=true (November 2018)

23. METABOLISM OF HEME - part 1 - picture completion *(Ac = acetate, Pr = propionate, Vi = vinyl)*

SYNTHESIS:



23. METABOLISM OF HEME - part 2 - tasks

DEGRADATION:

a) if heme (A, cyclic tetrapyrrole) is degraded, the linear tetrapyrroles are formed (B, C): state their names and write these names also to bottom formulas describing these molecules really as linear structures

b) name the functional groups and side chains (substituents) found in the structures of heme and both its degradation products

c) what is the fate of iron releasing during the heme degradation?

d) what is the fate of CO produced during the heme degradation?

e) what is the intracellular source of the cofactor necessary for the heme degradation?

f) state the names of enzymes (1) and (2) and notice how are the enzyme names related to the type of reaction (see structural differences of each reactant → product); describe the structural difference of the substances B and C

g) where in the body do the reactions (1) and (2) take place and what is their intracellular localization? what is the fate of the substance C?

h) bottom reactions: add the name of the second substrate to the reaction (b) (not pictured) and the name of the enzyme catalyzing the reaction

i) where in the body does the reaction (b) take place and what is the fate of its product? name the product, and state what type (by structure) of a substance it is

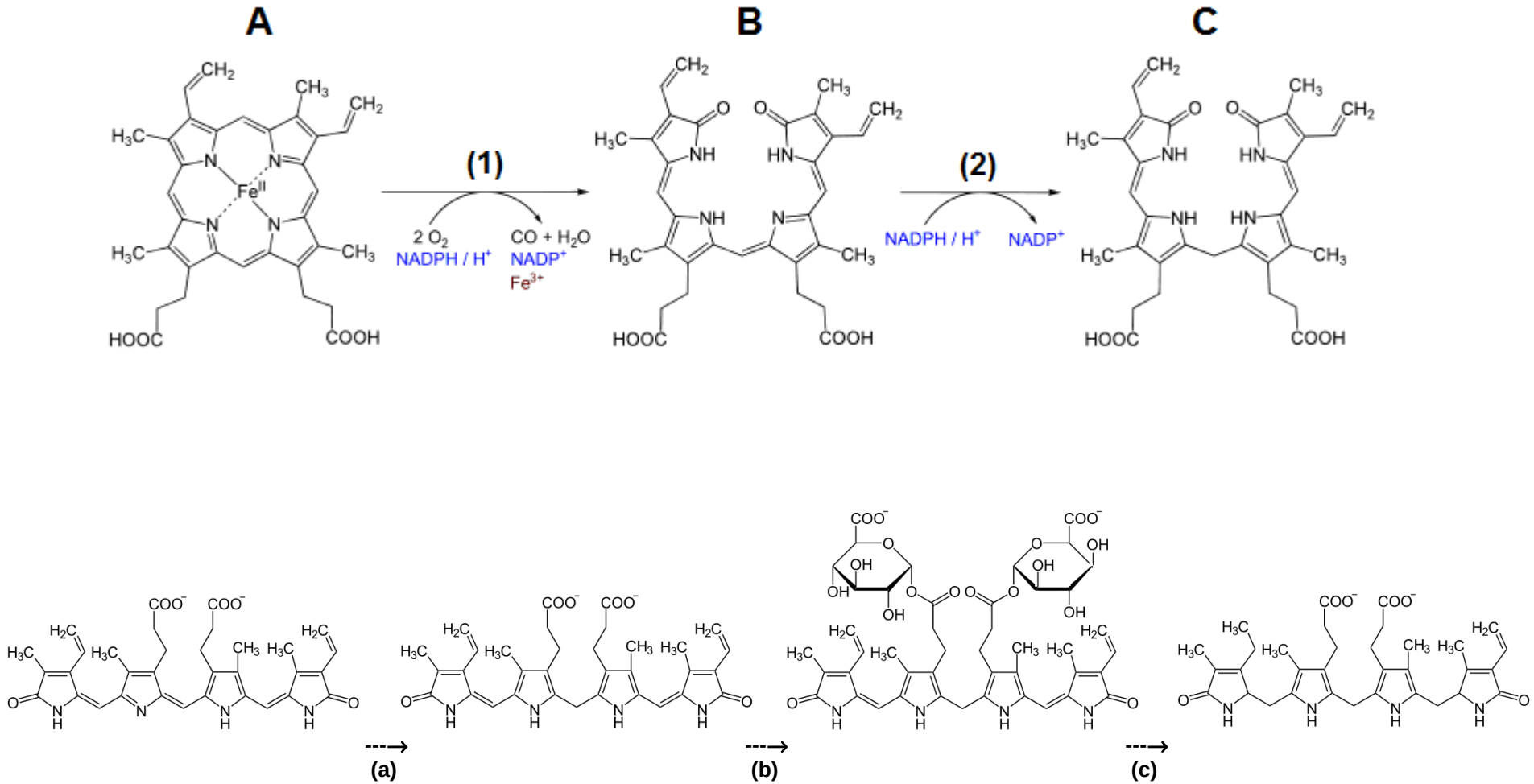
j) where in the body does the reaction (c) take place? (it includes more steps: deconjugation and reduction)

and what is the fate of its product? what is the name of the product? what is its structural difference from the substance C?

k) what can be the cause of increased blood concentrations of the substance C and/or its conjugated derivative? what are the pathophysiological connections?

23. METABOLISM OF HEME - part 2 - picture completion

DEGRADATION:



24. VITAMINS IN METABOLISM

a) The following table includes vitamins named by using big alphabet letters and numbers.
Use the alternative names of vitamins based on chemical composition *(see the list below)* and write the correct name for each vitamin into the table.

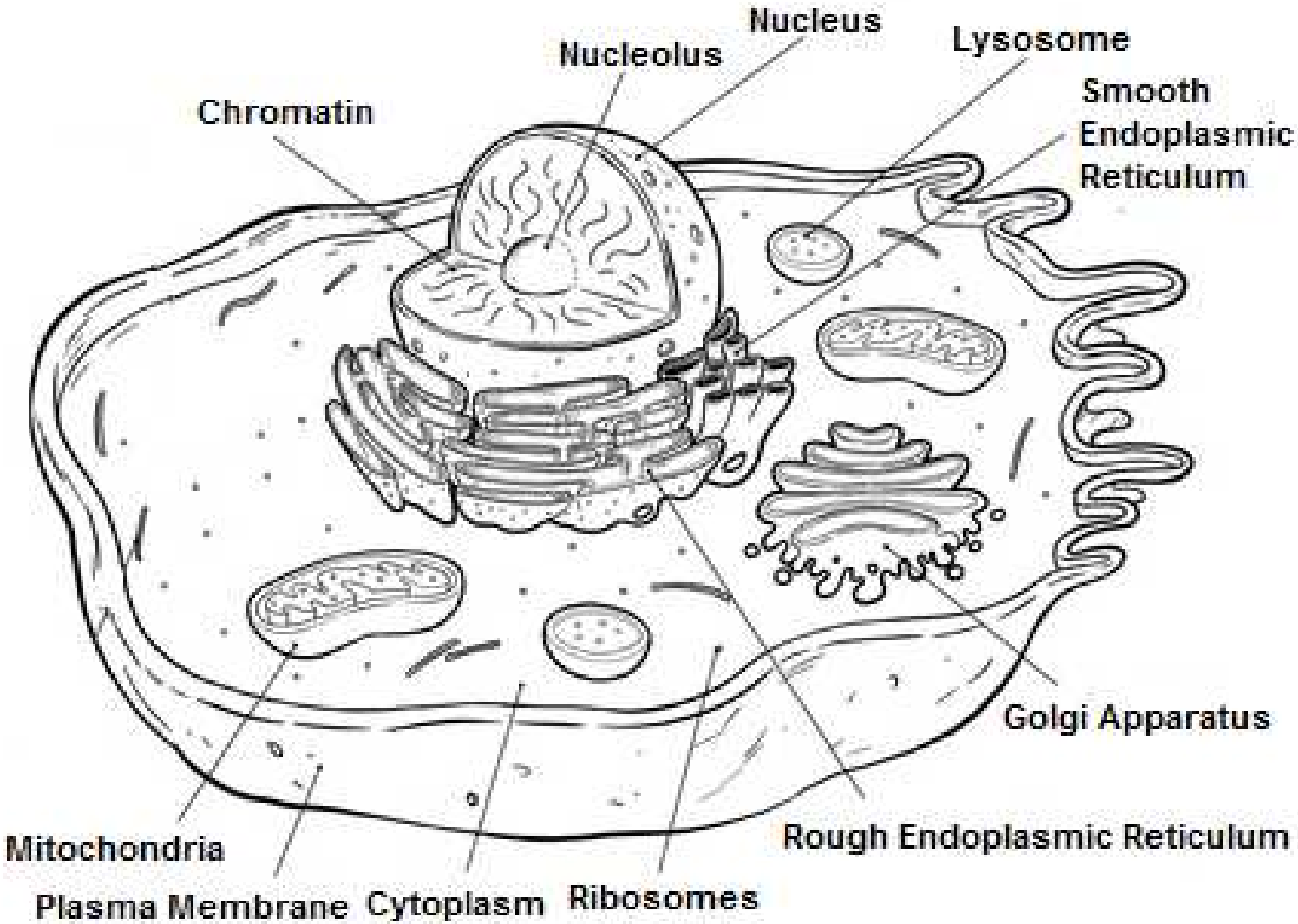
tocopherol, pyridoxine, thiamine, ascorbate, cobalamin, retinol, cholecalciferol, folate, riboflavin, phylloquinone, biotin, pantothenic acid, niacin (nicotinamide)

alphabet-using name of vitamin	chemical-composition-based name of vitamin	active form of vitamin	alphabet-using name of vitamin	chemical-composition-based name of vitamin	active form of vitamin
vitamin A			vitamin B9		
vitamin B1			vitamin B12		
vitamin B2			vitamin C		
vitamin B3			vitamin D3		
vitamin B5			vitamin E		
vitamin B6			vitamin K		
vitamin B7					

b) to vitamins, you know from energy metabolism, write the name (abbreviation) of their active forms (these that enter metabolic reactions)

c) use a coloured pencil and mark fat-soluble vitamins - what do they have common in their structures?

25. COMPARTMENTALIZATION of metabolic pathways write names of metabolic pathways into the picture according to their localization



picture adopted from www.timvandevall.com

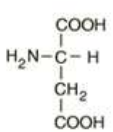
SACCHARIDES	alternative name	functional group / number of carbons	classification	bonds in the molecule	shape of the molecule	digestion products	notes
glucose	dextrose, grape sugar, blood sugar	aldohexose	monosaccharide		glucopyranose	glucose	fasting glycemia: 3.6 – 5.6 mmol/l
fructose	levulose, fruit sugar	ketohexose	monosaccharide		fructofuranose	fructose	sweetest naturally occurring monosacchar. (1.8 x sweeter than *)
galactose		aldohexose	monosaccharide	4-epimer of glucose	galactopyranose	galactose	found in glycoproteins
mannose		aldohexose	monosaccharide	2-epimer of glucose	mannopyranose	mannose	found in glycoproteins
ribose		aldopentose	monosaccharide		ribofuranose	ribose	only a little absorbed in GIT
saccharose or sucrose	beet sugar, cane sugar, "sugar"	12	disaccharide (non-reducing)	α-Glc (1→2) β-Fru		glucose and fructose	* sweet taste of sucrose is used as a standard to describe sweetness of other sugars
lactose	milk sugar	12	disaccharide (reducing)	β-Gal (1→4) β-Glc		galactose and glucose	synthesized in mammary gland
maltose	malt sugar	12	disaccharide (reducing)	α-Glc (1→4) β-Glc		2 glucoses	also formed during digestion of starch
glycogen	animal starch	polymer	polysaccharide (branched)	linear: α(1→4), branch: α(1→6)		glucose	glucan = glucose polymer; storage animal polysaccharide
starch	in Latin: Amylum	polymer	polysaccharide (linear amylose + branched amylopectin)	linear: α(1→4), branch: α(1→6)	amylose forms helix	glucose	glucan = glucose polymer; storage plant polysaccharide
cellulose	*cotton is almost pure cellulose*	polymer	polysaccharide (linear)	β(1→4)	forms fibres (H-bonds between chains)	not digested by humans	glucan = glucose polymer; structural plant polysaccharide, found in cell walls

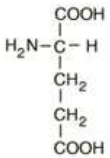
FATTY ACIDS	systematic name	abbreviated description	classification	class. in nutrition *	synthesized in humans	notes
butyric	butanoic	4:0	saturated short chain FA	SAFA	from acetyl-CoA	in Latin: Acidum butyricum, occurs in milk fat (together with 6:0 - 10:0)
caproic	hexanoic	6:0	saturated medium chain FA	SAFA	from butyric acid	intermediate of both synthesis and degradation of longer fatty acids
caprylic	octanoic	8:0	saturated medium chain FA	SAFA	from caproic acid	intermediate of both synthesis and degradation of longer fatty acids
capric	decanoic	10:0	saturated medium chain FA	SAFA	from caprylic acid	intermediate of both synthesis and degradation of longer fatty acids
lauric	dodecanoic	12:0	saturated medium chain FA	SAFA	from capric acid	intermediate of both synthesis and degradation of longer fatty acids
myristic	tetradecanoic	14:0	saturated long chain FA	SAFA	from lauric acid	intermediate of both synthesis and degradation of longer fatty acids
palmitic	hexadecanoic	16:0	saturated long chain FA	SAFA	from myristic acid	*the longest FA synthesized in cytosol*
stearic	octadecanoic	18:0	saturated long chain FA	SAFA	from palmitic acid	formed by elongation of *palmitic acid* in smooth endoplasmic reticulum
arachidic	eicosanoic	20:0	saturated long chain FA	SAFA	from stearic acid	formed by elongation of *stearic acid* in smooth endoplasmic reticulum
palmitoleic	*cis* hexadec-9-enoic	16:1;9	monounsaturated long chain FA	ω-7 MUFA	from palmitic acid	formed by desaturation of *palmitic acid* in smooth endoplasmic reticulum
oleic	*cis* octadec-9-enoic	18:1;9	monounsaturated long chain FA	ω-9 MUFA	from stearic acid	formed by desaturation of *stearic acid* in smooth endoplasmic reticulum
linoleic	*cis cis* octadeca-9,12-dienoic	18:2;9,12	polyunsaturated long chain FA	ω-6 PUFA **essential**	NOT	can serve as a substrate for the synthesis of *arachidonic acid*
γ-linolenic	*all cis* octadeca-6,9,12-trienoic	18:3;6,9,12	polyunsaturated long chain FA	ω-6 PUFA	from linoleic acid	formed by desaturation of *linoleic acid*
dihomo–γ-linolenic	*all cis* eicosa-8,11,14-trienoic	20:3;8,11,14	polyunsaturated long chain FA	ω-6 PUFA	from linoleic acid	formed by desaturation and elongation of *linoleic acid*; used for the synthesis of *eicosanoids* (= tissue hormones: prostaglandins, prostacyclins, tromboxanes and leukotrienes)
arachidonic	*all cis* eicosa-5,8,11,14-tetraenoic	20:4;5,8,11,14	polyunsaturated long chain FA	ω-6 PUFA	from linoleic acid	
α-linolenic	*all cis* octadeca-9,12,15-trienoic	18:3;9,12,15	polyunsaturated long chain FA	ω-3 PUFA **essential**	NOT	fish oil is rich in ω-3 FA
EPA	*all cis* eicosa-5,8,11,14,17-pentaenoic	20:5;5,8,11,14,17	polyunsaturated long chain FA	ω-3 PUFA	from α-linolenic	used for the synthesis of *eicosanoids*
DHA	*all cis* docosa-4,7,10,13,16,19-hexaenoic	22:6;4,7,10,13,16,19	polyunsaturated very long chain FA	ω-3 PUFA	from α-linolenic	formed by *desaturation and elongation of α-linolenic acid*

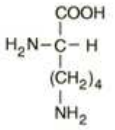
** SAFA = saturated fatty acid, MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid*

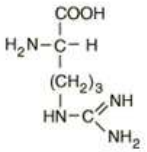
AMINO ACIDS	properties of side chain	type of side chain	charge of side chain	in nutrition	synthesized in humans	notes
glycine, Gly	no side chain	no side chain	no side chain		from serine	not chiral (neither L- nor D-); 1/3 of amino acids in collagen is Gly
alanine, Ala	nonpolar	methyl	NONE		from pyruvate	2nd most abundant amino acid in blood plasma
proline, Pro	nonpolar	cyclic	NONE		from glutamate	often hydroxylated in collagen (HyPro); (its N is incorporated in its cycle as an imino group -NH-)
valine, Val	nonpolar	branched	NONE	essential	NO	*involved in hydrophobic interactions,* one of BCAA (branched chain amino acids)
leucine, Leu	nonpolar	branched	NONE	essential	NO	*involved in hydrophobic interactions,* one of BCAA (branched chain amino acids)
isoleucine, Ile	nonpolar	branched	NONE	essential	NO	*involved in hydrophobic interactions,* one of BCAA (branched chain amino acids)
phenylalanine, Phe	nonpolar	aromatic, contains benzene	NONE	essential	NO	*involved in hydrophobic interactions*
tryptophan, Trp	nonpolar	aromatic, contains indol	NONE	essential	NO	*involved in hydrophobic interactions*
methionine, Met	nonpolar	contains sulfur (sulfide, -S-)	NONE	essential	NO	activated (SAM) acts as a donor of $-CH_3$ group
cysteine, Cys	nonpolar	contains sulfur (thiol, -SH)	NONE		from Ser + Met	2 Cys can be oxidized to cystine: form *disulfide bond* (Cys-S-S-Cys)
selenocysteine, SeCys	nonpolar	contains selenium	NONE		from serine	rare AA, formed during translation; same structure as Cys, with Se instead S
serine, Ser	polar	contains primary -OH group	NONE		from glycolytic intermediate	*involved in a formation of H-bonds*, can be glycosylated or phosphorylated
threonine, Thr	polar	contains secondary -OH group	NONE	essential	NO	*involved in a formation of H-bonds*, can be glycosylated or phosphorylated
tyrosine, Tyr	polar	aromatic, contains -OH (in phenol)	NONE		from phenylalanine	*involved in a formation of H-bonds*, can be phosphorylated

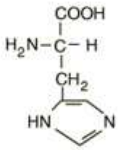
histidine, His	polar	aromatic, contains imidazole	POSITIVE (basic amino acid)	essential	NO	*involved in electrostatic interactions*, in muscle proteins can be methyl-His
arginine, Arg	polar	contains guanidine	POSITIVE (basic amino acid)	essential	(partially from glutamine)	*involved in electrostatic interactions*, urea cycle intermediate
lysine, Lys	polar	contains -NH_2	POSITIVE (basic amino acid)	essential	NO	*involved in electrostatic interactions*, in collagen can be hydroxylated (HyLys)
aspartic acid, Asp	polar	contains -COOH	NEGATIVE (acidic amino acid)		from oxaloacetate	*involved in electrostatic interactions*, anion: aspartate; dicarboxylic acid
glutamic acid, Glu	polar	contains -COOH	NEGATIVE (acidic amino acid)	*taste umami (meat taste)*	from α-ketoglutarate	*involved in electrostatic interactions*, anion: glutamate; dicarboxylic acid
asparagine, Asn	polar	contains -$CONH_2$ (amide group)	NONE		from aspartate	*involved in a formation of H-bonds*, can be glycosylated
glutamine, Gln	polar	contains -$CONH_2$ (amide group)	NONE		from glutamate	*involved in a formation of H-bonds*, most abundant amino acid in plasma

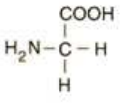

aspartic acid (Asp)

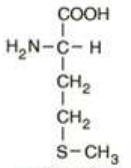

glutamic acid (Glu)

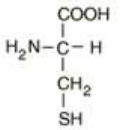

lysine (Lys)

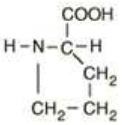

arginine (Arg)

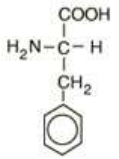

histidine (His)

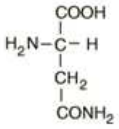

glycine (Gly)

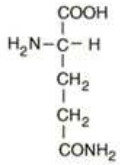

methionine (Met)

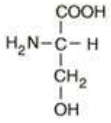

cysteine (Cys)

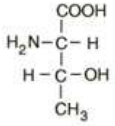

proline (Pro)

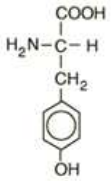

phenylalanine (Phe)

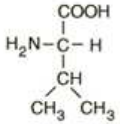

asparagine (Asn)

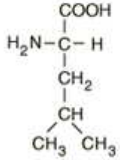

glutamine (Gln)

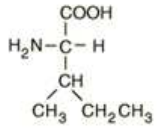

serine (Ser)

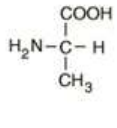

threonine (Thr)

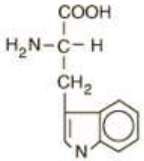

tyrosine (Tyr)


valine (Val)


leucine (Leu)


isoleucine (Ile)


alanine (Ala)


tryptophan (Trp)

ORGANIC ACIDS

number of carbons	systematic (IUPAC) name	common name	Latin name →	acyl name	anion name
saturated monocarboxylic acids					
1	methanoic acid	**formic acid**	Acidum formicum	formyl	formate
2	ethanoic acid	**acetic acid**	Acidum aceticum	acetyl	acetate
3	propanoic acid	**propionic acid**	Acidum propionicum	propionyl	propionate
4	butanoic acid	**butyric acid**	Acidum butyricum	butyryl	butyrate
5	pentanoic acid	**valeric acid**	Acidum valericum	valeryl	valerate
12	dodecanoic acid	**lauric acid**	Acidum lauricum	laur**o**yl	laurate
16	hexadecanoic acid	**palmitic acid**	Acidum palmiticum	palmit**o**yl	palmitate
18	octadecanoic acid	**stearic acid**	Acidum stearicum	stear**o**yl	stearate
saturated dicarboxylic acids					
2	ethandioic acid	**oxalic acid**	Acidum oxalicum	oxalyl	oxalate
3	propandioic acid	**malonic acid**	Acidum malonicum	malonyl	**malonate**
4	butandioic acid	**succinic acid**	Acidum succinicum	succinyl	succinate
5	pentandioic acid	**glutaric acid**	Acidum glutaricum	glutaryl	glutarate
6	hexandioic acid	**adipic acid**	Acidum adipicum	adip**o**yl	adipate
unsaturated monocarboxylic acids					
18:1, ω-9	*cis*-octadec-9-enoic acid	**oleic acid**	Acidum oleicum	ole**o**yl	oleate
18:2, ω-6	*cis*-octadeca-9,12-dienoic acid	**linoleic acid**	Acidum linoleicum	linole**o**yl	linoleate
18:3, ω-3	*cis*-octadeca-9,12,15-trienoic acid	**α-linolenic acid**	Acidum α-linolenicum	α-linolen**o**yl	α-linolenate
20:4, ω-6	*cis*-icosa-5,8,11,14-tetraenoic acid	**arachidonic acid**	Acidum arachidonicum	arachidon**o**yl	arachidonate
unsaturated dicarboxylic acids					
4	*cis*-butendioic acid	**maleinic acid**	Acidum maleinicum	maleinyl	**maleinate**
4	*trans*-butendioic acid	**fumaric acid**	Acidum fumaricum	fumaryl	fumarate
derivatives of carboxylic acids					
3	2-oxopropanoic acid	**pyruvic acid**	Acidum pyruvicum	pyruvyl	pyruvate
3	2-hydroxypropanoic acid	**lactic acid**	Acidum lacticum	lact**o**yl	lactate
4	3-oxobutanoic acid	**acetoacetic acid**	Acidum acetoaceticum	acetoacetyl	acetoacetate
4	3-hydroxybutanoic acid	**beta-hydroxybutyric acid**	Acidum **β**-hydoxybutyricum	β-hydroxybutyryl	β-hydroxybutyrate
4	2-hydroxybutandioic acid	**malic acid**	Acidum malicum	malyl	**malate**
4	2-oxobutandioic acid	**oxaloacetic acid**	Acidum oxaloaceticum	oxaloacetyl	oxaloacetate
5	2-oxopentandioic acid	**alfa-ketoglutaric acid**	Acidum α-ketoglutaricum	α-ketoglutaryl (2-oxoglutaryl)	α-ketoglutarate (2-oxoglutarate)
6	2-hydroxypropantricarboxylic acid	**citric acid**	Acidum citricum	citryl	citrate

DICTIONARY
- vitamins in metabolism-

vitamins	organic substances essential in the diet that human body needs in small quantities for the proper functioning (human cells are not able synthesize most of vitamins)	
lipophilic	= hydrophobic, nonpolar molecules, little soluble in water (they need a transporter for blood transport); these vitamins are absorbed from the diet and transported in the body together with other lipids, hence bile is essential for their absorption from the intestine (i.e. proper liver function); they are stored in the body (liver, adipose tissue), ant their excess is toxic (vitamins A and D)	vitamins: A, D, E, K *(all are isoprene derivatives)* stability in food: *often sensitive to oxidation*
hydrophilic	= polar substances, well water soluble; their excess is excreted with urine and their storage in tissues is short (exception: B12 stored in the liver for 3-5 years)	vitamins: C and B-complex (B1, B2, B3, B5, B6, B7, B9, B12) stability in food: *easily macerated into water*
synthesized in the human body	1) by intestinal bacteria: **vit. K, biotin**, **B12** 2) by sun light in the skin: **vit. D3** 3) only in excess of tryptophan: **vit. B3**	*this B12 is little absorbed* *D3 synthesis requires sun light (UV radiation)* *tryptophan is an essential amino acid*
haematopoietic *vitamis*	essential for haematopoiesis: **vit. B9** and **B12** *(folate is directly necessary for nucleotide synthesis, hence for cell division, B12 provides a regeneration of folate in a metabolism)*	their lack results in a megaloblastic anemia (megaloblasts in blood)
coagulation *vitamin*	essential for the synthesis of some clotting factors (in the liver): **vit. K** - it acts as a cofactor of an enzyme that incorporates an additional carboxylic functional group on gamma-carbon of glutamate in some proteins resulting in their ability to bind Ca^{2+} (enzyme: γ-glutamyl carboxylase) *vit. K-dependent clotting factors: II, VII, IX, X and anticoagulation proteins C and S*	similar use in bones: synthesis of osteocalcin *(= extracellular protein that binds Ca^{2+})*
vitamin ***regulating amount of calcium*** *in the body*	**vit. D** - it acts as a substrate from which calcitriol (D-hormone) is synthesized: calcitriol regulates an absorption of calcium in the intestine and in kidneys into the body, hence it increases the amount of calcium in the body and *also in blood*	
vitamin ***essential for connective tissues***	**vit. C** (cofactor needed for the synthesis of collagen)	*scurvy (scorbutus): in a lack of vit. C weakness, gum disease and subcutaneous bleeding develop*

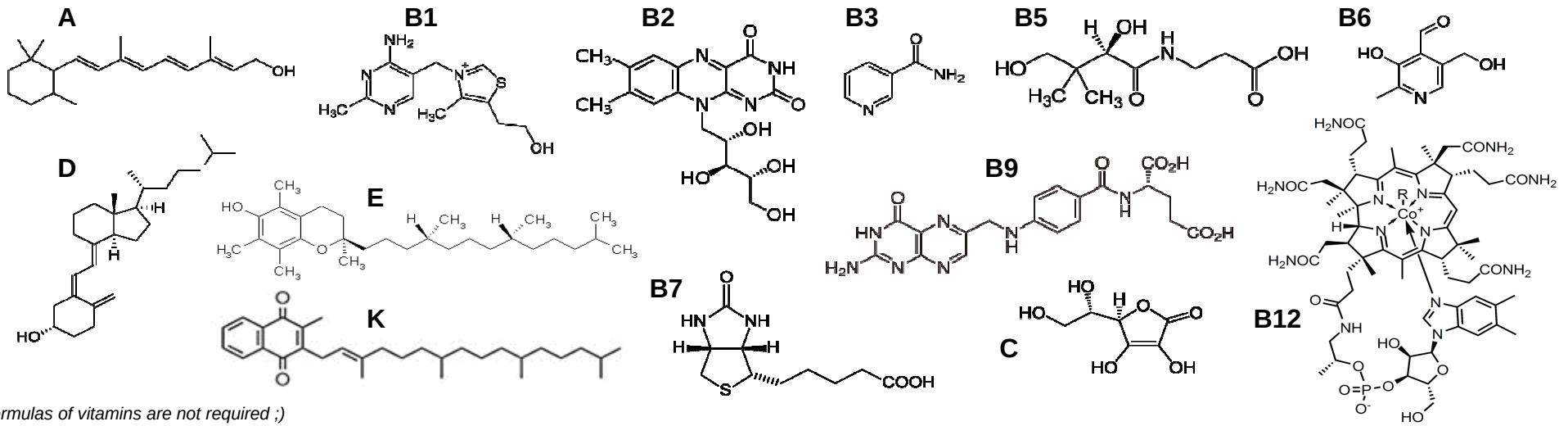
antioxidation *vitamins*	vitamins that serve as reducing agents - they inactivate radicals - **vit. C, E,** or participate in quenching singlet oxygen - **beta-carotene** *(provitamin of vitamin A, which alone is only a weak antioxidant)*	*vit. C is functioning in an aqueous solutions (cytosol, blood)* *vit. E is present in membranes* *beta-carotene occurs in subcutaneous fat*
vitamins ***of energy metabolism***	**vit. B1, B2, B3, B5, B6, B7** *their lack often affects functions of the nervous system (energy demanding), skin and mucous membranes (rapidly dividing cells)*	these vitamins are converted to enzyme cofactors in the body (coenzymes or prosthetic groups)
vitamin ***of amino acid metabolism***	**vit. B6** (transamination, decarboxylation, condensation reactions of amino acids)	*vitamin essential for the synthesis of neurotransmitters from amino acids*
teratogenic *vitamin*	**vit. A** - its excess during the first trimester of pregnancy causes malformations of fetus; i.e. the supplementation by vitamin A in pregnancy is dangerous *(β-carotene is not a problem: its amount converted into vit. A is regulated in the body)*	*active forms of vit. A, retinoic acid and other, serve as signaling molecules during fetal development*
vitamin A	active forms: *retinol, retinal, retinoic acid* *(= retinoids, conjugated C=C double bonds: all-trans or 11-cis)* retinol ↔ retinal → retinoic acid (oxidation) *the 2nd reaction step is irreversible* **retinol**: stored in the liver in a form of esters; necessary for a reproduction system **retinal**: necessary for vision (occurs in visual pigments) and for reproduction **retinoic acid**: involved in fetal development, differenciation and growth of epithelium (gene transcription) **! High doses are toxic !**	reserve capacity (body storage): for 1-2 years blood transport: transtyretin (= prealbumin) deficiency: impaired vision excess of vit. A in pregnancy: severe malformations *occurs in food of an animal orgin*
carotenes: **β-carotene**	*provitamin of vitamin A* (tetraterpene), its oxidative cleavage produces 2 retinals (this symetrical cleavage proceeds in enterocytes and in the liver); other carotenoids (α-carotene, γ-carotene) produce only 1 retinal by their cleavage **excess of β-carotene is not cleaved to vit. A**	yellow-orange pigment stored in adipose tissue lipophilic antioxidant *occurs in food of a plant origin*
vitamin D	active form: *calcitriol = D-hormone (= 1,25-dihydroxycholecalciferol)* cholecalciferol is formed from 7-dehydrocholesterol in skin exposed to UV radiation (it is a nonenzymatical process, 290-315 nm, for 15-20 min., longer time doesn´t produce more vit. D); stored and activated in the liver	vit. D3 = **cholecalciferol** (animal) vit. D2 = ergocalciferol (plant) blood transport: DBP (vit.D binding protein)

vitamin D *(continues)*	its active form **calcitriol** binds to intracellular receptors (= ligand-dependent transcription factors) *and stimulates the production of membrane transporters for Ca^{2+} in the intestine and kidneys: <u>it results in an increase of calcium amount in the body</u> and in blood;* *calcitriol is thus necessary for bone metabolism (99% of body calcium occurs in bones)* <u>other functions</u>*: important for cell differenciation and the immune system* **! High doses are toxic !** *(severe organ damage from hypercalcemia, calcification in the heart, lungs, kidneys)*	<u>predominating form in blood</u>: **25-hydroxycholecalciferol** *(formed in the liver by hydroxylation of vit. D3, then it is hydroxylated again in the kidneys to calcitriol; the hydroxylation in the kidneys is stimulated by parathormone in case of hypocalcaemia)* <u>deficiency:</u> rickets in children, osteomalacia in adults <u>excess:</u> accumulation of calcium in various organs (calcification)
vitamin E	<u>active form</u>: *α-tocopherol (the most efficient from all tocopherols)* *antioxidant of cell membranes* (including intracellular organelles and lipoproteins); higher amount of polyunsaturated fatty acids (PUFA, susceptible to oxidation) requires a higher amount of vit. E → **it protects membranes from lipoperoxidation** *(vit. E can be regenerated by vit. C, glutathione or urate)*	8 different **tocopherols** (β, γ, δ,….), they are formed only in plants (rich source: plant oils) synthetic vit. E is less effective: various enantiomers are included
vitamin K	<u>active form</u>: *hydroquinone form (= reduced form)* it acts as a cofactor of γ-glutamyl carboxylase, the enzyme that incorporates an additional carboxylic functional group on gamma-carbon of glutamate in some proteins resulting in their ability to bind Ca^{2+} *(this modified glutamate is called γ-carboxyglutamate - it contains two neighbouring -COOH groups in its side chain, and can thus bind calcium ion)* **in the liver:** *essential for the synthesis of clotting factors* II, VII, IX, X and anticoagulation proteins C and S **in bones:** *essential for the synthesis of an extracellular protein osteocalcin (binds* Ca^{2+}*)* <u>stored</u> and modified: in the liver **antivitamin K: coumarin derivatives (drug Warfarin)**	vit. K1 = **phylloquinone** (plants) vit. K2 = **menaquinone** (bacteria) vit. K3 and K4 = **menadione** or derivatives of menadiol (synthetic, some its forms are water soluble) „coagulation vitamin“ <u>deficiency:</u> impaired blood clotting (bleeding) *vit. K2 is synthesized by intestinal bacteria (half of its body requirement) ⇒ antibiotics cause a decreased amount of vit. K absorbed to blood*
vitamin B1	<u>active form</u>: *thiamine diphosphate (TDP) = thiamine pyrophosphate (TPP)* it serves as a cofactor of enzymes of an intermediary metabolism: 1) oxidative decarboxylation of α-keto acids *(pyruvate dehydrogenase = PDH, α-ketoglutarate dehydrogenase of the Krebs cycle, branched-chain-keto-acid dehydrogenase)* 2) transketolase *(pentose phosphate pathway)*	**thiamine** <u>essential for metabolism of saccharides</u>: its lack results in neurological problems due to a reduced amount of ATP in the nervous system

vitamin B2	active form: *FAD, FMN (prosthetic groups of enzymes)* it serves as a cofactor of oxidoreductases (binds 2 hydrogens = **2 H** = $2e^- + 2p^+$): 1) succinate dehydrogenase of the Krebs cycle = Complex II of the electron transport chain 2) acyl-CoA dehydrogenase (β-oxidation) 3) glycerol-3-phosphate dehydrogenase of the inner mitochondrial membrane (see transport of reducing equivalents into mitochondria)	**riboflavin** *reduced form: $FADH_2$* *its colour is dependent on the number of accepted electrons; riboflavin is yellow-green → when present in excess in the body it is excreted and stains urine* riboflavin contains ribitol instead of ribose *(on the left: only sample enzymes)*
vitamin B3	active form: *NAD^+, $NADP^+$ (coenzymes)* it serves as a cofactor of oxidoreductases (binds hydride ion = $\mathbf{H^-}$ = $2e^- + 1p^+$): 1) isocitrate dehydrogenase (DH), α-ketoglutarate DH, malate DH (Krebs cycle) 2) second oxidoreductase of β-oxidation 3) alcohol dehydrogenase, aldehyde dehydrogenase 4) glycerol-3-phosphate dehydrogenase (cytosolic) 5) glutamate dehydrogenase (GMD) 6) malate (malic) enzyme 7) fatty acid synthase	**niacin: nicotinic acid + nicotinamide** *reduced form: NADH or NADPH* *partially synthesized from tryptophan in the body (essential amino acid!)* *(on the left: only sample enzymes)*
vitamin B5	active form: *coenzyme A (CoA-SH), occurs also as a functional group of fatty acid synthase* binds to carboxylic acids (acyls) within cells: it forms their activated forms - *thioesters*, e.g.: 1) acyl-CoA 2) acetyl-CoA 3) succinyl-CoA 4) propionyl-CoA	**pantothenic acid** *ATP is needed for the attaching a fatty acid to coenzyme A*

vitamin B6	active form: *pyridoxal phosphate (PLP)* it serves as common cofactor of enzymes in metabolism of amino acids: 1) aminotransferases (ALT, AST, ...) 2) decarboxylases (glutamate decarboxylase, aromatic amino acid decarboxylase) 3) enzymes catalyzing a condensation of amino acids (cystathionine-β-syntase: synthesis of Cys from Met, delta-aminolevulinic acid synthase: heme synthesis)	**pyridoxine** = mix of various derivatives: pyridoxol, pyridoxal, pyridoxamine
vitamin B7	active form: *biotin covalently bound to a lysine side chain of enzyme (prosthetic group)* it serves as a cofactor of carboxylases (it incorporates CO_2 into a substrate) 1) pyruvate carboxylase 2) acetyl-CoA carboxylase 3) propionyl-CoA carboxylase	**biotin**, formerly vit. H *synthesized by intestinal bacteria* glycoprotein avidin from a raw egg white binds biotin from meal and thus protects its use in the body (but a lack of biotin in the body would be caused only by a higher number of raw eggs)
vitamin B9	active form: *tetrahydrofolate (THF)* *activation*: folate → dihydrofolate → tetrahydrofolate *(both steps are catalyzed by dihydrofolate reductase, NADPH)* it serves as a cofactor of enzymes transporting C1-fragments *(various ox. forms of rests of CH_4)* (methyl -CH_3, methylene -CH_2-, formyl -CHO, ...) the source of a C1-fragment (methylene) is for example the conversion of serine to glycine; methylene-THF can be then either irreversibly reduced to methyl-THFor it can be oxidized to other derivatives (e.g. formyl-THF) 1) synthesis of purine nucleotides (formyl-THF) 2) synthesis of thymidine (methylene-THF) 3) regeneration of methionine from homocysteine (methyl-THF); for this reaction vitamin B12 is also necessary (B9 and B12 work together in this reaction) !!! a lack of vit. B12 results in a „**functional lack of folate**“: folate is still present but accumulates in a form of methyl-THF, hence it is not more available for other reactions (e.g. nucleotide synthesis with consequent cell division); this situation is called „**folate trap“** ! Note that an **excess of folate** can cover a lack of vit.B12, because megaloblastic anaemia will not develop ! (it is normally coupled with either vit. B12 or vit. B9 deficiency)	**folate = folic acid** = folacin „ haematopoietic vitamin“ → its deficiency predominantly affects rapidly dividing cells including erythroblasts (they need a nucleotide synthesis for replication) dihydrofolate inhibitors are used as cytostatics (methotrexate) *vit. B9 is also essential during first weeks of pregnancy (supplementation)* deficiency: megaloblastic anaemia, defects in development of the neural tube **vit. B9 is often less present in a nutrition** (leaf vegetable)

vitamin B12	active form: *methylcobalamin (see the reaction 1)* or *deoxyadenosylcobalamin (see the reaction 2)* it serves as a cofactor of enzymes transporting C1-fragments 1) regeneration of methionine from homocysteine: it cooperates with vit. B9 !!! (B12 takes methyl from methyl-THF and incorporates it into homocysteine); this reaction is essential for regeneration of **free** folate (metyl-THF → THF) * 2) conversion of methylmalonyl-CoA to succinyl-CoA (isomerisation, methylmalonyl-CoA mutase) ** * a lack of vit. B12 can be covered by an excess of folate because one of problems developing in a deficiency of both vitamins is megaloblastic anaemia: in case of folate excess the anaemia doesn´t develop (see vit. B9) ** accumulation of methylmalonyl-CoA and its precursor propionyl-CoA causes neurological problems in case of vit. B12 deficiency (fatty acid synthesis and the formation of myelin sheath on axones of neurons are impared) **vit. B12 is synthesized only by bacteria** *(hence it is missing in plant food !!!)*	**cobalamin** *structurally it is one of corrinoids containing a tetrapyrrole ring (different from heme) with cobalt ion (in various oxidation states) bound by coordination-covalent bonds in the middle of its structure* synthetic B12: **cyanocobalamin** *(also formed in the body after a poisoning by cyanides)* absorbed in ileum only if bound to so called **intrinsic factor** (glycoprotein synthesized in the stomach) reserve in the liver: for 3 - 5 years (B12 is stored in the liver) deficiency: megaloblastic anaemia, neurological problems
vitamin C	active form: *ascorbic acid (reduced)* it serves as a cofactor of oxidoreductases, and works as an antioxidant (reducing power) 1) lysyl hydroxylase, prolyl hydroxylase (collagen synthesis) 2) dopamine β-hydroxylase (synthesis of epinephrine and norepinephrine)	**L- ascorbic acid, ascorbate** *as an antioxidant it participates in regeneration of vit. E;* *also reduces Fe^{3+} to Fe^{2+}(= prooxidation effect)* deficiency: worsen wound healing, fragile capilaries, scurvy



(formulas of vitamins are not required ;)

DICTIONARY
- names of enzymes -

oxidoreductases **EC 1**	catalyse oxidation-reduction reactions (redox reactions), i.e. transfer of electrons or hydrogen; electrons (or hydrogens) are transferred either to another organic substance (enzyme cofactor) or to oxygen; some enzymes incorporate oxygen(s) into a substrate	**$A_{ox} + B_{red} \rightarrow A_{red} + B_{ox}$**
* dehydrogenases (DH)	transfer hydrogen, or more precisely they transfer two electrons *in a form* of hydrogen (i.e. „reducing equivalents"); most of these reactions are reversible, but the enzymes are called according to the substrate that is dehydrogenated *cofactors*: NAD^+, $NADP^+$ (bind hydride ion H^-, i.e. 2 electrons and 1 proton), FAD, FMN (bind two hydrogen atoms, i.e. 2 electrons and 2 protons) some dehydrogenases catalyze a dehydrogenation coupled with another type of reaction: 1) oxidative *decarboxylation* of α-keto acids (irreversible) *5 cofactors*: TDP, CoA, lipoic acid, FAD, NAD^+ 2) oxidative *deamination* of an amino acid (NAD^+, $NADP^+$)	alcohol dehydrogenase (NAD^+) aldehyde dehydrogenase (NAD^+) lactate dehydrogenase (NAD^+) malate dehydrogenase (NAD^+) β-hydroxybutyrate dehydrogenase (NAD^+) succinate dehydrogenase (FAD) acyl-CoA dehydrogenase (FAD) glycerol-3-P dehydrogenase (NAD^+/FAD) pyruvate dehydrogenase (PDH) α-ketoglutarate dehydrogenase BCKA- dehydrogenase (Branched Chain Keto Acid dehydrogenase) glutamate dehydrogenase
* desaturases	are dehydrogenases converting saturated fatty acids to unsaturated ones: in this case the conversion of $-CH_2-CH_2-$ to $-CH=CH-$ is named „desaturation" (instead of dehydrogenation)	stearoyl-CoA-desaturase
* oxidases	transfer electrons to oxygen, which is thus reduced to H_2O or H_2O_2 *cofactors*: heme (transfer only 1 electron, not hydrogen: $Fe^{3+} + e^- \leftrightarrow Fe^{2+}$), FAD (binds 2 hydrogens, their electrons are subsequently transferred to oxygen)	cytochrome oxidase (see Complex-IV of ETC) glucose oxidase
* oxygenases	incorporate oxygen from O_2 into a substrate: *monooxygenases* incorporate 1 oxygen atom into a substrate, the other O is reduced to water; *dioxygenases* incorporate both oxygen atoms from O_2 into a substrate	cyclooxygenase (COX) lipoxygenase
* hydroxylases	are *monooxygenases*, which incorporate 1 oxygen atom as an alcohol (-OH, hydroxyl-) group into a substrate	cytochrome P450 (CYP)
* peroxidases	use hydrogen peroxide (H_2O_2) for an oxidation of a substrate or are involved in a conversion of an organic peroxide	catalase glutathione peroxidase
* reductases	catalyse reduction of a substrate, i.e. the direction of a reaction is specified in the name	HMG-CoA reductase (NADPH) glutathione reductase (NADPH)

transferases **EC 2**	transfer a part of one molecule (a functional group, a fragment of its carbon chain) into another molecule; they do not catalyse redox reactions (transfer of electrons or hydrogen)	**A-x + B → A + B-x**
* kinases	transfer phosphate from an „energy-rich" compound (ATP) into a substrate, they are named according to a substrate that is phosphorylated, their reactions are often irreversible; kinases are „phosphotransferases"	protein kinase glucokinase hexokinase (hexo- comes from the word hexose)
* phosphorylases	cleave a hydrolyzable substrate but use phosphate rather than water (as hydrolases do): phosphate restores the building blocs of the substrate after the cleavage, so one of products is a small phosphorylated molecule (e.g. glucose-1-phosphate, ribose-1-phosphate,…)	glycogen phosphorylase nucleoside phosphorylase
* transaminases	transfer amino group ($-NH_2$), hence their another name „aminotransferases", the reaction is often reversible this reaction looks as an *exchange of functional groups* between substrates (amino acid and keto acid): the amino acid is converted to new keto acid and the keto acid is converted to new amino acid - but their carbon skeletons remains unchanged *cofactor*: pyridoxal phosphate ***common pair** amino acid / keto acid in transamination reactions is **glutamate / α-ketoglutarate**, the other pair of acids depends on an enzyme specifity and bring the name to the enzyme (the enzyme is called according to this transaminated amino acid)*	*α-amino acid* + *α-keto acid* ↓↑ *α-keto acid* + *α-amino acid* alanine aminotransferase (ALT) aspartate aminotransferase (AST) BCAA aminotransferase (BCAA = Branched Chain Amino Acid)
* transketolases	transfer **two**-carbon-containing fragment (C2-fragment) *from a ketose to an aldose*; the ketose is thus shortened by 2 carbons and is converted to new aldose and the aldose is elongated by the 2 carbons and is converted to new ketose; the reactions are reversible *cofactor*: TDP = TPP (thiamine diphosphate = thiamine pyrophosphate)	transketolase (from the pentose phosphate pathway)
* transaldolases	transfer **three**-carbon-containing fragment (C3-fragment) *from a ketose to an aldose*; the ketose is thus shortened and converted to new aldose, the original aldose is simultaneously converted to new ketose longer by 3 carbons; the reactions are reversible	transaldolase (from the pentose phosphate pathway)
hydrolases **EC 3**	catalyse a hydrolytical cleavage of a hydrolyzable molecule (composed of two or more building blocs): released building blocs are transferred to water; organic *cofactor* is not common *the name of the cleaved molecule or bond is used to name the enzyme (e.g. peptidase)*	**A-B + H_2O → A-H + B-OH**
* nucleases	cleave nucleic acids	DNAases, RNAases
* nucleotidases	cleave nucleotides to nucleosides and phosphate; belong among esterases (phosphatases)	

* nucleosidases	cleave nucleosides to ribose and the N-containing heterocyclic base; they belong among glycosidases (cleave N-glycosidic bond)	
* proteases	cleave proteins; they are also called peptidases	
* peptidases	cleave peptide bonds in peptides and proteins; *exopeptidases* split off a terminal amino acid (aminopeptidases from N-terminus, carboxypeptidases from C-terminus of a peptide chain), *endopeptidases* split a peptide chain within, between amino acids to which they are specific	
* glycosidases	cleave O- or N-glycosidic bond between a saccharide and an alcohol- or amino- group of another substance; in oligosaccharides and polysaccharides they cleave O-glycosidic bonds between *monosaccharides to which they are specific (it is also a bond-specific cleavage)*	
* lipases	cleave neutral lipids, i.e. *triacylglycerols* (TAG) or *diacylglycerols* (DAG) or *monoacylglycerols* (MAG); they are more or less specific to a position of an ester bond in the substrate that is cleaved (at the 1st, 2nd or 3rd carbon of glycerol); belong among esterases	lipoprotein lipase adipocyte triacylglycerol lipase hormone-sensitive lipase monoacylglycerol lipase
* phospholipases	cleave glycerophospholipids and are specific to an ester bond they split: see 1) split off a terminal fatty acid (FA) 2) split off a FA from the middle carbon of glycerol 3) split of both FAs 4) split the bond between glycerol and phosphate 5) split the bond between phosphate and a terminal „polar head“ of a phospholipid	1) phospholipase A1 2) phospholipase A2 3) phospholipase B 4) phospholipase C 5) phospholipase D
* esterases	cleave ester bonds *(cholesterol esterase split off a fatty acid from cholesterol esters)*	cholesterol esterase
* phosphatases	cleave esters of phosphoric acid, i.e. it is a specific esterase; phosphoric acid is released as an anorganic phosphate (P_i = often pictured as „P in the ring“)	protein phosphatase glucose-6-phosphatase
* phosphodiesterases (PDE)	cleave an ester bond in a molecule in which phosphoric acid is bound by two ester bonds to two neighboring substances containing an alcohol group *(ester is formed by condensation of an acid with an alcohol, hence it can be cleaved by hydrolysis)*	nucleases phospholipase C phospholipase D cAMP phosphodiesterase cGMP phosphodiesterase

lyases **EC 4**	split off a small molecule (H_2O, CO_2) from a substrate - it is not a hydrolytical cleavage	**A-x ↔ B + x**
* dehydratases	split off water molecule from a substrate, this reaction is reversible, i.e. dehydratases also catalyse an addition of water to a double bond (hence their another name hydratases)	carbonate dehydratase *(= carbonic anhydrase)* fumarase = fumarate hydratase
* decarboxylases	split off CO_2 from a substrate, this reaction is irreversible *cofactor*: pyridoxal phosphate (PALP)	glutamate decarboxylase aromatic amino acid decarboxylase
isomerases **EC 5**	catalyse a conversion of isomers they are often called according to a type of isomerization in metabolism of saccharides the name „isomerase“ is used for enzymes converting an aldose to a ketose (or back) (glucose-6-phosphate ↔ fructose-6-phosphate)	**A ↔ isoA**
* epimerases	catalyse the conversion of epimers (isomers of saccharides differing in only one secondary -OH group spatial arrangement) (proceeds in a form of UDP-derivatives of monosaccharides, e.g. UDP-galactose ↔ UDP glucose)	UDP-glucose-4-epimerase
* mutases	catalyse a movement of a phosphate group within a molecule (glucose-1-phosphate ↔ glucose-6-phosphate)	phosphoglucomutase
* racemases	catalyse the conversion of one optical isomer to the other (D-amino acid ↔ L-amino acid)	racemase of amino acids
ligases **EC 6**	need energy from a simultaneous hydrolysis of ATP for their function; ATP is cleaved but products of this cleavage are not incorporated into a substarte (as it is in kinases) ATP is also cleaved by enzymes known as ATP-ases (pumps), but the energy released from ATP is used for transport of ions rather than for chemical conversion of substances, see the new class EC 7 *(e.g. Na/K-ATPase is not a hydrolase, nor ligase but translocase)*	**A + B + ATP** **→ A-B + ADP + P_i**
* carboxylases	elongate a carbon skeleton of a substrate by one carbon: they incorporate CO_2 into the substrate and thus form a new carboxylic acid functional group; the reaction is irreversible *cofactor*: biotin	pyruvate carboxylase acetyl-CoA carboxylase
* synthetases	some are also called synthases *(= general name for enzymes that synthesize something)*; if called synthetases they always need a simultaneous cleavage of ATP for energy, whereas synthases also catalyse reactions not ATP-dependent (the name *synthase* is used for enzymes of various other EC classes as well)	glutamine synthetase acyl-CoA synthetase aminoacyl-tRNA synthetase karbamoyl phosphate synthetase (or synthase)

translocases **EC 7**	are enzymes that catalyse the movement of ions or molecules across membranes or their separation within membranes (exchange transporters that are not dependent on enzyme-catalysed reactions, such as the exchange of ions across membranes, are not included) subclasses designate the types of ion or molecule translocated, sub-subclasses concern the reaction that provides the driving force for the translocation	**NEW ENZYME CLASS (from the year 2018)** **A (side 1) → A (side 2)**
* *miscelaneous names* *(based on a reaction catalysed by the enzyme, see names of the enzymes in EC 1 – EC 6)*	there are enzymes of various names, formerly included in previous EC classes (reductase, hydrolase, synthase, ...), now forming a new EC class of enzymes involved in transport processes through membranes and within membranes * enzymes of the electron transport chain (ETC) transferring H^+ into an intermembrane space of mitochondrion * enzyme producing most of ATP in cells by the process called oxidative phosphorylation (ATP-synthase) * enzymes catalysing an active transport of ions through membranes with simultaneous ATP cleavage (ATP-ases, pumps) * enzymes involved in transport of other substances through membranes (e.g. xenobiotics, proteins into mitochondria,….) * enzymes involved in rearrangement of membranes (flippases = transporters of phospholipids: they exchange phospholipids between outer and inner membrane leaflets with a simultaneous ATP cleavage)	*NADH-ubiquinone reductase* (see Complex I of ETC): transport of H^+ from the matrix into an intermembrane space of a mitochondrion *ubiquinol-cytochrome-c reductase* (see Complex III of ETC): transport of H^+ from the matrix into an intermembrane space of a mitochondrion *ATP-synthase*: transport of H^+ from the intermembrane space back into the matrix of a mitochondria (with a simultaneous synthesis of ATP in the process called oxidative phosphorylation) *Na^+/K^+ ATP-ase* (Na/K-pump): efflux of 3 Na^+ with influx of 2 K^+ againts their concentration gradients; participates in a maintaining of a membrane potential (negative inside) *H^+/K^+ ATP-ase*: an antiporter of the stomach mucosa *ABC-type xenobiotic transporter* (also called P-glykoprotein); ABC = ATP-Binding-Cassette *mitochondrial protein-transporting ATP-ase*: transport of proteins into a mitochondrion using a protein TIM complex
	(see sample enzymes on the right)	

NAMES OF ENZYMES – exercise: tasks to reactions found on **pages 77 - 79**

a) to all reactions draw the rational *structural formulas* of reactants and products (recommendation: draw the formulas vertically)

b) to each reaction write the *type of the reaction* – it is about the chemical change the reaction describes; mark *the changes by a colored pencil*

c) using the type of the reaction try to identify the *EC enzyme class* of the enzyme catalyzing the reaction

d) make a note whether the reaction is *reversible / irreversible* under physiological conditions

e) use the following *group common names* of enzymes and pair the proper enzyme name with each of the reactions:

aminotransferase	dehydrogenase	decarboxylase	phosphatase	phosphotransferase	lipase
hydratase	carboxylase	kinase	mutase	racemase	synthetase

f) create *the whole common enzyme name* of each enzyme catalyzing the individual reactions
(eg. dehydrogenase → *alcohol* dehydrogenase if alcohol is dehydrogenated)

g) from the following cofactors, choose the correct one used by the individual enzyme (only in case the enzyme uses some cofactor):

ATP	biotin	FAD	NAD^+	pyridoxal phosphate (PLP)

h) in case of cofactors derived from vitamins specify the *name of the vitamin*, from which the cofactor is derived

1) **lactate** → **pyruvate** **succinate** → **fumarate**

2) **alanine + α-ketoglutarate → pyruvate + glutamate** **glucose + ATP** *(don't draw)* → **glucose-6-phosphate + ADP**

3) **glucose-6-phosphate + H_2O → glucose + phosphate**

TAG + H_2O → DAG + fatty acid

4) **glutamate → γ-aminobutyrate + CO_2**

fumarate + H_2O → malate

5) **glucose-6-phosphate** → **glucose-1-phosphate**

L-serine → **D-serine**

6) **pyruvate + CO_2 + ATP** *(don't draw)* → **oxaloacetate + ADP + P_i**

glutamic acid + NH_3 + ATP *(don't draw)* → **glutamine + ADP + P_i**

Regulation of metabolic pathways

GLYCOLYSIS

regulatory enzymes	activation	inhibition
hexokinase		• glucose-6-phosphate
glucokinase	• insulin *(induction)* • fructose-1-phosphate *(liver)*	• fructose-6-phosphate
6-phosphofructo-1-kinase (6-PFK-1) *(key enzyme)*	• decreased ratio ATP / AMP • fructose-2,6-bisphosphate *(increased in increased ratio insulin / glucagon)* • insulin *(induction)*	• increased ratio ATP / AMP • citrate • acidic pH
pyruvate kinase	• insulin *(induction)* • fructose-1,6-bisphosphate *(feed forward regulation)*	• glucagon *(repression, inhibition by phosphorylation)* • increased ratio ATP / AMP • acetyl-CoA

GLUCONEOGENESIS

regulatory enzymes	activation	inhibition
pyruvate carboxylase	• acetyl-Co A • cortizol, glucagon *(induction)*	• insulin *(repression)*
phosphoenolpyruvate carboxykinase	• cortizol, glucagon *(induction)*	• insulin *(repression)*
fructose-1,6-bisphosphatase	• cortizol, glucagon *(induction)*	• decreased ratio ATP / AMP • fructose-2,6-bisphosphate *(increased in increased ratio insulin / glucagon)* • insulin *(repression)*
glucose-6-phosphatase	• cortizol, glucagon *(induction)*	• insulin *(repression)*

METABOLISM OF GLYCOGEN

regulatory enzymes	activation	inhibition
glycogen phosphorylase *(degradation of glycogen)*	• glucagon, adrenaline *(phosphorylation)* • decreased ratio ATP / AMP • Ca^{2+} *(muscle)*	• increased ratio ATP / AMP • glucose-6-phosphate • glucose
glycogen synthase *(synthesis of glycogen)*	• insulin *(induction)* • glucose-6-phosphate	• glucagon, adrenaline *(phosphorylation)*

PENTOSE PHOSPHATE PATHWAY

- increased ratio NADPH / $NADP^+$ inhibits glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase
  (= regulatory enzymes, they require $NADP^+$ for their function)

LIPOLYSIS

regulatory enzymes	activation	inhibition
hormone sensitive lipase *(adipocytes)*	• catecholamines, glucagon, ACTH *(phosphorylation)*	• insulin • prostaglandins
lipoprotein lipase *(endothelium surface)*	• insulin • apolipoprotein C-II	

BETA-OXIDATION

- increased supply of fatty acids (see regulation of lipolysis)
- velocity of fatty acid transport into a mitochondrion: carnitine-palmitoyltransferase I (= carnitine acyltransferase I) is inhibited by increased malonyl -CoA
- ratio of FAD/$FADH_2$ a NAD^+/NADH (more reduced forms → inhibition)
- consumption of acetyl-CoA by subsequent reactions

KETOGENESIS

- increased production of acetyl-CoA in the LIVER due to an increased concentration of FA in blood (it is related to the regulation of lipolysis and β-oxidation)

regulatory enzymes	activation	inhibition
hormon sensitive lipase *(lipolysis in fatty tissue)*	• decreased insulin • catecholamines	• increased insulin
carnitine acyltransferase I *(transport of FA into mitoch.)*		• malonyl-Co A • increased ratio insulin / glucagon

SYNTHESIS OF FATTY ACIDS

regulatory enzymes	activation	inhibition
acetyl-CoA carboxylase *(key enzyme)*	• citrate • insulin • low-fat, energy rich, high-saccharide diet *(induction)*	• acyl-CoA (C_{16} -C_{18}) • glucagon *(phosphorylation, repression)* • lipid rich diet, starvation *(repression)*
fatty acid synthase	• phosphorylated saccharides • low-fat, energy rich, high-saccharide diet *(induction)*	• glucagon *(phosphorylation, repression)* • lipid rich diet, starvation *(repression)*

SYNTHESIS OF TRIACYLGLYCEROLS (TAG)

- availability of acyl-CoA as a consequence of both fatty acid synthesis de novo and releasing of fatty acids from TAG

regulatory enzymes	activation	inhibition
phosphatidic acid phosphatase	• steroid hormones *(induction)*	
lipoprotein lipase *(related to storage of TAG in adipocytes)*	• insulin • apolipoprotein C-II	

UREA CYCLE

- allosterical regulation
- induction of the enzymes by protein-rich diet or by metabolic changes during starvation
- increased degradation of amino acids leads to an increased Gln synthesis, which results in adding more ornithine to the urea cycle (its capacity increases)
- in relation to acid-base balance (ABB): acidosis ⇒ inhibition of the urea cycle (salvage of HCO_3^-)

regulatory enzymes	activation	inhibition
karbamoylphosphate synthetase I	• N-acetylglutamate	
N-acetylglutamate synthetase	• arginine	

PYRUVATE DEHYDROGENASE COMPLEX (PDH)

- competitive inhibition by products (acetyl-CoA and NADH)
- interconversion of PDH: phosphorylated form is inactive, dephosphorylated form is active (= principal regulation mechanism)

regulatory enzymes	activation	inhibition
pyruvate dehydrogenase complex (PDH)	• Ca^{2+} • insulin *(adipocytes)* • catecholamines *(heart)*	• increased ratio NADH / NAD^+ • acetyl-CoA

KREBS CYCLE

- availability of substrates
- consumption of products by subsequent reactions

regulatory enzymes	activation	inhibition
citrate synthase		• increased ratio NADH / NAD^+ • succinyl-CoA
isocitrate dehydrogenase *(key enzyme)*	• decreased ratio ATP / ADP	• increased ratio NADH / NAD^+ • increased ratio ATP / ADP
2-oxoglutarate dehydrogenase	• Ca^{2+}	• increased ratio NADH / NAD^+ • increased ratio ATP / ADP • GTP • succinyl-CoA

RESPIRATORY CHAIN (ETC) AND ATP-SYNTHESIS

- availability of O_2
- ratio of NADH / NAD^+ (oxidation of NADH to NAD^+ in ETC leads to an increase of proton gradient ⇒ more ATP can be synthesized)
- availability of ADP for ATP synthesis
- uncoupling proteins: they uncouple ETC from ATP-synthesis by decreasing a proton gradient: ETC is activated, but ATP-synthesis inhibited

SYNTHESIS OF HEME

- **in erythrocyte precursors the heme synthesis is controlled by iron availability**

regulatory enzymes	activation	inhibition
delta-aminolevulinic acid synthase (ALA-synthase)	• many drugs and metabolites *(induction) – in the liver*	• heme, hematin *(repression, inhibition of activity) – in the liver*

SYNTHESIS OF CHOLESTEROL

regulatory enzymes	activation	inhibition
HMG-CoA reductase	• insulin, tyroxine *(induction)*	• cholesterol • glucagon *(repression)* • oxosterols *(repression)*

SYNTHESIS OF NUCLEOTIDES *(see Course 4)*

- **PRDP-synthetase:** inhibited by both purine and pyrimidine nucleotides (nucleoside diphosphates, nucleoside triphosphates) = *feed back inhibition*
- **nucleoside diphosphate reductase**: activated by nucleoside triphosphates, inhibited by deoxyadenosine triphosphate (dATP)
- **key regulatory enzymes**:

regulatory enzymes	activation	inhibition
glutamine-PRDP-amidotransferase (purines)	• PRDP	• IMP, GMP, AMP *(allosterical inhibition)*
carbamoylphosphate synthetase II (pyrimidines)	• PRDP • ATP	• UTP

Mechanisms of metabolic pathways regulation taken from these textbooks:
Devlin, T. M. (editor): Textbook of Biochemistry with Clinical Correlations, 4th ed. Wiley-Liss, Inc., New York, 1997. ISBN 0-471-15451-2
Koolman, J.; Röhm, K., H.: Color Atlas of Biochemistry. Thieme, Stuttgard, 1996. ISBN 3-13-100371-5

APPENDIX

The solution to the tasks is provided in the appendix so that you can check your own answers. I do not recommend copying the answers to the blank maps from the study materials or from the appendix. If you first think about the questions yourself and fill in the blank maps from memory based on what you've already learned, this workbook will serve its purpose: it will help you understand in context what knowledge you should have about each metabolic pathway. And if you don't understand something enough to be able to solve the tasks in the workbook yourself, ask questions in the biochemistry seminars: your teachers will be happy to explain what will be needed.

Organic acids in metabolism / picture 1 / pages 4 - 5

a) acetyl-CoA, acetic acid, acetate, lactate, pyruvate, alanine, glycine, succinyl-CoA, succinate, fumarate, malate, oxaloacetate, aspartate, asparagin, malonyl-CoA, acetone, acetoacetate, β-hydroxybutyrate, α-ketoglutarate, glutamate, glutamine

b) C2/ acetyl-CoA, acetic acid, acetate (+ *glycine, but it is not formed from* acetic acid in the body): condensation of acetic acid with coenzyme A (CoA-SH, where the -SH group enters the reaction with acid) produces acetyl-CoA and water (*thioester bond is formed between the acetic acid residue, the so-called acetyl and coenzyme A, in this reaction ATP is consumed)*, the opposite reaction to condensation is hydrolysis, i.e. acetyl-CoA is hydrolytically cleaved into acetic acid and coenzyme A / acetic acid in water releases a proton (H^+), i.e. the conversion of acetic acid to acetate is dissociation

C3/ lactate, pyruvate, alanine: lactate is oxidized to pyruvate, which is converted to alanine by transamination / alanine is converted by transamination to pyruvate, which is reduced to lactate (*this is how these substances are mutually converted into each other in metabolism, it is a connection between glucose and protein metabolism)* / oxidation of lactate to pyruvate is referred to as dehydrogenation (two hydrogens are lost), the opposite reaction is hydrogenation of pyruvate to lactate / transamination requires two reactants: a keto acid and an amino acid, when these substances sort of exchange functional groups, i.e. the keto acid gives rise to an amino acid with the same number of carbons as was in the keto acid, and the amino acid gives rise to a keto acid with the same number of carbons as was in the amino acid *(glutamate is a source of the amino group for the conversion of pyruvate to alanine, from glutamate α-ketoglutarate is formed in this reaction; analogously, alanine is converted to pyruvate in the reaction with α-ketoglutarate, from which glutamate is formed in this reaction)*

C4/ succinyl-CoA, succinate, fumarate, malate, oxaloacetate, aspartate, asparagine *(in this order, these substances are also converted into each other in metabolism)*: succinyl-CoA is hydrolysed and converted to succinic acid, which dissociates to succinate *(both a weak acid and its anion are always present in an aqueous solution, so in biochemistry we usually speak only of an anion and its name and formula are used, thus, the name of the acid is "interchangeable" with the name of its anion);* succinate is converted to succinyl-CoA by condensation with CoA-SH (*see analogy between acetyl-CoA and acetate above*) / succinate is oxidized to fumarate (dehydrogenation); fumarate is reduced to succinate (hydrogenation) / fumarate is converted to malate by addition of water (hydration); malate is transformed into fumarate by elimination of water (dehydration) *(only this trans isomer of butenedioic acid is formed enzymatically)* / malate is converted to oxaloacetate by oxidation (dehydrogenation); oxaloacetate is converted to malate by reduction (hydrogenation) / oxaloacetate is converted to aspartate by transamination; aspartate is converted by transamination to oxaloacetate / aspartate (more precisely aspartic acid) is converted by condensation with ammonia to asparagine, which is an amide of aspartic acid *(asparagine is not formed in metabolism by the reaction of aspartate with ammonia, but glutamine is the source of amide nitrogen);* asparagine is hydrolytically broken down into ammonia and aspartic acid, which dissociates to aspartate in water *(POINT: asparagine is converted to aspartate, so both of these amino acids are connected with the Krebs cycle: aspartate transaminates to oxaloacetate, which is an intermediate of the Krebs cycle; oxaloacetate is formed in the Krebs cycle by a sequence of reactions: succinyl-CoA - succinate - fumarate - malate - oxaloacetate and oxaloacetate can then form aspartate and asparagine, which are amino acids found in proteins)*

C3/ malonyl-CoA, acetone: metabolically unrelated; malonyl-CoA is an acyl (= acid rest) of malonic acid, formed in metabolism from acetyl-CoA by carboxylation (i.e. reaction with CO_2 leading to the elongation of the molecule by the -COOH group); acetone, on the other hand, is formed by decarboxylation of acetoacetate (loss of the carboxyl group, which is released as CO_2)

C4/ acetoacetate, β-hydroxybutyrate: acetoacetate is reduced to β-hydroxybutyrate (hydrogenation), while β-hydroxybutyrate is oxidized (dehydrogenation) to acetoacetate; as an unstable so-called beta-keto acid, it spontaneously decarboxylates to acetone, so acetone is actually formed from β-hydroxybutyrate in two steps (POINT: this is why these three substances are referred to in medicine as "ketone bodies", because acetone is a ketone)

C5/ α-ketoglutarate, glutamate, glutamine *(similar to the triad of oxaloacetate, aspartate, asparagine):* α-ketoglutarate is converted to glutamate (and vice versa) by transamination, glutamate is converted to glutamine (= glutamate amide) by condensation with ammonia, glutamine is hydrolytically broken down into glutamate and ammonia *(this is how glutamine and glutamate are converted to α-ketoglutarate, which is an intermediate of the Krebs cycle, they can also be formed from α-ketoglutarate)*

c) 1) hydoxy-derivatives: lactate, malate, β-hydroxybutyrate; 2) keto-derivatives: pyruvate, oxaloacetate, acetoacetate, α-ketoglutarate; 3) amino acids: alanine, glycine, aspartate and glutamate; 4) amides: asparagine and glutamine; 5) acetyl-CoA, succinyl-CoA, malonyl-CoA; 6) acetone is a ketone

a) the inner mitochondrial membrane is shown: the intermembrane space is at the top, the mitochondrial matrix at the bottom

b) complex I regenerates (reoxidizes) NADH to NAD^+, electrons enter the respiratory chain, H^+ moves to the intermembrane space; NADH is formed mainly in the Krebs cycle and β-oxidation of fatty acids, also during the oxidation of β-hydroxybutyrate to acetoacetate (degradation of ketone bodies), during the degradation of branched-chain amino acids and alcohol, etc.; NADH originating from glycolysis (or generally from the cytosol) cannot be – as the whole molecule - transported into mitochondria, instead shuttle mechanisms are used (the malate-aspartate shuttle also transfers electrons to complex I)

c) complex II = succinate dehydrogenase (= enzyme of the Krebs cycle), its cofactor is FAD (prosthetic group permanently bound in the enzyme molecule), the reduced enzyme contains $FADH_2$, which is regenerated by transferring electrons directly to coenzyme Q (ubiquinone), protons (H^+) from complex II do not move to the intermembrane space

d) oxidized form: ubiquinone, reduced form: ubiquinol

e) 1) mitochondrial glycerol-3-phosphate dehydrogenase (found in the inner membrane of the mitochondria), its substrate is glycerol-3-phosphate and its product is dihydroxyacetone phosphate; it is part of the glycerol phosphate shuttle that connects glycolysis to the respiratory chain: it brings electrons from NADH formed in glycolysis; (2) ETF-dehydrogenase, transfers electrons from ETF (Electron-Transferring-Flavoprotein), which takes electrons from acyl-CoA dehydrogenase, which is the first enzyme of β-oxidation of fatty acids

f) cytochromes contain heme with Fe^{3+} as a prosthetic group: it is reduced to heme-Fe^{2+} by accepting an electron and is regenerated to heme-Fe^{3+} after the electron is passed on; thus, cytochrome c can accept only one electron from complex III in one cycle, and then transfers the electron to complex IV

g) complex IV = cytochrome oxidase (cytochrome c oxidase); cyanide (CN^-)

h) $2\ e^- + 2\ H^+ + ½\ O_2 \rightarrow H_2O$ ($4\ e^- + 4\ H^+ + O_2 \rightarrow 2\ H_2O$)

i) see lecture

j) ATP-synthase works like an inverted proton pump: it contains a channel through which H^+ ions return from the intermembrane space (i.e. from a higher concentration) to the matrix, the energy of this proton transport (along a gradient) is used to synthesize ATP from ADP and phosphate; ATP/ADP translocase is necessary for ATP synthesis: it transfers ADP to the matrix in exchange for ATP; UCP is an ion (H^+) channel, i.e. it allows protons to pass along a gradient from the intermembrane space into the matrix: the energy released in this way is converted into heat, resulting in reduced ATP production by ATP-synthase (the proton gradient created by the respiratory chain decreases, UCP thus "uncouplers" the respiratory chain from ATP production)

Krebs cycle / picture 3 / pages 8 - 9

a) acetyl-CoA is a molecule drawn above reaction No. 1 at the very top, it is formed during the breakdown of lipids, carbohydrates and proteins: it is a product of β-oxidation of fatty acids and pyruvate dehydrogenase complex (i.e. it is formed from pyruvate produced by glycolysis, by oxidation of lactate and from degradation of certain amino acids), it is also a product of the degradation of ketone bodies and ketogenic amino acids, and is also formed during the breakdown of ethanol (i.e. alcohol)

b) succinate (E) → fumarate (F) → malate (G) → oxaloacetate (H); reactions:
8) succinate dehydrogenase (= complex II), dehydrogenation: FAD → $FADH_2$
9) fumarase (fumarate hydratase), hydration: + H_2O
10) malate dehydrogenase, dehydrogenation: $NAD^+ \rightarrow NADH + H^+$

c) shortening of the molecule by one carbon: reactions No. 5 + 6; No. 4 + 5 = isocitrate dehydrogenase, oxidative decarboxylation: isocitrate (B) + NAD^+ → (oxalosuccinate) + NADH + H^+ → α-ketoglutarate (C) + CO_2
No. 6 = α-ketoglutarate dehydrogenase, oxidative decarboxylation of α-keto acid, this reaction is similar to pyruvate dehydrogenase reaction, contains the same cofactors *(thiamine diphosphate = TDP, lipoic acid, coenzyme A, FAD, NAD^+)*: α-ketoglutarate (C) + NAD^+ + CoA-SH → succinyl-CoA (D) + CO_2 + $NADH+H^+$

d) reactions 4, 6, 10; NADH enters the respiratory chain through complex I, where it transfers two electrons, while transporting H^+ to the intermembrane space, NAD^+ returns to the mitochondrial matrix

e) α-ketoglutarate (C) is formed by transamination of glutamate (Glu), oxaloacetate (H) is formed by transamination of aspartate (Asp), *formulas on page 63*

f) oxaloacetate (H), formed by carboxylation of pyruvate, pyruvate carboxylase: pyruvate + CO_2 + ATP $\rightarrow$ oxaloacetate + ADP + phosphate; cofactor biotin, pyruvate: CH_3-CO-COO^-

g) citrate synthase (1), isocitrate dehydrogenase (4+5), α-ketoglutarate dehydrogenase (6); general regulation of the Krebs cycle: inhibition by an increased NADH/NAD^+ ratio (it occurs also when there is a lack of oxygen in the cell, if the respiratory chain is inhibited) and an increased ATP/ADP ratio (i.e. when there is enough ATP in the cell); citrate synthase is also regulated at the level of substrate availability (oxaloacetate and acetyl-CoA): if there is a lack of oxaloacetate (unstable keto acid), acetyl-CoA cannot be degraded in the Krebs cycle

h) reaction No. 7: during the cleavage of CoA-SH from succinyl-CoA (D) (thioester bond is cleaved), the released energy is used for the synthesis of GTP from GDP and phosphate (or directly ATP from ADP and phosphate: depending on the type of isoenzyme); if GTP is formed, it can subsequently enter into the reaction: GTP + ADP $\rightarrow$ GDP + ATP

i) succinate dehydrogenase (8) is also referred to as complex II of the respiratory chain; it is a flavoenzyme of the Krebs cycle, its prosthetic group FAD is reduced to $FADH_2$ during the conversion of succinate (E) to fumarate (F), and then is regenerated back to FAD by transferring electrons to the ubiquinone of the respiratory chain (*complex II thus participates only in the KC: it can obtain electrons only from succinate, $FADH_2$ is not released from the enzyme like NADH)*

<u>Reactions of pyruvate</u> / picture 4 / page 10 - 11

a) reaction D: pyruvate + NADH + H^+ $\rightarrow$ lactate + NAD^+ *(formulas on page 5)*; it allows to regenerate (reoxidize) NADH in the cytosol (without the need to use the respiratory chain); proceeds if NADH / NAD^+ ratio is increased, i.e. even under anaerobic conditions, NAD^+ is again available for glycolysis, which thus ends not with pyruvate, but with lactate

b) reaction C: pyruvate + CoA-SH + NAD^+ $\rightarrow$ acetyl-CoA + CO_2 + NADH + H^+; pyruvate must first be transported from the cytosol to the mitochondrial matrix, where the reaction is catalyzed by the pyruvate dehydrogenase complex (PDH), containing 5 cofactors: thiamine diphosphate = TDP, lipoic acid, CoA-SH, FAD, NAD^+; acetyl-CoA can be further degraded to 2 CO_2 in the Krebs cycle, NADH enters the respiratory chain and participates in the formation of ATP by aerobic phosphorylation (= the respiratory chain + ATP synthase)

c) reaction A: pyruvate + *glutamate* $\rightarrow$ alanine + *α-ketoglutarate (formulas on page 4),* alanine aminotransferase, cofactor: pyridoxal phosphate, cytosol

d) reaction C (formation of acetyl-CoA) and reaction B (formation of oxaloacetate), both the products are substrates of citrate synthase (reaction 1 of the Krebs cycle)

e) reaction B: pyruvate + CO_2 + ATP $\rightarrow$ oxaloacetate + ADP + phosphate, pyruvate carboxylase (regulatory enzyme of gluconeogenesis from pyruvate, i.e. also from some amino acids, or from lactate), cofactor biotin, mitochondria

f) reaction A (formation and degradation of alanine), reaction B (aspartate is formed from oxaloacetate by transamination and the other way round)

g) reaction E: the product is acetaldehyde, which is enzymatically reduced to ethanol by NADH + H^+ *(alcoholic fermentation of glucose)*

h) reactions A and D take place in the cytosol (they are reversible), reactions B and D take place in the mitochondrial matrix (under physiological conditions they are irreversible)

i) oxaloacetate enters the Krebs cycle (the conversion of pyruvate to oxaloacetate is the main anaplerotic reaction of KC) or is a substrate for amino acid synthesis (transamination produces aspartate and subsequent amidation produces asparagine), it is also an intermediate of gluconeogenesis, i.e. it can be converted to glucose

j) acetyl-CoA enters the Krebs cycle (where it is degraded to 2 CO_2), or it can be converted to fatty acids, cholesterol or ketone bodies

k) pyruvate is a product of glycolysis, it is also formed during the degradation of some amino acids and also by oxidation of lactate

Glycolysis / picture 5 / pages 12 - 13

a) substrate phosphorylation (also substrate-level phosphorylation) is a reaction in which ATP is produced independently of the respiratory chain, which is necessary for oxidative phosphorylation (i.e. ATP synthesis by ATP synthase), the substrate of the substrate-level phosphorylation is some "macroergic = energy rich") compound, during the cleavage of which the released energy is used to form ATP: *1,3-bisphosphoglycerate = 1,3-BPG* (reaction No. 7, the products are 3-phosphoglycerate and ATP) and *phosphoenolpyruvate = PEPA* (reaction No. 10, the products are pyruvate and ATP)

b) reaction No. 6: glyceraldehyde-3-phosphate + NAD^+ + phosphate $\rightarrow$ 1,3-bisphosphoglycerate + NADH + H^+ *(during oxidation of the substrate carboxylic acid is formed from aldehyde, the phosphate is bound by an anhydride bond, i.e. 1,3-BPG is a "macroergic" compound from which ATP is formed in a subsequent reaction*); regeneration of NADH takes place in any reaction in which NADH serves as a source of reduction equivalents (in the form of hydrogens, i.e. it is a donor of two electrons that reduce the substrate): the three main examples are 1) *product of glycolysis* pyruvate + NADH + H^+ $\rightarrow$ lactate + NAD^+ (cytosol, lactate dehydrogenase), or 2) *an intermediate of glycolysis* dihydroxyacetone phosphate (DHAP) + NADH + H^+ $\rightarrow$ glycerol-3-phosphate + NAD^+ (cytosol, glycerol-3-P-dehydrogenase, which is involved in the glycerol phosphate shuttle), or an *intermediate of metabolism (e.g. of amino acids)* oxaloacetate + NADH + H^+ $\rightarrow$ malate + NAD^+ (cytosol, malate dehydrogenase, involved in the malate-aspartate shuttle); shuttles are used to transport reduction equivalents into the respiratory chain proceeding in the mitochondria under aerobic conditions

c) reaction No. 1 (hexokinase or glucokinase): glucose + ATP $\rightarrow$ glucose-6-P + ADP; reaction No. 3 (6-phosphofructo-1-kinase): fructose-6-P + ATP $\rightarrow$ fructose-1,6-bisphosphate + ADP

d) reaction No. 4: fructose-1,6-bisphosphate $\rightarrow$ dihydroxyacetone phosphate = DHAP (top) + glyceraldehyde-3-phosphate

e) reaction No. 2: glucose-6-P $\rightarrow$ fructose-6-P (aldose/ketose); reaction No. 5: dihydroxyacetone phosphate$\rightarrow$ glyceraldehyde-3-P (ketose/aldose); reaction No. 8: 3-phosphoglycerate $\rightarrow$ 2-phosphoglycerate (mutase, i.e. it transfers phosphate within the molecule); in the reaction No. 10 phosphate is first cleaved off: the product enolpyruvate is unstable, therefore it isomerizes to a stable keto derivative pyruvate, these two molecules are tautomers (keto/enol isomers)

f) DHAP (one of two aldolase products) is converted by isomerization to the second glyceraldehyde-3-P, which acts as a substrate of reaction No. 6, i.e. for this reaction there are two substrates available

g) 1,3-BPG **(**product of reaction No. 6): if there is a lack of oxygen (hypoxia) in a tissue, 1,3-BPG is isomerized to 2,3-BPG in erythrocytes; this isomer serves as a regulatory molecule: it binds to hemoglobin and thus reduces its affinity for oxygen $\rightarrow$ the consequence is the release of oxygen from the erythrocyte into the hypoxic tissue; during the degradation of 2,3-BPG, ATP is not formed, because both phosphates are bound by an ester bond in this molecule (in 1,3-BPG, the *first phosphate is bound by an anhydride bond), so 2,3-BPG* is not "macroergic" and the subsequent reaction does not work as substrate-level phosphorylation - ATP is not formed, only phosphate is cleaved off

(h) reaction No 1: glucokinase (GK), found in the liver and pancreatic B-cells, it has a lower affinity for glucose than hexokinase, which occurs in other cells; GK will therefore start phosphorylate glucose to a greater extent only at a higher concentration of glucose in the cell, which occurs only at a higher concentration of glucose in the blood, i.e. higher glycemia

i) glucose-6-P enters the pentose cycle, fructose-6-P and glyceraldehyde-3-P exit the pentose cycle (or enter in the opposite direction); for this reason, the pentose cycle can proceed either as a branch of glycolysis or as a closed cycle *(fructose-6-P and glyceraldehyde-3-P can be converted back to glucose-6-P, using some reactions of gluconeogenesis)*

j) reactions No. 1, 3 and 10 are strongly exergonic, i.e. they are only unidirectional under physiological conditions

k) *in general*, glycolysis is activated by insulin (after a meal), but also by a lack of energy in the cell (i.e. an increased AMP/ATP ratio) and is inhibited by glucagon (in the liver), but also by sufficient energy in the cell (increased ATP/AMP ratio); reaction No. 1: hexokinase, HK, is (unlike glucokinase, GK) inhibited by excess glucose-6-P (glucokinase is inhibited only by excess of fructose-6-P and is activated by fructose-1-P produced during the breakdown of fructose in the liver); reaction No. 3 is *a key regulatory reaction*: 6-phosphofructo-1-kinase (6-PFK-1) is inhibited by an increased ATP/A MP ratio, increased concentration of citrate and H^+ ions (i.e. decreased pH) and activated by increased AMP/ATP ratio and insulin; reaction No. 10: pyruvate kinase is inhibited by increased ATP/AMP ratio, increased acetyl-CoA concentration and glucagon, it is activated in a "step forward" mode by increased concentrations

of fructose-1,6-bisphosphate (an intermediate of glycolysis) and by insulin; regulation by insulin leads, among other things, to the synthesis of fructose-2,6-bisphosphate (it works as an "insulin messenger"), which activates 6-phosphofructo-1-kinase (6-PFK-1); both mentioned hormones affect the activity of regulatory enzymes that are already present in the cell, as well as regulate the transcription of genes encoding the regulatory enzyme of glycolysis (increasing or decreasing their amount in the cell)

Gluconeogenesis / picture 6 / pages 14 - 15

a) reactions C and B**,** catalyzed by kinases (HK or GK / 6-PFK-1) in glycolysis, are replaced by phosphatases in gluconeogenesis: kinases consume ATP, but phosphatases do not form ATP – they only release phosphate from the intermediate as inorganic phosphate (P_i); reaction C: glucose-6-phosphatase hydrolytically breaks down glucose-6-phosphate into glucose and P_i; reaction B: fructose-1,6-bisphosphatase hydrolytically breaks down fructose-1,6-bisphosphate to fructose-6-phosphate and P_i

b) glucose-6-phosphatase is found in the membrane of the endoplasmic reticulum of those cells that can deliver glucose to the blood: hepatocytes, renal tubular cells, enterocytes; hepatocytes produce glucose in gluconeogenesis or obtain it by breaking down glycogen (glucose-6-phosphatase is absent in muscles), gluconeogenesis also takes place in the kidneys, tubular cells of the kidneys and enterocytes let pass glucose through themselves: absorption of glucose from the primary urine or lumen of the intestine into the blood

c) reaction A is catalyzed in glycolysis by pyruvate kinase (irreversible reaction, as well as reactions catalyzed by HK/GK and 6-PFK-1), so in gluconeogenesis the conversion of pyruvate to phosphoenolpyruvate (PEP) is provided by two other enzymes, the intermediate product of this conversion is oxaloacetate:
1) pyruvate carboxylase (found only in mitochondria) catalyzes the reaction pyruvate + CO_2 + ATP $\rightarrow$ oxaloacetate + ADP + P_i; oxaloacetate must then cross the inner mitochondtrial membrane using a shuttle, where it is first converted to aspartate by transamination (mitochondrial isoenzyme of AST), in the cytosol aspartate is then transaminated back to oxaloacetate (cytosolic isoenzyme of AST); 2) phosphoenolpyruvate carboxykinase catalyzes the reaction oxaloacetate + GTP $\rightarrow$ PEP + CO_2 + GDP *(oxaloacetate structure on p. 5),* the second enzyme occurs in both the cytosol and the mitochondria (if the reaction takes place in the mitochondria, PEP is transported to the cytosol)

d) the Cori cycle is an interorgan cycle where lactate is extrahepatically produced by anaerobic glycolysis from glucose, 1 glucose $\rightarrow$ 2 lactates (e.g. in muscles, erythrocytes), lactate is then transported through the blood to the liver, where glucose is synthesized from 2 lactates, the new glucose is released to blood and can again be used in extrahepatic tissues (cycle: glucose $\rightarrow$ lactate $\rightarrow$ glucose); gluconeogenesis from lactate first requires conversion of lactate to pyruvate (lactate dehydrogenase in cytosol, cofactor NAD^+), then transport of pyruvate into the mitochondria, its conversion to oxaloacetate and subsequently to PEP (see point c); so if gluconeogenesis originates from lactate or pyruvate (pyruvate can be formed from some amino acids, e.g. alanine), part of it takes place in the mitochondria (pyruvate carboxylase + mitochondrial AST), other enzymes are found in the cytosol, only glucose-6-phosphatase in the endoplasmic reticulum – on the luminal side of its membrane *(the formula of lactate is on page 5)*

e) after lipolysis (= cleavage of triacylglycerols into fatty acids and glycerol) in adipose tissue, glycerol HO-CH_2-CH(OH)-CH_2-OH is released into the blood, from which it is taken by the liver and is first phosphorylated in hepatocytes by glycerol kinase to glycerol-3-phosphate HO-CH_2-CH(OH)-CH_2-O-P_i (glycerol + ATP $\rightarrow$ glycerol-3-phosphate + ADP), which is then oxidized in the cytosol by glycerol-3-phosphate dehydrogenase (NAD^+) to dihydroxyacetone phosphate: HO-CH_2-CO-CH_2-O-P_i (it is an intermediate product of gluconeogenesis); i.e. two glycerols form up 1 glucose, all reactions take place in the cytosol (except for the conversion of glucose-6-P $\rightarrow$ glucose, since the enzyme glucose-6-phosphatase is localized in the ER membrane)

f) 1) pyruvate is formed from alanine, alanine aminotransferase (ALT) (alanine + alpha-ketoglutarate $\rightarrow$ pyruvate + glutamate), 2) oxaloacetate is formed from aspartate (aspartate + alpha-ketoglutarate $\rightarrow$ oxaloacetate + glutamate), aspartate aminotransferase (AST)

g) all amino acids that are degraded into one of the intermediates of the Krebs cycle can be converted by this cycle up to oxaloacetate, which is also an intermediate product of gluconeogenesis, see point c); malate can also be transported from KC to the cytosol (the conversion of malate $\leftrightarrow$ oxaloacetate is catalyzed by malate dehydrogenase, which is the enzyme found in both the mitochondria and in the cytosol, cofactor NAD^+)

h) factors that activate glycolysis, inhibit gluconeogenesis and vice versa (insulin, glucagon, ATP/AMP ratio); acetyl-CoA activates pyruvate carboxylase

Degradation of glycogen / picture 7 / pages 16 -17

a) in the glucose formula, the C1 carbon is always on the right, i.e. the C4 carbon is on the left, and the bond that connects the adjacent glucose molecules is referred to as the α(1→4) glycosidic bond (α- indicates that the hemiacetal -OH group goes below the plane of the ring)

b) + c) reaction No. 1 is catalyzed by glycogen phosphorylase (= regulatory enzyme of glycogenolysis), its second substrate (a) is inorganic phosphate, because this enzyme is a transferase that catalyzes the so-called phosphorolytic (not hydrolytic) cleavage, when released glucose is transferred to an inorganic phosphate (not to water) and forms glucose-1-phosphate: α(1→4) bond was cleaved, therefore the phosphate binds to the -OH group on C1 of glucose; the enzyme is activated by glucagon and increased AMP/ATP ratio (in muscle also by increased Ca^{2+} concentration), and is inhibited by insulin, increased ATP/AMP ratio and by increased concentration of glucose-6-phosphate or glucose in the cell (i.e. "end" products)

d) reaction No. 2 is isomerization of glucose-1-phosphate to glucose-6-phosphate, in the molecule the phosphate has moved to another -OH group, enzyme: phosphoglucomutase

e) reaction No. 3 is the hydrolytic cleavage of glucose-6-phosphate into glucose and inorganic phosphate (D), enzyme: glucose-6-phosphatase, it is a hydrolase (EC 3) found on the luminal membrane of the ER, i.e. glucose-6-phosphate must be transferred to the ER, the products of its cleavage are then again transported out into the cytosol; the enzyme is found in hepatocytes, tubular cells of the kidneys and enterocytes, and is absent in muscles (therefore the cleavage of glycogen by glycogen phosphorylase in muscle does not provide free glucose which can be released into the blood: instead glucose-6-phosphate enters glycolysis in muscle, thus saving 1 ATP in glycolysis, which would otherwise be used for glucose phosphorylation by hexokinase)

f) if already phosphorylated glucose enters glycolysis, the net gain of such glycolysis is 1 ATP more than if glycolysis starts with free glucose (glycolysis from free glucose – e.g. taken from blood – produces 4 ATP, but 2 ATP are consumed at the beginning of glycolysis: the net gain is only 2 ATP; in case that glucose-6-phosphate enters the glycolysis directly, net gain are 3 ATP)

(g) substances labelled (a) and (d) are both 'inorganic phosphate', which is the term used for an equilibrium mixture of $H_2PO_4^-$ with HPO_4^{2-} that acts as a buffer: $H_2PO_4^- \leftrightarrow HPO_4^{2-} + H^+$

h) glycogenosis is one of the so-called storage disorders of metabolism, where a hereditary or acquired deficiency of one of the enzymes of glycogen metabolism leads to the accumulation of glycogen in cells; the consequence is damage to the tissues (liver, muscles) from excess glycogen and at the same time from the lack of a quick source of energy, i.e. glucose, released by the breakdown of glycogen

i) glucose-6-phosphatase deficiency (enzyme No. 3): phosphorylated glucose cannot be released from cells into the blood, therefore hypoglycemia occurs (liver glycogenolysis is the main source of blood glucose in fasting)

j) muscle glycogen phosphorylase deficiency (enzyme No. 1 and 4): during intensive muscle work, glucose released from muscle glycogen is the main source of energy for skeletal muscle; ATP deficiency leads to cramps and damage to muscle fibers, the myoglobin contained in them is released into the blood and can cause a kidney damage

k) reaction No. 5 (transfer of trisaccharide from the branching site to the linear part of the glycogen molecule, where glycogen phosphorylase then continues in degradation of glycogen) and reaction No. 6, that is a cleavage of free glucose from the branching site: α(1→6) is cleaved hydrolytically (not phosphorolically), so not phosphorylated but free glucose is formed

l) free glucose is the product of hydrolase activity of the debranchig enzyme; while glucose-1-phosphate is formed by phosphorylase activity of glycogen phosphorylase, which cannot cleave branching sites

m) reactions No. 4 and 7 are catalyzed by glycogen phosphorylase: from the non-reducing glycogen ends (more than 4 glucose residues away from the branching site) it cleaves glucose from glycogen and transfers it to inorganic phosphate to form glucose-1-phosphate

Synthesis of glycogen / picture 8 / pages 18 - 19

a) glucose (A): the carbon on the right is No. 1, then it is numbered clockwise, so the primary alcohol group (top left) is on carbon No. 6

b) glucose (A) + ATP (a) → glucose-6-phosphate **(**B) + ADP (b), glucokinase in the liver (also in the β-cells of the islets of Langerhans of the pancreas), in other tissues (e.g. muscles) it is a hexokinase that has a higher affinity for glucose: hence glucokinase (having a lower affinity) acts as a "glycemic sensor" and begins to

catalyze the reaction only if glycemia is increased and more glucose enters the cells (the liver thus stores only excess of glucose in the form of glycogen)

c) isomerization of glucose-6-phosphate (B) to glucose-1-phosphate (C), phosphoglucomutase

d) substance (c) is UTP **(**nucleotide uridine triphosphate): glucose-1-phosphate + UTP → glucose-1-UDP (= UDP-glucose) + pyrophosphate (diphosphate)

e) reaction No. 3 is catalyzed by uridyltransferase, which transfers only UMP from UTP to glucose-1-phosphate, released pyrophosphate is hydrolytically cleaved by pyrophosphatase into two phosphates in the subsequent reaction No. 3´, which shifts the equilibrium of reaction No. 3 in the direction of product formation, i.e. the resulting UDP-glucose is then more available for glycogen synthesis

f) UDP-glucose (D) is the active form of glucose: after cleavage of UDP (d) from UDP-glucose, the new glycosidic bond α(1→4) is formed between the -OH group on C1 of the free glucose and the so-called non-reducing end of the glycogen chain (free glucose binds to the C4 of glucose at the end of glycogen)

g) reaction No. 4 is catalyzed by glycogen synthase: it is activated by glucose-6-phosphate (i.e. by the substrate of reaction No. 2) and by insulin, and in the liver the enzyme is inhibited by glucagon

h) glycogen has one reducing end (in the middle), all other ends (branches) are non-reducing: it can be synthesized and degraded rapidly at all these ends at the same time; the reducing end has free hemiacetal hydroxyl group, the non-reducing ends have hemiacetal hydroxyl blocked by α(1→4) bond; the new glucose thus binds to the C4 terminal glucose of the glycogen molecule

(i) glycogenin, which first glycosylates itself on the -OH group of its tyrosine side chain, a further increase in the glycogen molecule is catalyzed by glycogen synthase; glycogenin is bound to the reducing end of glycogen

j) branching enzyme, it transfers a part of the linear chain elsewhere, where it forms new α(1→6) bond

k) bottom and on the right α(1→6) bonds at the branching points, top on the left α(1→4) bonds

l) * denotes non-reducing ends at which the elongation of the glycogen molecule takes place (as well as its degradation), i.e. glycogen metabolism is fast and can take place at several sites of the molecule at the same time

Pentose phosphate pathway / picture 9 / pages 20 - 23

*Aldoses: glucose / glucose-6-phosphate (*hexoses), ribose-5-phosphate (pentose), glyceraldehyde-3-phosphate (triose) + another aldose of the pentose cycle is erythrose (tetrose)

Ketoses: fructose-6-phosphate (hexose), ribulose-5-phosphate (pentose) + other ketoses of the pentose cycle are xylulose-5-phosphate (pentose) and sedohephtulose-7-phosphate (heptose)

a) NADPH is used for various reduction syntheses (synthesis of fatty acids, cholesterol), for the degradation of foreign substances (xenobiotics), for antioxidant protection (glutathione reductase), for the synthesis of various signaling molecules (NO, steroid hormones)

b) glucose-6-phosphate →→ ribulose-5-phosphate →→ glyceraldehyde-3-phosphate or fructose-6-phosphate →→ glucose-6-phosphate (includes enzymes of pentose cycle and gluconeogenesis)

c) glucose-6-phosphate →→ ribulose-5-phosphate → ribose-5-phosphate or glyceraldehyde-3-phosphate/ fructose-6-phosphate →→ ribose-5-phosphate (as a branch of glycolysis or gluconeogenesis)

d) glucose-6-phosphate →→ ribulose-5-phosphate →→ fructose-6-phosphate / glyceraldehyde-3-phosphate →→ pyruvate

e) glucose-6-phosphate dehydrogenase: glucose-6-phosphate + $NADP^+$→ 6-phosphogluconolactone (= cyclic ester of gluconic acid) + $NADPH+H^+$

(lactonase): 6-phosphogluconolactone + H_2O → 6-phosphogluconate

(6-phosphogluconate dehydrogenase):
6-phosphogluconate + $NADP^+$ → ribulose-5-phosphate + CO_2 + $NADPH+H^+$

briefly: glucose-6-phosphate + 2 $NADP^+$ → ribulose-5-phosphate + CO_2 + 2 $NADPH+H^+$

regulatory enzyme: glucose-6-phosphate dehydrogenase, regulated by the $NADP^+$ / NADPH ratio, its activity is also increased by insulin

f) hereditary glucose-6-phosphate dehydrogenase deficiency: reduced activity of the oxidative phase of the pentose cycle leads to insufficient production of NADPH in the cell (the pentose cycle is its main source); there is excessive oxidative stress in erythrocytes (thy need NADPH for protection), leading to damage to the erythrocyte membrane and their breakdown = hemolysis (shorter lifespan); anemia is defined as a reduced ability of the blood to carry oxygen due to reduced hemoglobin concentration (here due to increased breakdown of erythrocytes)

g) glyceraldehyde-3-phosphate (triose), ribose-5-phosphate (pentose), fructose-6-phosphate (hexose)

h) 3x ribulose-5-phosphate (ketopentose)

i) 2x xylulose-5-phosphate (ketopentose), 2x fructose-6-phosphate (ketohexose)

j) transketolase transfers C2-fragments, transaldolase transfers C3-fragments

k) fructose-6-phosphate and glyceraldehyde-3-phosphate: they can again enter glycolysis or gluconeogenesis, i.e. they can be broken down to pyruvate or form glucose-6-phosphate

From 3 glucose-6-phosphate molecules (3x6=18 carbons) 3 *CO_2* + 3 *ribulose-5-phosphates are formed, from the 3 ribulose-5-phosphates 2 fructose-6-phosphates + 1 glyceraldehyde-3-phosphate can be created (total: 18 carbons)*

If we look at the pentose cycle as an alternative pathway of glucose oxidation in the cell, the previous stoichiometry shows that we need 6 cycles to break down 1 molecule of glucose-6-phosphate to 6 molecules of CO_2: when 6 molecules of glucose-6-phosphate enter 6 turns of the pentose cycle, 6 CO_2 molecules are gradually formed, and 5 molecules of glucose-6-phosphate are regenerated in this sequence of reactions.

Metabolism of fructose / picture 10 / pages 24 - 25

a) the first formula on the top left is fructose: the carbon on the top right is C1, then it is numbered clockwise, on the top left is C6 (full name: α-fructofuranose); the product of the reaction is fructose-1-phosphate (cyclic formula), on the bottom left is the linear formula of fructose-1-phosphate, and the products of the reaction are dihydroxyacetone phosphate and glyceraldehyde (below); the disaccharide on the top right is sucrose (= saccharose): alpha-glucopyranose (top) (1→2) beta-fructofuranose (below)

b) "hepatic fructokinase": fructose + ATP → fructose-1-phosphate + ADP (liver / cytosol of hepatocyte); essential fructosuria: fructose is excreted in the urine because it cannot be adequately metabolized, asymptomatic disease (fructose is also phosphorylated by hexokinase to fructose-6-phosphate = intermediate of glycolysis)

c) aldolase B (= fructose-1-phosphate aldolase, i.e. it breaks down this substrate in addition to fructose-1,6-bisphosphate from glycolysis, it occurs in the liver, kidneys and intestines): fructose-1-phosphate → dihydroxyacetone phosphate + glyceraldehyde (liver / cytosol of hepatocyte); hereditary fructose intolerance (fructose intolerance): the presence of fructose, sucrose or sorbitol in food can lead to vomiting, flatulence, abdominal pain, hypoglycemia – fructose must be completely exluded from the diet, otherwise liver damage and other problems may occur (fructose-1-phosphate accumulates, it activates glycolysis, phosphate is missing for ATP synthesis,...)

d) dihydroxyacetone phosphate is isomerized (by triose phosphate isomerase) to glyceraldehyde-3-phosphate, which is further converted by glycolysis to pyruvate, or reduced (glycerol-3-phosphate dehydrogenase, NADH) to glycerol-3-phosphate, from which triacylglycerols are formed

e) + f) glyceraldehyde: 1) by triose kinase to glyceraldehyde-3-phosphate (glycolysis intermediate) or 2) by aldehyde dehydrogenase (NADH) to glycerol, which is phosphorylated by glycerol kinase (ATP) to glycerol-3-phosphate, from which triacylglycerols are formed or can be oxidized to dihydroxyacetone phosphate (glycerol-3-phosphate dehydrogenase) and then isomerized to glyceraldehyde 3-phosphate (glycolysis intermediate)

g) the metabolism of fructose by hepatic fructokinase (fructolysis) bypasses the regulatory reaction of glycolysis (6-PFK-1, which is the slowest step of glycolysis), i.e. fructose is broken down faster and its metabolism is not so strongly regulated; in addition, fructose-1-phosphate activates glycolysis, to which products of fructose-1-phosphate cleavage can enter; so that in the presence of fructose carbohydrates are broken down faster (both glucose and fructose) to pyruvate → acetyl-CoA → fatty acids → TAG (= stored fat)

h) sucrose = α-glucopyranose (1→2) β-fructofuranose; in the small intestine it is hydrolytically broken down by the brush border enzyme sucrase, both monosaccharides are absorbed into enterocytes (glucose by secondary-active transport with sodium, fructose by facilitated diffusion);

fructose is metabolized mainly in the liver, but it can also be phosphorylated by hexokinase in other cells (adipocytes) to fructose-6-phosphate (and enters glycolysis); however, hexokinase has a lower affinity for fructose than for glucose

Metabolism of galactose / picture 11 / pages 26 – 27

a) first row from left: galactose / galactose-1-phosphate / UDP-galactose; second row from left: glucose-6-phosphate / glucose-1-phosphate / UDP-glucose; bottom right: galactose / galactitol

b) galactokinase (A), cytosol of hepatocytes; in case of galactokinase deficiency, galactose accumulates in the blood (galactosemia, type II) and is excreted in the urine, as it is not sufficiently degraded: if galactose is not excluded from the diet, it is reduced to galactitol to an increased extent (aldose reductase), galactitol accumulation in the lens of the eye causes cataracts

c) phosphoglucomutase (B), isomerase (EC 5); its substrate (glucose-6-phosphate) is formed by phosphorylation of glucose by glucokinase: glucose + ATP → glucose-6-phosphate + ADP (cytosol of hepatocyte)

d) (EC 2.7.7.9) glucose-1-phosphate *uridyltransferase* (C): glucose-1-phosphate + UTP → glucose-1-UDP (= UDP-glucose) + pyrophosphate (= diphosphate); the source of UDP of this uridyltransferase is UTP (the reaction produces pyrophosphate, which is why this enzyme is sometimes also referred to as "pyrophosphorylase"); UDP-glucose is an "activated form of glucose", serving as a substrate in glycogen synthesis, UDP-glucuronate synthesis, and as a source of UDP in galactose metabolism (see this picture)

e) UDP-galactose-4-epimerase = galactose epimerase (D), it catalyzes the isomerization of UDP-galactose to UDP-glucose (during the degradation of galactose) or the isomerization of UDP-glucose to UDP-galactose during the synthesis of galactose from glucose

(f) (EC 2.7.7.12) the common name of the enzyme is galactose-1-phosphate *uridyltransferase* (E): UDP-glucose + galactose-1-phosphate → glucose-1-phosphate + UDP-galactose (= galactose-1-UDP); the source of UDP of this uridyltransferase is UDP-glucose (not UTP as uses the enzyme from the subquestion d); this reaction serves to activate galactose (to UDP-galactose), which can then isomerize to UDP-glucose (for galactose degradation) or can be used in another way (e.g. for the synthesis of lactose or polysaccharides); the hereditary deficiency of this enzyme is *galactosemia* (type I, classic galactosemia), it is more serious than galactosemia from galactokinase deficiency (type II); if not exluded from the diet in time (galactose main source is milk and dairy products that contain lactose, which is a disaccharide made from galactose and glucose), severe liver damage occurs from the accumulation of galactose-1-phosphate, unprocessed galactose also accumulates and galactitol is formed, all of which also leads to brain damage

g) galactose → glucose: reactions A → E (first line of formulas) → D → E (second line of formulas, where UDP glucose serves as a UDP donor for further degradation of galactose) → B + reaction catalyzed by glucose-6-phosphatase → glucose

h) aldose reductase (cytosol): galactose → galactitol (the same enzyme catalyzes the conversion of glucose to glucitol = sorbitol); the velocity of the reaction depends on the concentration of the substrate: when the concentration of galactose increases, more galactitol is formed, which increases the osmolarity inside the cell → in the eye lens it leads to the formation of cataracts

i) galactose is a component of the disaccharide lactose (milk, dairy products), but also occurs as a component of various glycoproteins; lactose in the digestive tract is broken down (hydrolyzed) by the enzyme lactase (on the brush border of the small intestine), the products of cleavage (galactose and glucose) are absorbed into the enterocyte; if lactase is not sufficiently functional (lactose intolerance), diarrhea and flatulence occur after ingestion of dairy products (due to the lactose present in the intestines): lactose is polar and binds water + intestinal bacteria produce gases through their metabolism (the amount of lactase also decreases during life)

Beta-oxidation of fatty acids / picture 12 / pages 28 - 31

a) acyl-CoA (= common name for the rest of the fatty acid) / stearoyl-CoA (this acyl, bound to CoA, has a total of 18 carbons)

b) acyl-CoA-dehydrogenase (A), FAD → $FADH_2$, it is connected with the respiratory chain via coenzyme Q (CoQ, ubiquinone): $FADH_2$ is a prosthetic group of the enzyme, i.e. it is not releasable; $FADH_2$ is regenerated to FAD by the enzyme ETF (= Electron-Transferring Flavoprotein), from which electrons

are transferred to ETF-dehydrogenase, which then transfers them to ubiquinone; reaction (A) is dehydrogenation (= elimination of 2 hydrogen atoms) when the saturated chain of acyl changes to unsaturated, in which the double bond is formed in the *trans* configuration between C2 and C3

c) reaction (B) is hydration (= addition of water), substance (b) is H_2O, one hydrogen binds to C2, -OH group binds to C3, which is the β-carbon: hence the name beta-oxidation

d) the cofactor of the enzyme (C) is NAD^+, which leaves the reaction as NADH + H^+; NADH is then released from the enzyme and can be regenerated in the respiratory chain by Complex 1, to which it transfers two electrons and is released as NAD^+; enzyme (C) catalyzes dehydrogenation (= elimination of 2 hydrogen atoms), in which the primary alcohol group on the β-carbon is converted to a keto- group: hence the name beta-oxidation

e) enzyme (D) is thiolase: it catalyzes thioclastic cleavage, in which acetyl is released and then binds via sulfur ("thio" from theion = sulfur) to the molecule of coenzyme A (CoA-SH) forming a thioester bond; (c) is CoA-SH, (d) is acetyl-CoA (CH_3-CO-S-CoA), which can then enter the Krebs cycle, or (in the liver) be converted to ketone bodies

f) it is shorter by 2 carbons, i.e. by the cleaved acetyl-, which has cleaved off as acetyl-CoA

g) stearoyl-CoA → palmitoyl-CoA (that can enter the next cycle turn), butyryl-CoA → acetyl-CoA (it does not enter further cycle of beta-oxidation, i.e. butyryl-CoA gives rise to 2 acetyl-CoA after one turn, which are the end products of beta-oxidation)

h) in order to be stearoyl-CoA completely degraded to acetyl-CoA, it must go through 8 cycles of beta-oxidation: C18 / 2 = 9 acetyl-CoA molecules are formed (1 acetyl-CoA in each of the 7 cycles, but in the last - the 8^{th} cycle 2 acetyl-CoA are formed); 1 $FADH_2$ and 1 NADH are formed in each cycle, i.e. a total of 8 $FADH_2$ and 8 NADH are produced (for n = number of carbons of fatty acid: n/2 acetyl-CoA molecules, (n/2 – 1) $FADH_2$ molecules, (n/2 – 1) NADH molecules)

i) propionyl-CoA (C3) is formed in beta-oxidation of a fatty acid with an odd number of carbons = odd chain FA (it is formed instead of the last acetyl-CoA), i.e. one such fatty acid produces 1 acetyl-CoA in each cycle, and 1 acetyl-CoA + 1 propionyl-CoA in the last cycle

j) propionyl-CoA-carboxylase (E), coenzyme is biotin: propionyl-CoA + CO_2 + ATP → methylmalonyl-CoA + ADP + phosphate

k) methylmalonyl-CoA-mutase (F), the coenzyme is a derivative of vitamin B12 (deoxyadenosylcobalamin): it is an isomerization, the product of which is succinyl-CoA, i.e. an intermediate product of the Krebs cycle (KC), to which propionyl-CoA (after conversion to methylmalonyl-CoA and succinyl-CoA) enters in this way; propionyl-CoA is also formed during the degradation of some amino acids, which also enter the KC in this way (valine, isoleucine, methionine, threonine) and since glucose can be formed from the KC intermediates, propionyl-CoA also provides carbons for gluconeogenesis

l) left: oleic acid (ω-9), linoleic acid (ω-6), α-linolenic acid (ω-3), γ-linolenic acid (ω-6); right: eicosapentaenoic acid = EPA (ω-3), arachidonic acid (ω-6), docosahexaenoic acid = DHA (ω-3)

m) unsaturated fatty acids are already partially oxidized (their chains have double bonds and thus fewer hydrogen atoms), so that less energy is obtained by their oxidation than from saturated fatty acids; double bonds at the *cis* position must isomerize to *trans* during beta-oxidation (see first beta-oxidation reaction, "A") and must be in the correct position (between C2 and C3), i.e. the oxidation of unsaturated fatty acids requires extra steps (and enzymes), sometimes even with the consumption of NADPH; *conclusion*: unsaturated fatty acids are less advantageous as a source of energy than saturated ones, but they have other important uses in the body (for the structure of membranes, synthesis of signaling molecules)

Metabolism of ketone bodies / picture 13 / pages 32 - 35

SYNTHESIS

a) 2x acetyl-CoA → acetoacetyl-CoA + CoA, thiolase (A); it is actually the last step of beta-oxidation running in the opposite direction; acetyl-CoA entering the synthesis of ketone bodies comes mainly from beta-oxidation of fatty acids (during starvation, when the concentration of fatty acids in the blood increases)

b) acetyl-CoA (CH_3-CO-S-CoA) → HS-CoA

c) HMG-CoA (β-hydroxy-β-methylglutaryl-CoA), it is produced in the mitochondrial matrix; it is also an intermediate of cholesterol synthesis

d) HMG-CoA-lyase (C): HMG-CoA → acetoacetate + acetyl-CoA (c)

(e) acetoacetate + (d) NADH + H^+ → β-hydroxybutyrate + NAD^+;
enzyme: β-hydroxybutyrate dehydrogenase (D)

(f) reaction (E) is the spontaneous decarboxylation of acetoacetate to form acetone and CO_2 (e); since β-hydroxybutyrate can be converted by reaction (D) to acetoacetate that breaks down to acetone (which is a ketone) all three substances are referred to as "ketone bodies"

g) acetone is a waste product of metabolism and is normally excreted from the body (urine, breath, sweat)

h) ketone bodies are synthesized mainly in the liver, in the mitochondria of hepatocytes

DEGRADATION

a) β-hydroxybutyrate + NAD^+ → acetoacetate + NADH + H^+; β-hydroxybutyrate dehydrogenase (D); it is important to note that the β-hydroxybutyrate / acetoacetate ratio is influenced by the NADH/NAD^+ ratio in the cell

b) succinyl-CoA (f) + acetoacetate → succinate (g) + acetoacetyl-CoA; substances (f) and (g) belong to the intermediates of the Krebs cycle (KC), from where (f) is used as a source of coenzyme A for the activation of ketone bodies, substance (g) then returns to the KC; it is a branch of the KC in which GTP (ATP) is not formed in the KC, but instead the energy of the thioester bond cleavage (when CoA is split off) is used to transfer CoA to acetoacetate, activating acetoacetate for degradation in subsequent steps

c) SCOT = succinyl-CoA-transferase (F): the absence of this enzyme in the liver is important because the liver is the main site of ketone bodies synthesis, which are secreted to the blood as a source of energy for extrahepatic tissues; if ketone bodies were also broken down in the liver, the liver would not be such efficient in the production of ketone bodies

d) thiolase (A): acetoacetyl-CoA + CoA (a) → 2x acetyl-CoA

e) it is also the last reaction of the last cycle of β-oxidation, providing not one, but two acetyl-CoA (see page 93, point h)

f) acetyl-CoA is subsequently degraded in the Krebs cycle, i.e. ketone bodies serve as an alternative source of energy for cells during starvation (in addition to glucose, fatty acids and some amino acids, the degradation of which also produces acetyl-CoA)

g) mitochondrial matrix; therefore, ketone bodies cannot be a source of energy for erythrocytes (they do not have mitochondria)

Synthesis of fatty acids / picture 14 / page 36 - 37

a) C2: acetyl- (picture on the left), C4: butyryl- (picture on the right)

b) in both pictures the formula at the top right; malonyl- is the rest (acyl) of dicarboxylic malonic acid: HOOC-CH_2-COOH (acid) / HOOC-CH_2-CO– (acyl); the carboxyl group is cleaved off during fatty acid (FA) synthesis, i.e. malonyl- will provide only 2 carbons for the synthesis

c) CH_3-CO-S-CoA + CO_2 + ATP → HOOC-CH_2-CO-S-CoA + ADP + P_i

acetyl-CoA + carbon dioxide (usually in the form of HCO_3^-) + ATP → malonyl-CoA + ADP + phosphate; here, ATP is the energy source for the endergonic reaction; CO_2 occurs in body fluids dissolved: it forms bicarbonate (CO_2 + H_2O → H_2CO_3 → HCO_3^- + H^+); the synthesis of malonyl-CoA is catalyzed by the enzyme acetyl-CoA carboxylase, its cofactor is biotin; it is a regulatory enzyme of the entire synthesis of fatty acids, it occurs in the cytosol and is activated by insulin and citrate, inhibited by glucagon and the final product of FA synthesis, i.e. palmitoyl-CoA

d) CO_2 originates from malonyl- bound to ACP (HOOC-CH_2-CO-S-ACP): its carboxyl group (HOOC-) was split off (in the form of CO_2) and to the remaining -CH_2-CO-S-ACP group was transferred acetyl- (CH_3-CO-) from the peripheral site of the enzyme, resulting in the formation of acetoacetyl- bound to the catalytic site of the enzyme (CH_3-CO-CH_2-CO-S-ACP); the left part of the previous formula (CH_3-CO-) comes from acetyl, the right part of the formula (-CH_2-CO-S-ACP) comes from malonyl

e) CH_3- group (on the left in the formulas) has the number C4, i.e., C1 is a carbon bonded through sulfur to the enzyme: α- carbon is C2, β-carbon is C3; the synthesis of FA is referred to as "reduction synthesis", where the reduction will occur at β-carbon

f) the cofactor of reduction reactions (B/D) is NADPH (+ H^+), originating mainly from the pentose cycle; NADPH is also formed in a reaction catalyzed by malic enzyme: malate + $NADP^+ \rightarrow$ pyruvate + CO_2 + NADPH (+ H^+), where malate was previously formed from oxaloacetate, originating from the reaction: citrate + CoA $\rightarrow$ oxaloacetate + acetyl-CoA (after transport of citrate from mitochondria to cytosol, which is the way how is "acetyl-CoA" (acetyl) transported through the inner membrane out of the mitochondria)

The first reduction (B) is the conversion of the keto- group to the secondary alcohol group (addition of 2 hydrogens to the C=O group), the second reduction reaction (D) is the conversion of the unsaturated chain to the saturated one (addition of 2 hydrogens to the -CH=CH- group); it is the same type of B / C / D reactions *(but in the opposite direction) than in β-oxidation of fatty acids.*

g) butyryl-ACP (product of reaction D from the 1st FA synthesis cycle); butyryl is then deposited from the catalytic site (ACP) to the peripheral binding site (upper left in the right picture) and the second malonyl- from malonyl-CoA is bound to the ACP (upper right in the right picture) and the 2nd cycle of the "spiral" of FA synthesis begins

h) from malonyl, see point (d); however, in the 2nd cycle, acetyl- was not transferred from the peripheral site, but by 2 carbons longer butyryl-, which is a product of the 1st cycle of the synthetic spiral; therefore, the product of the A2 reaction contains 6 carbons (not 4 C as a product of the A reaction)

i) CH_3- group (on the left in the formulas) has the number C6, i.e., C1 is a carbon bonded through sulfur to an enzyme: α- carbon is C2, β-carbon is C3; FA synthesis is referred to as "reduction synthesis", where the reduction will occur on β-carbon (as in the 1st cycle of a synthetic spiral)

j) NADPH + $H^+ \rightarrow NADP^+$ (2 hydrogen atoms = 2 electrons + 2 H^+ were transferred to the intermediate of FA synthesis, thus reducing it); subsequently, $NADP^+$ is used in reactions in which NADPH is formed from $NADP^+$ (mainly in the pentose cycle, also in the reaction catalyzed by the malic enzyme)

k) hexanoyl- (acyl of hexanoic acid), its C6 (-CH_3 on the left) is referred to as ω-carbon (as well as the methyl end of any FA); this methyl group originates from acetyl, bound to the peripheral site of the enzyme at the very beginning of the synthesis of FA (in the 1st cycle); it follows that in the synthesis of FA, the FA chain is elongated at the carboxyl end of the molecule (not at the ω-end); so if an elongation of some essential FA (e.g. linoleic acid) proceeds in the cell, its ω-end does not change, i.e. ω-6 FA can give rise to another ω-6 FA, only having a longer chain (analogously from ω-3 FA only another ω-3 FA can form)

l) palmitoyl-CoA (C16) is the longest product formed by this FA synthetic spiral: a total of 1 acetyl-CoA and 7 malonyl-CoA molecules enter the reaction of its synthesis (i.e. a total of 7 cycles of the FA synthetic spiral); palmitoyl-CoA can be used directly for the synthesis of TAG or phospholipids, or it is elongated and/or desaturated (by desaturases) to form other fatty acids (all subsequent reactions take place in the smooth ER)

*For the synthesis of a fatty acid with an even number of carbons (n), n/2 acetyl-CoA molecules are needed, of which only one acetyl-CoA enters the synthesis directly (and its methyl end becomes the ω-end of FA), all other two-carbon containing substrates enter the synthetic spiral as malonyl-CoA (*after the conversion of acetyl-CoA *to malonyl-CoA in the regulatory reaction of the entire synthesis, catalyzed by acetyl-CoA carboxylase).*

Metabolism of triacylglycerols / picture 15 / pages 38 - 39

a) glycerol (A) is formed during lipolysis in adipose tissue (it is released from adipocytes into the blood and new TAGs can be formed from it only in the liver, where it is phosphorylated by glycerol kinase to glycero-3-phosphate); glycerol is also formed by the reduction of glyceraldehyde, which is produced during the metabolism of fructose in the liver (by aldolase B from fructose-1-phosphate); dihydroxyacetone phosphate (B) is an intermediate of glycolysis, it is also formed from fructose by aldolase B (together with glyceraldehyde)

b) glycerol-3-phosphate (C): it is formed from glycerol by glycerol kinase (glycerol + ATP $\rightarrow$ glycerol-3-phosphate + ADP) found only in the liver; it is also formed from dihydroxyacetone phosphate (DHAP) by glycerol-3-phosphate dehydrogenase (DHAP + NADH + $H^+ \rightarrow$ glycerol-3-phosphate + NAD^+) in various cells (it is part of the so-called glycerol phosphate shuttle)

c) glycerol-3-phosphate + acyl-CoA → monoacylglycerol-3-phosphate + CoA / monoacylglycerol-3-phosphate + acyl-CoA → diacylglycerol-3-phosphate + CoA / diacylglycerol-3-phosphate = phosphatidic acid is further broken down by phosphatidic acid phosphatase: diacylglycerol-3-phosphate + H_2O → 1,2-diacylglycerol (DAG) + phosphate / 1,2-DAG + acyl-CoA → triacylglycerol (TAG) + CoA; all enzymes that carry acyl- from acyl-CoA to the substrate are called acyltransferases

d) phosphatidic acid (D) is also an intermediate product of glycerophospholipid synthesis

e) triacylglycerol = TAG (E); in the liver, it is incorporated into the structure of the lipoprotein VLDL, which is released into the blood and serves to transport TAG through the blood to the target tissues: lipoprotein lipase is found on the endothelium of blood vessels (mainly in muscle and adipose tissue), which breaks down the TAG present in VLDL in the blood; the released fatty acids are then used in muscle cells (as a source of energy) or adipose tissue (to synthesize storage TAGs); TAG synthesized in adipose tissue adipocytes are released from the smooth ER (where TAG synthesis takes place) as lipid droplets enveloped by a single layer of phospholipids and proteins: in this form, TAG are stored in adipocytes (see histology)

f) during lipolysis, 3 ester bonds between glycerol (its -O) and 3 fatty acids (their -CO-R) are hydrolytically cleaved in TAG: O–CO (right) / CO–O (left)

g) in the small intestine it is mainly pancreatic lipase (from pancreatic juice), in blood it is lipoprotein lipase (bound to the surface of endothelial cells), in fat cell (adipocyte) it is adipocyte TAG lipase (cleaves TAG and DAG), hormone-sensitive lipase (cleaves TAG, DAG and MAG, mainly it splits off the FA from TAG at positions 1- and 3-), MAG lipase (cleaves the last FA from MAG)

h) 2) the amount of lipoprotein lipase (LPL), bound to the endothelium of blood vessels, is increased by insulin, LPL is activated by apoprotein CII, which is located on the surface of lipoproteins (chylomicrons, VLDL), whose TAG is cleaved by LPL; 3) in adipocytes, lipolysis is regulated by hormone-sensitive lipase (HSL), its activity increases when the intracellular concentration of cAMP in the adipocyte is increased (e.g. by the effects of the hormones glucagone and adrenaline), the activity of HSL is inhibited by insulin (i.e. in case of insulin deficiency in diabetics, excessive lipolysis occurs in adipose tissue, the concentration of fatty acids in the blood increases, which is why an excess of ketone bodies (ketoacidosis) subsequently develops)

i) the products of lipolysis are fatty acids and glycerol: 1) from the blood supplying the muscle, the fatty acids go to the muscle fibers (where they serve as a source of energy), 2) from the blood supplying the adipose tissue, the fatty acids go to the adipocytes (where they form a storage TAG), 3) from adipocytes, fatty acids are released to the blood (and various tissues can then obtain energy from them), in case of their excess in blood, the liver converts FA to ketone bodies; glycerol, released by lipolysis (anywhere), reaches the liver through the blood, where it can serve to synthesize TAG or glucose (gluconeogenesis)

<u>Urea cycle</u> / picture 16 / pages 40 - 41

a) urea (= carbonic acid diamide) is a product of reaction No. 3

b) *ammonia*, in aqueous solutions: $NH_3 + H_2O \leftrightarrow NH_4^+ OH^-$ (equilibrium concentrations of ammonia and ammonium cation depend on pH, at pH of body fluids the ammonium cation predominates); the second substrate is *carbon dioxide*: it dissolves in water: $CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+$ (in body fluids it predominates as bicarbonate); ammonia is formed during the breakdown of amino acids (it is produced mainly by glutaminase and glutamate dehydrogenase) and some other N-containing substances; CO_2 is the end product of oxidation of all nutrients (carbohydrates, lipids, proteins)

c) carbamoyl phosphate synthetase I (2): regulatory enzyme of urea synthesis; 1 ATP serves as a substrate (see phosphate incorporated in the structure of carbamoyl phosphate), the cleavage of the second ATP only supplies energy to the endergonic reaction (catalyzed by "synthetase", which is a ligase); all reactions in which ATP is used require Mg^{2+} ions, they bind to the negative charge of phosphates present in the ATP molecule; carbamoyl is an acyl (rest) of carbamic acid (carbonic acid amide), which has an unstable structure (H_2N-CO-OH), it is formed directly as anhydride with phosphate (carbamoyl phosphate) – the cleavage of phosphate in the next step, when entering the urea cycle, allows the condensation of carbamoyl with ornithine (just as CoA is cleaved from acetyl-CoA upon entry into the Krebs cycle)

d) reaction in the upper left over the urea cycle is an important regulatory mechanism: it describes the formation of the regulatory molecule N-acetylglutamate, which is an allosteric activator of the regulatory enzyme carbamoyl phosphate synthetase I; from left: glutamic acid + acetyl-CoA → N-acetylglutamic acid (this reaction is activated by increased concentration of arginine by activation of N-acetylglutamate synthetase = enzyme 1, arginine is formed from glutamine during increased

degradation of proteins in the body, i.e. when there is an increased need to detoxify ammonia in the urea cycle)

e) aspartic acid / aspartate (Asp) enters the cycle from the right and brings the second -NH_2 group into the structure of the emerging urea: transamination of oxaloacetate (an intermediate of the Krebs cycle) produces aspartate: oxaloacetate + glutamate → aspartate + α-ketoglutarate, enzyme: AST (aspartate aminotransferase, it has both a mitochondrial and a cytoplasmic isoenzyme); glutamate is a common second product formed by transamination of various amino acids, i.e. it carries their amino nitrogen, which thus indirectly gets into the urea cycle

f) arginine (Arg), substrate of reaction No. 3; arginine is only semi-essential because a certain amount of it is produced in the body

g) arginase: it breaks down arginine into urea and ornithine; with increased breakdown of proteins in the body, a lot of arginine is produced in the kidneys, which gets to the liver through the blood, enters the urea cycle as an intermediate and thus increases the capacity of the cycle: in the urea cycle it is converted to ornithine, and thus more ornithine molecules are available, with which carbamoyl phosphate can condense and so detoxify more ammonia
(it is similar to the anaplerotic reaction of the Krebs cycle, where oxaloacetate is added in this way)

h) carbamoyl phosphate condenses with ornithine to form citrulline; citrulline binds with aspartate forming argininosuccinate, which is then cleaved to arginine and fumarate, that is released from the cycle (see box)

(i) fumarate / fumaric acid: (*trans*) HOOC-CH=CH-COOH, formed from the carbon skeleton of aspartate, whose amino group became part of the arginine structure; fumarate is an intermediate of the KC, into which it can join

j) in the mitochondria the urea cycle begins and ends, in the cytosol only the following reactions take place: citrulline + aspartate → argininosuccinate → fumarate + arginine → urea + ornithine, i.e. citrulline and ornithine must be transported from / to the mitochondria (fumarate can also return to the mitochondria)

k) 2 ATP are used to form carbamoyl phosphate (1 phosphate is incorporated into the substrate, 1 phosphate is released) + 1 ATP for the condensation of citrulline with aspartate (but here AMP + diphosphate are formed, which is further broken down into 2 phosphates), i.e. in total 4 ATP equivalents are consumed (4 free phosphates are formed from the splitting of 4 so-called "macroergic bonds")

l) *analogy* urea cycle / Krebs cycle: both are cycles, i.e. they run in a cycle (= regeneration of ornithine / oxaloacetate in a cycle); the same number of carbons enter and exit the cycle (carbamoyl phosphate → urea / acetyl-CoA → 2 CO_2); the substance that enters the cycle is "activated" (carbamoyl phosphate → phosphate is cleaved off / acetyl-CoA → CoA is cleaved off when entering the cycle); the carbon that leaves the cycle is a waste product (urea is excreted in the urine / CO_2 is exhaled); both cycles start with a molecule at C and end with a molecule at O (citrulline / citrate and ornithine / oxaloacetate); both have anaplerotic reactions (arginine / oxaloacetate) and both cycles are interconnected (oxaloacetate – aspartate – fumarate)

The main difference between the urea cycle and the Krebs cycle is that the urea cycle is actually an anabolic pathway (urea synthesis) and consumes ATP, while the Krebs cycle is mainly catabolic (breaks down acetyl-CoA), produces ATP (GTP) and reduced cofactors due to which ATP is formed.

Transamination / picture 17 / pages 42 - 43

a) α-ketoglutarate (A), glutamate (B)

b) aminotransferase = transaminase, the cofactor is pyridoxal phosphate (PLP), which is a derivative of vitamin B6 (pyridoxine)

c) ALT = alanine aminotransferase: alanine (C1) / pyruvate (D1), formulas on p. 5

d) AST = aspartate aminotransferase: aspartate (C2) / oxaloacetate (D2), p. 5

e) branched-chain amino acid transaminase: valine (C3) / α-keto acid (D3), which has the same carbon skeleton as valine (= principle of transamination); valine on page 63

f) the reason is that the second pair (α-keto acid / α-amino acid) is the same for most common transamination reactions (α-ketoglutarate / glutamate)

g) transamination reactions are reversible under physiological conditions (they run in both directions)

h) from glutamate (B) glutamine is formed by condensation with ammonia ("amidation") catalyzed by glutamine synthetase (ligase): glutamate + NH_3 + ATP → glutamine + H_2O + ADP + phosphate; glutamate can be formed from glutamine (which is an amide of glutamate, having an amide group -$CONH_2$ instead of a

carboxyl group in the side chain) by hydrolytic cleavage (hydrolysis) catalyzed by glutaminase (hydrolase): glutamine + $H_2O \rightarrow$ glutamate + ammonia

i) asparagine is an amide of aspartate, formed by the reaction of aspartate with glutamine (not with ammonia), where glutamine is a source of amide nitrogen: aspartate + glutamine + ATP $\rightarrow$ asparagine + glutamate + AMP + diphosphate

j) glutamate (B) is converted to α-ketoglutarate (A) (and vice versa, the reaction is reversible) also by glutamate dehydrogenase (GMD), which catalyzes the so-called oxidative deamination of glutamate (= central reaction of amino acid metabolism), when amino nitrogen is released from the molecule as ammonia (in transaminations it is only transferred from one molecule to another): glutamate + $NAD^+ \rightarrow NH_3$ + α-ketoglutarate + NADH + H^+ (in the opposite direction of the reaction, when glutamate is formed, NADPH is the cofactor instead of NADH); the reaction takes place in the mitochondrial matrix and either delivers or withdraws α-ketoglutarate to/from the Krebs cycle (referred to as one of the anaplerotic / cataplerotic reactions of the Krebs cycle)

Metabolism of tyrosine / picture 18 / pages 44 - 45

a) tyrosine (Tyr) is the molecule (B), it is not essential, because it can be formed in the body from phenylalanine (Phe) (A), which is an essential AA

b) the conversion of phenylalanine (A) to tyrosine (B) is catalyzed by phenylalanine hydroxylase: Phe + O_2 + THB $\rightarrow$ Tyr + H_2O + DHB, its cofactor is tetrahydrobiopterin (THB, in the reaction it is oxidized to dihydrobiopterin, DHB; it is not a vitamin derivative, but is synthesized in the body from GTP); this reaction is both a pathway of tyrosine synthesis and a pathway of degradation of excess phenylalanine in the body; when this enzyme (or its cofactor) is missing, phenylalanine accumulates in the blood (hyperfenylalaninemia), a disease due to hereditary deficiency in fenylalanine hydroxylase is referred to as phenylketonuria

c) an alternative route of degradation of phenylalanine is its transamination to phenylpyruvate (C): phenylalanine + α-ketoglutarate $\rightarrow$ phenylpyruvate + glutamate (for comparison: transamination of alanine produces pyruvate); phenylpyruvate is a keto acid and can be reduced by analogy with pyruvate to phenyllactate (hydroxy acid) or it can be converted to phenylacetate (these by-products of phenylalanine metabolism give urine a musty smell – the "smell of mice")

d) DOPA = dihydroxyphenylalanine is an intermediate product of the synthesis of catecholamines (= dopamine, noradrenaline, adrenaline), which are signaling molecules; tetrahydrobiopterin (THB) is needed as a cofactor for both hydroxylations of phenylalanine to DOPA (Phe $\rightarrow$ Tyr $\rightarrow$ DOPA), i.e. in case of THB metabolism disorder phenylalanine accumulates (hyperphenylalaninemia) and not only tyrosine but also catecholamines are missing

e) melanins are pigments produced by melanocytes, if they are missing, albinism develops; the synthesis of melanins from tyrosine requires tyrosinase (not tyrosine hydroxylase, which produces DOPA during the synthesis of catecholamines); this pathway also includes DOPA as an intermediate, but it differs from the synthesis of catecholamines, one of other intermediates is dopachinone (melanins are more complex quinones – they have more cycles - with conjugate system of double bonds, therefore they are colored)

f) tyrosine (B) is degraded by a pathway that produces homogentisic acid, which is further converted by homogentisate-1,2-dioxygenase = homogentisate oxidase, a disease caused by a deficiency of this enzyme is called alkaptonuria: colourless homogentisic acid accumulates, spontaneously oxidizes to quinone, from which polymerization the dark pigment alkaptone is produced (it is excreted not only in the urine, but also accumulates in the joints, where it causes arthritis, and also ochronosis = brown / ochre discoloration of some tissues, especially cartilage)

g) tyrosine (and phenylalanine) degradation products: fumaric acid (D) enters the Krebs cycle (fumarate is one of the KC intermediates), acetoacetic acid (E) is one of ketone bodies (acetoacetate); that is, both of these aromatic amino acids (Phe and Tyr) are both glucogenic (break down to the KC intermediate and after its conversion to oxaloacetate can enter gluconeogenesis) and ketogenic (their degradation also produces acetoacetate, i.e. one of the ketone bodies)

Metabolism of methionine / picture 19 / pages 46 - 47

a) methionine (A) is one of the essential amino acids, S-adenosylmethionine = SAM (B) is an activated form of methionine that serves as a cofactor of some methyl ($-CH_3$) transporting enzymes, S-adenosylhomocysteine (C) is a product of the SAM cofactor after methyl transfer to the substrate, homocysteine (D) is a non-essential, non-proteinogenic sulfur-containing amino acid, differing from cyteine in that it is longer by one $-CH_2-$ group (homo-)

b) in the SAM molecule (B), the methyl group is bound to a "triple-bonded sulfur" (S with a positive charge), thus being "activated" for the methylation of a substrate; methyl transfer is catalyzed by methyltransferases; substance X can be ethanolamine in the synthesis of choline, noradrenaline in the synthesis of adrenaline, DNA bases in case of their methylation (a way of regulating gene transcription), SAM is also used in the synthesis of carnitine, creatine, melatonin, and during the degradation of some signaling molecules,...

c) homocysteine (D) is used, if necessary, to synthesize cysteine: homocysteine + serine → cystathionine + H_2O (condensation is catalyzed by cystathionine-β-synthase, the amino acids are linked through their side chains by sulphur (-S-), its enzyme cofactor is pyridoxal phosphate); cystathionine is then broken down into cysteine, α-ketobutyrate and ammonia; thus the source of the carbon skeleton for cysteine synthesis is serine, and only the sulfur comes from homocysteine (i.e. originally from methionine ingested through food) and it forms the -SH group in the cysteine side chain; this process is called *transsulfuration*

d) it is the regeneration of methionine (A) from homocysteine (D): the so-called salvage reaction restores essential methionine in the cycle; the source of the methyl group is methyl-tetrahydrofolate (methyl-THF), which is a derivative of folic acid (folate = vit. B9) – its methyl is first transferred to cobalamin (vit. B12), which is the cofactor of the enzyme methionine synthase: methylcobalamin then transfers methyl to homocysteine to form methionine; this cooperation of vitamins B9 and B12 is also important in the clinic (both vitamins are administered together during supplementation); if B12 is missing, folate remains in the form of methyl-THF (so called "folate trap"); folate is thus regenerated to free THF in this *remethylation* of homocysteine to methionine

Another vitamin of sulphur amino acid metabolism is vit. B6: its active form is pyridoxal phosphate (PLP), and it is used for the synthesis and subsequent cleavage of cystathionine, which is an intermediate in the synthesis of cysteine from methionine; in order to reduce the pathological concentration of homocysteine in the blood, all these three vitamins are sometimes administered together (B6 + B9 + B12), as they are necessary for the metabolism of homocysteine (conversion of homocysteine either to cysteine or methionine)

e) with homocystinuria (cystathionine-β-synthase deficiency): if homocysteine metabolism is disturbed, it accumulates in the blood (hyperhomocysteinemia) and is oxidized: 2 homocysteine molecules form homocystine disulfide, which is excreted in the urine; *hyperhomocysteinemia* is associated with atherosclerosis, it occurs in patients with diseases of the cardiovascular system

Metabolism of branched-chain amino acids / picture 20 / pages 48 - 49

a) leucine (A), isoleucine (B), valine (C), all of which are essential

b) enzyme: aminotransferase of branched-chain amino acids, i.e. it has a broader substrate specificity, its cofactor is pyridoxal phosphate (a derivative of vit. B6); the enzyme occurs mostly in skeletal muscle (little is found in the liver), i.e. branched-chain amino acids from food go mainly to the muscles, where they, among other, serve as a source of nitrogen for the synthesis of other amino acids; branched amino acids + α-ketoglutarate → branched α-keto acid + glutamate (glutamate can then be a source of amino groups in other transaminations, in which various other amino acids are formed); for formulas see page 5

c) reaction No. 2 is oxidative decarboxylation of α-keto acids (similar to PDH reaction), i.e. this enzyme complex also has 5 cofactors: TDP, CoA, lipoic acid, FAD, NAD^+; CoA and NAD^+ enter the reaction, NADH + H^+ is produced, α-keto acid is converted to acyl-CoA by 1 carbon shorter than was the α-keto acid, carbon dioxide (CO_2) was split off; the reaction is catalyzed by branched-chain α-keto acid dehydrogenase = BCKA (branched-chain-keto-acid) dehydrogenase, it occurs in mitochondria; the enzyme has the highest activity after meal in the liver, where BCKA get from the muscle through the blood, during starvation the reaction also takes place in the muscle

d) the end products of leucine (A) degradation are acetyl-CoA (D) and acetoacetic acid (acetoacetate), which is a ketone body, formed by the cleavage of HMG-CoA by the enzyme HMG-CoA lyase (as in the synthesis of ketone bodies), i.e. leucine is a purely ketogenic amino acid, glucose is not normally formed from the products of its degradation; acetoacetate is degraded to 2 acetyl-CoA (see degradation of ketone bodies), i.e. both products of leucine degradation enter the KC via acetyl-CoA, where they are oxidized to CO_2

e) isoleucine (B) and valine (C) are converted to propionyl-CoA by similar reactions as occur in β-oxidation (i.e. including the formation of reduction equivalents); propionyl-CoA is then metabolized in the same way as when it is

formed by β-oxidation of fatty acids with an odd number of carbons, see the answers on page 93 (j) (k); (F) is succinyl-CoA

f) leucinosis = maple syrup urine disease (MSUD), the enzyme BCKA dehydrogenase is deficient, leading to the accumulation of branched-chain keto acids (caramel smell of urine), as well as branched-chain amino acids; brain damage and developmental disorders occur

Synthesis of cholesterol / picture 21 / pages 50 – 51

a) reaction A: 2x acetyl-CoA → acetoacetyl-CoA + CoA-SH, enzyme: thiolase that catalyzes the reaction also in the opposite direction, see the last step of beta-oxidation; the same reaction and enzyme also occurs in the synthesis of ketone bodies (first reaction), but ketogenesis takes place only in the mitochondria of hepatocytes, while the beginning of cholesterol synthesis is localized in the cytosol, mainly of hepatocytes, but also in other cells *(cholesterol is also synthesized by cells in the brain for their own use)*

b) reaction B: acetoacetyl-CoA + acetyl-CoA → 3-hydroxy-3-methylglutaryl-CoA (= HMG-CoA) + CoA-SH; the same reaction also occurs in the synthesis of ketone bodies (as a second reaction of ketogenesis, but only in mitochondria of hepatocytes)

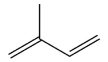
c) reaction C: HMG-CoA + 2 NADPH + H^+ → mevalonate + 2 $NADP^+$ + CoA-SH; enzyme: HMG-CoA reductase; the primary alcohol group was formed by reduction (using two NADPH molecules) of the carboxyl group, which has been formed before by hydrolytic cleavage of HMG-CoA molecule (yielding the -COOH group and CoA-SH), see carbon on the right (glutaryl is an acyl derived from glutaric acid = pentanedioic acid); HMG-CoA reductase is bound to the smooth endoplasmic reticulum from the cytosolic side

d) HMG-CoA reductase is a key regulatory enzyme in cholesterol synthesis; it is mainly regulated by a feedback: an intracellular cholesterol decreases or increases the enzyme activity as needed (according to the amount of cholesterol in the cell); the enzyme is also inhibited by glucagon, lack of ATP in the cell (via AMP-dependent kinase) and pharmacologically by drugs "for high cholesterol" called statins; the enzyme is activated by insulin (i.e. cholesterol is synthesized after a meal from excess acetyl-CoA, which comes from the breakdown of excess glucose and other nutrients)

e) reaction D: mevalonate + 2 ATP → mevalonate-5-pyrophosphate + 2 ADP *pyrophosphate = diphosphate*

f) reaction E: mevalonate-5-pyrophosphate + ATP → isopentenyl diphosphate + carbon dioxide + ADP + P_i; CO_2 comes from the decarboxylation of the substrate (carbon on the left)

g) reaction F: two forms of the so-called "activated isoprene": dimethylallyl diphosphate (left) and isopentenyl diphosphate (right), their subsequent polymerization produces various isoprenoids, i.e. derivatives of isoprene (its polymers are called terpenes)

h) isoprene = 2-methylbut-1,3-diene: 

i) reaction product G: geranyl diphosphate (C10, belongs to monoterpenes), reaction product H: farnesyl diphosphate (C15, belongs to sesquiterpenes), reaction product I: squalene (C30, belongs to triterpenes)

j) carbon-chain, i.e. hydrophobic chain of geranyl occurs in the form of its dimer, as geranylgeranyl- (C20) in membranes, where it forms so-called lipid anchors of proteins (also "prenyl anchors"), which fix some membrane proteins in the plasma membrane; another such anchor is farnesyl- (C15) (so-called prenylation is one of the posttranslational modifications of some membrane proteins)

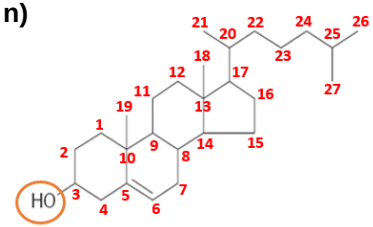
This means that during the synthesis of cholesterol, other substances that are very important for cells are produced: if necessary, they are removed from this metabolic pathway before cholesterol is created (another such isoprenoid substance is dolichol, which is necessary for the synthesis of glycoproteins)

k) coenzyme Q = ubiquinone; it is a mobile electron carrier dissolved in the inner mitochondrial membrane, and it is an essential part of the electron transport chain (ETC): after its reduction to ubiquinol it transfers electrons to complex III during the cellular respiration necessary for ATP production

l) C30 (belongs to triterpenes), see point i)

m) the main source of the reduced form of NADPH for cells is the pentose cycle (= pentose phosphate pathway, PPP)

n)



o) the fatty acid binds by ester bond to the designated -OH group;

Esterified cholesterol is stored in cells in the form of lipid droplets, it is also found in lipoproteins of blood plasma (in their hydrophobic core, because if esterified it is non-polar).

Free cholesterol is an important amphiphilic component of the plasma membrane, with the polar -OH group oriented towards the outside or inside of the cell (it is equally present in both sheets of the membrane, thus reducing the permeability of the membrane to small polar molecules). Free cholesterol is also found in the surface layer of lipoproteins of blood plasma.

p) steroid hormones (glucocorticoids, mineralocorticoids, sex hormones) and bile acids, which are necessary for the digestion of lipids in the GIT, are synthesized from cholesterol; the last intermediate produced in the cholesterol synthetic pathway is 7-dehydrocholesterol (not shown in the picture 21), from which cholesterol is enzymatically formed, or in the skin this intermediate is converted by UV radiation to vitamin D3 (called cholecalciferol), from which the hormone calcitriol is synthesized in the body

Cholesterol cannot be broken down by human cells, its excess is excreted from the body through bile, mainly in the form of bile acids, which are produced from cholesterol in the liver.

Metabolism of lipoproteins / picture 22 / pages 52 – 53

a) free fatty acids (FFA), 2-monoacylglycerol (2-MAG), which are formed by hydrolysis of TAG catalyzed by pancreatic lipase; cholesterol and free fatty acid (CHOL and FFA), formed by hydrolysis of cholesterol esters by cholesterol esterase; lysophosphatidylcholine and free fatty acid (LPC and FFA), which are formed by hydrolysis of phosphatidylcholine (lecithin) by phospholipase A2 (phospholipids make up 1-10% of lipids ingested in food, lecithin predominates: it brings choline and unsaturated FA to the body)

b) from left / top to bottom: chylomicron (CHM, it is the largest) and chylomicron remnant (CHM-remnant), which contain structural Apo-B48 that is synthesized in enterocytes; HDL-nascent (tiny, discoidal in shape) and HDL-mature (lipid-filled) containing specific Apo-AI; VLDL (the second largest), which is produced in the liver, IDL (formed from VLDL, it is „VLDL-remnant“) and LDL (formed from IDL), all three contain Apo-B100

CHM changes to CHM-remnant by decreasing the amount of its TAGs, which are hydrolyzed by lipoprotein lipase (LPL) that is located in capillaries bound to the surface of endothelial cells, mainly in muscle and adipose tissue

HDL-nascent is converted into HDL-mature by taking in free cholesterol from the surface of cells (by ABCA1 transporter that uses ATP); HDL is associated with the enzyme LCAT (lecithin-cholesterol-acyl-transferase), which uses the phospholipid lecithin (from the HDL surface layer) and splits off one acyl (i.e. the rest of the fatty acid) from the sn-2 position (forming a lysophospholipid) and transfers the acyl to cholesterol to form esterified cholesterol – which, due to its hydrophobic nature, is then moved from the surface to the HDL core

VLDL is converted to IDL by LPL (as well as CHM to CHM-remnants), LPL splits off fatty acids from TAG in lipoproteins, these fatty acids enter surrounding cells via CD36, which is a transloccase that carries FFA into the cell; when VLDL meets HDL in the circulation, there is a lipid exchange between VLDL and HDL: some esterified cholesterol (CE) is transferred from HDL to VLDL by CETP (cholesterol-ester-transfer protein) and some TAGs are transferred from VLDL to HDL; this changes the composition of both the lipoproteins HDL and VLDL

IDL is converted to LDL by HTGL (hepatic-triglyceride-lipase), which is found on endothelial cells in the liver

c) free cholesterol is located in the surface layer of lipoproteins, with its polar -OH group oriented towards the aqueous environment of the blood plasma

d) the LDL-receptor (= Apo-B/E receptor) occurs on the plasma membrane of cells in target tissues, which thus receive cholesterol (LDL) from the blood

An inherited disorder called *familial hypercholesterolemia* leads to the accumulation of cholesterol in the blood and the early occurrence of cardiovascular diseases; the cause of this disorder is an impaired LDL-receptor synthesis leading to its dysfunctional structure (the receptor is not formed properly, the lipoprotein does not bind or is not transferred to the cell, or the receptor is not recycled); another possible cause is an impaired synthesis of the ligand of this receptor (Apo-B100), or of other protein(s) that participate in the entire process of transporting LDL into the cell.

The LDL-receptor is incorporated into the cell membrane according to the amount of cholesterol in the cell: if there is enough intracellular cholesterol, fewer receptors are incorporated into the membrane; if there is little cholesterol in the cell, the cell will expose more receptors on its surface.

e) the LDL-receptor is activated by Apo-B100 occuring in the surface layer of the lipoprotein (Apo-B100 is a ligand of this receptor, like the "key to the lock on the door to the cell"); it is also called the Apo-B/E receptor because it also binds ApoE; the LDL-receptor binds cholesterol-rich lipoproteins (mainly LDL) and thus allows the cell to remove the lipoproteins from the blood by endocytosis of the entire receptor with the bound lipoprotein; LDL-receptor is found in tissues that take cholesterol from blood, including endocrine glands, which synthesize steroid hormones from cholesterol

f) cholesterol is taken from the surface of cells by HDL, in which it is subsequently esterified by LCAT and then can be transferred (by CETP) to other circulating lipoproteins (VLDL) or taken up by the liver

g) LCAT is activated by Apo-AI (in HDL)

h) lipoprotein lipase (LPL) is activated by Apo-CII (in CHM and VLDL)

i) the liver enzyme cholesterol-7-α-hydroxylase is a regulatory enzyme for the synthesis of bile acids (cholic acid and chenodeoxycholic acid), which are formed in hepatocytes from cholesterol and are the main form of how the body can get rid of excess cholesterol; they are secreted into bile, which reaches the duodenum and is necessary for the digestion of lipids in the small intestine (it emulsifies fats: turns large fat droplets into many small ones - thus increasing the surface area, to which digestive enzymes then bind); bile acids are partially reabsorbed from the gastrointestinal tract and returned to the liver (where their excess inhibits cholesterol-7-α-hydroxylase)

Sufficient content of dietary fibre contributes to the reduced resorption of bile acids from the gastrointestinal tract into the blood: if less of them is reabsorbed, more is excreted in the faeces. As a result, the liver has to convert additional cholesterol into bile acids, which helps to reduce the concentration of cholesterol in the blood.

Metabolism of heme / picture 23 / pages 54 - 57

SYNTHESIS

a) synthesis begins and ends in the mitochondria, reactions on the right take place in the cytosol

b) succinyl-CoA (A) + glycine (B) → δ-aminolevulinate (C) = ALA = delta-aminolevulinic acid); regulatory enzyme of the whole synthesis: ALA-synthase, cofactor: pyridoxal phosphate (PLP) = vit. B6 derivative; -NH_2 group of the product (C) is located in position 5 (it is 5-aminolevulinic acid; C5 is referred to as delta-carbon according to the Greek alphabet because C2 is always alpha-carbon, C3 is beta-carbon, etc.)

c) in the bone marrow (a) heme is synthesized for hemoglobin in erythrocyte precursors, the synthesis is regulated mainly by the availability of iron; in the liver (b), where a lot of hemoproteins occur (mainly cytochromes), the synthesis is inhibited by an excess of the product, i.e. heme (= feedback inhibition) and is stimulated by various xenobiotics (foreign substances), the metabolism of which is catalyzed by hemoproteins (cytochromes P450)

d) ALA is transported to the cytosol, where two of its molecules condense to form porphobilinogen (D), which is a derivative of pyrrole; enzyme: ALA-dehydratase = porphobilinogen synthase (contains zinc, so it is sensitive to heavy metals); lead poisoning leads to an accumulation of delta-aminolevulinic acid, which, due to its similarity to the neurotransmitter gamma-aminobutyrate, (GABA) leads to neurological disorders

e) uroporphyrinogen III (E) is formed by asymmetrical connection of 4 porphobilinogens catalyzed by uroporphyrinogen III synthase (without this enzyme, another isomer, symmetrical uroporphyrinogen I, would spontaneously form)

f) coproporphyrinogen III (F), differs from substance (E) by side chains: instead of 4 residues of "acetate" (more precisely carboxymethyl rests) contains 4 methyls (4 CO_2 has been cleaved)

g) substance (F) is converted into protoporphyrinogen III (G) after transport to the mitochondria; it has 2 vinyl residues instead of 2 "propionate" residues (more precisely carboxyethyl rests); the transformation of a substance (G) to (H) is an oxidation that produces a conjugated system of double bonds, so that the product protoporphyrin IX is colored (Roman numerals indicate different isomers); if porphyrinogens accumulate, they oxidize spontaneously (in light) to different colored porphyrins, which cause photosensitivity (see the disorder called porphyria)

h) the incorporation of iron is catalyzed by ferrochelatase, which is inhibited by heavy metals (lead); ferrous protoporphyrin IX = heme is formed (it is metalloporphyrin)

i) porphyrias can be acquired or inherited; depending on which enzyme is deficient, the manifestations vary, in general: if the deficiency is at the beginning of the synthetic pathway, delta-aminolevulinate accumulates and causes neurological disorders, if later enzymes are missing, porphyrinogens also accumulate and cause photosensitivity; some porphyrinogens are excreted in the urine (where they are oxidized by light to porphyrins, giving the urine pink color); at the same time, anemia occurs (low hemoglobin); *so imagine a vampire: he's bloodthirsty, he doesn't go out in broad daylight, he behaves strangely...*

j) conjugated system of double bonds, see g)

DEGRADATION

a) biliverdin (B) is green, bilirubin (C) is yellow-orange (see the color of the bruise: at first it is purple-red from hemoglobin/heme from the blood, later it turns green to yellow); the color depends on the type of conjugate system of double bonds in the tetrapyrrole molecule

b) CH_2=CH- / vinyl, -CH_3 / methyl, HOOC-CH_2-CH_2- / carboxyethyl, =O / keto-

c) if heme is free, it is easily oxidized to hemin (contains Fe^{3+}), the released iron is stored in the cell in the form of Fe^{3+} in ferritin (storage protein), or as Fe^{2+} it is transported by DMT-1 (divalent metal transporter) through the membrane out of the cell, where it is oxidized again to Fe^{3+} (by an enzyme with ferroxidase activity: by hephaestin in the cell membrane – mainly of enterocyte, or by ceruloplasmin in the blood), then binds to transferrin (transport protein) and is transported in the blood to the heme synthesis sites or for further use (it is a cofactor of many metalloenzymes), or it is stored in the liver in ferritin

d) carbon monoxide (CO) is formed from one methine bridge (=CH-) of heme (cyclic tetrapyrrole) after its cleavage into a linear tetrapyrrole; it acts as a signaling molecule, in the blood it binds with a high affinity to hemoglobin which serves as its transport protein, then it is gradually exhaled (there is much less of CO in the blood than oxygen)

e) NADPH is formed mainly in the pentose cycle (in the cytosol)

f) heme oxygenase (1) oxidatively breaks down heme into biliverdin, biliverdin reductase (2) reduces the double bond in the middle of the biliverdin molecule (the methine bridge changes to methylene, the double bonds in the neighboring pyrrole are also rearranged) to form bilirubin, which therefore has a different color than biliverdin

g) degradation of heme from destructed erythrocytes (i.e. from hemoglobin) takes place in the endoplasmic reticulum in cells of the reticuloendothelial system (RES, mainly in the spleen), bilirubin is released from them into the blood, where it binds to albumin (bilirubin itself is poorly soluble in water); this bilirubin is also called "indirect bilirubin" because it does not react directly with the chemical agent during laboratory determination, but only after it has been released from albumin; it is the predominant form of bilirubin occurring in the blood (physiological concentration up to 17 µmol/l)

h) UDP-glucuronate (it is "activated" form of glucuronate so that it can be conjugated with bilirubin): 2x UDP-glucuronate + bilirubin $\rightarrow$ conjugated bilirubin (= bilirubin diglucuronide = bilirubin diglucosiduronide); enzyme: glucuronosyltransferase; the product of the conjugation is well soluble in water

i) the conjugation of bilirubin takes place in the liver (in the same way as various other substances that are poorly soluble in water – endogenous or exogenous, the so-called xenobiotics); conjugates are excreted into the bile by active transport, enter the intestines with the bile and can thus be excreted from the body in the feces; a small amount of conjugated bilirubin also enters the blood, where it is referred to as "direct bilirubin", because it does not have to be transported by albumin (the conjugate is water-soluble), so during laboratory determination, the conjugated bilirubin reacts directly with the chemical reagent (physiological concentration up to 5 µmol/l); in medicine, the total concentration of bilirubin in the blood (physiological concentration up to 22 µmol/l) is commonly determined

The proportion of direct and indirect bilirubin is assessed in order to find out where in the metabolism of bilirubin a disorder has occurred: in front of the liver / in the liver / in the bile ducts (this is a differential diagnosis of jaundice = icterus that is caused by an increased concentration of bilirubin in the blood); therefore, it is important to understand bilirubin metabolism.

j) in the large intestine, this transformation is done by bacteria (their enzymes): they deconjugate bilirubin and reduce it to urobilinogen (product of reaction “c”), which is colorless because it no longer has a conjugated system of double bonds; part of urobilinogen is resorbed into the blood and returned to the liver via entero-hepatic circulation; from the blood it also gets into the urine (see “uro-“ in the name), with which it is excreted; part of urobilinogen is further metabolized in the intestine to other so-called urobilinoids (e.g. stercobilinogen), these are excreted in the feces; since in light "-bilinogens" are oxidized to "-bilins" (e.g. urobilinogen to urobilin), these substances give color to urine and feces

k) increased breakdown of erythrocytes (= hemolytic or prehepatic jaundice, indirect bilirubin is elevated), impaired bilirubin metabolism in the liver (= hepatic jaundice, both indirect and direct bilirubin are elevated), obstruction of the bile ducts, e.g. by a stone (= obstructive or posthepatic jaundice, direct bilirubin is elevated); it is a differential diagnosis of jaundice

a) *tocopherol* = *vit. E*, pyridoxine = vit. B6, thiamine = vit. B1, ascorbate = vit. C, cobalamin = vit. B12, *retinol* = *vit. A*, *cholecalciferol* = *vit. D*, folate = vit. B9, riboflavin = vit. B2, *phylloquinone* = *vit. K*, biotin = vit. B7 = vit. H, pantothenic acid = vit. B5, niacin (nicotinamide) = vit. B3

b) pyridoxal phosphate = PLP (vit. B6), thiamine diphosphate = TDP = thiamine pyrophosphate = TPP (vit. B1), deoxyadenosylcobalamin or methylcobalamin (vit. B12), tetrahydrofolate = THF (vit. B9), flavinadenine dinucleotide = FAD / $FADH_2$ (vit. B2), carboxybiotin (vit. B7), coenzyme A = CoA-SH = CoA (vit. B5), nicotinamide adenine dinucleotide = NAD^+ / $NADH + H^+$ or nicotinamide adenine dinucleotide phosphate = $NADP^+$ / $NADPH + H^+$ (vit. B3)

c) see point a) *in italics*: *fat-soluble vitamins (A, D, E, K), others* are water-soluble; hydrophobic = lipophilic = water-insoluble vitamins (but fat-soluble): all have at least part of their structure derived from isoprene: $CH_2=C(CH_3)-CH=CH_2$, i.e. they are isoprenoids (derivatives of isoprene, which forms polymers by polyaddition)

Compartmentalization of metabolic pathways / picture 25 / page 59

Mitochondria: Krebs cycle (KC), aerobic phosphorylation (= respiratory chain + ATP synthase), pyruvate dehydrogenase complex (PDH), β-oxidation, synthesis (only in the liver) and degradation (not in the liver) of ketone bodies, beginning and end of synthesis of heme and urea* (urea cycle = ornithine cycle occurs only in the liver), beginning of gluconeogenesis** (if it starts from pyruvate, lactate and KC intermediates, i.e. also from amino acids that are degraded into them), glutamate dehydrogenase (= central enzyme of amino acid metabolism), some transaminases (AST)

Cytosol: glycolysis, gluconeogenesis** (the whole from oxaloacetate or from DHAP, i.e. when it starts from glycerol), pentose cycle, synthesis and degradation of glycogen, metabolism of fructose and galactose, synthesis of fatty acids (from C4 to C16, i.e. up to palmitate), various transaminases (ALT, AST), part of the synthesis of heme and urea*

Smooth endoplasmic reticulum: synthesis of triacylglycerols, elongation and desaturation of fatty acids, glucose-6-phosphatase (only in hepatocytes, tubular renal cells and enterocytes)

** gluconeogenesis takes place only in the liver (90%) and kidneys (and also enterocytes)

Names of enzymes / exercises / pages 76 - 79

a) see Pictures in this workbook; γ-aminobutyrate: $H_2N-CH_2-CH_2-CH_2-COO^-$; *for answers with formulas see next page*

b) (1) dehydrogenation (NAD^+), dehydrogenation (FAD); (2) transamination, phosphorylation (phosphate transfer); (3) hydrolysis, hydrolysis; (4) decarboxylation, hydration; (5) isomerization, isomerization; (6) carboxylation, condensation ("amidation")

c) (1) EC 1 = oxidoreductases, (2) EC 2 = transferases, (3) EC 3 = hydrolases, (4) EC 4 = lyases, (5) EC 5 = isomerases, (6) EC 6 = ligases

d) *reversible* under physiological conditions: (1) both, (2) transamination only, (4) hydration only, (5) both; *irreversible* under physiological conditions: (2) only phosphorylation (ATP is split), (3) both, (4) decarboxylation only, (6) both (ATP is split)

e) (1) dehydrogenase, dehydrogenase, (2) aminotransferase, phosphotransferase = kinase, (3) phosphatase, lipase, (4) decarboxylase, hydratase, (5) mutase, racemase, (6) carboxylase, synthetase

f) (1) lactate dehydrogenase / NAD^+; succinate dehydrogenase / FAD, (2) alanine aminotransferase (ALT) / PLP; glucokinase or hexokinase, (3) glucose-6-phosphatase, lipase, (4) glutamate decarboxylase / PLP; fumarate hydratase, (5) phosphoglucomutase; serine racemase / PLP, (6) pyruvate carboxylase / ATP, biotin; glutamine synthetase / ATP

g) see (f)

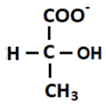
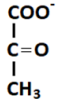
h) NAD^+ / vit. B3, FAD / vit. B2, PLP / vit. B6

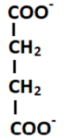
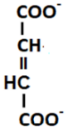
I have worked out the solutions to all the tasks in as much detail as possible, with the intention of helping you understand the metabolic context, which may be useful to you in the future. If you come across any inaccuracies or ambiguities in the solution, I will be happy for your feedback. Thank you, Vladimíra Kvasnicová.

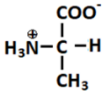
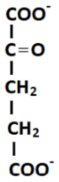
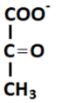
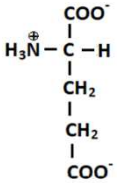
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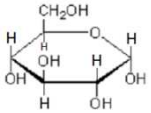
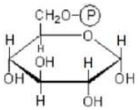
see task **a)**

Exercise

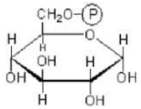
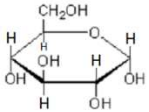
1)  + NAD^+ →  + $NADH + H^+$

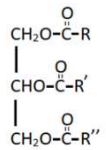
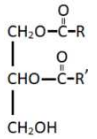
 + FAD →  + $FADH_2$

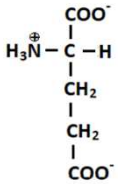
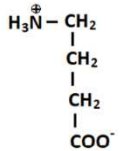
2)  +  →  + 

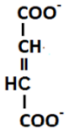
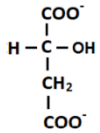
 + ATP →  + ADP

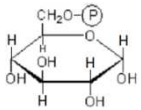
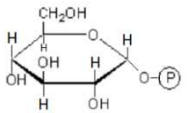
3)

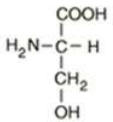
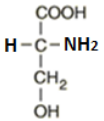
 + H_2O →  + phosphate

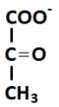
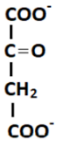
 + H_2O →  + HOOC-R´´

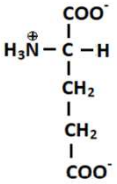
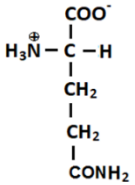
4)  →  + CO_2

 + H_2O → 

5)  → 

 → 

6)  + CO_2 + ATP →  + $ADP + P_i$

 + NH_3 + ATP →  + $ADP + P_i + H_2O$