UNIVERSITÉ PARIS-EST CRÉTEIL

TUTORIALS

BIOCHEMISTRY

Energy metabolism

FACULTY OF SCIENCES AND TECHNOLOGY

L2 SVT - CB

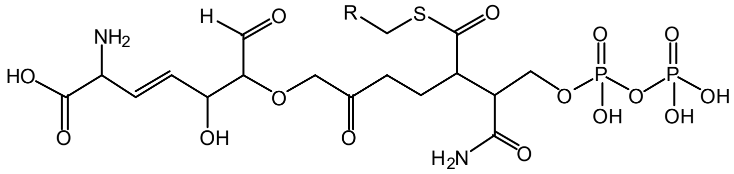
2016-2017

LIST OF TUTORIAL SESSIONS

Redox reactions	**1 session**
Mitochondrial respiratory chain	**1 session**
Hydrolysis/condensation reactions (transfer)	**1 session**
Energetic catabolism	**1 session**
Reading metabolic paths	**1 session**
Predicting metabolic pathways	**2 session**

REMINDER OF SOME CHEMICAL FUNCTIONS

Identify the different chemical functions in the following compound:



REDOX REACTIONS

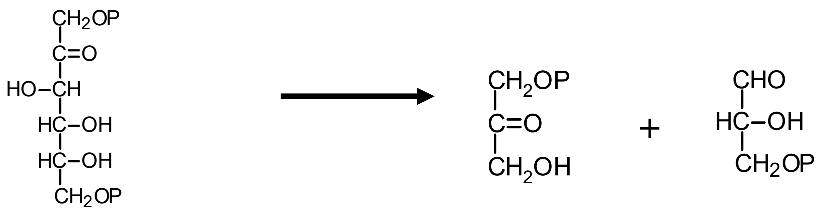
The next steps are part of important metabolic pathways in living organisms. For each step identify whether a redox reaction is involved (be aware that other reactions might occur in the same path) and determine whether it is an oxidation or a reduction.
Equilibrate the redox reactions with redox co-enzymes and specify the corresponding transferred e^- and H^+ in each half-complementary reaction.

a) Glycolysis steps

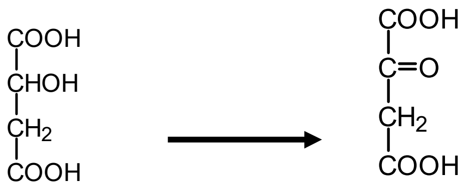
- Glyceraldehyde (glyceral) → Glycerol
  $CH_2OH\text{-}CHOH\text{-}CHO \longrightarrow CH_2OH\text{-}CHOH\text{-}CH_2OH$

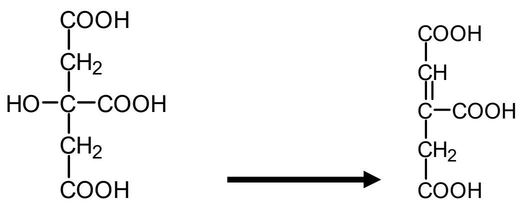
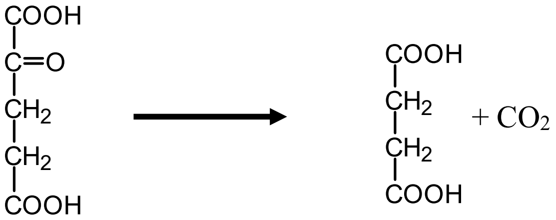
- Glyceraldehyde (glyceral) → Glyceric acid
  $CH_2OH\text{-}CHOH\text{-}CHO \longrightarrow CH_2OH\text{-}CHOH\text{-}COOH$

- D-fructose-1-6 phosphate   Dihydroxyacetone phosphate + glyceraldehyde-3-phosphate



b) Krebs cycle (citric acid cycle or tricarboxylic acid [TCA] cycle)

- L-Malate  Oxaloacetate

- Citrate  Cis-aconitate α-ketoglutarate  Succinate + CO_2

c) Fatty acid metabolism (Lynen helix)

- Lauryl-CoA $\longrightarrow$ Lauric acid + Coenzyme A

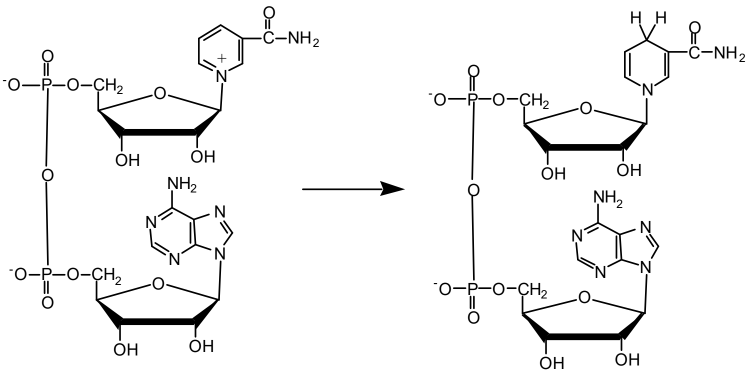
$$CH_3\text{–}(CH_2)_{10}\text{–CO–S–CoA} \longrightarrow CH3\text{–}(CH_2)_{10}\text{–COOH} + \text{HS–CoA}$$

- 3-Ketocaproyl-CoA + Coenzyme A $\longrightarrow$ Butyryl-CoA + Acetyl-CoA

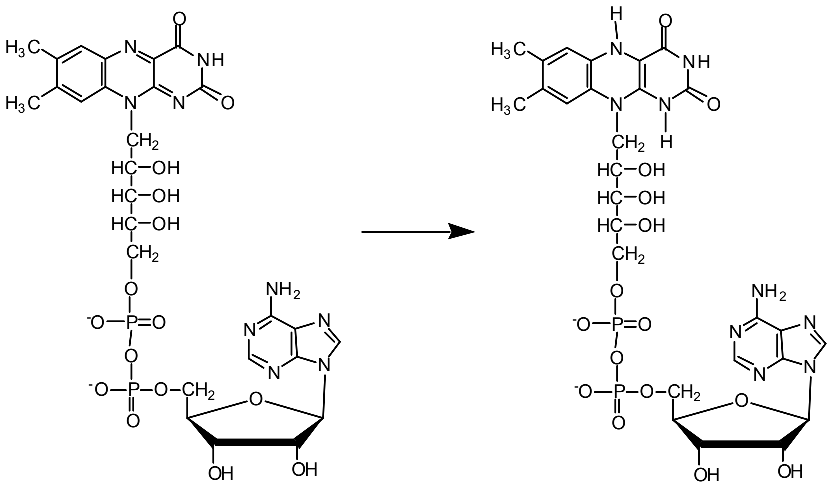
$$CH_3\text{–}(CH_2)_2\text{–CO–}CH_2\text{–CO–S–CoA} + \text{HS–CoA} \longrightarrow CH_3\text{–}(CH_2)_2\text{–CO–S–CoA} + CH_3\text{–CO–S–CoA}$$

d) Redox coenzymes

The NAD^+/NADH and FAD/$FADH_2$ redox coenzymes couples are frequently involved in redox metabolic reactions. In the following structures, identify the redox moiety for each coenzyme as well as the number of electrons and protons that are exchanged.



NAD^+ NADH



FAD $FADH_2$

MITOCHONDRIAL RESPIRATORY CHAIN
(Electron transport chain)

<u>Part 1)</u> Respiratory chain transporters

Mitochondrial respiratory chain involves electron transporters (coenzymes) including: FMN, Coenzyme Q, cytochromes a, b, c, c1 and a3. In order to study these transporters, isolated mitochondria were incubated in a survival medium and the percentage of oxidation r(T) of each transporter in the preparation was determined.

In a first experiment, oxygen was injected to the preparation and NADH was then added. The following results were obtained:

(i) r(FMN) $<$r(Q)

(ii) r(cyt c1) $<$ r(cyt c) $<$ r(cyt a).

Question 1.1) *How do you interpret these results?*

In a second experiment, O_2 was injected to the preparation until complete oxidation of the transporters, then successively an inhibitor and NADH were added.
NB: The inhibitor is necessarily added before NADH.

The next table gives the r(T) values for the different transporters measured in the presence of different inhibitors.

	Antimycine	Amytal	Cyanure
Coenz Q	0	0	0
FMN	0	0	0
Cyt c	100	100	0
Cyt c_1	100	100	0
Cyt a	100	100	0
Cyt a_3	100	100	100
Cyt b	0	100	0

Question 1.2) *Determine the order of the transporters in the respiratory chain.*

An excess of electron donor AH2 is added to a preparation of totally oxidized mitochondria, and r(T) is measured:

Transporter	FMN	Coenz Q	Cyt b	Cyt c	Cyt a
r(T)	100	100	100	30	70

Question 1.3) *How do you interpret these results?*
Question 1.4) *How many ATP are produced by mitochondrial preparation?*

<u>Part 2.</u> Mitochondrial respiratory chain inhibitors

The influence of two compounds **X** and **Y** on the respiration of a mitochondrial preparation is studied.

<u>First experiment:</u> NADH, ADP, phosphate, and oxygen are added to the mitochondrial suspension. Mitochondrial respiration is confirmed.

Question 2.1) *How can we confirm mitochondria respiration and ATP production?*

Then **X** is added to the suspension: Mitochondrial respiration is still ongoing, but ATP production is arrested.

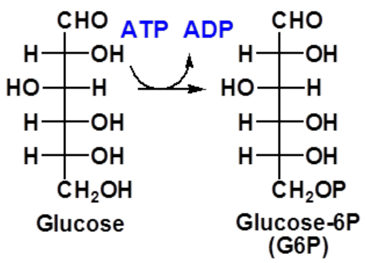
2nd experiment: NADH, ADP, phosphate, oxygen and compound Y are added to the mitochondrial suspension. Mitochondrial respiration stops. Then X compound is added : mitochondrial respiration does not restart.

3rd experiment: Succinate, ADP, phosphate, and oxygen are added to the mitochondrial suspension. Mitochondrial respiration restarts. Then Y compound is added and mitochondria keep respiring.

Question 2.2) Using observations *from these experiments, indicate what can be the actions of X and Y compounds in the respiratory chain?*

HYDROLYSIS/CONDENSATION REACTIONS
(transfer reactions)

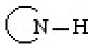
I. The first step of glycolysis, transforming glucose into glucose-6-phosphate (G6P), requires ATP as follows:



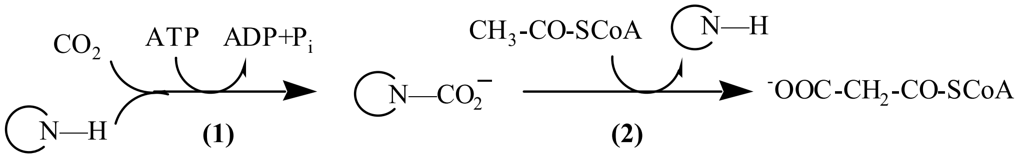
Ia) *Indicate whether this is a single or double transfer reaction and decompose it into the corresponding hydrolysis and condensation reactions.*

II. Carboxylation reactions in animal cells occur by introduction of CO_2 in a series of steps always involving biotin (VitaminB7) as cofactor. ATP provides energy for this type of reactions. For instance the synthesis of malonyl-CoA from acetyl-CoA can be summarized as:

$$CH_3\text{-CO-SCoA} + CO_2 \xrightarrow[\text{Biotin}]{\text{ATP}\quad\text{ADP+P}_i} {}^-\text{OOC-}CH_2\text{-CO-SCoA}$$

Biotin is here symbolized by: 

Indeed, this metabolism occurs in two successive steps:



II.a) *Determine whether step 1 corresponds to a simple or double transfer reaction. Justify your answer.*

II.b) *If step 1 is a double transfer reaction, split it up in two plausible simple transfer reaction and indicate the double transfer intermediates.*

III. From the following reaction:

$$RCOO^- + HSCoA \xrightarrow{ATP \;\; AMP + PPi} RCOSCoA$$

III.a) *Determine whether this corresponds to a simple or double transfer reaction. Justify your answer.*

III.b) *If you have identified a double transfer reaction, split it up into two plausible simple transfer reactions and define the double transfer intermediates.*

ENERGETIC CATABOLISM

I. GLYCOLYSIS

1) AEROBIC AND ANAEROBIC GLYCOLYSIS BALANCE ASSESSMENT

Virtually all tissues have a requirement for glucose to function normally. Glycolysis is the major pathway of glucose metabolism and occurs in the cytosol of all cells. It can occur aerobically or anaerobically depending on whether oxygen is available. This is clinically significant because oxidation of glucose under aerobic conditions results in higher ATP production.

a) *Write the substrate-product balance of the conversion of D-glucose into pyruvate*

- *Considering molecules involved in the glycolysis pathway including cofactors.*
- *Considering molecules involved in the glycolysis pathway after cofactors regeneration by the respiratory chain.*

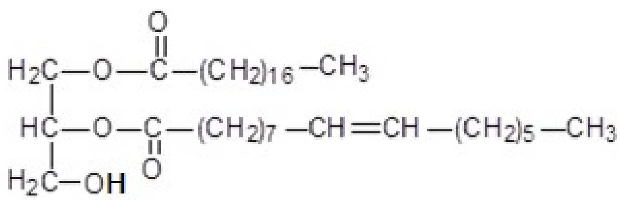
The majority of ATP in a cells is formed by the electron transport chain, which requires a large input of oxygen. However, many organisms have developed strategies to carry out metabolism without oxygen, or can switch from aerobic to anaerobic cell respiration when oxygen is scarce.

b) *Write the reaction at pH 7 which allows a cell to regenerate the redox cofactors used in the transformation of glucose to pyruvate in anaerobic conditions and determine corresponding ATP yield.*

c) *Compare ATP yields in aerobic vs anaerobic conditions and comment.*

II. FATTY ACID / TCA CATABOLISM

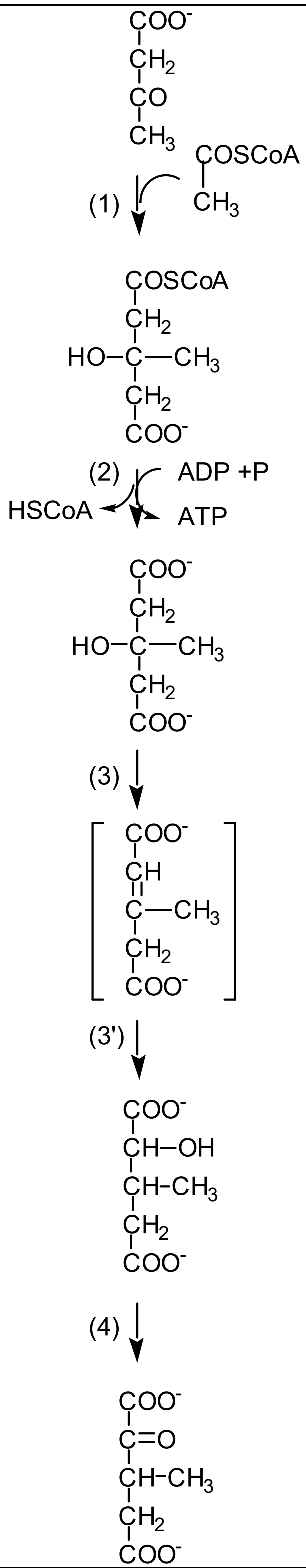
1) *How many CO_2 molecules will be generated by the complete degradation of the following diglyceride (present in circulation) by the Lynen helix and the TCA cycle ? Determine ATP yield after cofactors regeneration by the respiratory chain?*



READING METABOLIC PATHS

I. VALINE DEGRADATION

Complete the following pathway: for each step indicate the involved reaction type(s) (major processes), enzyme, coenzyme (s) and reversibility. Write the missing structure between (6) and (7).



<u>Reaction type(s):</u>
<u>Enzyme:</u>
<u>Coenzyme(s):</u>
<u>Reversibility:</u>

<u>Reaction type(s):</u>
<u>Enzyme:</u>
<u>Coenzyme(s):</u>
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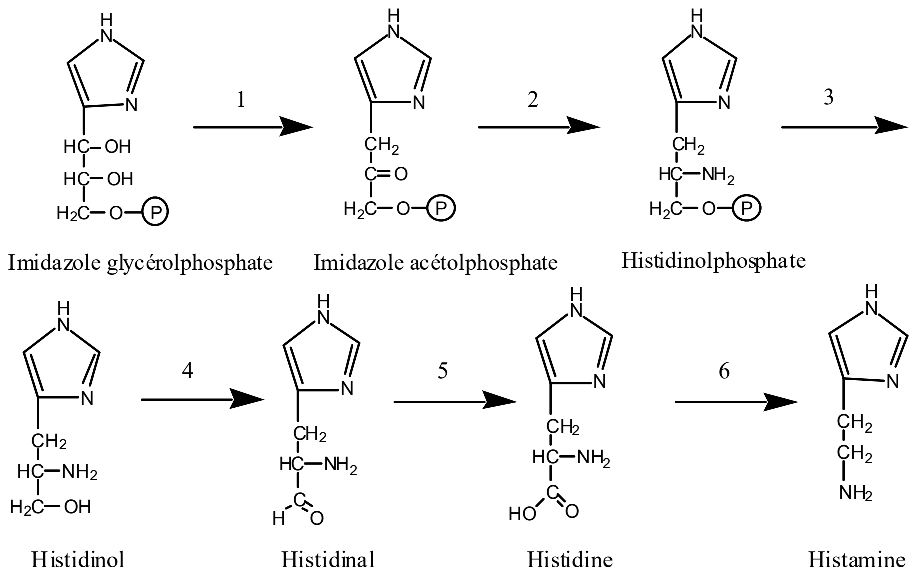
<u>Reaction type(s):</u>
<u>Enzyme:</u>
<u>Coenzyme(s):</u>
<u>Reversibility:</u>

II. Histidine biosynthesis

Histidine biosynthesis involves the transformation of precursor *N'*-(5'-prosphoribosyle-ATP) into imidazole-glycerolphosphate, which is then transformed into histidine by the sequence of reactions indicated below. Histidine can then form histamine, an important hormone of the immune system.
(Ⓟ symbolizes phosphate groups).



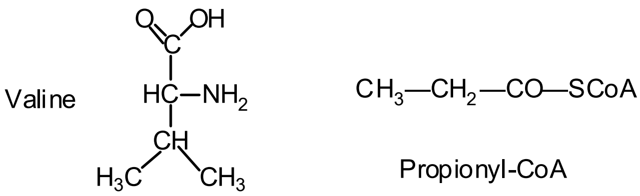
PREDICTING METABOLIC PATHWAYS

I. VALINE CATABOLISM

Valine degradation results in the production of propionyl-CoA and CO_2.

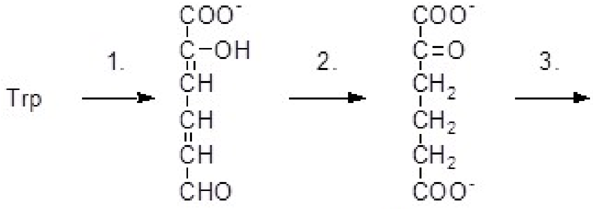
a) *Determine the number of predictable major processes (reactions) that could be involved in the pathway. Justify your answers.*

b) *Propose a sequence of reactions allowing this degradation. Include enzymes, coenzymes and reversibility* Necessary for each step.



II. CATABOLISM OF TRYPTOPHAN

Tryptophan catabolism involves the formation of 2-hydroxymuconate-6-semialdehyde as intermediate. This is in turn transformed into α-ketoadipate, which finally yields Acetyl-CoA and CO_2:

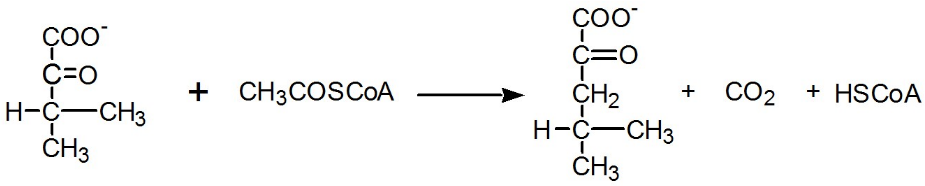


2-hydroxymuconate-6-semialdehyde α-ketoadipate

Propose a metabolic pathway for sequences 2 and 3. For each step indicate the involved reactions, enzyme, coenzymes and reversibility.

III. BIOSYNTHESIS OF ∝-KETOISOCAPROIC ACID

α-Ketoisocaproate is an intermediate in the metabolism of leucine. In this pathway, a mole of ∝-ketoisovalerate can react with a mole of acetyl-coA to yield ∝-ketoisocaproate and CO_2, as follows :



α–ketoisovalerate α–ketoisocaproate

Propose a sequence of reactions to build this path. Include enzymes, coenzymes and reversibility necessary for each step.