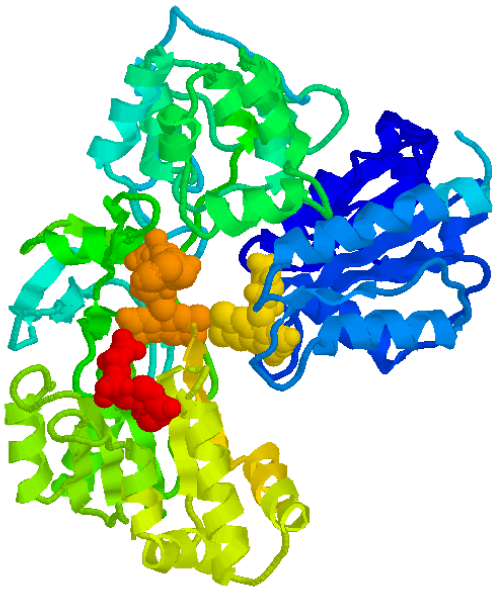
BIOCHEMISTRY I

BY

CRISTINA COSTA



by Cristina Costa

cooperative effect → in proteins with many subunits – conformation change in one subunit is transferred to other subunits. (explains how oxygen binding occurs)

1 Structure of haemoglobin; structure/function relationships; the oxygen saturation curve; movement of haemoglobin saturation and oxygen transport

Hb A: major Hb type – is a heterotetramer: 2 α-chains + 2 β-chains;

Each of the 4 subunits carries a heme* group (prosthetic): protoporphyrin IX + Fe^{2+} ion

(oxidation of Fe^{2+} to Fe^{3+} is v rare; methaemoglobin values in blood are about 1-2%.)

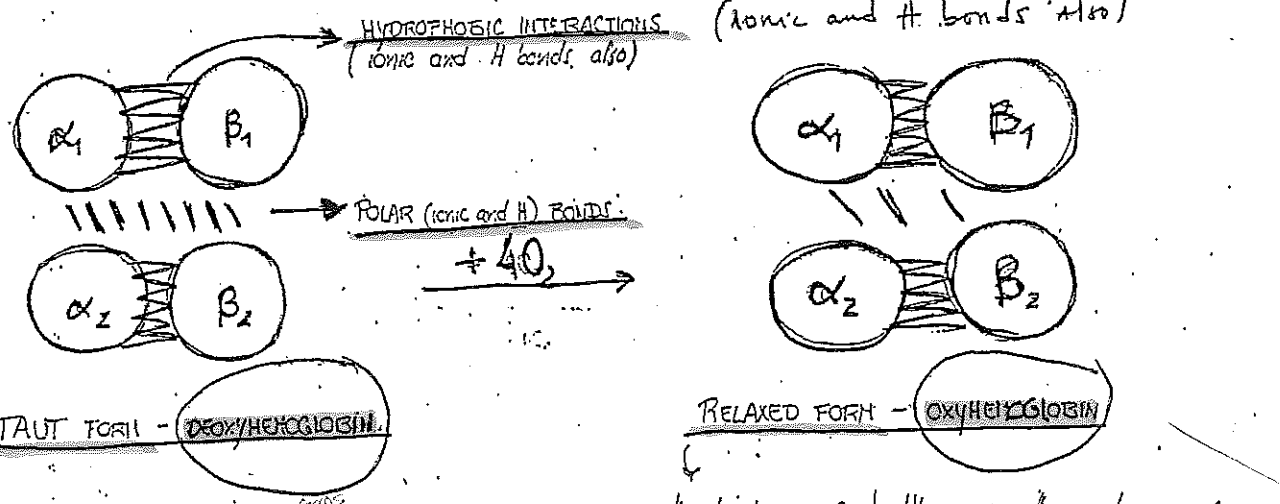
↓ iron of heme group is in the Fe^{3+} form, doesn't carry oxygen

4 of the 6 coordination sites of Fe^{2+} are occupied by N atoms of the pyrrole rings and another one is occupied by a histidine residue.

The 6th site is for oxygen (oxyhemoglobin) or H_2O (deoxyhemoglobin)

↳ QUATERNARY STRUCTURE OF HB:

→ Hb tetramer is composed of 2 homodimers $(\alpha\beta)_1$ and $(\alpha\beta)_2$



forms the network of ionic and H bonds. constrain the movement of the polypeptide strains

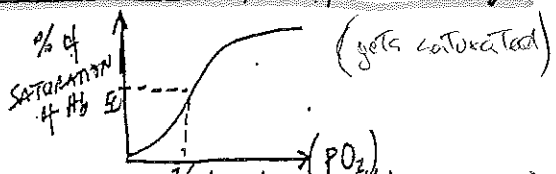
↳ the binding of O_2 to Hb causes the rupture of some of the ionic and H bonds. The polypeptide chains have more freedom of movement.

LOW OXYGEN-AFFINITY FORM

(stabilized by 2,3-bisphosphoglycerate** / 2,3-bisphosphoglycerate)

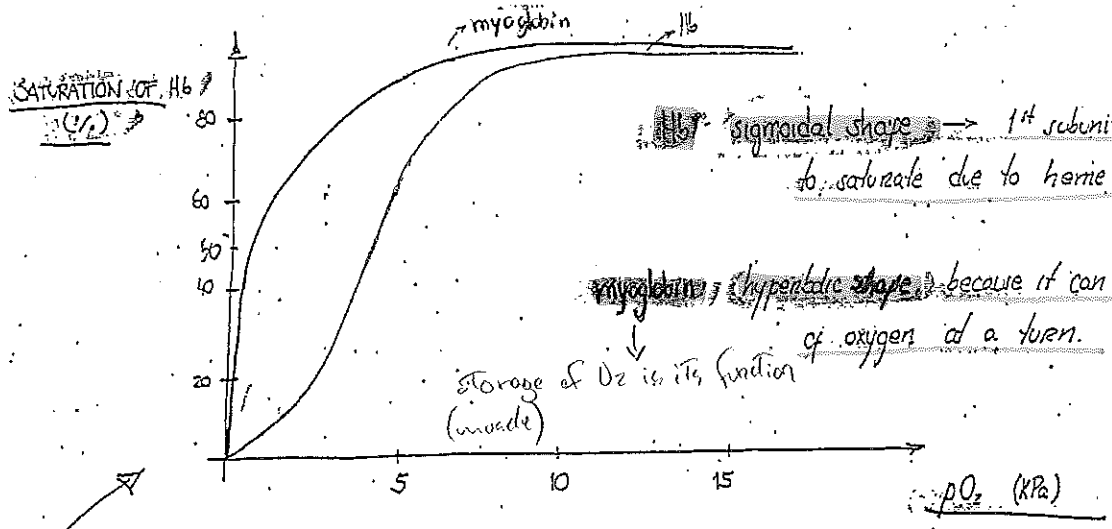
HIGH OXYGEN-AFFINITY FORM

FUNCTION OF HB: transports CO_2 from tissues to the lungs and carries 4 mol of O_2 from the lungs to the tissues, one per each heme group.



↳ OXYGEN DISSOCIATION / SATURATION CURVE – a plot of Y (degree of saturation of Hb) measured at different partial pressures of O_2 (pO_2)

→ The pO_2 needed to achieve half saturation of binding sites (P_{50}) is aprox 26mm Hg!



Hb - sigmoidal shape → 1st subunit is more difficult to saturate due to heme-heme interactions

myoglobin - hyperbolic shape - because it can only bind 1 mol of oxygen at a turn.

OXYGEN TRANSPORT

ALLOSTERIC EFFECTORS → substances that induce conformational changes in the enzyme (in a different site of the one where the effector binds.) (in this case Hb is not an enzyme)

OF Hb:

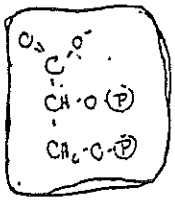
- pO_2 - positive effect → high pO_2 promotes oxygen binding, due to HEME-HEME INTERACTIONS: the binding of the first O increases the affinity for it in the other heme groups. This effect explains the sigmoidal shape of the saturation curve.
(if it were hyperbolic, Hb would have maximum affinity for oxygen throughout the whole pO_2 range, thus it wouldn't release oxygen in the tissues!)
- pH, pCO_2 } BOHR EFFECT → an increase of pCO_2 or a lowering of pH decrease the affinity of Hb for oxygen, thus a shift to the right in the saturation curve
(↑ pCO_2 → ↓ pH → release of O_2)
(the protons for the decrease of pH can be obtained by the blood buffer:
$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$)

Bohr effect scheme:

$HbO_2 + H^+ \rightleftharpoons HbH + O_2$
(oxyhemoglobin) (deoxyhemoglobin) — BOHR EFFECT

→ - (m. tissues) - ↓ pH → ↑ $[H^+]$ → release of O_2
← - (in lungs) - ↑ pO_2 → Hb binds oxygen

- 2,3-bisphosphoglycerate - decreases oxygen affinity by binding to deoxyhemoglobin and thus stabilizing the taut conformation of HbH.



SITE OF BINDING: positively charged cavity formed by the β-chains (in the centre!)

HbO_2 + 2,3-BPG ⇌ Hb-2,3-BPG + O_2
(oxyhemoglobin) (deoxyhemoglobin)

→ 2,3-BPG reduces oxygen affinity, shifting the saturation curve to the right.
→ This decrease enables oxygen to be released in the tissues.

2 Normal hemoglobin types in blood, hemoglobin concentration. Others forms (glycohemoglobin, methemoglobin, carboxyhemoglobin) and abnormal hemoglobins.

Hb values...

→ Hb can bind a max. of 220mL (O_2) per liter.

→ Hb content in blood { men: 140 - 180 g/L; women: 120 - 160 g/L

→ CONCENTRATION OF Hb IN BLOOD: 2,15 - 2,65 mmol/L

M_r(hemo) = 16 000

NORMAL HUMAN HEMOGLOBINS

		fraction of total Hb
• HbA (appears only after 8th month)	$\alpha_2\beta_2$	90%
• HbA_{1c}	$\alpha_2\beta_2$-glucose	3-9%
• HbF	$\alpha_2\gamma_2$	< 2%
• HbA_2	$\alpha_2\delta_2$	2-5%

HbA_{1c} - part of HbA that becomes glycosylated (increased amounts are found in diabetes mellitus patients because their HbA is in contact with higher blood glucose concentrations).

HbF - fetal Hb - major Hb found in fetus and newborn, HbA only starts being synthesized at the 8th month, it gradually replaces HbF.
→ HbF has higher affinity for oxygen because it transports O_2 to the fetus

HbA_2 - appears 12 weeks after birth (3 months)

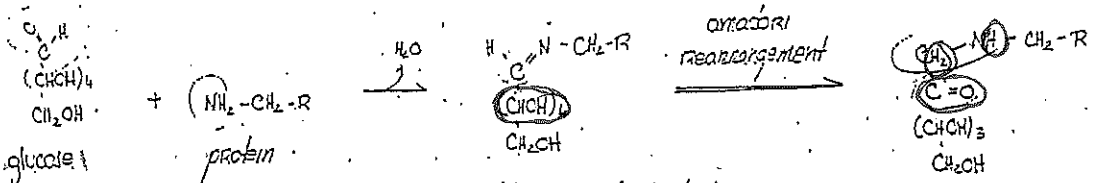
OTHER FORMS OF Hb

→ CARBOXYHEMOGLOBIN = Hb combined with CO (monoxide carbonyle), which will bind strongly to the sites oxygen should bind. (→ Avoids O_2 binding)

values (in total Hb): NON-SMOKERS: 3%; HEAVY SMOKERS: 12% (symptoms start at 20%)

→ non-enzymatic

→ GLYCOHEMOGLOBIN – Hb that suffers glycation by exposure to glucose in blood.



GLUCATED PROTEIN !!

→ METHEMOGLOBIN = hemiglobin — Hb in which the iron ion has oxidation no. 3+ instead of 2+ (caused by nitrate or NO!)
→ not able to transport O_2 ⇒ tissue hypoxemia.
Met-Hb values are kept low in blood (1-2%) by reduction of the iron ion.

ABNORMAL Hbs — caused: { - point mutation – Hb S ($\alpha_2\beta_2^{6Glu\to Val}$); Hb C ($\alpha_2\beta_2^{6Glu\to Lys}$); etc.
- absent / defective synthesis of Hb chains { α – α-thalassemia; β – β-thalassemia }

- Hb S ($\alpha_2\beta_2^{6Glu\to Val}$) disease – SICKLE-CELL DISEASE → due to a nucleotide alteration (valine instead of glutamate) in the 6th position of β-globin chain in the β-globin gene. It's a recessive disorder, occurs in individuals with 2 inherited mutant alleles. Resulting Hb: $\alpha_2\beta^S_2$ (Hb S)
(Heterozygotes contain both Hb S and Hb A ⇒ sickle-cell trait (no symptoms))
Misshapen erythrocytes often block blood flow in narrow capillaries → causes anoxia (O_2 deprivation) of tissues and can lead to cell death.

- Hb C disease ($\alpha_2\beta_2^{6Glu\to Lys}$) – single amino acid substitution (lysine instead of glutamate)
→ patients have a mild, chronic hemolytic anemia.

- β-THALASSEMIAS – synthesis of β-chains absent (β°-thalassemia) or defective:
there are 2 copies of the β-globin gene
  - one defective gene: β-thalassemia trait (minor)
  - 2 defective genes: β-thalassemia major – severe anemia → blood transfusions!

- α-THALASSEMIAS – synthesis of α-chains is absent (α°-thalassemia) or defective:
there are 4 copies of the α-globin gene
  - 1 defective gene: silent carrier
  - 2 " genes: α-thalassemia trait
  - 3 " " : Hb H disease – sorrowful severe hemolytic anemia.
  - 4 " " : hydrops fetalis – fetal death because α-chains are needed in Hb F

protein catalysts which increase the rate of reactions without being changed in the overall process.

p53 (3) **Enzymes** – structure and catalytic function, characteristics of biocatalysis; Enzyme-substrate interaction, examples of mechanisms of enzyme-catalyzed reactions. The term "isoenzymes".

→ Characteristics of biocatalysis:
- the enzymes remain unchanged
- the reaction equilibrium (K) is not altered
- enzymes increase the rate at which a reaction approaches equilibrium by lowering the free energy of activation – ΔG

Enzymes → nearly all are proteins; they can also have:
- organic coenzymes (coenzymes)
- metal ions (cofactor)
- non-protein prosthetic groups – covalently bound coenzymes

} needed for catalytic activity

⇒ many enzymes are restrained to certain organelles (compartments) in the cell; the principle of compartmentalization facilitates the control of the different metabolic pathways.

⇒ enzymes give to the reactions reaction rates of several orders of magnitude greater. (10^6 - 10^{17}) Higher reaction rate

⇒ enzymes are very unstable and are subjects of biodegradation.

⇒ " are highly specific catalysts: catalyze a single type of reaction or a closely related set of reaction (absolute substrate specificity is rather rare)

⇒ **enzymes** are regulated catalysts

All these can be changed or regulated:
- catalytic activity can be inhibited or increased (by binding of molecules or covalent modifications)
- the specificity of few enzymes can be changed
- the amount of enzymes in the cell can be controlled (eg. by gene expression)

Enzyme-substrate interaction → INDUCED-FIT MODEL: the enzyme changes its shape on substrate binding. The active site becomes complementar to the substrate only after its binding.

2 types of COFACTORS:
- **PROSTHETIC GROUPS** – joined tightly (by covalent bond) to the protein
- **CO-ENZYMES** – freely attached to the protein (is able to exist without it)

CATALYTIC MECHANISMS – depend on the nº of substrates!

transition state!

① **MONOSUBSTRATE** (monomolecular) **REACTIONS** (rare...) → $E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$

② **BISUBSTRATE** (bimolecular) **REACTIONS** → $E + S_A + S_B \rightleftharpoons ES_{AB} \rightleftharpoons EP_{AB} \rightleftharpoons E + P_A + P_B$

③ **MULTIPLE SUBSTRATE REACTIONS**

- **SEQUENTIAL DISPLACEMENT** – all S must bind to E before P is release
  - type ordered – substrates bind to E in a defined sequence
  - type random – the order of addition of S/release of P is random
- **DOUBLE-DISPLACEMENT** (PING-PONG) – one or more products are released before all S bind to the E

↳ the decrease of free E of activation ΔG is caused by facilitating the formation of the transition state

examples of different types of catalytic mechanisms:

- covalent catalysis – formation of transitory covalent bonds between E and S
- acid-base catalysis – protonization of S or catalytic groups of E
- (many others...)

ISOENZYMES → enzymes that differ in aa sequence but catalyse the same chemical reaction

(isoforms of def. enzyme)

↳ close variants

example: glucokinase is a variant of hexokinase that is not inhibited by glucose-6-P

different alleles of the same gene

- **alloenzymes** – enzymes from different alleles of the same gene
- **isoenzymes** – " from different genes that catalyse the same reaction

different genes, same reaction

→ isoenzymes may contain different nº of charged aa and may, therefore, be separated from each other by electrophoresis.

As different organs have characteristic proportions of different isoenzymes, the pattern of isoenzymes found in the plasma may serve as a means of identifying the site of tissue damage.

4) Kinetics of enzyme-catalyzed reactions: the term reaction rate, factors affecting the rates of enzyme catalyzed reactions. The progress curves, the Michelis-Menten plot (saturation curve). The Km value and its significance.

FUNDAMENTALS OF REACTION KINETICS

- studies the rates of chemical reactions
- can be expressed by velocity when we consider the changes in the [reactants]

Simple reaction: $S \rightarrow P$

$$v = -\frac{1}{\nu}\frac{\Delta[S]}{\Delta t} = \frac{1}{\nu}\frac{\Delta[P]}{\Delta t}$$ mol · l⁻¹ · s⁻¹

(ν = stoichiometric coefficients)

factors affecting velocity:
- T (temperature)
- [x] (reactant's quantity)
- catalysts or inhibitors

→ THE RATE or velocity of a reaction is the no. of substrate molecules converted to product per unit of time; usually expressed by mmol/min → with

VELOCITY EQUATION ($aA + bB \rightarrow cC$)

$$v = K[A]^{\alpha}[B]^{\beta}$$ (K = kinetic constant)

$\alpha + \beta$ = reaction order → the sum of all exponents ($a, b, \ldots$) gives the REACTION ORDER

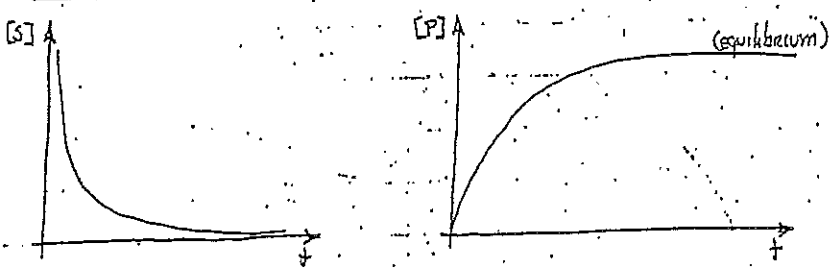
$v = K[A]^{\alpha} \cdot K[B]^{\beta}$

by this we can say that in closed systems, due to decreasing [x]s of reactants, the reaction velocity will gradually decrease till it reaches equilibrium.

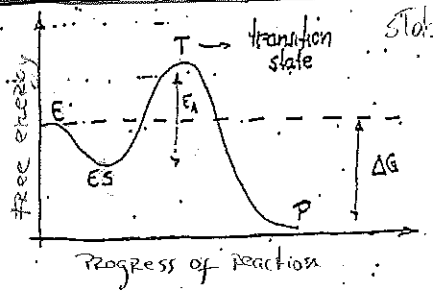
→ PROGRESS CURVES (KINETIC CURVES) - plot of [S] or [P] against time

$S \rightarrow P$ (1st order reaction)

$v = K \cdot [S]$



KINETICS OF ENZYME-CATALYZED REACTIONS



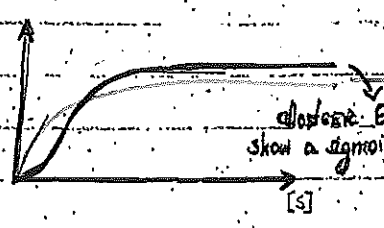
Stability

at constant [E]:

V_0 rises linearly as [S] increases, then it begins to level till it reaches a limit value (when all the active sites are occupied)

FACTORS AFFECTING THE RATES OF ENZYME CATALYZED REACTIONS

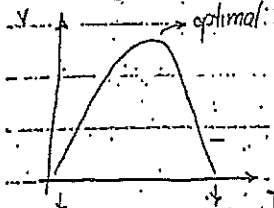
① **SUBSTRATE CONCENTRATION** → high [S] increases reaction rate until a maximal velocity (V_{max}) (until there is saturation of all the active sites)



E following Michaelis-Menten kinetics show hyperbolic curve

allosteric E show a sigmoidal curve

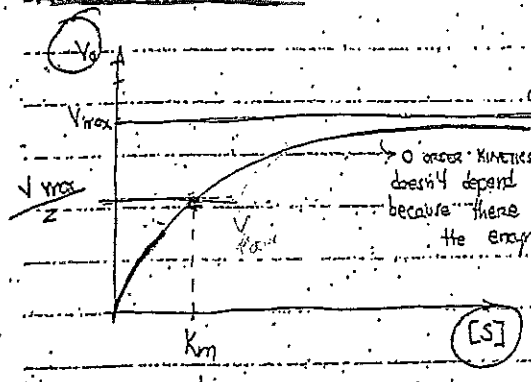
② **TEMPERATURE** → the increasing of T increases the reaction rate until a peak velocity is reached (at the so-called optimal T), at higher T the reaction rate will gradually decrease and it may cause enzyme denaturation



③ **pH** → the optimum pH varies for different enzymes; extremes of pH can lead to enzyme denaturation, because its structure depends on the ionic character of the amino acid side chains.

The catalytic processes require that the E and S have specific chemical groups in either ionized or unionized state in order to react

MICHAELIS-MENTEN PLOT → a plot of V_0 against [S] OR **SATURATION CURVE**



Michaelis constant

MICHAELIS-MENTEN EQUATION:

$$V_0 = V_{max} \frac{[S]}{[S] + K_m}$$

describes dependence of V_0 on [E] and [S] in monosubstrate reactions

concentration of substrate that gives half of the V_{max}

IF...

- $K_m \gg [S]$ → $V_0 = K \cdot [S]$ → 1ST ORDER KINETICS
- $K_m = [S]$ → $V_0 = \frac{1}{2} V_{max}$ → defines the Michaelis constant
- $[S] \gg K_m$ → $V_0 \cong K$ → 0 ORDER KINETICS

THE K_M VALUE AND ITS SIGNIFICANCE

↳ the [S] which gives half of the V_{max}

K_M → is independent of [E] and defines the [S] range that an enzyme requires in order to work efficiently

↓

is inversely related to the affinity of the enzyme for its substrate. Thus the substrate with least value of K_M is the best for the enzyme

Low K_M → high affinity and vice versa

↳ because a low [S] is needed to half-saturate the enzyme (achieve $\frac{V_{max}}{2}$)

→ FACTORS AFFECTING THE RATES OF ENZYME CATALYZED REACTIONS

1. SUBSTRATE CONCENTRATION
2. TEMPERATURE
3. PH

MICHAELIS-MENTEN EQUATION

$$V_0 = V_{max} \frac{[S]}{[S] + K_m}$$

$K_m > [S] \rightarrow V_0 = k[S] \rightarrow 1^{ST}$ ORDER KINETICS

$K_m = [S] \rightarrow V_0 = \frac{1}{2} V_{MAX} \rightarrow$ DEFINES THE MICHAELIS CONSTANT

$[S] > K_m \rightarrow V_0 = k \rightarrow$ 0 order kinetics

They define the efficiency of an enzyme: catalytic activity = $\frac{\Delta P}{\Delta t}$ | catalytic concentration = $\frac{\text{catalytic activity}}{V}$

5 The enzyme activity (the term, units of enzyme activity - U and Katals, catalytic concentration) and arrays of enzymes - (the conditions used in enzyme arrays, the kinetics arranged by the substrate concentration, the kinetic and/or constant-time method).

→ CATALYTIC (enzyme) ACTIVITY → the increase in the rate of a reaction that the enzyme produces in a specific assay system

↓

SI unit: Catal / kat

1 cat = 1 mol/s

1 IU = 16(6) n kat

older unit → international unit (IU) = μmol/min

→ CATALYTIC CONCENTRATION → is the catalytic activity estimated in certain volume of a liquid

↳ units: cat/L / μcat/L / ncat/L

ENZYME ASSAYS: laboratory methods for measuring enzymatic activity:

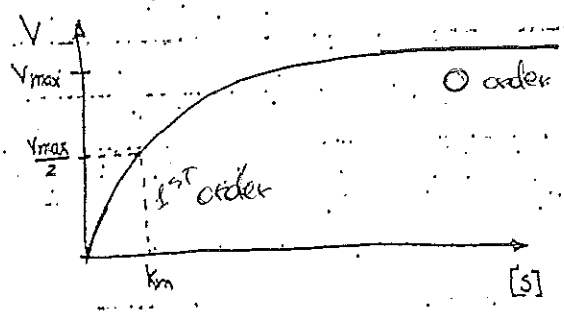
ASSAYS OF ENZYMES → immunochemical methods are the most convenient (use of antibodies)

→ determination of the amount of an enzyme in a complex mixture by measuring the velocity of the reaction catalysed, assuming that this velocity is proportional to the amount of E present

CONDITIONS FOR IN ENZYME ASSAYS:
- nearly optimal T and pH
- presence of necessary cofactors
- absence of inhibitory factors

→ The ZERO-ORDER KINETICS IS PREFERRED (high substrate concentrations)

THE KINETICS ARRANGED BY SUBSTRATE CONCENTRATION:
- if [S] << Km → $V_0 = K[S]$ → 1ST ORDER KINETICS
- if [S] = Km → $V_0 = \frac{1}{2} V_{max}$
- if [S] >> Km → $V_0 = K$ → ZERO-ORDER KINETICS



METHODS FOR ESTIMATION OF ENZYME ACTIVITY

1. **CONSTANT TIME METHOD** — reactions proceed for a fixed time (Δt), then stopped by inactivation. The [P] is measured. An average velocity is calculated: $\frac{[P]}{\Delta t}$

2. **KINETIC METHOD** (1st ORDER) — changes in substrate / product are measured continually in the course of the reaction (eg. by spectrophotometers)

(if only the 1st order state can be arranged, kinetic methods are preferred)

6 Factors affecting catalytic activity of enzymes (the optimal conditions, activators and inhibitors, basal types of inhibitors, the distinguishing competitive from noncompetitive inhibition using saturation curves. The roles of metal ions in enzymatic catalysis (cofactors, metals as activators and inhibitors, examples of metalloenzymes)

OPTIMAL CONDITIONS FOR ENZYME ACTIVITY → enzymatic reactions may be affected by (T) (pH) [S] and [E]
Each enzyme has an optimal set of conditions at which maximum reaction rate occurs

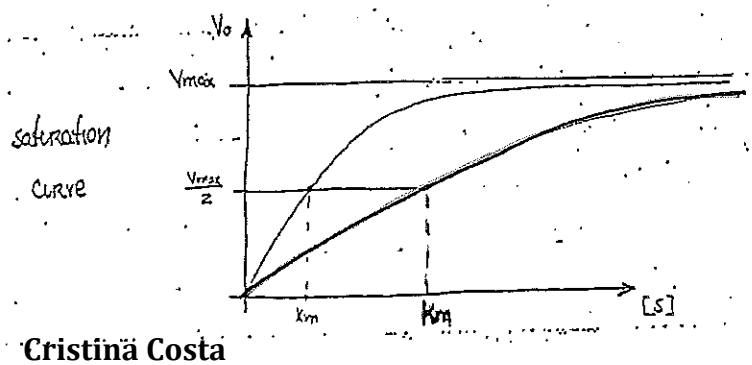
INHIBITOR → substance that reduces enzyme activity
ACTIVATOR → " " increases " "

INHIBITION
- IRREVERSIBLE
- REVERSIBLE
  - competitive
  - noncompetitive

IRREVERSIBLE INHIBITION
↳ usually the inhibitors are not from biological origin and bind to the enzyme covalently, turning substrate binding impossible
↳ eg: (heavy metal ions, penicillin, 5-fluorouracil, allopurinol)

REVERSIBLE INHIBITION
↳ usually inhibitors bind to the enzyme loosely and can rapidly dissociate from the enzyme-inhibitor complex

→ **COMPETITIVE INHIBITORS** – resemble the substrates and bind to the active sites; they compete with normal substrate for the active sites

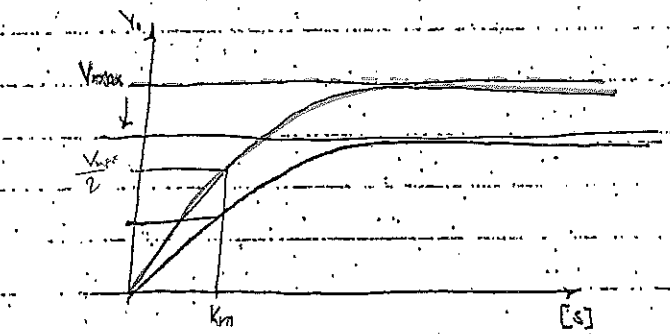


saturation curve

Competitive inhibitors INCREASE THE Km value without changing the Vmax
↓
Vmax can be reached, but at much higher [S]s

↳ examples of competitive inhibitors: malonate

→ **NONCOMPETITIVE INHIBITORS** — bind to both free enzyme and enzyme-substrate complex but not in the active site. This inhibition cannot be overcome by increasing [S], only by increasing [E]



non-competitive inhibitors decrease Vmax without changing Km

Dual poison

Ethylenediaminetetraacetic acid

example of noncompetitive inhibitor: EDTA and other metalloenzymes

(UNCOMPETITIVE INHIBITORS bind only to the enzyme-substrate complex - decrease both Km and Vmax)

METALLOENZYMES → enzymes with a metal as a cofactor or incorporated in the molecule.

↳ examples: superoxide dismutase (Zn and Cu), cytochrome oxidase (Fe and Cu)

METALS AS COFACTORS — metal ions are common cofactors. Ions like Fe, Co (↓ in vit. B_{12}), Ni, Cu are very important cofactors in nutrition

Cu^{2+} and Zn^{2+} are the most common enzyme cofactors

↳ eg. in cytochromes (oxidizing enzymes)

are the most common prosthetic groups (⅓ of all enzymes)

METALS AS ACTIVATORS / INHIBITORS

↳ Metal ions that participate in redox reactions generally are complexed to prosthetic groups such as heme or iron-sulfur clusters

↳ they can facilitate the binding and orientation of substrates, the formation of covalent bonds with reaction intermediates (Co^{2+} in coenzyme B_{12}-cobalamin); or interaction with substrates to render them more electrophilic (electron-poor) or nucleophilic (electron-rich)

7. Regulation of the catalytic activity of enzymes by covalent modification (namely conversions of proenzymes, reversible phosphorylations, activation of protein kinases). Allosteric proteins and enzymes (positive and negative cooperativities, allosteric activation and inhibition)

REGULATION OF ENZYMES BY COVALENT MODIFICATION

① **REVERSIBLE PHOSPHORYLATIONS**: addition / removal of phosphate groups from specific serine, threonine or tyrosine residues in the enzyme. Phosphorylation reactions are catalyzed by protein kinases that use ATP as a phosphate donor. PO_4^{3-} groups are cleaved by phosphoprotein phosphatases.
↳ the phosphorylated form may be more or less active, depending on the type of enzyme

② **CONVERSIONS OF PROENZYMES** (ACTIVATION): proenzyme (zymogen) is an inactive enzyme percursor, it requires a biological change (such as a hydrolysis) to turn into an active enzyme. They are stored in secretory granules and covalently activated in lysosomes.
examples of proenzymes: pepsinogen, trypsogen,

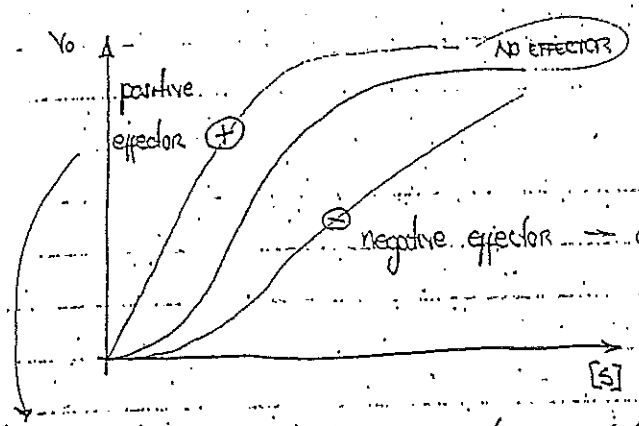
③ **ACTIVATION OF PROTEINKINASES**: protein kinases are enzymes that modify other proteins by chemically adding phosphate groups to them (phosphorylation). Their activity is highly regulated; they are turned on or off by phosphorylation (sometimes by the own kinase = autophosphorylation), by binding of activator or inhibitor proteins, or small molecules, or by controlling their location in the cell relative to their substrates
↓ Activated by phosphorylation

ALLOSTERIC PROTEINS AND ENZYMES

Not all enzymes obey the M-M equation. Allosteric enzymes are usually oligomers, composed of more than 1 subunit (protomer), often regulatory and catalytic.

Allosteric E = Regulatory E → because they have regulatory functions in metabolism.

↓ composed of 2 parts:
- active centre: for binding of the substrates
- allosteric site: " " " " allosteric effectors



→ allosteric enzymes exhibit sigmoidal satur. curves.
→ the binding of allosteric effectors may either stimulate or inhibit the enzyme activity

negative effector ⟹ allosteric inhibition

allosteric activation ⟹ increased affinity for substrate !

Allosteric effectors – regulatory molecules, allosteric (distinct) to the substrate, that bind to allosteric sites in the E.
↳ usually low molecular compounds

COOPERATIVE EFFECT
↳ the binding of the S to one active site in the 1st subunit affects the S binding to the other subunits by inducing changes in conformation.
→ POSITIVE – when binding to 1st subunit facilitates the next bindings.
→ NEGATIVE – " " " " " difficults " " "

That's how he does it !!

BOHR EFFECT – (from question no.2) – enables oxygen transport and delivery by Hb

an increase of pCO_2 (in the tissues) ⟹ decrease in pH → increases $[H^+]$ thus

$HbO_2 + H^+ \rightleftharpoons HbH + O_2$

oxygen is released

Hb affinity for O_2 decreases

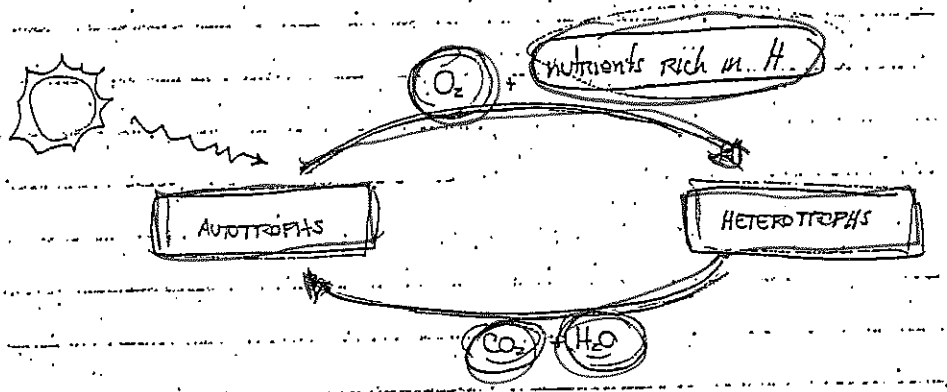
8 _ The roles of Hydrogen and Oxygen in the Energy exchange of living systems (goodstuffs for chemotrophs, three stages in the extraction of energy from nutrients: reducing equivalents), production of ATP by oxidative phosphorylation and by phosphorylation on the substrate level.

CHEMOTROPHS = organisms that derive E from inorganic reactions

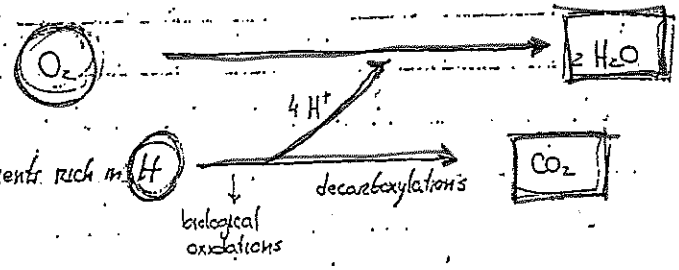
Photosynthetic organisms (phototrophs) use the E from sunlight to convert light energy into chemical energy. Chemotrophs, which include animals, obtain chemical energy through the oxidation of goodstuffs generated by phototrophs.

AUTOTROPHIC CELLS = green leaf cells of plants and photosynthetic bacteria = utilize CO_2 from atmosphere as only source of carbon for the production of all the C-containing biomolecules. They absorb radiant E from the sun. The synthesis of organic compounds is essentially the reduction (hydrogenation) of CO_2 by means of hydrogen atoms, produced by the photolysis of water. (O_2 is generated and released)

HETEROTROPHIC CELLS = cells of higher animals and most microorganisms = must obtain carbon in the form of relatively complex organic molecules (nutrients such as glucose) formed by other cells. They obtain their E from the oxidative (mostly aerobic) degradation of organic nutrients made by autotrophs and return CO_2 to the atmosphere.



IMPORTANCE OF H and O in the E exchange of living systems



$\Delta G^\circ = -474,3$ kJ

MOST OF GIBBS FREE E in the body originates in the exergonic synthesis of water: $2\ H_2 + O_2 \rightleftharpoons 2\ H_2O$

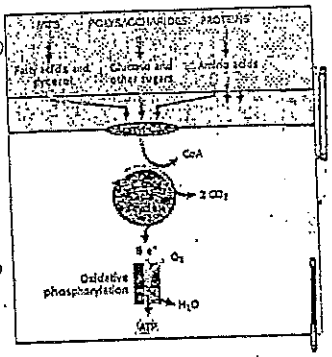
CH7

STAGES OF EXTRACTION OF ENERGY FROM FOODSTUFFS = CATABOLISM

1° large biomolecules are broken down into smaller units

2° degradation to some amphibolic intermediates (mainly acetyl-CoA)

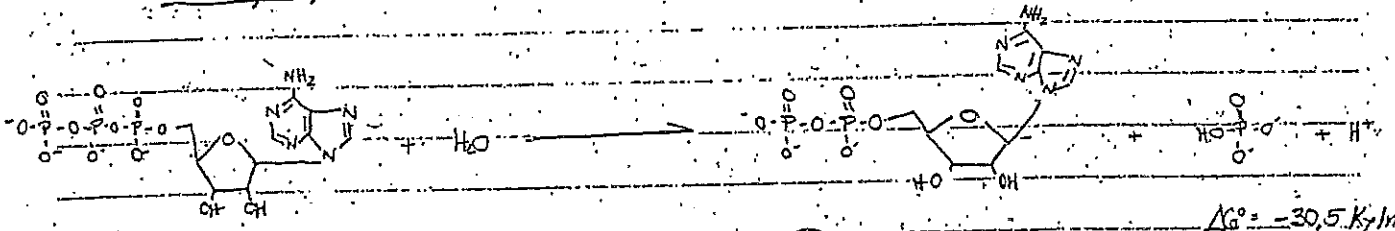
3° FINAL COMMON PATHWAYS: most ATP comes from the oxidation of acetyl-CoA



→ REDUCING EQUIVALENTS = 2 electrons of NADH

ADENOSINE TRIPHOSPHATE (ATP) = high-energy compound that serves as "universal currency" of free E. in biological systems

ATP hydrolysis drives metabolism by shifting the equilibrium of coupled reactions.



$\Delta G^\circ = -30,5$ Kj/mol

ATP + H_2O → ADP + P_i

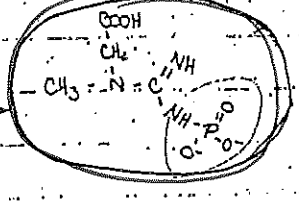
⇒ GTP, CTP, UTP, TTP are quite analogous to ATP

as well

⇒ GDP, CDP, UDP, TDP are analogous to ADP

different types of HIGH-ENERGY COMPOUNDS

- ANHYDRIDES
  - di- or triphosphates = ATP, ADP
  - phosphosulfates = phosphoadenosyl-phosphosulfate
  - acylphosphates = 1,3-biphosphoglycerate
- ESTER = phosphoenolpyruvate
- THIOESTERS = acyl-CoA
- AMIDES = phosphocreatine

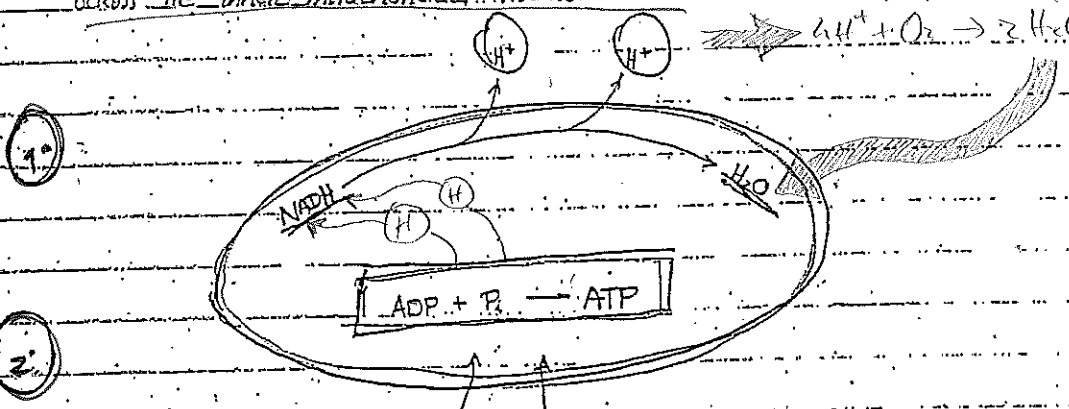


SYNTHESIS OF ATP

(1) BY OXIDATIVE PHOSPHORYLATION — IN MITOCHONDRIA (generates more than 90% of the ATP)

The synthesis of ATP is coupled to the oxidation of H to water in the terminal respiratory chain — H atoms, as NADH+H⁺ or FADH₂, are oxidized to water.

↳ the synthesis of ATP is driven by the electrochemical potential of proton gradient across the inner mitochondrial membrane



ionic potential ← H⁺ ... H⁺ ... → oxigenation of hydrogen by O₂ is coupled with ATP synthesis

for the phosphate binding (not direct; intermediate)

(2) BY PHOSPHORYLATIONS OF ADP ON THE SUBSTRATE LEVEL — few reactions in which a nucleoside triphosphate (ATP) is synthesized by utilization of the free E of hydrolysis of a soluble high-energy compound.

↳ E released by some carbon oxidations can be converted into high phosphoryl-transfer potential → these oxidations couple with the synthesis of ATP!

↳ eg. high phosphoryl transfer potential of phosphoenolpyruvate arises from the keto-enol conversion

9 **Transport across membranes** (various types of passive and active transport mechanisms, characters of transporters and ionophores, examples) → see other side

→ **FREE DIFFUSION** (Passive diffusion) ↑concentration → ↓concentration

Size: only small, uncharged molecules — gases, H_2O, NH_3, glycerol or urea

Polarity: apolar molecules — benzene, ethanol, diethyl ether and some narcotic agents

→ **FACILITATED TRANSPORT** → Facilitated diffusion — through:

- **CHANNEL PROTEINS** — proteins with a polar pore, through which ions and other hydrophilic compounds can pass. Eg: ion channels and porins (passage limited to size)
- **TRANSPORTERS** — recognize and bind the molecule to be transported and help it passing through conformational changes

→ Any of these 3 processes DOESN'T REQUIRE ENERGY, they all follow a concentration gradient (and charge gradient)

→ **ACTIVE TRANSPORT** — can run against a concentration or charge gradient

→ requires an input of E, supplied by the hydrolysis of ATP

→ the transporter first binds its "cargo" onto one side of the membrane;

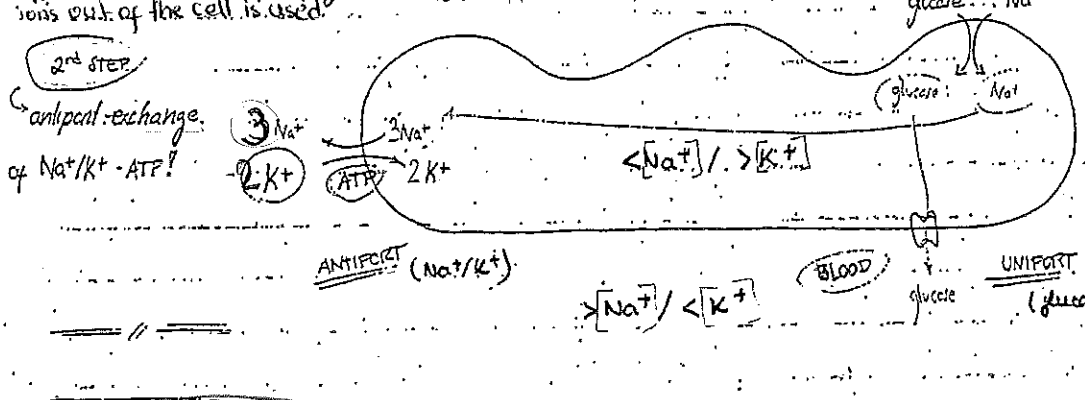
→ ATP-dependent phosphorylation then causes a conformational change that releases the cargo on the other side of the membrane

→ **PRIMARY ACTIVE TRANSPORT** → directly uses E to transport molecules across a membrane

→ **SECONDARY ACTIVE TRANSPORT** → 2 coupled transports!

There is no direct coupling to ATP. An electrochemical potential difference created by pumping ions out of the cell is used.

→ Eg: SODIUM PUMP – in intestine: Sodium/Potassium pump



↑ [Na+] – outside
↑ [K+] – inside
↑ [glucose] – inside

TRANSPORT SYSTEMS help cells regulate their volume, internal pH and ionic environment

→ import metabolites important for biopracesses and export toxic substances

ESTABLISH ION GRADIENTS, which are required for oxidative phosphorylation and stimulation of muscle and nerve cells, for example.

ANOTHER CLASSIFICATION OF TRANSPORT PROCESSES — based on no. of particles and direction of transport

- **UNIPORT** = a single particle passes the membrane, with help of a channel or transporter
  - eg: transport of glucose into liver cells

- **SYMPORT** = simultaneous transport of 2 different particles in the same direction (no ATP consumption, because they derive the needed E for the movement of one molecule from the movement of the other.)
  - Eg: transport of glucose/aa with Na^+ ions into intestinal epithelial cells (previous pages!)

- **ANTIPORT** = simultaneous transport of 2 different molecules in opposite directions (ATP NEEDED!)
  - often process ions that are similarly charged → electroneutral process
  - Eg: K^+/Na^+ antiport (previous page); HCO_3^- (out) / Cl^- (in) antiport of erythrocyte membrane

CHARACTERS OF TRANSPORTERS AND IONOPHORES

1. **TRANSPORTERS** — examples:
   - **GLUCOSE TRANSPORTERS (GLUT)**
     - Glut 1 and Glut 3 = in nearly all cells; ensure continuous glucose uptake
     - Glut 2 = only in liver and pancreas (β cells)
     - Glut 4 = mainly in muscle and fat cells — controlled by insulin
     - Glut 5 = mediates secondary reabsorption of glucose in intestines and kidneys
   - **AQUAPORINS** = hydrophilic pores that allow only water to pass
     - they are important in kidneys, where they promote the reuptake of water
   - **TRANSPORT ATPases** = "ion pumps" that transport cations
     - F type: use H^+ transport for ATP synthesis (eg: mitochondrial ATP synthase)
     - V type: pump protons into lysosomes or other acidic cell compartments
     - P type: undergo covalent phosphorylation during the transport
       - Eg: Ca^{2+}-ATPase = in muscle = pumps Ca^{2+} released in the cytoplasm to trigger muscle contraction back into the sarcoplasmic reticulum

2. **IONOPHORES** = ion channels, facilitate the diffusion of ions through biological membranes
   - **VOLTAGE-GATED CHANNELS** = open and close depending on the membrane potential
     - Eg: voltage-gated Na^+ channel: conducts electrical impulses in the nervous system due to high equilibrium potential for Na^+, an influx of Na^+ occurs, resulting in local depolarization of the membrane, which propagates by activation of neighbor Na^+ channels. A spreading depolarization wave of this type is known as action potential.

→ K+ channels are involved in the repolarization of the membrane.

- **LIGAND-GATED CHANNELS** — open and close in response to specific ligands.
  - → Eg: nicotinic receptors for acetylcholine — 5 subunits, with 4 transmembrane helices each. Acetylcholine binds to the α-subunits, opening the pore shortly. Binding of neurotransmitter changes subunits position, expanding the pore and dissociating acetylcholine.
- **PASSIVE CHANNELS** — transport depends only on concentration gradient.
  - → Eg: K+ channel in Streptomyces lividans

REDUCING EQUIVALENT ——— e⁻ transferred in ... Role

10 Mitochondria (general structure, overview of the main roles in metabolism). Transporter systems in the inner mitochondrial membrane (transport and transporter types, examples).

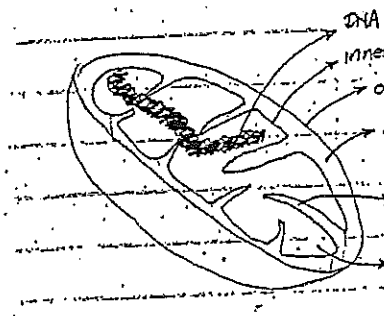
Mitochondria ... (1-2 μm) are found in almost all ... eukaryotic cells ... ~~1-2 μm~~ 0.5-10 μm they vary greatly in size and number per cell.

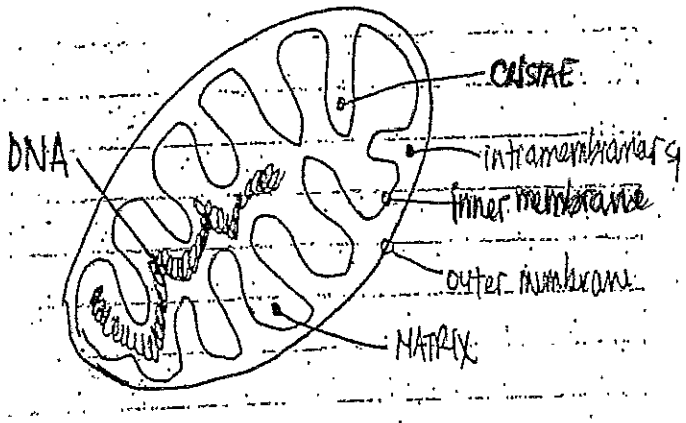
2 membranes:

- OUTER MEMBRANE — quite permeable for small molecules and ions. — contains many copies of mitochondrial porin (voltage-dependent anion channel — VDAC)
- INNER MEMBRANE — impermeable to nearly all ions and polar molecules, but there are many specific transporters which shuttle metabolites and protons across the membrane.

ENDOSYMBIONT THEORY tells that mitochondria were aerobic bacteria that entered in symbiosis with anaerobic eukaryotes. This is supported by many findings: mithocondria have a ring-shaped DNA (4 incl. per mitochondria) and have their own ribosomes.

the outer membranes (from the cell forming the phagocytose)





METABOLIC FUNCTIONS:

- OXIDATIVE PHOSPHORYLATION = produce most of cellular ATP
- IN THE MATRIX: tricarboxylic acid cycle (citric acid cycle), β-oxidation of fatty acids and parts of the urea cycle.
- IN THE INNER MEMBRANE: respiratory chain, ATP synthesis, and enzymes involved in heme biosynthesis.
  - enables oxidative phosphorylation, as it establishes a proton gradient when the respiratory chain pumps protons from the matrix to the intermembranar space.

- oxidation of pyruvate
- oxidation of a.a.
- β-oxidation
- TCA
- synthesis of urea
- synthesis of a.a.
- partial synthesis of heme

↳ mitochondria also functions as calcium reservoir

↳ " " plays an essential role in programmed cell death: apoptosis

MITOCHONDRIAL TRANSPORT SYSTEMS

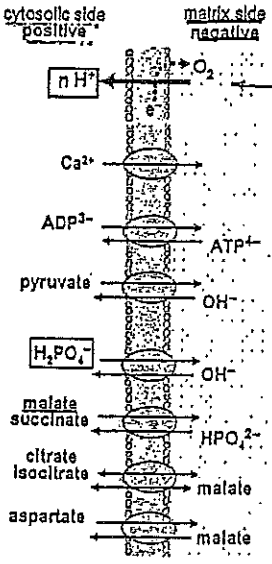
The outer membrane has pores, which allow small molecules to pass. But the inner membrane doesn't and thus it is impermeable to all substances (except for H_2O, O_2, CO_2 and NH_3). is impermeable to small ions

is permiable to O_2, CO_2, H_2O, NH_3, acetic acid, O_2, 3-C or 4-C sugars

TRANSPORT SYSTEMS

- UNIPORT = metabolites are transported alone
- SYMPORT = " " " " with a second substance
- ANTIPORT = " " " " in exchange for another molecule (ATP-driven)
- ACTIVE TRANSPORT
  - PRIMARY ACTIVE PROTON TRANSPORT = forms the proton motive force (directly uses energy)
  - SECONDARY ACTIVE TRANSPORTS = driven by the proton motive force (uses 2 transport types)

Transport through the inner mitochondrial membrane – examples:



- Free diffusion of O_2, CO_2, H_2O, NH_3
- Primary active H^+ transport forms the proton motive force (the primary gradient)
- Secondary active transports driven by a H^+ gradient and dissipating it:
  - ATP/ADP translocase
  - pyruvate transporter
  - phosphate permease – forms a (secondary) phosphate gradient
  - dicarboxylate carrier
  - tricarboxylate carrier
  - the malate shuttle for NADH + H^+

(transporters)

6

TRANSPORT OF REDUCING EQUIVALENTS FROM CYTOPLASM INTO MITOCHONDRIA BY REDOX SHUTTLES

There are 2 substrates that supply e⁻ to the TERMINAL RESPIRATORY CHAIN

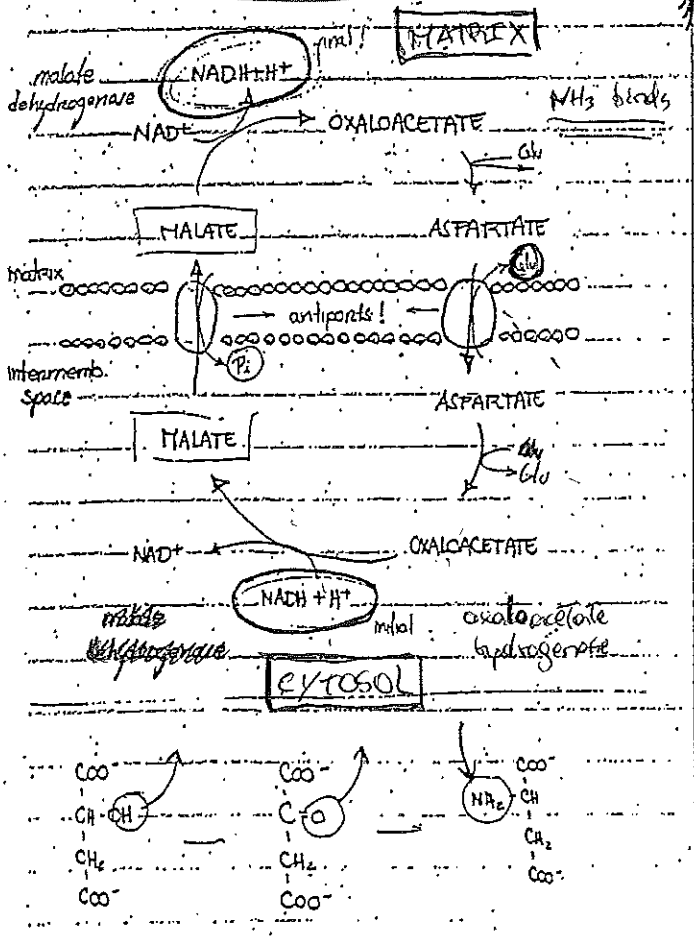
1. $NADH + H^+$ → which is reoxidized to NAD^+ by the complex I of the chain. → MALATE SHUTTLE

IN THE MATRIX OF MITOCHONDRIA, $NADH + H^+$ is the product of many dehydrogenase-catalyzed reaction (from oxidative decarbox. of pyruvate, β-oxidation of FA, Krebs cycle and deamination of glutamate)

IN THE CYTOSOL, $NADH + H^+$ is also product of dehydrogenations (1,3-bisphosphoglycerate → to 3-phosphoglycerate; lactate → to pyruvate)

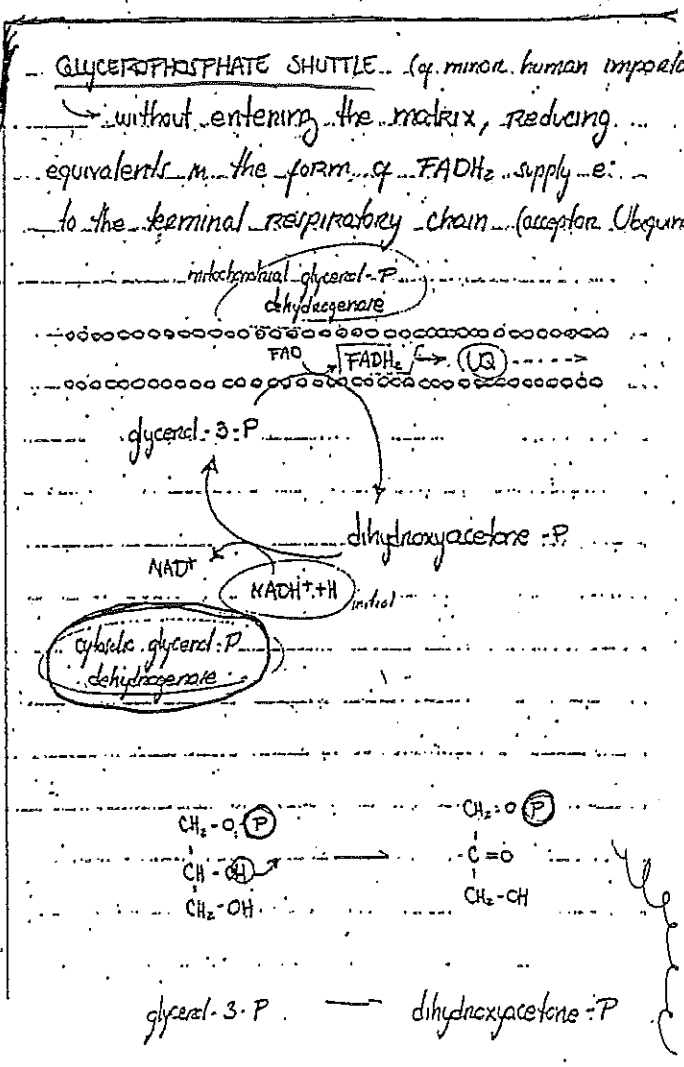
Because the inner mit membrane is impermeable, the reducing equivalents have to be transported by redox shuttles INTO THE MITOCHONDRIA

MALATE SHUTTLE (universal)



GLYCEROPHOSPHATE SHUTTLE (of minor human importance)

→ without entering the matrix, reducing equivalents in the form of $FADH_2$ supply e⁻ to the terminal respiratory chain (acceptor Ubiquinone)



malate → oxaloacetate → aspartate

glycerol-3-P → dihydroxyacetone-P

(2) SUBSTRATES FOR FLAVIN DEHYDROGENASES OF THE COMPLEX II → GLYCEROPHOSPHATE SHUTTLE

(the reduced $FADH_2$ supply e⁻ to Ub.)

USE OF $FADH_2$

IN THE MITOCHONDRIAL MATRIX the substrates are FATTY ACYL-CoAs from the β-oxidation pathway and SUCCINATE from the citrate cycle

IN THE CYTOSOL = glycerol-3-P is reoxidized by glycerol-P dehydrogenase to dihydroxyacetone-P

⇓

GLYCEROPHOSPHATE SHUTTLE

fatty acyl-CoAs —(acyl-CoA dehydrogenase: introduction of double bond)→

succinate —(succinate dehydrogenase)→ fumarate

⇓

$FADH_2$ gets the hydrogens that are released from those reactions showed above

11 Pyridine-nucleotide dependent dehydrogenases (structure & coenzymes function)

→ The pyridine nucleotides: NAD^+ and $NADP^+$ are widely distributed as coenzymes of dehydrogenases!

(They transport HYDRIDE IONS (H^-) and always act on SOLUBLE ENZYMES (unbound to)

NAD^+ → transfer reducing equivalents from catabolic pathways to the respiratory chain → ENERGY METABOLISM

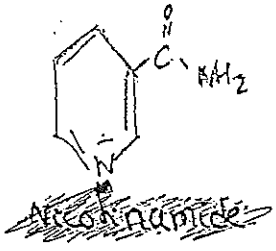
$NADP^+$ → most important reductant in anabolic pathways (biosynthesis) (mainly formed in the pentose phosphate pathway)

→ no radical intermediate steps occur.

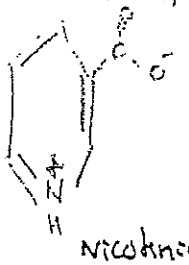
Structures:

NAD^+ - NICOTINAMIDE ADENINE DINUCLEOTIDE

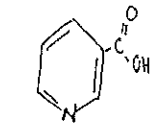
the constituent of NAD^+ (as well as of $NADP^+$) is nicotinamide:



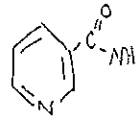
Nicotinamide



Nicotinic acid (niacin, PP)

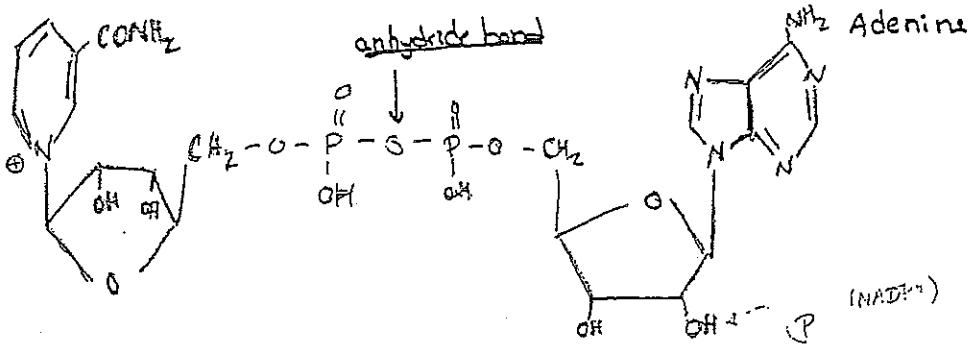


nicotinic acid



nicotinamide

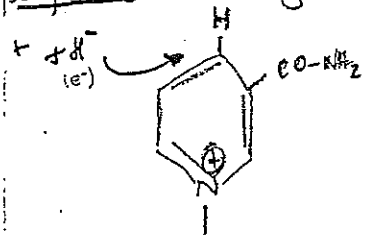
niacin (vit. B_3)



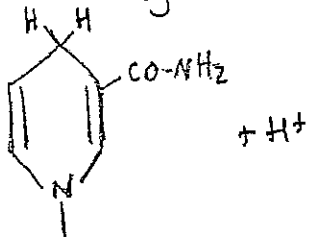
NAD^+ - is the coenzyme of dehydrogenases:

. It acts as an **OXIDANT** that takes off 2 atoms of Hydrogen from the substrate.

one Atom plus on e⁻ (hydride anion H^-) is added to the para-position of a pyridinium ring, the remaining H^+ binds to the enzyme.



Oxidized form NAD^+
(aromatic ring, ⊕ charge)



Reduced form NADH + H
(quinoid ring, no charge)

example: Dehydrogenation of ethanol by alcohol dehydrogenase

$$H_3C-CH_2-OH + NAD^+ \longrightarrow H_3C-\overset{O}{\overset{\|}{C}}-H + NADH + H^+$$

$NADPH+H^+$ → Nicotinamide adenine dinucleotide phosphate ⇒ $NADPH+H^+$

acts as an **REDUCTANT** that supplies 2 atoms of hydrogen

- to the substrates in reductive syntheses of FA or cholesterol
- the hydroxylating enzymatic systems (e.g. synthesis of bile acid and other steroids, biotransformation of drugs).

schematic representation of the hydroxylations various biomolecules catalyzed by the hydroxylating monooxygenases.

$$R-H + O_2 + NADPH + H^+ \longrightarrow R-OH + H_2O + NADP^+$$

SCHEME OF HYDROXYLATIONS CATALYZED BY HYDROXYLATING MONOOXIGENASES

12 Flavoproteins (structures and function of the flavin prosthetic group, function of flavin dehydrogenases)

- FMN and FAD (Flavin mononucleotide, Flavine adenine dinucleotide) → contain isoalloxazine → transfer $2e^-$ and $2H^+$
- FMN = isoalloxazine + ribitol
- FAD = FMN + AMP (Adenosine monophosphate)

FUNCTION: They act as OXIDANTS in certain types catalyzed by dehydrogenases

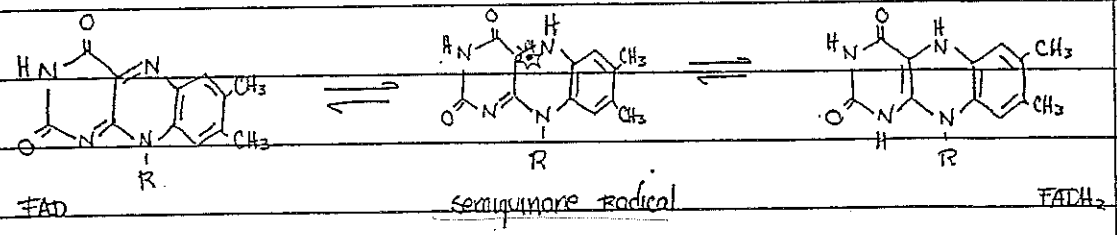
FMN and FAD are functionally similar

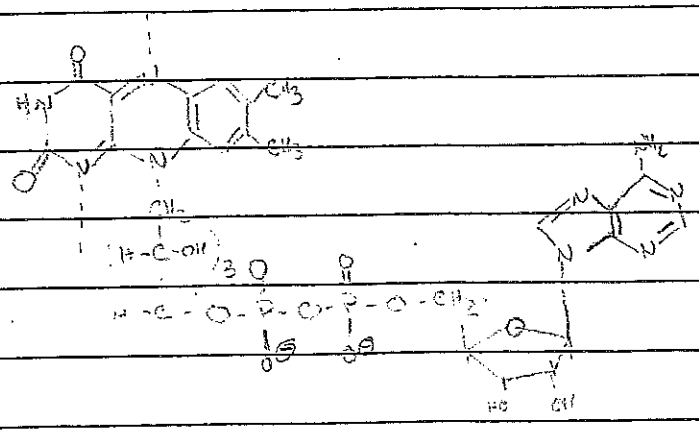
→ FOUND IN DEHYDROGENASES, OXIDASES and MONOOXYGENASES

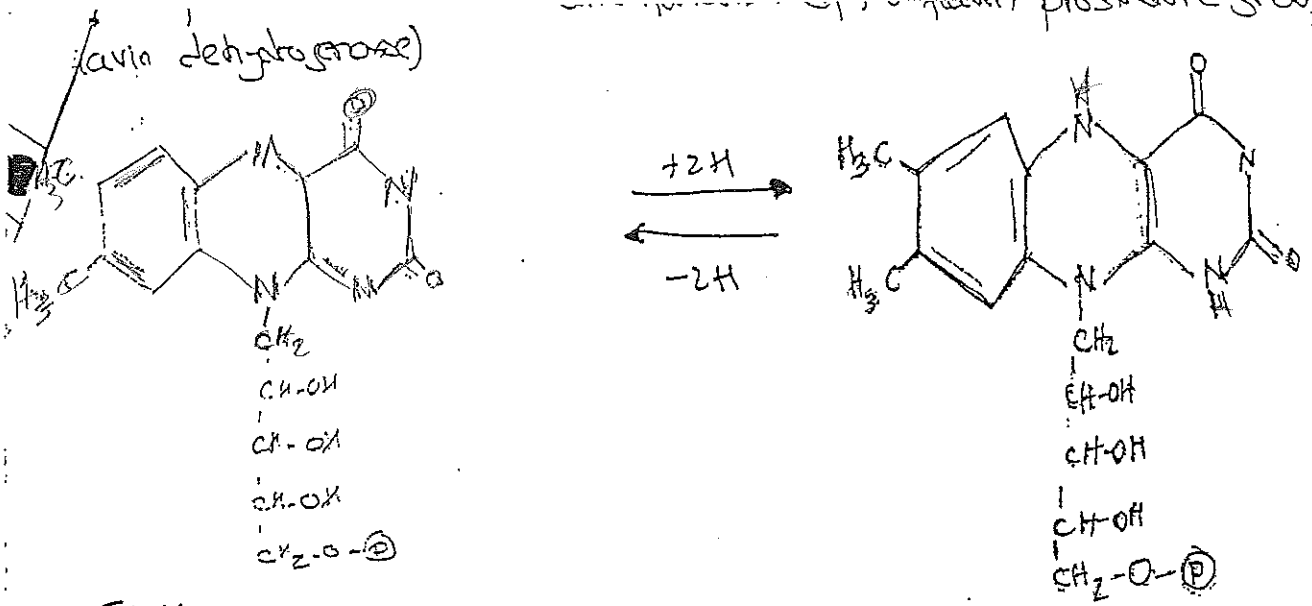
It's aim is to take out hydrogens

(in contrast to pyridine nucleotides), they give rise to RADICAL INTERMEDIATES (semiquinone radical)

to prevent damage to cell components they remain bound as prosthetic groups in the enzyme protein.







- **flavoproteins:**
  - contain flavin prosthetic group either as flavin mononucleotide (FMN, component of complex I)
  - as a flavin Adenine dinucleotide (FAD, dehydrogenases, - components of complex II)

coenzyme FMN (as well as FAD) ⟹ transfers 2 atoms of hydrogen;

FMN and FAD ⟹ are formed i.e. ... only from the vitamin riboflavin

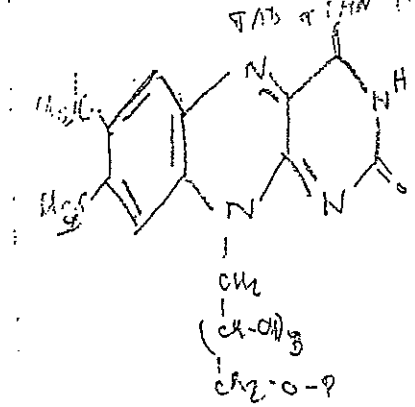
L-amino acid oxidase: an FMN-linked enzyme found in kidney. w/general specificity for the oxidative deamination of the naturally occurring L-amino acids;

xanthine oxidase: which contains molybdenum and plays an important role in the conversion of purine bases to uric acid, and is of particular significance / uricotelic* animals.

FAD and FMN are derived from riboflavin (vitamin B_2)

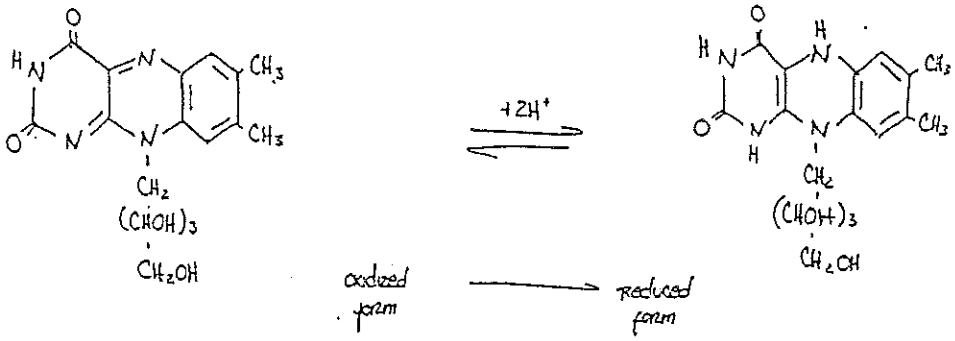
they can be partially reduce to the semiquinone radical, by the addition of 1H or fully w/ 2 hydrogen, dihydroflavin.

usually flavin prosthetic group is bound to protein tightly but non-covalently, but in some important ex, succinate dehydrogenase, FAD is covalently attached.



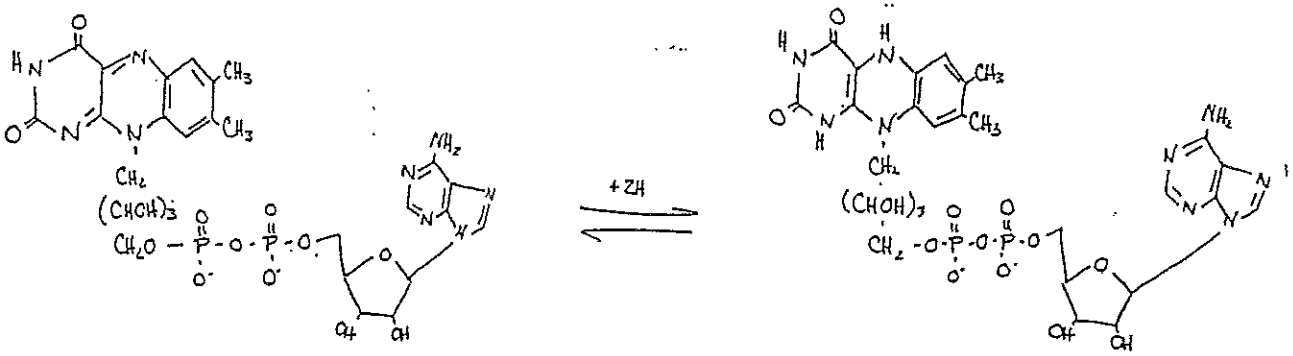
* URICOTELIC → excreting uric acid as the chief component of nitrogenous wastes

FMN (flavin mononucleotide) ⟶ $FMNH_2$



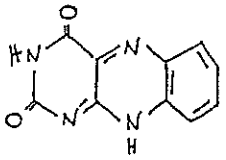
oxidized form ⟶ reduced form

FAD + 2H ⟶ $FADH_2$ (flavin adenine dinucleotide)



Oxidized form

Reduced form



isoalloxazine (flavin) → characteristic redox-active group of flavin coenzymes

13 Cytochromes of the mitochondrial respiratory chain (main structural features, their Roles in mitochondrial complexes) and of monooxygenase hydroxylating systems (cytochrome P450)

CYTOCHROMES = heme-containing proteins that are one-electron carriers due to reversible oxidation of the iron atom: $Fe^{2+} \rightleftharpoons Fe^{3+} + e^-$

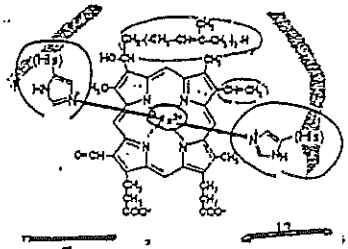
3 TYPES OF MAMMALIAN CYTOCHROMES: a; b and c
→ they differ in the substituents attached to the porphin ring
→ ALL OF THEM OCCURR IN MITOCHONDRIAL RESPIRATORY CHAIN

→ CYTOCHROME TYPE (a) → heme has one formyl and one 15-C farnesyl side chain, it is known as heme A
→ CYTOCHROME aa_3 - Mr 170000 - central Fe ion attached to 2 histidyl residues and has 2 substituents: 1 isoprenoid chain and a formyl group
→ Its function is inhibited by: CO; CN^-, HS^- and N_3^- anions

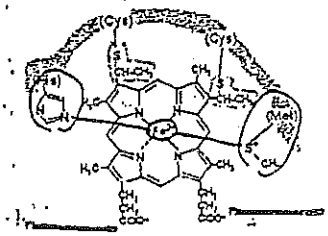
→ CYTOCHROME TYPE (b) → the prosthetic group is heame bound non-covalently to proteins
→ they include class P-450
→ they occurr also in membraner of ER and in the outer mit. memb

→ CYTOCHROME TYPE (c) → have same prosthetic group as b, but 2 vinyl side chains are reduced and linked by thioester bonds to cysteine side chains in the protein
→ CYTOCHROME c - mw 12000 - Central Fe ion attached to N of His_{18} and to S of Met and 2 vinyl groups bind covalently S atoms of cysteine side chains
→ the heme is unable to bind O_2, CO and CN^- ion.
Cyt c = peripheral protein that moves on the outer side of the inner mit. mem

Haem a of cytochrome aa_3



Haem of cytochrome c



CYTOCHROME P450 MONOOXYGENASE SYSTEM

Monooxygenases: incorporate one atom from molecular oxygen into a substrate (creating -OH) with the other atom being reduced to water.

NADPH provides the necessary reducing equivalents ($2e^-$)

overall reaction catalyzed by a cytochrome P450 enzyme:

$$R-H + O_2 + NADPH + H^+ \longrightarrow R-OH + H_2O + NADP^+$$

(where R may be a steroid, drug or other chemical)

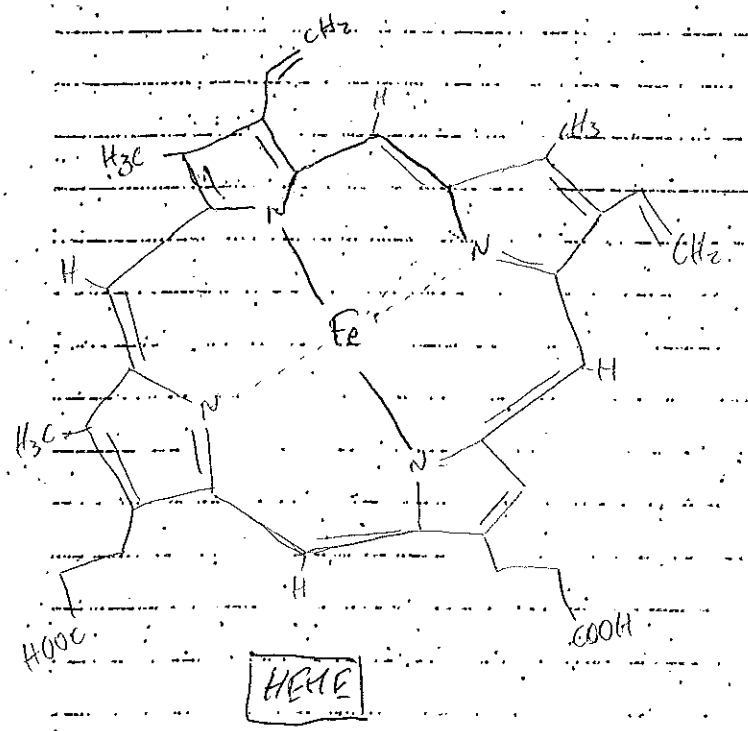
2 IMPORTANT FUNCTIONS OF CYT. P450 monooxigenase system

1° MITOCHONDRIAL cytochrome P450 ms. ⇒ participates in hydroxylation of steroids a process to make them more water soluble.

2° MICROSOMAL cytochrome P450 ms → associated with membranes of smooth ER (particular in the liver) it participates in detoxification of xenobiotics (foreign compounds)

If hydroxylates these toxins, also using NADPH as the source of electrons.

↳ this hydroxylation will either activate/inactivate the drug or make it more soluble, facilitating its excretion from the body.



HEME

14. The mitochondrial respiratory chain (function, main components of the mitochondrial complexes, the proton-motive force, the respiratory control)

THE MITOCHONDRIAL TERMINAL RESPIRATORY CHAIN REOXIDIZES $NADH+H^+$ OR $FADH_2$ by transporting e^- to the terminal acceptor O_2, which is reduced to form water

↓

the free E of the oxidation of NADH OR $FADH_2$ IS USED TO PUMP PROTONS TO THE OUTSIDE OF THE INNER MITOCH. MEMBRANE

↓

THE PROTON GRADIENT across the inner mit membrane is the PROTON MOTIVE FORCE

↓

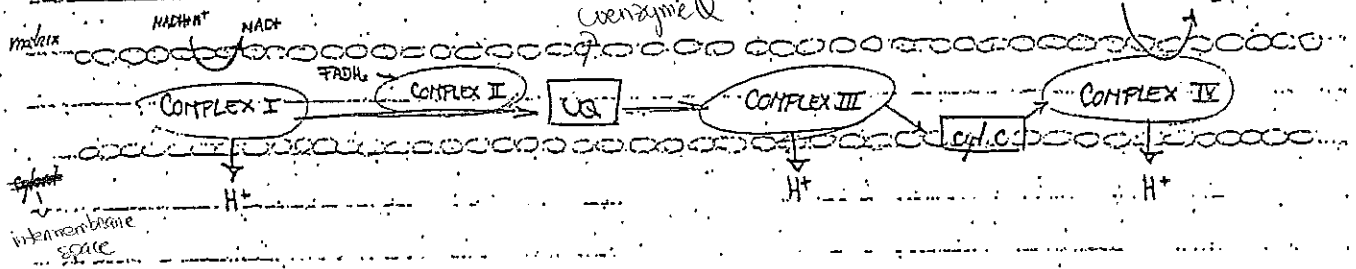
is going to couple the terminal respiratory chain with the PHOSPHORYLATION OF ADP — ATP synthesis. The protons re-entry the matrix through an ATP-synthase comp.

MAIN COMPONENTS OF THE RESPIRATORY CHAIN:
- 4 large protein complexes
- ubiquinone
- cytochrome c

(small transporters!)

NADH dehydrogenase
cytochrome b-c1
ATP synthase
" oxidase
ubiquinone
cytochrome c

NADH and e^- from: H^+ molecular O_2 and H_2O, ADP and Pi



- COMPLEX I = NADH dehydrogenase
- cyt bc_1 COMPLEX III = cyt bc_1 reductase ← (ubiquinone) CoQ
- COMPLEX IV = cyt. c (oxidase) ← (cyt. c)

→ catalyze active, electrogenic H^+ transport!

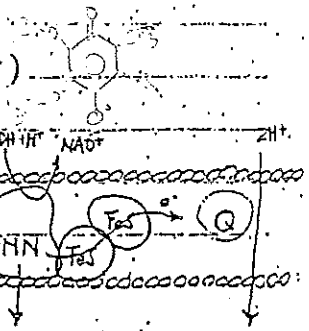
COMPLEX II:
- succinate dehydrogenase
- acyl-CoA "
- glycerolphosphate "

→ transfers e^- from $FADH_2$ to Q (not protons!)

in detail

→ COMPLEX I – NADH dehydrogenase ; – more than 30 subunits :

- one subunit with prosthetic group FMN – accepts 2H atoms from NADH to give $FMNH_2$ and 2 e⁻ transfer to FeS-proteins ;
- many FeS-proteins – transfer 2e⁻ to 2 semiquinones ($\cdot Q^-$)



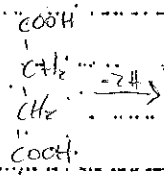
$NADH + H^+ + FMN \longrightarrow NAD^+ + FMNH_2$

$FMNH_2 \longrightarrow FMN + 2H^+ + 2e^-$

2 H⁺ are transferred through the membrane (mechanism not yet clear...)

→ COMPLEX II – succinate / acyl-CoA / glycerol-P dehydrogenases, 3 independent flavin dehydrogenases that act in similar way, but only SUCCINATE DEHYDROGENASE is mentioned:

- components: (conformer)
  - one FAD as prosthetic group
  - 3 FeS-proteins
  - one cyt. b_{560} → transfers e⁻ to semiquinone ($\cdot Q^-$)

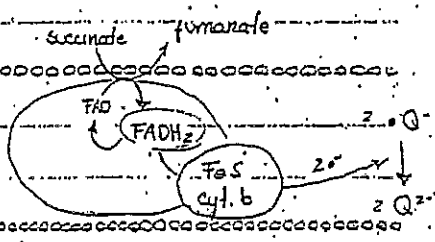


(succinate DH – participates in the citrate cycle, in which is the only integral membrane protein (the others are in the matrix)

↳ complex II transports e⁻ from $FADH_2$ to Q

it doesn't carry protons across the inner membrane,

so the oxidation of $FADH_2$ produces less proton gradient (less ATP) than oxidation of NADH+H⁺.



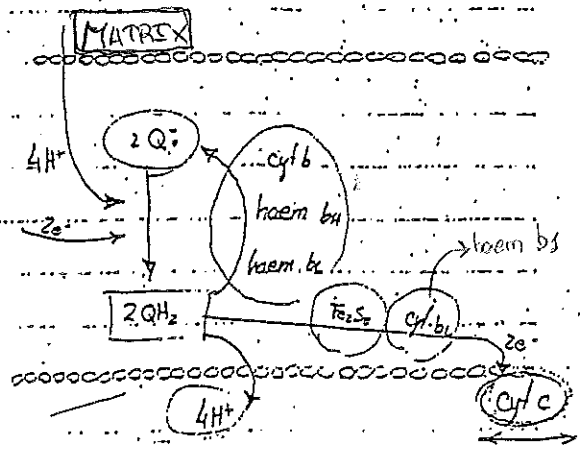
→ COMPLEX III – cytochrome c reductase – consists of 11 subunits, the most important are:

- cytochrome b – contains 2 haems: haem b_L (low affinity) and haem b_H (high affinity)
- one FeS-protein (Fe_2S_2)
- cytochrome c_1 ← e⁻ (cyt. c) ← e⁻ = cyt. c_1 (complex IV)

→ the anion Q^{2-} binds 4 protons from the matrix and QH_2 moves within the lipid bilayer

↳ then 2e⁻ are transferred from it to the cyt. c, one by Rieske Fe_2S_2 protein and the other by haem b_L of the cyt. b

(·Q⁻ will be regenerated – Q cycle)



↳ the 2e⁻ transferred translocate 4 protons across the membrane!

lateral dynamics

transfers e⁻ from cyt. c_1 of complex III to complex IV

cytochrome c → soluble peripheral membrane haemeprotein → bound to the outer side of the inner mitochondral membrane through weak electrostatic interactions, so it can move along it.)

→ COMPLEX IV = cytochrome c oxidase (also called cytochrome aa_3)

→ catalyzes the 4-electron reduction of O_2 to H_2O

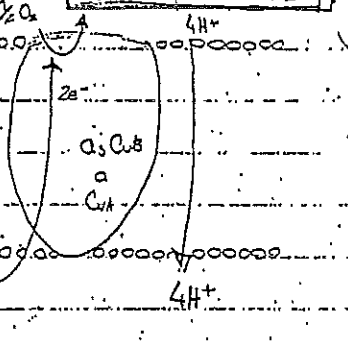
→ consists of 13 subunits:

2 haems: a + a_3

3 Cu atoms: 2 centres Cu_A + 1 centre Cu_B

(Cu_B + haem a_3 ⇒ binuclear centre = site of O_2 reduction!)

$O^{2-} + 2H^+ \rightarrow H_2O$



→ only way in which this reaction occurs without release of partly reduced intermediates (superoxides, peroxides, ...

THE PROTON MOTIVE FORCE Δp is the quantity expressed in terms of potential

↓

represents the E available for ATP synthesis, as well as for other endergonic processes (secondary active transport of ions) or production of heat (dissipation of $\Delta\mu_{H^+}$ in uncoupling)

millivolts per mole of H^+ transferred: $\Delta p = \frac{\Delta G_{H^+}}{F}$ — ΔG_{H^+} = E that results from ≠ [x]s and charges between the 2 sides of the membrane

THE RESPIRATORY CONTROL = inhibitors of the terminal respiratory chain

- COMPLEX I = blocked by insecticide ROTENONE (a limited synthesis of ATP occurs due to con
- COMPLEX III = inhibited by ANTIMYCIN A. Ascorbate restores respiration
- COMPLEX IV = blocked by CO, CN^-, H_2S (refinery intoxication) and N_3^- (azide ion)

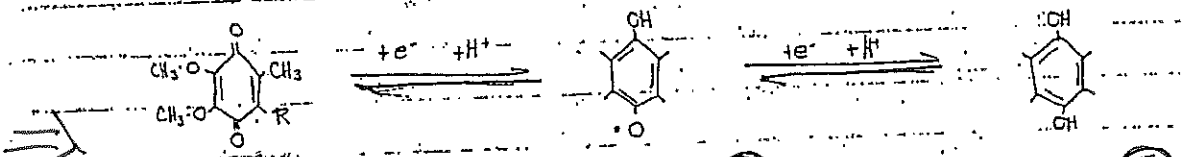
uncoupling (4)

three / isoforms / ATP synthase blocker / (ATP/ADP translocase)

15 Ubiquinone (structure, function) and iron sulphur proteins (the term, functions)

UBIQUINONE (COENZYME Q) acts as a free hydrogen transporter.

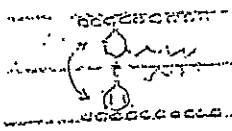
↳ it accepts stepwise 2 e⁻ (one from the complex I or II and the other from the cyt. b) and 2 protons (from the mitochondrial matrix) ⟹ completely reduced to ubiquinol



UBIQUINONE (Q) SEMIQUINONE (QH) UBIQUINOL (QH₂)

R = $-(CH_2-CH=\overset{CH_3}{C}-CH_2)_{10}-H$ Isoprenoid chain ⟹ extremely lipophilic: is anchored within the lipid bilayer

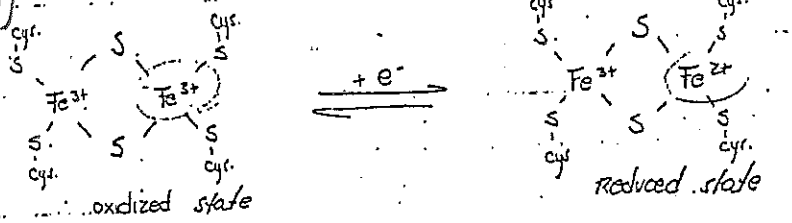
⟹ the ring of ubiquinone or ubiquinol (not semiquinone) can move from the membrane matrix side to the cytosolic side and is able to translocate e⁻ and protons



IRON-SULPHUR PROTEINS (FeS-proteins, non-haem iron proteins)

↳ each cluster accepts or donates ONLY one e⁻

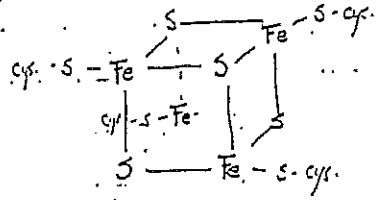
• Fe_2S_2 CLUSTER



oxdized state reduced state

function?

• Fe_4S_4 CLUSTER



16 Energetics of the respiratory chain, oxidative phosphorylation (structure and function of the ATP synthase, coupling of phosphorylation to electron transport, respiratory control, uncouplers).

ENERGETICS OF THE RESPIRATORY CHAIN

→ complex 1, 3 and 4 drive 4 H^+ across the membrane; each, so in one cycle: 12 H^+ are pumped out the membrane (4 for each 4 H^+ 1 ATP is synthesized, 12 will be synthesized:

- COMPLEX I → COMPLEX III → COMPLEX IV ⇒ results in 3 ATP mol (one for each 4 H^+)
- COMPLEX II → " → " ⇒ " 2 ATP mol

Stoichometry of the ATP synthesis is not exactly recognized, but we presume that the re-entry of 4 protons drives the synthesis of 1 ATP

⇒ Transfer of 2e⁻ from NADH to O_2 results in 3ATP, from $FADH_2$ only 2 ATP. Ex: citric acid cycle

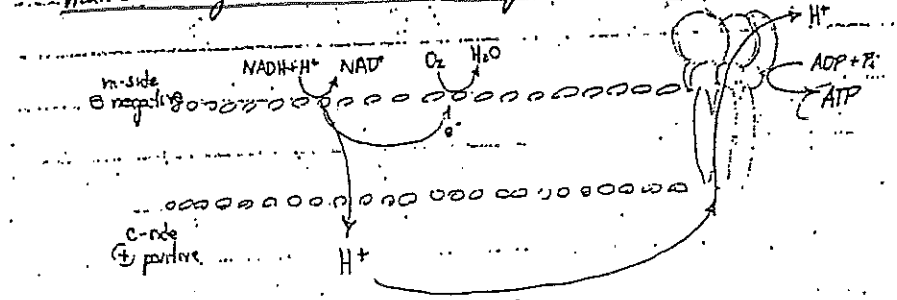
OXIDATIVE PHOSPHORYLATION

COUPLING OF PHOSPHORYLATION TO TERMINAL RESPIRATORY CHAIN → the link between the 2 processes is the PROTON MOTIVE FORCE.

Translocation of protons across the inner mitochondrial membrane results in formation of an electrochemical gradient: $\Delta\mu_{H^+}$

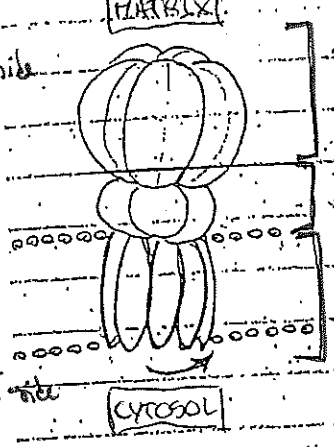
A proton motive force represents the E available for ATP synthesis, as well as for other endergonic processes (secondary active transport of ions across the membrane) or production of heat (dissipation of $\Delta\mu_{H^+}$ by re-entry of protons through thermogenin in the brown adipose tissue).

ATP SYNTHASE - phosphorylation of ADP is driven by the flux of protons back into the matrix along the electrochemical gradient through ATP synthase.



Doesn't take part in the reaction?

ATP synthase consists of 3 parts: 2 subunits



1. F_1 (head) = projects into the matrix, 5 subunit types (α_3, β_3, rotating γ, δ that catalyze ATP synthesis in coupled system, or ATP hydrolysis in uncoupled mitochondria
2. CONNECTING SECTION
3. F_0 (segment) = inner membrane component, 3 subunit types (a, b_2, c_{10}), that form a rotating proton channel

CONTROL OF THE OXIDATIVE PHOSPHORYLATION = Synthesis of ATP depends on (Question 8)
- supply of substrates (mainly $NADH+H^+$)
- supply of O_2
- the energy output of the cell: hydrolysis of ATP increases the [ADP] in the matrix ↑ which activates ATP production

↓

the higher the [ADP], the higher the uptake of O_2 by mitochondria, for ATP production

↓

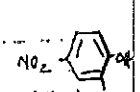
THIS MECHANISM IS CALLED RESPIRATORY CONTROL

UNCOUPLING OF RESPIRATORY CHAIN AND PHOSPHORYLATION = is the wasteful oxidation of substrates without ATP synthesis = protons are pumped across the membrane but they somehow re-enter the matrix with no ATP synthesis.

The free energy derived from these oxidations appears as heat.

TYPES OF UNCOUPLERS:

1. TRUE UNCOUPLERS = compounds that transfer protons through the membrane
   eg: 2,4-dinitrophenol (DNP)

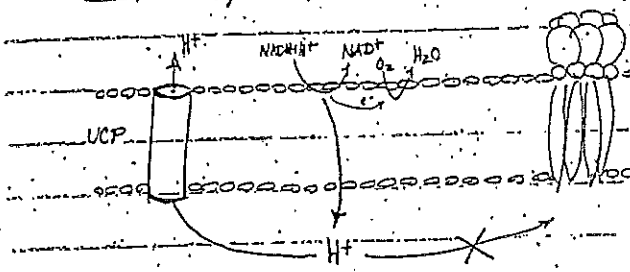


2. IONOPHORS = don't disturb the chemical potential of protons, but diminish the electric potential by enabling free re-entry of K^+ (valinomycin) or both K^+ and Na^+ (antibiotics): they enable ions to penetrate through the membrane: gradient becomes un
3. INHIBITORS OF ATP SYNTHASE = oligomycin
4. INHIBITORS OF ATP/ADP TRANSLOCASE: plant and mold toxins: bongkrekic acid binds ADP to the translocase; atractylate binds ATP to the translocase. ATP synthase then lacks its substra

THERMOGENIN (uncoupler protein, UCP) ∴ NATURAL UNCOUPLER

↓

an inner mitochondrial membrane protein that transports protons back to the matrix, bypassing ATP synthase



UCP occurs in brown adipose tissue of newborn children and hibernating animals.

Its activity is stimulated by fatty acids!

17 Transport of glucose into cells. Glucose transporters - types

Glucose cannot diffuse directly into cells, but enters by two types of transport mechanism:

① Na+ - independent, facilitated diffusion transport system

② Na+ - monosaccharide co-transporter system.

① Na+ independent, facilitated diffusion transport

→ This system is mediated by GLUT-1 to GLUT-14 (glucose transporter isoforms 1 to 14)
These transporters exist in the membrane in two conformational states. Extracellular glucose binds to the transporter, which then alters its conformation, transporting glucose across the cell membrane.

① Tissue specificity of GLUT gene expression: the glucose transporter display a tissue-specific pattern of expression
Example:
GLUT-3 → is the primary glucose transporter in neurons
GLUT-1 → is abundant in erythrocytes and brain.
GLUT-4 → is abundant in adipose tissue and skeletal and cardiac muscle is increased with insulin.
↳ Transports glucose into muscle and fat cells, the presence of insulin, which signals the fed state leads to a rapid increase in the number of GLUT-4 transporters in the plasma membrane. Hence, insulin promotes the uptake of glucose by muscle and adipose tissue

② Specialized functions of GLUT isoforms: In facilitated diffusion, glucose movement follows a concentration gradient that is, from a high glucose concentration to a lower one.

Example:
GLUT-1, GLUT 3 and GLUT 4 are primarily involved in glucose uptake from the blood. In contrast GLUT-2, which is found in the liver, kidney, and β cells of the pancreas, can either transport glucose into these cells when blood glucose levels are high, or transport glucose from the cells to the blood when blood glucose levels are low. (during fasting)

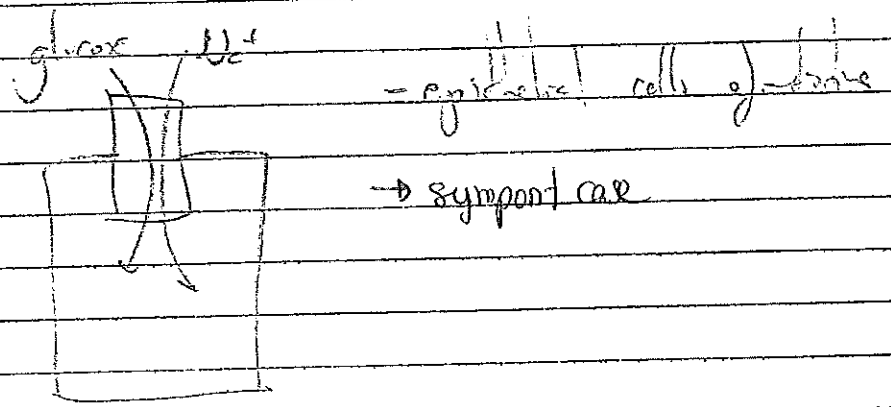
Blood [glucose] < high: [illegible]
low: [illegible]

GLUT-5 → primary transporter of Fructose, in small intestine

• ② Na^+ - monosaccharide cotransporter system

this is an energy requiring process that transports glucose "against" a concentration gradient — that is, from low glucose concentrations outside the cell to higher concentrations within the cell. low [ ] → high [ ]

this system is a carrier-mediated process in which the movement of glucose is coupled to the concentration gradient of Na^+, which is transported into the cell at the same time. this type of transport occurs in the epithelial cells of the intestine, renal tubules, and choroid plexus.



18 The glycolitic pathway - localization, reaction and regulation of glycolysis.

Glycolysis → ...in all tissues ...for the breakdown of glucose to provide ATP and other ... intermediates for other metabolic pathways.

- pyruvate is the end product (in cells with mitochondria and oxygen supply) → AEROBIC glycolysis
  - ↳ then: OXIDATIVE DECARBOXYLATION OF PYRUVATE TO acetyl Co.A → for CITRIC ACID cyc
- pyruvate is reduced to lactate - in non-oxygenated conditions → ANAEROBIC GLYCOLYSIS - in tissues with no mitochondria (erythrocytes) or in cells with few O_2

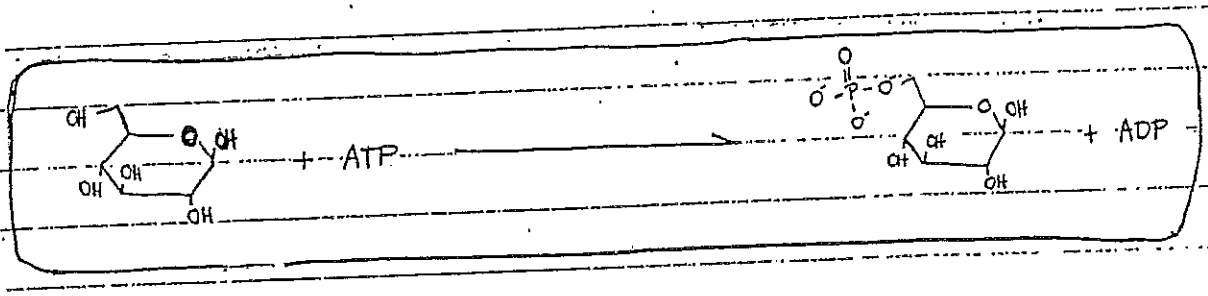
REACTIONS OF GLYCOLYSIS ... 2 phases
- investment phase = first 5 reactions
- E generation phase

Glycolysis occurs in the cytosol of all tissues

Glycolysis occurs in the cytosol of all tissues

IRREVERSIBLE

STAGE 1 - PHOSPHORYLATION OF GLUCOSE → to GLUCOSE-6-PHOSPHATE
↳ so that it can penetrate cell membranes ! (there are no transmembrane carriers for glucose and it is too polar to pass by diffusion).

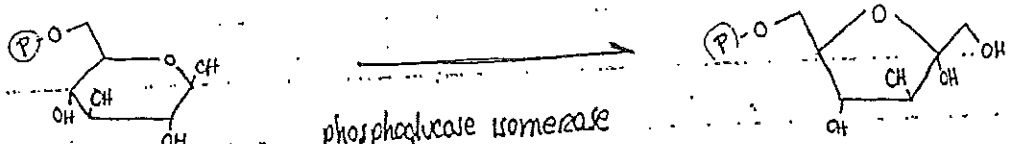


ENZYMES for this process:

- **HEXOKINASE** - broad substrate specificity (phosphorylates many hexoses)
  - in most tissues
  - Low K_M ⇒ high affinity for glucose (concentration that gives half of the MAX. VEL)
  - inhibited by glucose-6-P
- **GLUCOKINASE** - induced by insulin (works better after meals)
  - function: provides glucose for synthesis of glycogen and F.A.
  - in liver cells and in pancreas islets cells
  - higher K_M ⇒ requires a bigger [S] for its half-saturation (low affinity) → works better in hyperglycemia
  - inhibited by fructose-6-P (in equilibrium with glucose-6-P)

In abr... patients with diabetes causes a deficiency in hepatic glucokinase
↳ the patient is unable to decrease blood glucose levels !

STAGE 2 – ISOMERIZATION OF GLUCOSE-6-P ⟶ to fructose-6-P

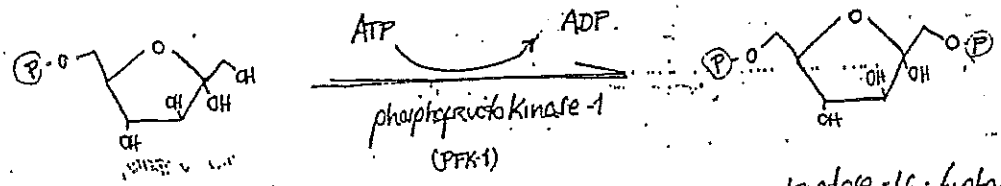


Wrong X ⇒ Slowest step of glycolysis - the velocity of all the process depends on the vel. of this ste

L3S30.

STAGE 3 – PHOSPHORYLATION OF FRUCTOSE-6-P. ⟶ to fructose-1,6-biphosphate

IRREVERSIBLE



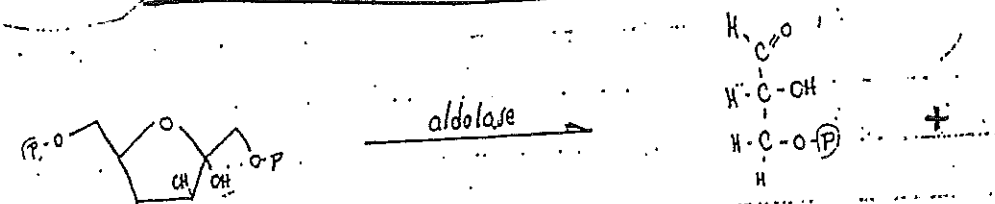
(phosphofructokinase-1)

PFK-1 ⟶ is regulated by Allosteric control

- ATP (and citrate) high levels of ATP INHIBIT allosterically PFK-1, and high levels of AMP or ADP activate it!
- Fructose-2,6-biphosphate (from gluconeogenesis) : activator of PFK-1

? END of investment phase

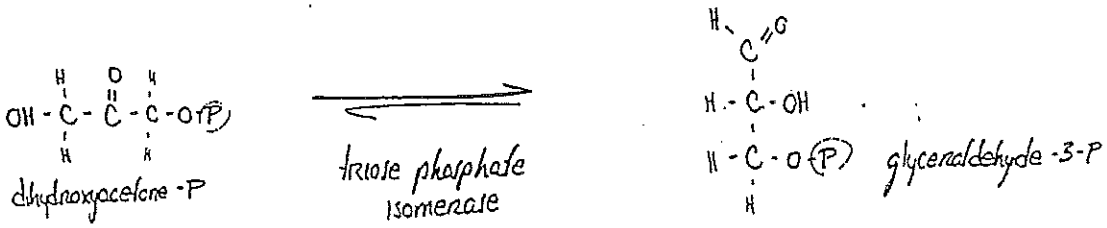
STAGE 4 – CLEAVAGE OF FRUCTOSE-1,6-BIPHOSPHATE ⟶ to dihydroxyacetone phosphate and glycera... 3-Phosp



This process is an aldocondensation!

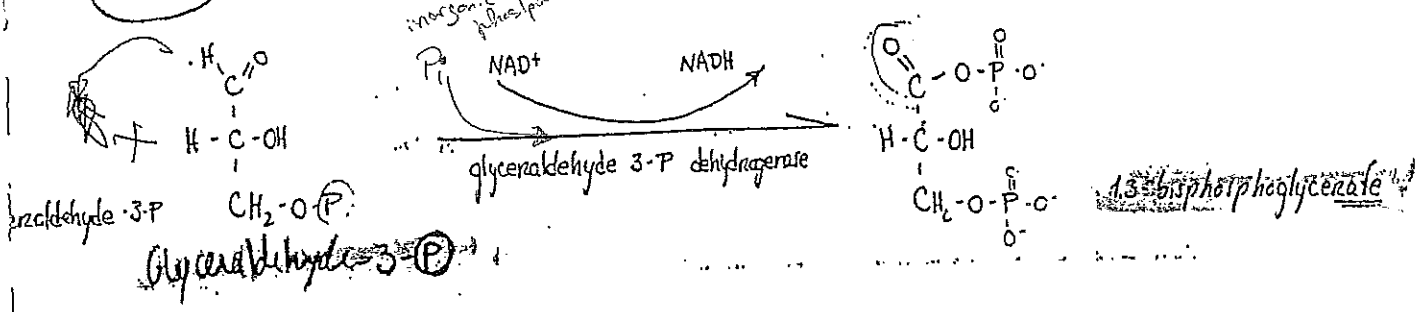
⇒ the reaction is not regulated

STAGE 5 – ISOMERIZATION OF DIHYDROXYACETONE PHOSPHATE → to glyceraldehyde-3-P!

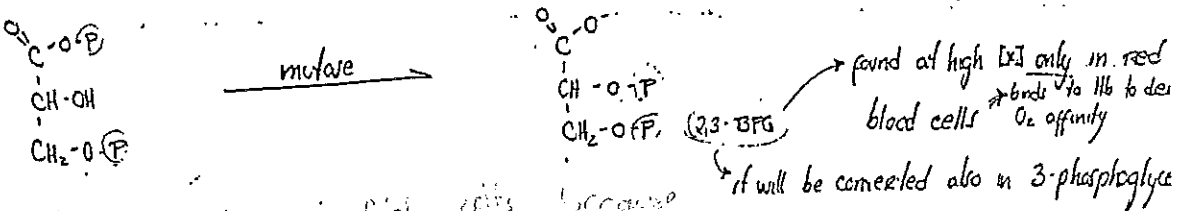


↳ So we end up with 2 molecules of glyceraldehyde-3-P from the metabolism of fructose-1,6-

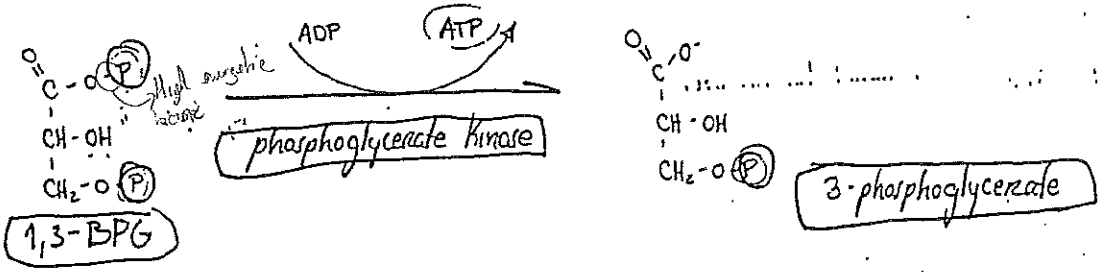
STAGE 6 – OXIDATION OF GLYCERALDEHYDE-3-P → to 1,3-bisphosphoglycerate



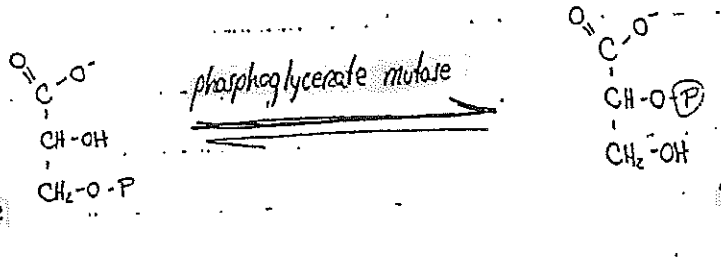
NOTE: SOME OF THE 1,3-bisphosphoglycerate IS CONVERTED TO 2,3-bisphosphoglycerate (2,3-BPG):



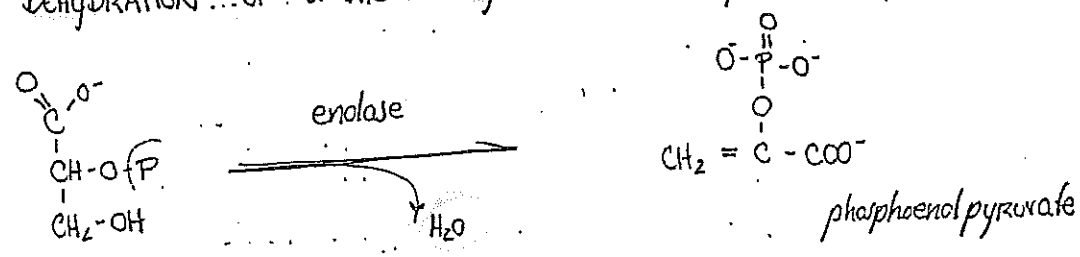
STAGE 7 – SYNTHESIS OF 3-PHOSPHOGLYCERATE producing ATP!



STAGE 8 - SHIFT OF (P) GROUP FROM C3 to C2

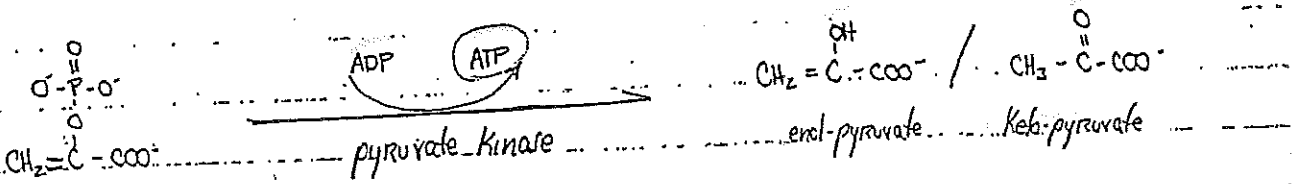


STAGE 9 - DEHYDRATION OF 2-PHOSPHOGLYCERATE → to phosphoenolpyruvate



IRREVERSIBLE

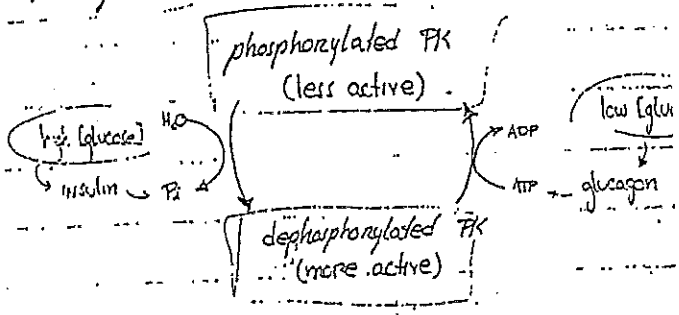
STAGE 10 - FORMATION OF PYRUVATE PRODUCING ATP



This reaction is a substrate phosphorylation

REGULATION OF PYRUVATE KINASE

- activated by fructose-1,6-bisphosphate (+)
- inhibited by glucagon (in liver) by phosphorylation (−)



IN WELL-FED STATE

↓ GLUCAGON

↑ INSULIN

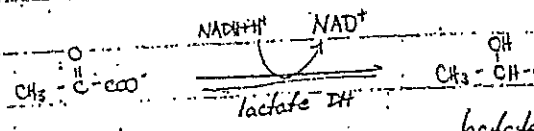
DURING STARVATION

↑ GLUCAGON → because released when [glucose] is low, ... causes liver to degrade glyco

↓ INSULIN → released when [glucose] is high, ... uptake of glucose by cells!

19 The glycolysis under anaerobic conditions: the role of lactate dehydrogenase reaction, the Cori cycle, the LD isoenzymes.

ANAEROBIC Glycolysis



Role of lactate dehydrogenase:

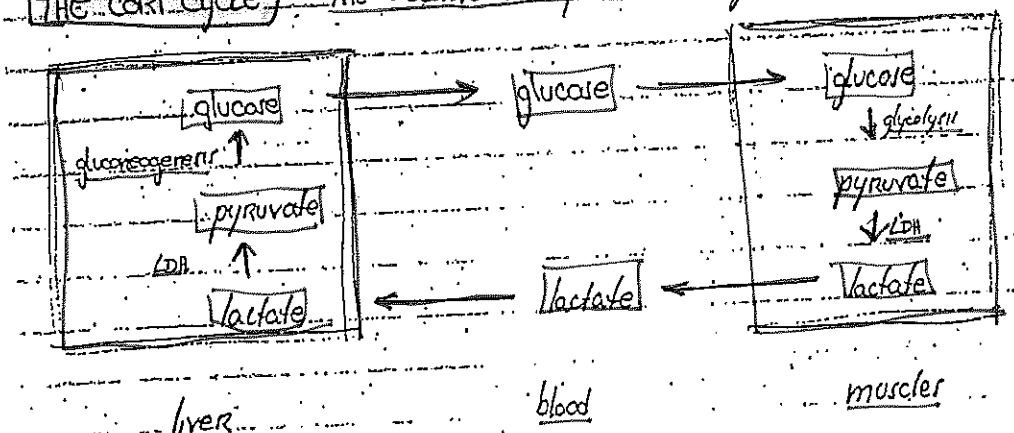
The purpose of this final reaction is to regenerate NAD^+ consumed in dehydrogenation of 3-phosphoglyceraldehyde to 1,3-bisphosphoglycerate.

It also occurs when we want to save as much E as possible: the lactate goes to liver where it is oxidized to pyruvate → gluconeogenesis (converted to glucose) → again glycolysis

This reaction may occurr in 2 situations:

1) when the oxygen supply is limited, as in muscle during intensive exercise
2) in tissues with few or no mitochondria, such as the medulla of the kidney, mature erythrocytes, leukocytes, and cells of the lens, cornea and testes.

THE CORI CYCLE – the reconversion of lactate to glucose in the liver



(next page)

ISOENZYMES OF LACTATE DEHYDROGENASE – are used to detect myocardial infarctions

↳ L-lactate DH is a tetrameric enzyme where 4 subunits occurr in 2 isoforms, designated H (for heart) and M (for muscles)

LDH ISOENZYME	SUBUNITS (4)	
I_1	HHHH	→ predominates in heart
I_2	HHHM	
I_3	HHMM	
I_4	HMMM	
I_5	MMMM	→ predominates in muscle

Following a myocardial infarction or in liver diseases, the damaged tissues release characteristic LDH isoenzymes into the blood.

20. Energetic yield of glycolysis under anaerobic and aerobic conditions.

ANAEROBIC GLYCOLYSIS: (2 mol) of ATP generated for each mol. of glucose
→ there is no NET production OR consumption of NADH.
→ although it releases only a small fraction of E, it is a valuable source of E in some conditions:
1) when oxygen supply is limited, as in muscles during intensive exer.
2) for tissues with few or no mitochondria, such as the medulla of the kidney, mature erythrocytes, leukocytes and cells of the lens, cor and testes.

AEROBIC GLYCOLYSIS: net gain of (2) ATP per mol. of glucose
(2) mol. of NADH are also produced per mol. of glucose. ; ongoing aerobic glycolysis requires oxidation of most this NADH by the electron transpor chain, producing 3 mol. ATP per mol. of NADH.

So in the end we get: $2 + 2 \times 3 =$ 8 mol. of ATP per mol. of glucose.

Reactions where NADH is produced:
- glyceraldehyde 3-P $\xrightarrow[\text{GLYCERALDEHYDE-3-P DH}]{P_i + NAD^+ \rightarrow NADH}$ 1,3-bisphosphoglycerate
- pyruvate + CoA $\xrightarrow[\text{PYRUVATE DH COMPLEX}]{NAD^+ \rightarrow NADH}$ acetyl-CoA + CO_2 → only in aerobic glycolysis

1 + 1 = 2

Reaction where NADH is consumed → pyruvate $\xrightarrow[\text{LACTATE DH}]{NADH \rightarrow NAD^+}$ lactate → only in anaerobic glycolysis

1 - 1 = 0

→ glyceraldehyde 3-P → 1,3 bisphosphoglycerate ($NAD^+ \rightarrow NADH$)

METABOLISM OF PYRUVATE

1) IN AEROBIC GLYCOSIS ⇒ oxidative decarboxylation

CoA + pyruvate —(NAD+ → NADH + H+, CO2)→ acetyl-CoA ⇒ used for citric acid cycle

2) IN ANAEROBIC GLYCOSIS ⇒ when we want to save as much E source as possible!

↳ occurs in the muscles, sometimes brain (during starvation) and in the erythrocytes (don't have mitochondria)

pyruvate —(NADH+H+ → NAD+)→ lactate → goes to liver where it will oxidized to pyruvate

$CH_3-\overset{O}{\overset{\|}{C}}-COO^-$ ... $CH_3-\underset{OH}{CH}-COO^-$

gluconeogenesis

converted to glucose

AGAIN GLYCOLYSIS (cyclic process)

1,3 bi(P)glycerate → 3(P)glycerate

(P)enolpyruvate → pyruvate

ENERGETIC GAIN:

- ANAEROBIC GLYCOLYSIS → 2 mol of ATP generated for each mol of glu
- AEROBIC GLYCOLYSIS → for each mol glucose = 2 mol ATP + 2 mol NADH (previous page, ongoing glycolysis) → 3 mol ATP for each

SO IN THE END WE GET 8 mol ATP!

ANAEROBIC GLYCOLYSIS:

1) when O_2 supply is limited — as in muscles during intensive exercise

2) in tissues with few or no mitochondria — i.e.: medulla of kidney, mature erythrocytes, leukocytes and cells of the lens, cornea and

21 Oxidative decarboxylation of pyruvate and other 2-oxoacids (location, roles of particular coenzymes in the pyruvate and 2-oxoglutarate dehydrogenase complexes energetics significance).

The oxidative decarboxylation of pyruvate takes place in the matrix of mitochondria

Pyruvate + CoA + NAD$^+$ $\xrightarrow{\text{PYRUVATE DH COMPLEX}}$ acetyl-CoA + CO_2 + NADH

→ The oxidative decarboxylation of pyruvate is the link between glycolysis and the citric acid cycle

PYRUVATE DH COMPLEX - 3 enzymes
- E_1 - decarboxylating component
- E_2 - transacetylase - core
- E_3 - dihydrolipoyl DH

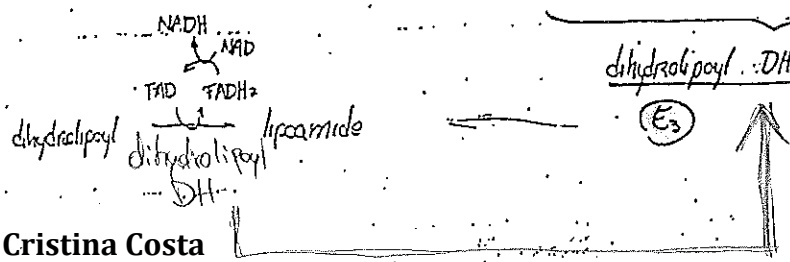
requires 5 cofactors:
- Thiamine diphosphate — prosthetic group of E_1
- Lipoamide — " " of E_2
- Coenzyme A
- FAD — " " of E_3
- NAD$^+$

STEPS IN THE OXIDATIVE DECARBOXYLATION OF PYRUVATE

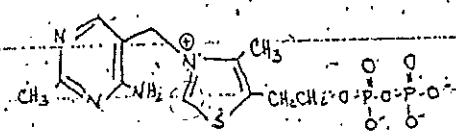
$CH_3-\overset{O}{\overset{\|}{C}}-COO^-$ (pyruvate) $\xrightarrow{-CO_2}$ $CH_3-\overset{O}{\overset{\|}{C}}-H$ (acetaldehyde (bound to TDP)) $\xrightarrow[-2H]{\text{oxidation}}$ $CH_3-\overset{O}{\overset{\|}{C}}-$ (acetyl (bound to lipoamide)) $\xrightarrow{CoA}$ $CH_3-\overset{O}{\overset{\|}{C}}-S-CoA$ (acetyl-CoA)

decarboxylating component (E_1) — transacetylase (E_2)

dihydrolipoyl DH (E_3) → Reoxidation of dihydrolipoamide to lipoam (2H$^+$ accepted by FAD and then by NAD$^+$)

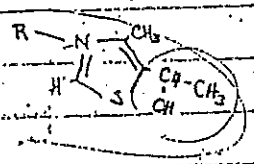


E_1 = DECARBOXYLATING COMPONENT → has TDP bound:



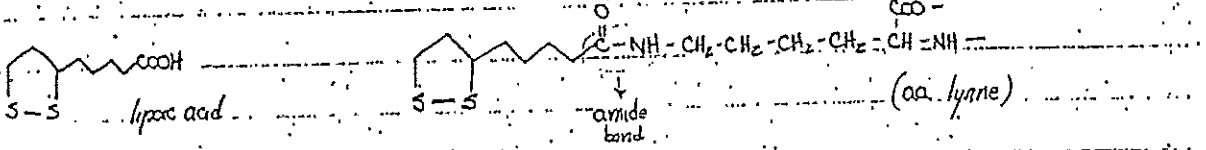
→ Coenzyme thiamine diphosphate = binds pyruvate; the product of decarboxylation is acetaldehyde (bound to TDP as hydroxyethyl)

E_1 catalyzes the transfer of the (α-hydroxyethyl

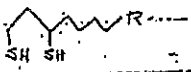


to the lipoyl arm of transacetylase E_2

E_2 = TRANSACETYLASE → has (LIPOIC ACID attached to its lysyl residue) = LIPOAMIDE



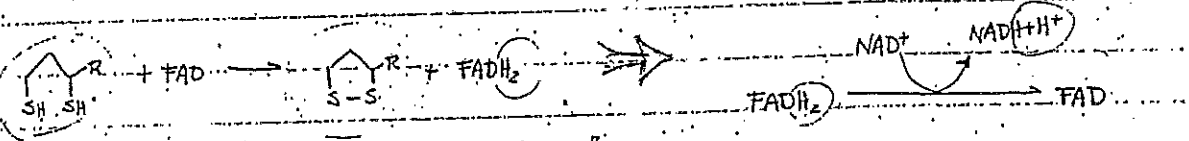
↳ lipoamide acepts hydroxyethyl group from TDP and then oxidizes it to acetyl being reduced to dihydrolipoamide:



↳ the acetyl is then transferred to Coenzyme A

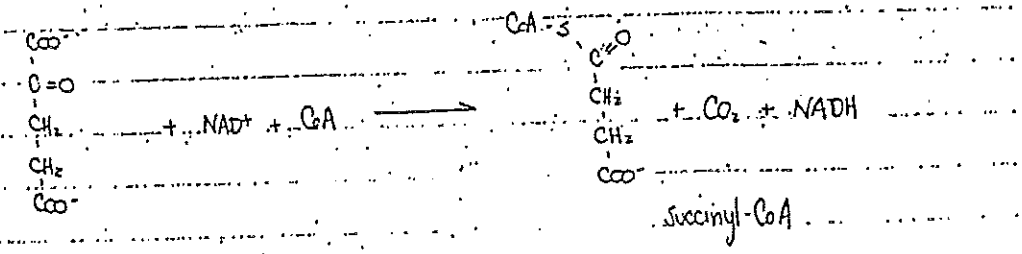
↳ the dihydrolipoyl swings to E_3 to be reoxidized:

E_3 = DIHYDROLIPOYL DH = has FAD bound to it, that accepts 2H atoms which are passed to NAD^+



↳ the role of E_3 is to reoxidize dihydrolipoyl!

OXIDATIVE DECARBOXYLATION OF 2-OXOGLUTARATE = in the citrate cycle - similar to the pyruvate



COMPONENTS OF (2-OXOGLUTARATE DH COMPLEX): E_1 + E_2 are different but homologous to the pyruvate; E_3 of the two complexes are identical

THE OXIDATIVE DECARBOXYLATION OF PYRUVATE is an irreversible step!

↳ draws 1 mol. of NADH, which will be used in respiratory chain ⇒ 3 mol. ATP

REGULATION OF THE PYRUVATE DH COMPLEX

(−) INHIBITION
- by NADH and acetyl-CoA (immediate products)
- by ATP
- by phosphorylation

(+) ACTIVATION ⇒ by dephosphorylation

22 The Citric acid cycle – location, Reactions of the cycle, the amphibolic Role of the cycle (the final pathway for the oxidation of nutrients and the pathways originating from the cycle).

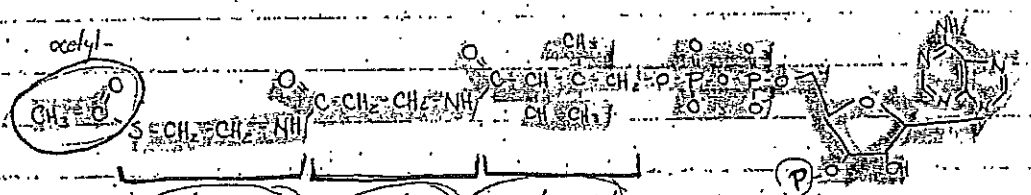
CITRIC ACID CYCLE = occurs in the matrix of mitochondria

(tricarboxylic / Krebs cycle)

↳ is the final COMMON pathway for the oxidation of nutrients (oa, f.a, saccharid

↳ MOST OF THE INTERMEDIATES ENTER THE CYCLE AS ACETYL-CoA

ACETYL-CoA = formed from the breakdown of saccharides (oxid. decarboxylation of pyruvate), fatty acids (β oxidation) and Ketone bodies, and many amino acids.



CoA

→ The acetyl group of acetyl-CoA is oxidized ←

leave the cycle

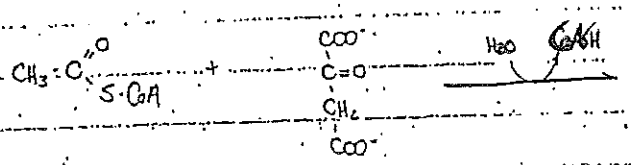
$CH_3-CO-S-CoA + 3H_2O \longrightarrow (2\ CO_2) + (8H^+ + CoASH)$

serve to form 3 mol. of NADH + H⁺ and a mol. of $FADH_2$

REACTIONS OF THE CYCLE (8 RT. Reac.)

1. condensation of acetyl-CoA and oxaloacetate : 1. group – CITRATE
2. isomerization of citrate into isocitrate
3. isocitrate is oxidized and decarboxylated to 2-oxoglutarate
4. oxidative decarboxylation of 2-oxoglutarate to succinyl-CoA
5. cleavage of succinyl-CoA is coupled to the phosphorylation of GDP
6. oxidation of succinate to fumarate
7. oxaloacetate is regenerated by hydration of fumarate and oxidation
8. of malate.

(1) CONDENSATION OF ACETYL-CoA AND OXALOACETATE → catalyzed by CITRATE SYNTHASE



acetyl-CoA — oxaloacetate — Citrate

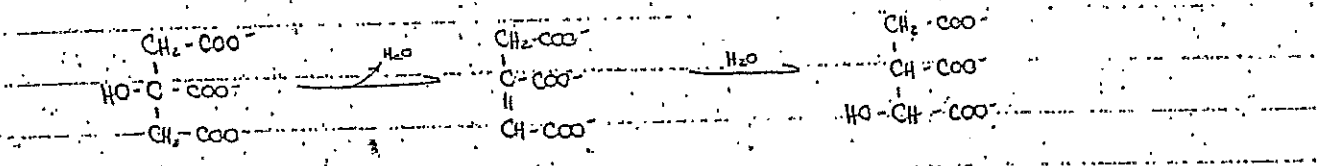
↳ this reaction is an aldol condensation and it is IRREVERSIBLE IN MITOCHONDRIAL MATRIX.

⇓

enolate ion + aldehyde/Ketone —H₂O→ β-hydroxyaldehyde/Ketone

(2) ISOMERIZATION OF CITRATE TO ISOCITRATE → catalyzed by ACONITASE (cofactor Fe-S-protein)

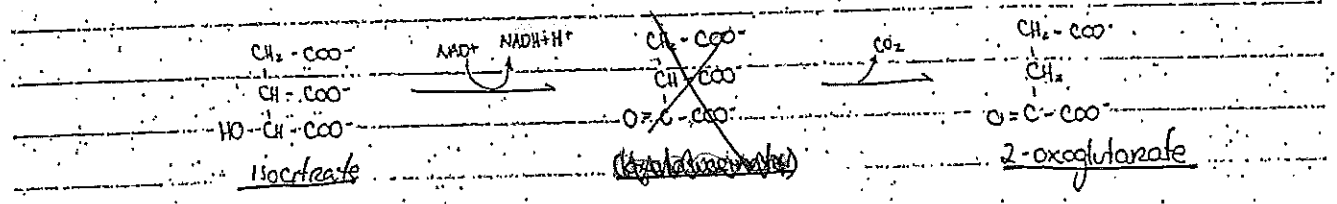
↳ dehydration followed by hydration!



Citrate — aconitate — isocitrate

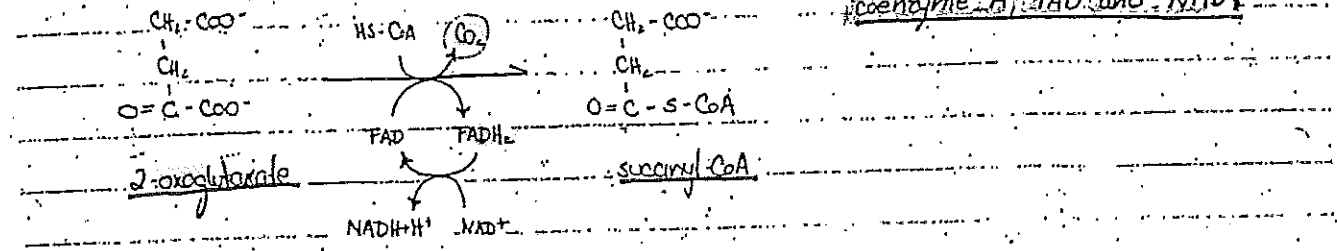
(3) ISOCITRATE IS OXIDIZED AND DECARBOXYLATED TO 2-OXOGLUTARATE → ISOCITRATE DEHYDROGEN (cofactor NAD⁺)

(first of 4 oxidation reactions) THIS REACTION IS IRREVERSIBLE!



Isocitrate — (oxalosuccinate) — 2-oxoglutarate

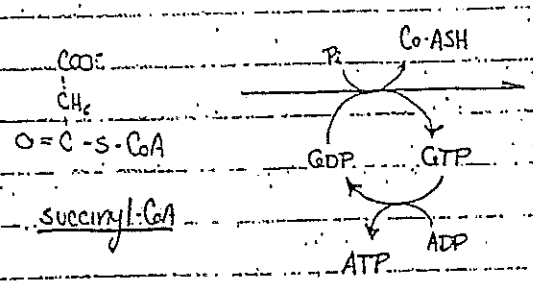
(4) OXIDATIVE DECARBOXYLATION OF 2-OXOGLUTARATE TO SUCCINYL-CoA → catalyzed by 2-OXOGLUTARATE DEHYDROGENASE COMPLEX → 5 cofactors: TDP, lipoic coenzyme A, FAD and NAD⁺



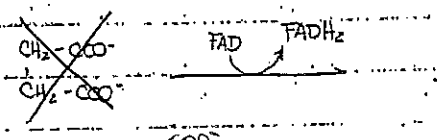
2-oxoglutarate — succinyl CoA

5. CLEAVAGE OF SUCCINYL-CoA COUPLED TO PHOSPHORYLATION OF GDP ! – catalyzed by SUCCINYL-CoA SYNTHASE

→ the only step in which is directly yeld an high-E compound

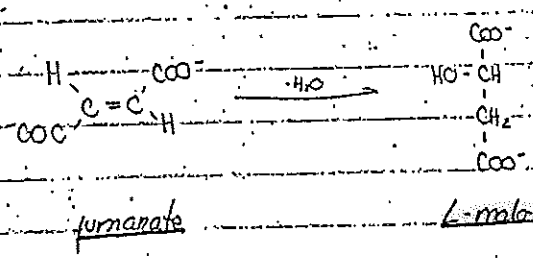


6. OXIDATION OF SUCCINATE TO FUMARATE ! catalyzed by SUCCINATE DEHYDROGENASE (cofactor FAD)

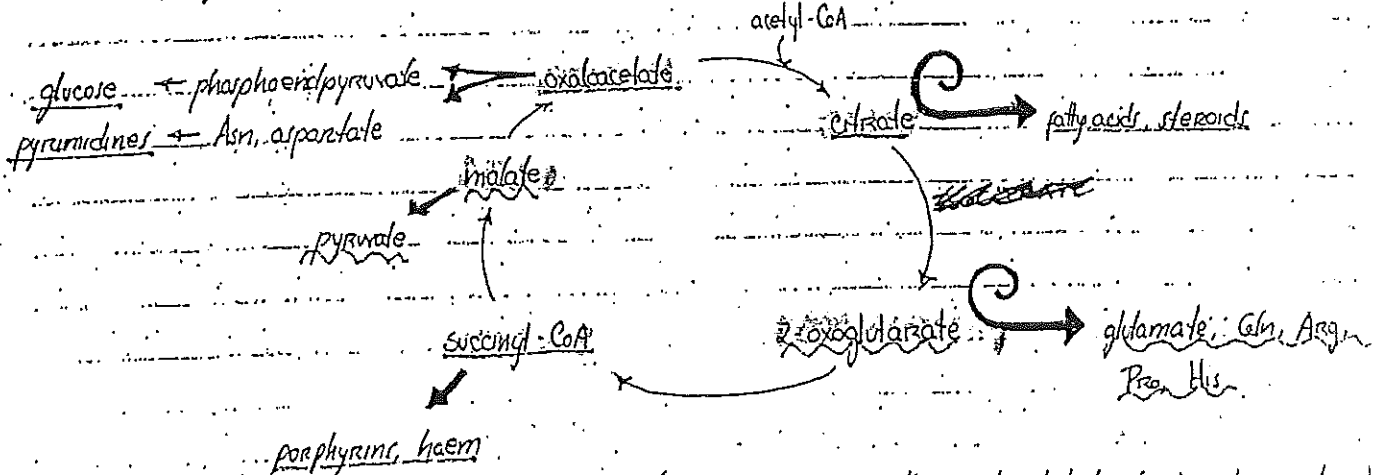


→ the only enzyme in the cyc
that is embedded in the inner mit.
→ the enzyme is associated with th
complex II

7. and 8. OXALOACETATE IS REGENERATED BY HYDRATION OF FUMARATE AND OXIDATION OF MALATE ! – catalyzed by FUMARASE and MALATE DEHYDROGENASE

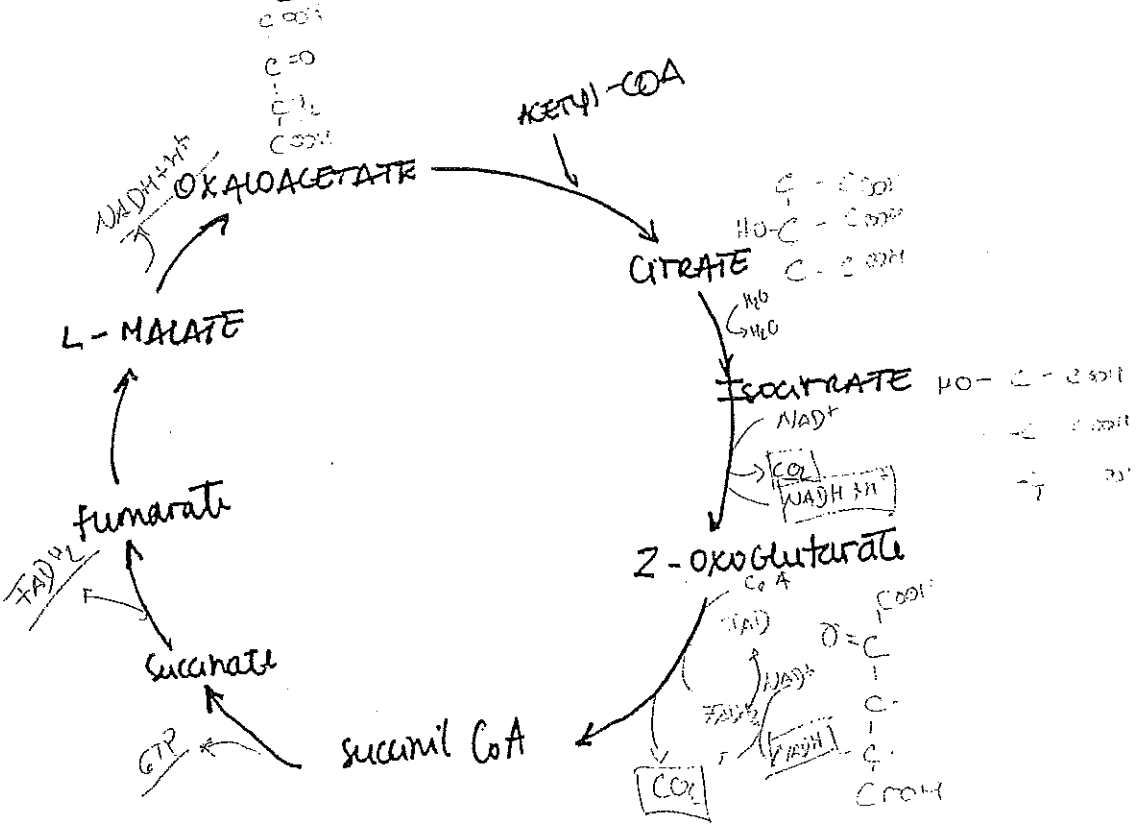


THE AMPHIBOLIC ROLE OF THE CYCLE – citrate cycle provides intermediates for biosynthesis



(the intermediates are then replenished by anapleurotic reactions)

CITRIC ACID cycle



23 The energetic yield and regulation of the citric acid cycle. The anaplerotic reactions (replenishing the intermediates of the cycle)

ENERGETIC YELD OF CITRATE CYCLE → See scheme on the back of next page!

1. oxaloacetate —(acetyl-CoA)→ citrate
2. citrate → isocitrate
3. isocitrate —(NAD+)→ 2-oxoglutarate → 3 ATP
4. 2-oxoglutarate —(NAD+)→ succinyl-CoA → 3 ATP
5. succinyl-CoA —(GTP)→ succinate → 1 GTP → 1 ATP
6. succinate —(FAD)→ fumarate → 2 ATP
7. fumarate → malate
8. malate —(NAD+)→ oxaloacetate → 3 ATP

↳ TOTAL = approx. 12 ATP mol from the oxidation of 1 acetyl-CoA

↳ (11 due to reoxidation of reduced coenzymes - NAD, FAD)!

↳ (1 GTP = direct yield from a substrate level phosphorylation)

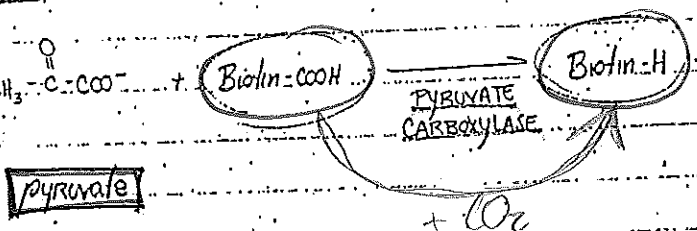
REGULATION OF THE CITRATE CYCLE:

↳ Though O_2 doesn't participate directly in the cycle, it can only operate under aerobic conditions because it requires a supply of oxidized NAD^+ and FAD.

6. pyruvate → acetyl-CoA → inhibitors of pyruvate DH:
   - ATP
   - NADH
   - Acetyl-CoA
- isocitrate → 2-oxoglutarate → inhibitors of isocitrate DH:
  - ATP
  - NADH
  - → activator of isocitrate DH: ADP
- 2-oxoglutarate → succinyl-CoA → inhibitors of 2-oxoglutarate DH:
  - ATP
  - NADH
  - Succinyl-CoA

THE ANAPLEUROTIC REACTIONS → lead to the net synthesis or replenishment of pathway components. → form intermediates of a metabolic pathway – many reactions

The most important is the FORMATION OF NEW OXALOACETATE BY CARBOXYLATION OF PYRUVATE



pyruvate — oxaloacetate

- use these molecules as substrates

OTHER ANAPLEUROTIC REACTIONS

- reductive carboxylation of pyruvate to malate
- transamination of aspartate to oxaloacetate
- " of glutamate to 2-oxoglutarate
- etc.

24 ~~Irreversible reactions in glycolysis and gluconeogenesis (location, substrates and the course of gluconeogenesis, regulation)~~

Gluconeogenesis (localization, substrates and the course of gluconeogenesis, regulation) relationship between gluconeogenesis and glycolysis.

GLUCONEOGENESIS → synthesis of glucose from non-saccharide precursors

- major nonsaccharide precursors:
  - lactate
  - glucogenic aa
  - glycerol

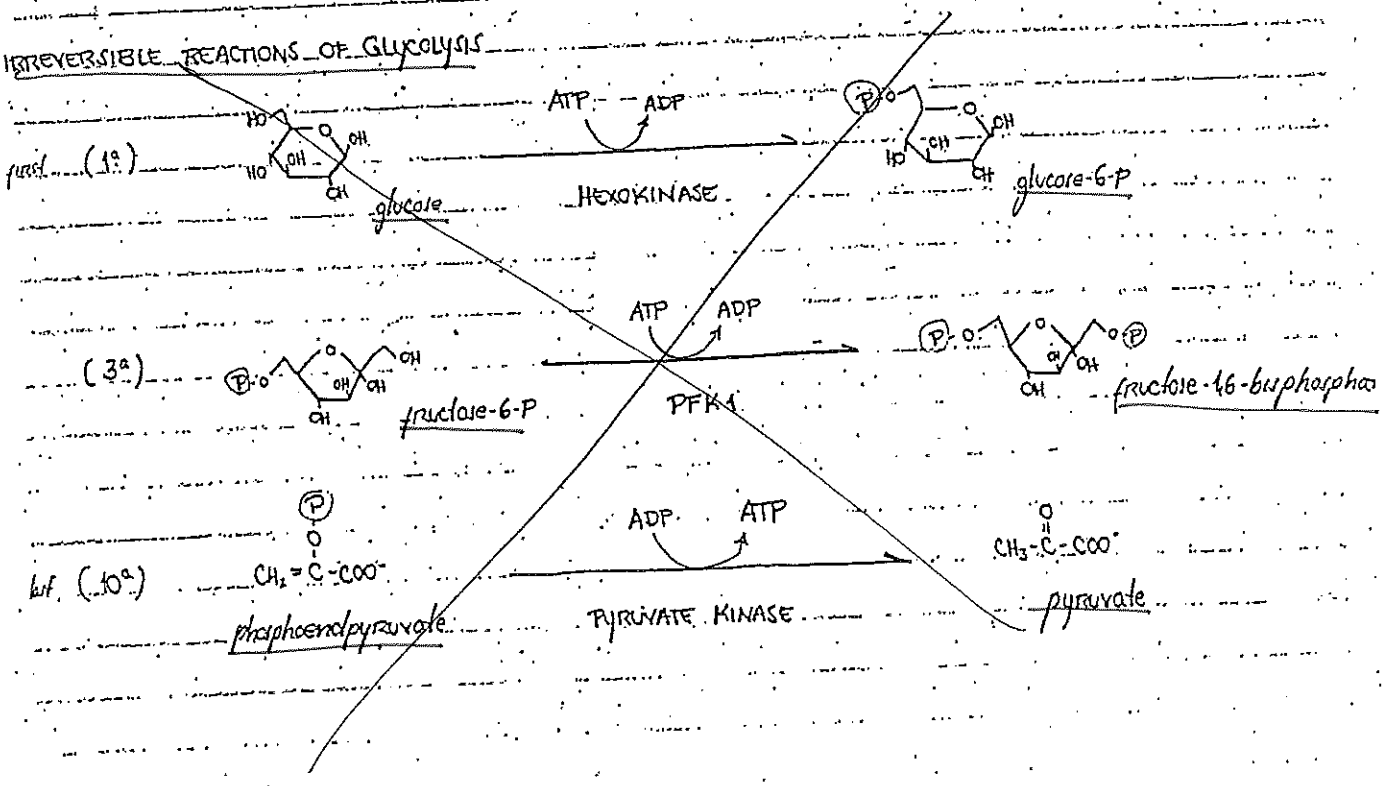
  → they should be firstly converted to pyruvate (or enter the pathway as the intermediates oxaloacetate or dihydroxyacetone)

IS NOT A REVERSAL OF GLYCOLYSIS (reversão) – because 3 reactions of GLYCOLYSIS are IRREVERSIBLE.

So we get 3 CONTROL POINTS:
- HEXOKINASE: glucose → glucose-6-P
- PHOSPHOFRUCTOKINASE 1 (PFK 1): fructose-6-P → fructose-1,6-biP
- PYRUVATE KINASE: phosphoenolpyruvate → pyruvate

allosterically inhibited:
- PFK 1: inhibited by ATP, low pH (⊖); activated by fructose-2,6-bisphosphate (⊕)
- PYRUVATE KINASE: activated by insulin; inhibited by glucagon

IRREVERSIBLE REACTIONS OF GLYCOLYSIS



GLUCONEOGENESIS = IRREVERSIBLE REACTIONS

(biotin coenz)

(1) $CH_3-C(=O)-COO^-$ (pyruvate) —ATP, ADP— PYRUVATE CARBOXYLASE → $^-OOC-C(=O)-CH_2-COO^-$ (oxaloacetate) —GTP, GDP, CO_2— PHOSPHOENOLPYRUVATE CARBOXYKINASE → $CH_2=C(-O-P)-COO^-$ (phosphoenolpyruvate)

in mitochondria | in cytosol

when pyruvate is the source of C atoms for gluconeogenesis:

- mitochondrial OOA is reduced to malate by MALATE DEHYDROGENASE
- malate is then transported to cytosol and reoxidized to OOA (this reaction will yield NADH for glycolysis)
- THIS STEP IS NEEDED TO TRANSPORT OOA OUT OF MITOCHONDRIA !

(2) phosphoenolpyruvate —$+H_2O$, ENOLASE→ 2-phosphoglycerate —(3) PHOSPHOGLYCERATE MUTASE→ 3-phosphoglycerate

(4) 3-phosphoglycerate —ATP, ADP— PHOSPHOGLYCE KINASE → 1,3-buphosphoglycerate

(5) 1,3-buphosphoglycerate —NAD+, NADH— GLYCERALDEHYDE-3-P DH → glyceraldehyde-3-P

(6) glyceraldehyde-3-P —TRIOSE-P ISOMERASE→ dihydroxyacetone-P

(7) dihydroxyacetone-P + glyceraldehyde-3-P —ALDOLASE→ fructose-1,6-biphosphate

(8a) IRREVERSIBLE

fructose-1,6-biphosphate —H_2O, P_i, FRUCTOSE-1,6-BIPHOSPHATASE→ fructose-6-P

(9) PHOSPHOGLUCOSE ISOMERASE

fructose-6-P —P_i, H_2O, GLUCOSE-6-PHOSPHATASE→ glucose

(10a) IRREVERSIBLE

→ Only in the liver and kidneys the final product is free glucose, glucose-6-P is converted to glucose in the ER.

REGULATION OF GLUCONEOGENESIS

(1) Glucagon stimulates gluconeogenesis by 3 mechanisms:

↳ it lowers the level of fructose-2,6-bisphosphate → activation of fructose-1,6-biphosphatase and inhibition of PFK-1

(fructose-2,6-bisP acts as an intracellular signal, indicating that glucose is abundant, thus it increases the rate of glycolysis.)

↳ it stimulates, via elevations in cAMP levels and cAMP-dependent protein kinase, the phosphorylation of pyruvate kinase to its inactive form. This stops phosphoenolpyruvate from its conversion to pyruvate, what diverts PEP to the synthesis of glucose

↳ increases the transcription of the PEP carboxykinase gene, what will help to deal with the high levels of oxaloacetate during fasting

(In the well-fed state, insulin has the opposite effect.)

(2) Availability of glucogenic precursors. Decreased levels of insulin favor mobilization of aa from the muscle protein to support gluconeogenesis

(3) During fasting, acetyl-CoA allosterically activates pyruvate carboxylase. (acetyl-CoA accumulates in the body due to large lipolysis).

(4) fructose-1,6-bisphosphatase is inhibited by AMP = compound that stimulates PFK1. Elevated AMP thus stimulates pathways that oxidize nutrients to provide E for the cell.

SUMMARY

STIMULATION OF GLUCONEOGENESIS

- Glucagon stimulates gluconeogenesis, because it inhibits pyruvate kinase and PFK-1.
- Low [insulin] → mobilizes aa from the muscle to support gluconeogenesis
- In fasting, acetyl-CoA activates pyruvate carboxylase
- ↑[AMP] → inhibition of fructose-1,6-bP → inhibition of PFK-1

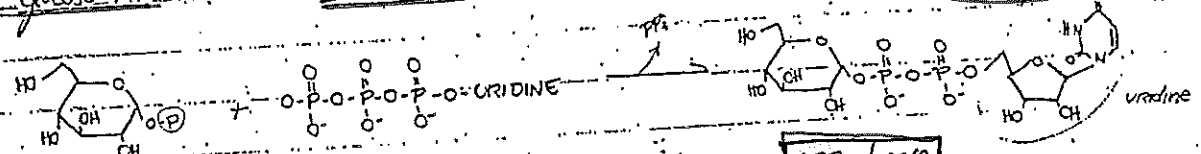
25 GLYCOGENESIS – location, reactions of glycogen synthesis, control mechanisms.

GLYCOGEN = large and branched polymer present in animal cells as granules – Ø 10-40 nm

MAIN STORAGE PLACES: cytosol of liver and skeletal muscle

GLYCOGEN SYNTHESIS

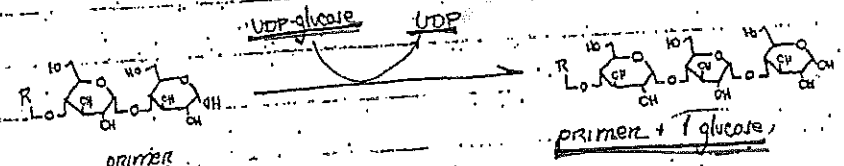
1. glucose-6-P is isomerized to glucose-1-P by PHOSPHOGLUCOMUTASE
2. then glucose-1-P should be activated to UDP-GLUCOSE – catalyzed by UDP-glucose phosphorylase



glucose-1-P + uridine triphosphate (UTP) → UDP-glucose

3. ENZYMES THAT CATALYSE SYNTHESIS OF GLYCOGEN

- **GLYCOGEN SYNTHASE** – Key regulatory enzyme in glycogenesis
  - catalyzes formation of α-1,4-glycosidic bonds by transfer of glucosyl from UDP-glucose to an existing chain (primer):



- **BRANCHING ENZYME** – forms α-1,6-glycosidic bonds – branches of glycogen.
  - branching is important: increases solubility and velocity of synthesis/degradation of glycogen.
  - the branching enzyme is AMYLO-(α-1,4 → α-1,6)-TRANSGLUCOSYLASE

REGULATION OF GLYCOGEN SYNTHESIS

→ The liver serves as glucose supplier for the whole body (the muscles keep the energy for the own n
  - it responds to changes in blood [glucose] – mediated by insulin, or glucagon and adrenalin

The control acts through phosphorylations (by protein kinases) or dephosphorylations (by phosphoprotein phosphatases) of key enzymes and some regulatory proteins.

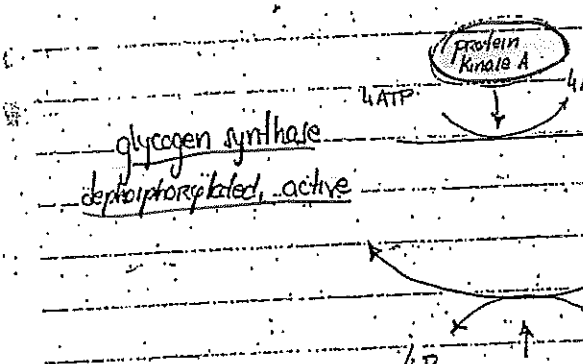
⇒ PHOSPHORYLATED GLYCOGEN SYNTHASE IS INACTIVE !

• ALLOSTERIC REGULATION of glycogen synthesis → In the well-fed state, glycogen synthase is allosterically activated by glucose-6-P present in high concentrations

→ glucose-6-P will then isomerase to glucose-1-P by phosphoglucomutase

• INHIBITION OF GLYCOGEN SYNTHESIS BY cAMP-DIRECTED PATHWAY

1. The binding of hormones glucagon/epinephrine to hepatocyte/muscle cell receptors activates ADENYLYL CYCLASE
   → this enzyme catalyzes synthesis of cAMP
2. cAMP activates **cAMP-dependent protein kinase A**
3. Protein Kinase A then phosphorylates (INACTIVATES) glycogen synthase



exists in 2 forms: { active "a" dephosphorylated; inactive "b" phosphorylated

→ can convert glycogen synthase to its active form, removing hydrolytically the phosphate groups

SUMMARY

REGULATION OF GLYCOGEN SYNTHESIS on glycogen synthase !

- glycogen synthase is allosterically activated by GLUCOSE-6-P (in the well-fed state!)
- ↑ [x] of adrenaline/glucagon → synthesis of cAMP → activates protein kinase A → phosphorylates (INACTIVATES!) glycogen synth

↓ [glucose]

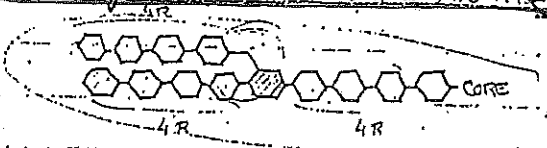
26 GLYCOGENOLYSIS – (degradation of glycogen) in the liver and skeletal muscles – the steps and control of glycogen degradation, inherited disorders

GLYCOGEN BREAKDOWN IN CELLS = cooperation of 2 enzymes: { - glycogen phosphorylase; - debranching enzyme }

1. GLYCOGEN PHOSPHORYLASE = the key regulatory enzyme in glycogen degradation

→ catalyzes the sequential phosphorolysis of α-1,4-glycosidic bonds, from the non-reducing ends and only if they are more distant than 4 residues from a branching point

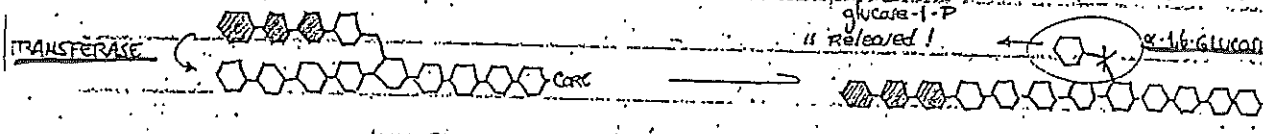
→ SO IN THE END IT WILL PRODUCE MANY MOLS OF GLUCOSE-1-P AND A LIMIT DEXTRIN



2. GLYCOGEN DEBRANCHING ENZYME = bifunctional enzyme:

→ converts the limit dextrin into a linear one

- TRANSFERASE activity = shifts a block of 3R from an outer branch to the other
- α-1,6-GLUCOSIDASE activity = hydrolyses α-1,6-glycosidic bonds



→ PHOSPHORYLASE CAN NOW ATTACK THE REMAINING CHAIN !

→ PHOSPHOGLUCOMUTASE converts glucose-1-P into glucose-6-P = intermediate of glycolysis

REGULATION OF GLYCOGEN DEGRADATION

The LIVER serves as glucose supplier for the whole body ! (the muscles keep the glucose in the)

→ it responds to changes in the blood [glucose] – mediated by insulin, or by glucagon and adrenaline

The control acts through phosphorylations (by protein kinases) or by dephosphorylations (by phosphoprotein phosphatases) of key enzymes and some regulatory proteins.

phosphorylated glycogen phosphorylase is the ACTIVE form

→ phosphorylated glycogen phosphorylase is ACTIVE

↳ serves for breakdown

ALLOSTERIC REGULATION (in liver)

1. IN THE WELL-FED STATE = glycogen phosphorylase is allosterically inhibited by glucose-6-P and ATP

2. ACTIVATION OF GLYCOGEN DEGRADATION BY CALCIUM IN MUSCLES = in muscle contraction, nerve impulses cause membrane depolarization → Ca^{2+} are released

4 mol of Ca^{2+} bind to calmodulin → conformational change → activated Ca^{2+}-calmodulin complex! → this will activate phosphorylase Kinase, which will phosphorylate glycogen phosphorylase. ⇒ glycogen degradation.

3. ACTIVATION OF GLYCOGEN DEGRADATION BY cAMP IN MUSCLES = muscle glycogen phosphorylase is active in the presence of high [AMP], that occurs under conditions of anoxia and ATP depletion.

(in this case there is no phosphorylation of glycogen phosphorylase)

ACTIVATION OF GLYCOGEN DEGRADATION BY cAMP-DIRECTED PATHWAY

1º = activation of protein Kinase = binding of glucagon or epinephrine to cell-membrane receptors results in activation of cAMP-dependent protein Kinase

inactive protein Kinase A —cAMP→ active PROTEIN KINASE A

2º = activation of phosphorylase Kinase = phosphorylation by

↳ exists in 2 forms:
- active "a" form
- inactive "b" form

phosphorylase Kinase (inactive "b" form) —active PROTEIN KINASE A→ phosphorylase Kinase (active "a" form)

3º activation of glycogen phosphorylase

↳ also exists in 2 forms:
- phosphorylated, active "a" form
- dephosphorylated, inactive "b" form

glycogen phosphorylase b —phosphorylase Kinase, 4 ATP → 4 ADP→ glycogen phosphorylase "a" → begins glycogen breakdown

INHERITED DISORDERS = group of genetic diseases that result from a defect in an enzyme required for glycogen synthesis or degradation.

↳ they result in formation of abnormal glycogen or in accumulation of excessive amounts of normal glycogen as a (result of defective degradation)

An enzyme may be defective in a single tissue or affect the liver, muscles, kidney, intestines and myocardium.

Glycogen Storage Diseases (GSDs) may be fatal in infancy.

⇓

examples:

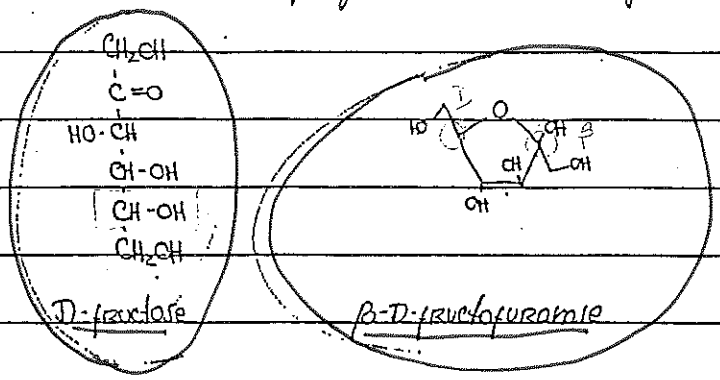
- affect liver and kidney:
  - Type Ia: Von Gierke disease (glucose-6-phosphatase deficiency) → activator of glycogen synthesis
  - Type Ib: glucose-6-phosphatase translocase deficiency
- affects all organs ← Type II: Pompe disease (α-1,4-glucosidase deficiency)
- muscle and liver ← Type III: Cod disease (debranching enzyme deficiency)

SUMMARY OF REGULATION OF GLYCOGEN DEGRADATION

1. Glucose-6-P and ATP (well-fed state) inhibit glycogen phosphorylase = synthesis of glycogen
2. In muscle contraction: release of Ca^{2+} → binding to calmodulin → activated Ca^{2+}-calmodulin complex → activates phosphorylase kinase → phosphorylation (ACTIVATION) of glycogen phosphorylase
3. low [ATP] in muscles → high [AMP] → activates glycogen phosphorylase
4. ↑ [glucagon] → activation of protein kinase A → activation of phosphorylase kinase → activation of glycogen phosphorylase

27 METABOLISM OF FRUCTOSE AND GALACTOSE. DEFECTS

⇒ **FRUCTOSE** – mainly ingested as SUCROSE (α-glucose —anomeric bond→ fructose)



METABOLIZED mostly IN LIVER, not subjected to insulin control

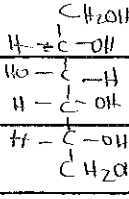
fructose —(ATP → ADP, fructokinase)→ fructose-1-P —(aldolase B)→ dihydroxyacetone-P + glyceraldehyde —(ATP → ADP)→ glyceraldehyde-3-P

Glycolysis intermediates

(IN THE INTESTINAL MUCOSA, MUSCLE, ADIPOSE TISSUE: fructose → fructose-6-P ⇒ GLYCOLYSIS straight away...)

Synthesis of fructose from glucose: POLYOL PATHWAY

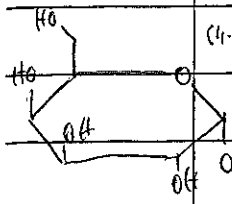
glucose —(NADPH+H⁺ → NADP⁺, aldose reductase)→ D-glucitol —(NAD⁺ → NADH+H⁺, alditol dehydrogenase)→ D-fructose



DEFECTS IN FRUCTOSE METABOLISM

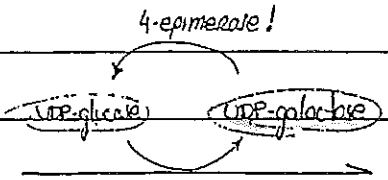
- ESSENTIAL FRUCTOSURIA – lack of fructokinase – high [fructose] in blood
- HEREDITARY FRUCTOSE INTOLERANCE – low activity of aldolase B
  fructose-1-P accumulates in liver → removal of inorganic (P) from cytosol
  oxidative phosphorylation inhibited!
  hypoglycaemia

GALACTOSE → occurs as lactose (β-galactose $\xrightarrow{1\text{-}4\text{-}\kappa\text{-}gly.}$ κ-glucose)



(4-epimer of glucose)

METABOLISM IN LIVER to glucose:



galactose $\xrightarrow[\text{galactokinase}]{\text{ATP ADP}}$ galactose-1-P → glucose-1-P

gal-1-P uridyltransferase

DEFECTS IN GALACTOSE CATABOLISM

- GALACTOSAEMIA — hereditary deficiency of galactokinase or Gal-1-P uridyltransferase
- LACTOSE INTOLERANCE — deficiency in lactase in intestinal mucosa (normal to about 5-10% when reaching adulthood)

PENTOSE - PHOSPHATE PATHWAY (phosphogluconate pathway)

↳ one of secondary pathways of glucose catabolism

2 purposes:

- production of NADPH (as carriers of E in form of REDUCING POWER in reactions of synthesis and hydroxylations catalyzed by monooxygenases)
- " " Ribose-5-P ⟹ biosynthesis of nucleic acids!

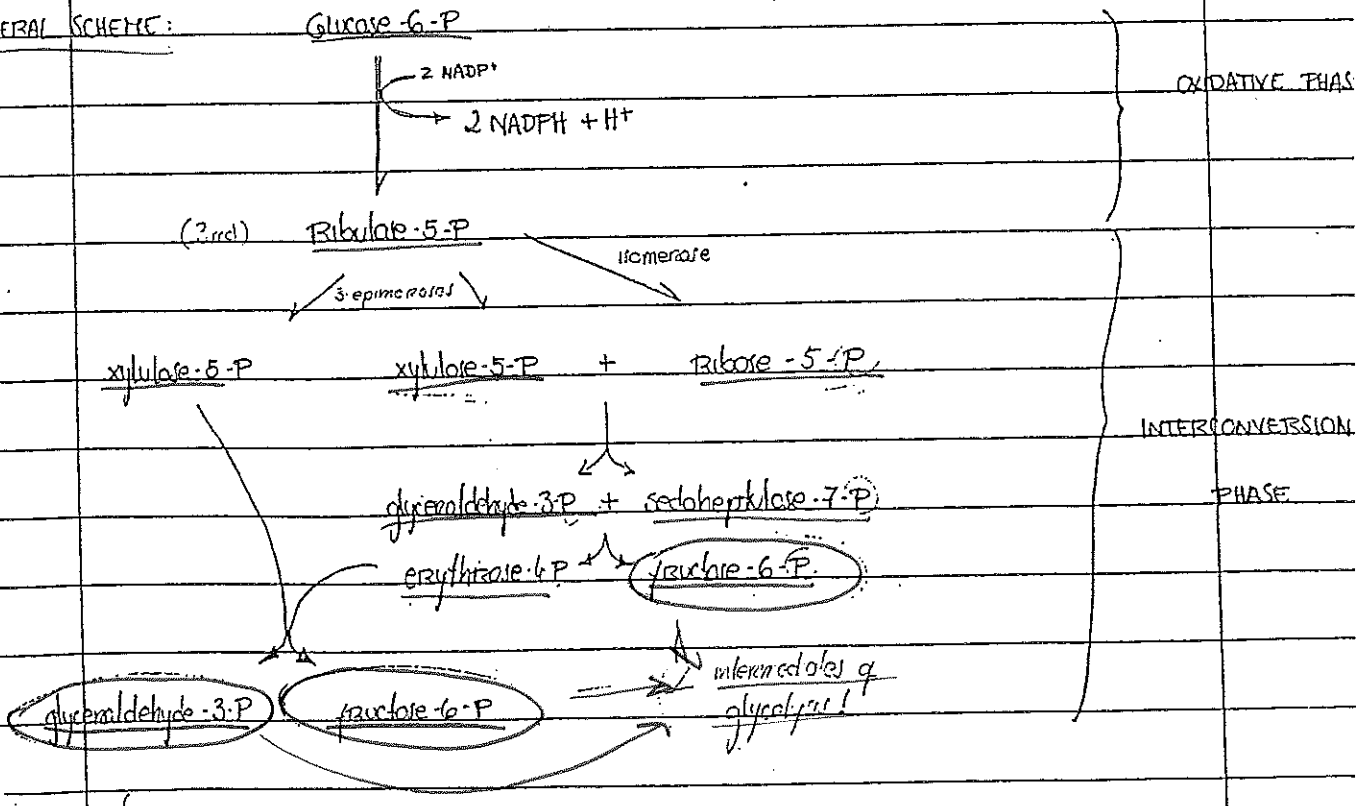
⇒ it does NOT SERVE to generate ATP !

LOCATION → (in cytosol) of:

highly active in:
liver, adipose tissue, mammary gland, adrenal cortex
less active in:
- skeletal muscle

- "F" pathway → fat cells
- "I" pathway → liver (or other) cells

GENERAL SCHEME:

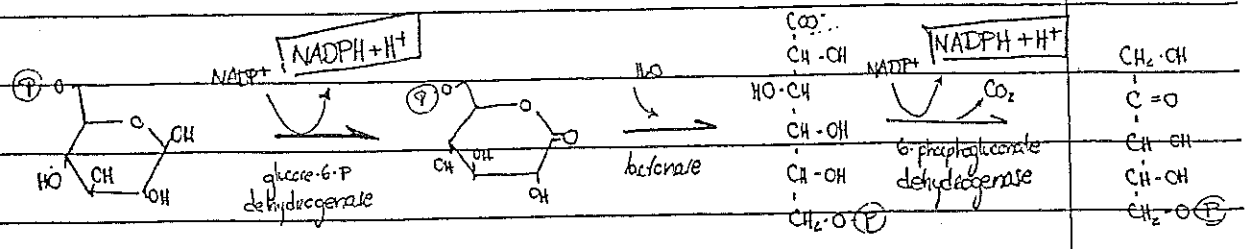


SUMMARY

- 3 GLUCOSE-6-P + 6 NADP+ ⟶ 6 NADPH+H+ + 2 FRUCTOSE-6-P + GLYCERALDEHYDE-3-P + 3CO
- 3 GLUCOSE-6-P + 6 NADP+ ⟶ 6 NADPH+H+ + 3 RIBOSE-5-P + 3CO

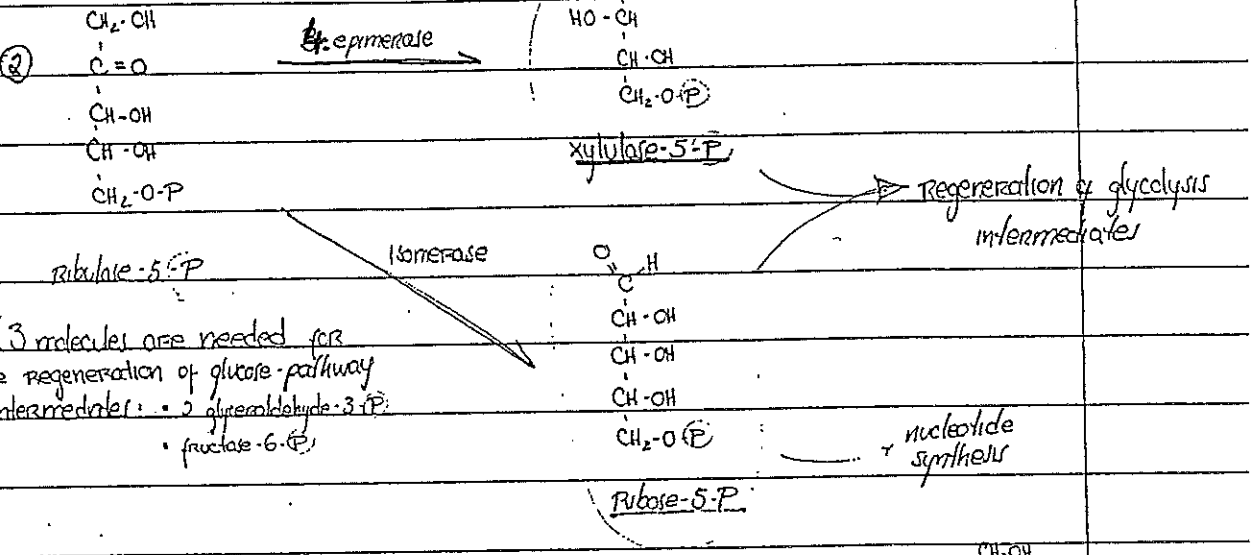
PENTOSE PHOSPHATE CYCLE

OXIDATIVE PHASE (IRREVERSIBLE)



glucose-6-P → glucono-6-P-lactone → 6-phosphogluconate → Ribulose-5-P

INTERCONVERSION PHASE (Reversible)



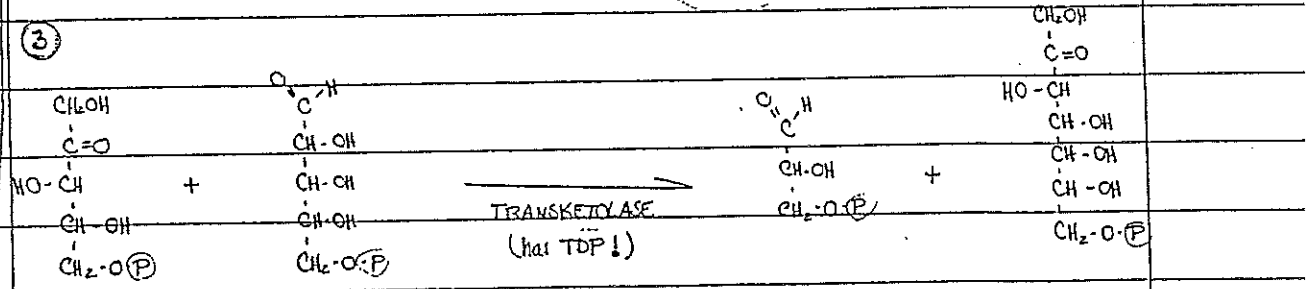
Ribulose-5-P → Xylulose-5-P

Ribulose-5-P → Ribose-5-P → nucleotide synthesis

(3 molecules are needed for the regeneration of glucose-pathway intermediates: • 2 glyceraldehyde-3-P • fructose-6-P)

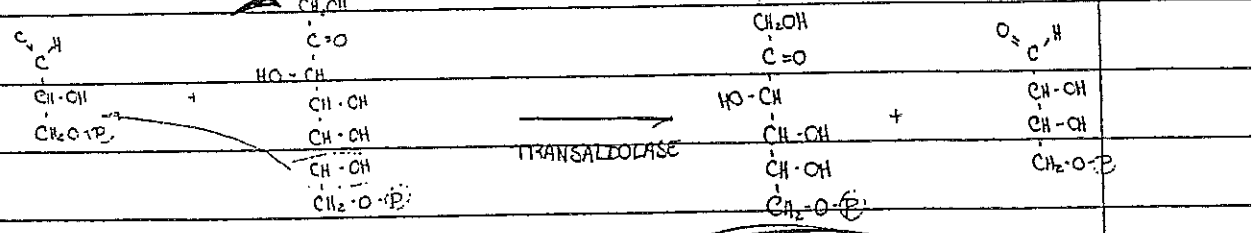
Xylulose-5-P → Regeneration of glycolysis intermediates

③



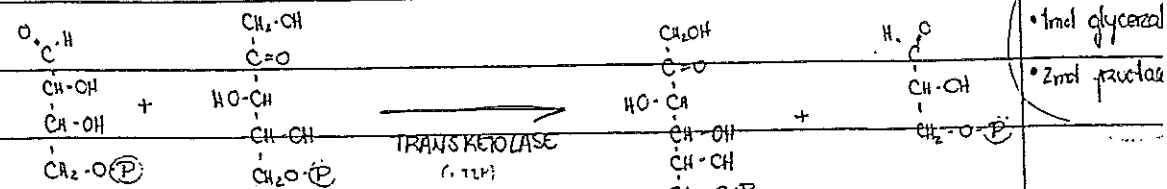
xylulose-5-P + Ribose-5-P → glyceraldehyde-3-P + sedoheptulose-7-P

④



glyceraldehyde-3-P + sedoheptulose-7-P → fructose-6-P + erythrose-4-P

⑤



erythrose-4-P + xylulose-5-P → fructose-6-P + glyceraldehyde-3-P

• 1mol glyceral
• 2mol fructo

29 The origin of Reactive Oxygen Species (ROS), oxygen radicals detoxification (enzymes and natural antioxidants)

O_2 constantly gives rise to small quantities of toxic substances: Reactive Oxygen Species (ROS), powerful oxidation agents or extremely reactive free radicals which damage cellular structures and functional molecules.

(due to their role in O_2 transport, erythrocytes are particularly at risk from ROS)

ORIGIN OF ROS → from the partial reduction of molecular oxygen: (ver oneiical chemistry)

$O_2 \xrightarrow{e^-} O_2^{-\bullet} \xrightarrow{e^-} H_2O_2 \xrightarrow{e^-} OH^{\bullet} \xrightarrow{e^-} H_2O$

SUPEROXIDE — HYDROGEN PEROXIDE — HYDROXYL RADICAL ⇒ Reactive oxygen species

These compounds are formed continuously as by-products of aerobic metabolism, through reactions with drugs and environmental metabolism or when the level of antioxidants is low, ALL CREATING THE CONDITION OF OXIDATIVE STRESS

The ROS can cause serious chemical damage to DNA, proteins and unsaturated lipids, leading to cell death.

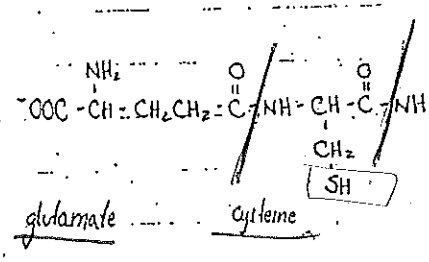
The cell has protective mechanisms from these compounds: enzymes and antioxidants

- ENZYMES that catalyze antioxidant reactions → reduced GLUTATHIONE can detoxify hydrogen peroxide, in a reaction catalyzed by glutathione peroxidase. It gives oxidized glutathion which no longer has protective properties, so the cell will have to regenerate it in a reaction catalyzed by glutathione reductase, using (NADPH) as cofactor. Thus, NADPH indirectly provides e^- for the reduction of hydrogen peroxide.

- ANTIOXIDANT CHEMICALS → intracellular reducing agents such as ascorbate, vit. E and β-caro: (vit C) (Beta-carotene?)
  These are able to reduce and, thus, detoxify oxygen intermediates.
  Consumption of food rich in these antioxidants is correlated with a decreased number of health problems.

30 Glutathione - structure, function (reducing effect, conjugation with GSH)

GLUTATHIONE (γ-glutamyl-cysteinyl-glycine)



(Reduced form).

FUNCTIONS:

1. Conjugation with lipophilic compounds (detoxyfication of reactive electrophiles)

2. Transport of aa into cells with concomitant attachment of γ-glutamyl group (through group translocation, γ-glutamyl cycle)

3. Reduced GSH confronts oxidative stress:

- reduces methaemoglobin to Hb and disulfides to thiols: R-SS-R + 2 GSH ⟶ 2 R-SH + GSSG
- reduces peroxides in the reaction:

2 GSH (reduced) + HOOH —(GLUTATHIONE PEROXIDASE)→ GSSG + 2H2O

↓ oxidized form = no protective properties

THE CELL REGENERATES IT:

Comes from glucose 6-P generated in pentose phosphate pathway

GSSG —(NADPH+H+ → NADP+; GLUTATHIONE REDUCTASE)→ 2GSH

NOTE: In erythrocytes NADPH is needed from the pentose-P cycle. If glucose-6-P DH is compromised in some way, NADPH levels will fall and oxidized glutathione won't be reduced

→ As a result, H peroxide will accumulate, threatening membrane stability and causing red cell lysis.

→ On the other hand, Glucose 6-P deficiency protect against falciparum malaria (The parasite needs reduced glutathione and the products of pentose phosphate cycle for optimal growth)

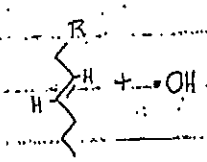
31 Lipid peroxidation. Tocopherols and other lipophilic antioxidants.

LIPID PEROXIDATION = oxidative degradation of lipids = the process in which free radicals take e⁻ from the lipids in cell membranes → cell damage.

- It is a chain reaction mechanism
- often affects PUFA (polyunsaturated fatty acids) because they contain multiple double bonds and also methylene $-CH_2-$ groups, with especially reactive hydrogens.
- MAY CAUSE CANCER, INFLAMMATORY DISEASES, ATHEROSCLEROSIS AND AGING

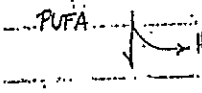
3 steps:

1. **INITIATION** = initiators react with PUFA and a fatty acid radical is produce (Reactive Oxygen Species)

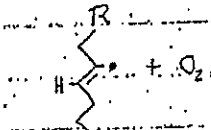


Initiators are mostly ROS such as •OH

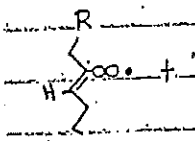
($X\bullet + RH \longrightarrow R\bullet + XH$)



2. **PROPAGATION** = as the fatty acid radical is very unstable, it reacts readily with oxygen, creating a peroxyl-fatty acid radical



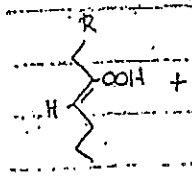
This is also an unstable species that reacts with another free fatty acid, producing a different fatty acid radical and an hydrogen peroxide!



This process continues in a cycle

($R\bullet + O_2 \longrightarrow ROO\bullet$)

($ROO\bullet + RH \longrightarrow ROOH + R\bullet$)



3. **TERMINATION** = The radical reaction stops when 2 radicals react and produce a non-radical species

3 possibilities:

- $ROO\bullet + ROO\bullet \longrightarrow ROOR + O_2$
- $ROO\bullet + R\bullet \longrightarrow ROOR$
- $R\bullet + R\bullet \longrightarrow RR$

To control and reduce lipid peroxidation ⟹ ANTIOXIDANTS

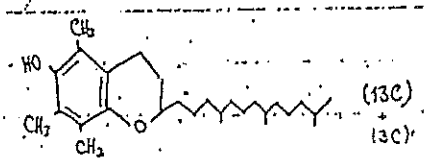
FOOD ADDITIVES
- Butylated Hydroxyanisole (BHA)
- Butylated Hydroxytoluene (BHT)

NATURAL-OCCURRING ANTIOXIDANTS
- vit. E – tocopherols – lipid-soluble → trap ROO· radicals!
- urate (water-soluble)
- vit. C (water-soluble)

(β-carotene is an antioxidant at a low pO_2)

2 types of antioxidants
- PREVENTIVE – reduce the rate of chain initiation
  - eg: catalase and other peroxidases, and chelators such as EDTA
- CHAIN-BREAKING – interfere with chain propagation
  - eg: superoxide dismutase, urate and vit. E, which acts in the lipid phase to trap ROO· radicals

OTHER LIPOPHILIC ANTIOXIDANTS

α-tocopherol ⟹ 

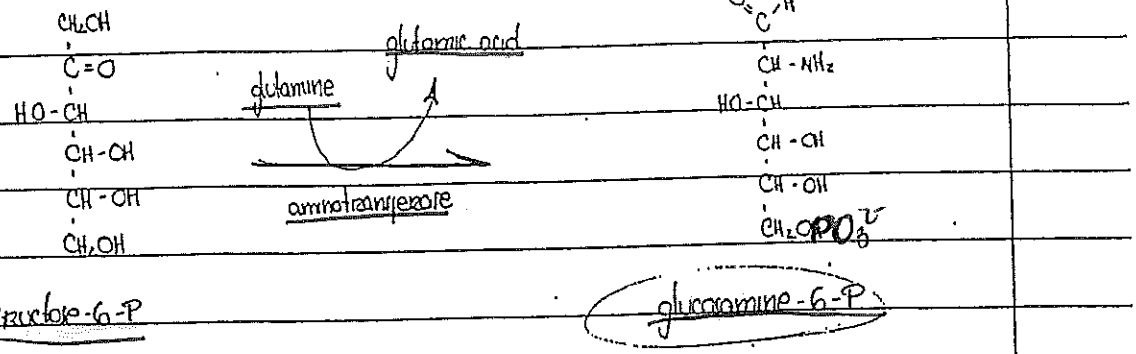
(derivative of chromah).

~~Synthesis and metabolism of glucuronic acid (uronic acid pathway) - Synthesis of amino sugars and sialic acids, significance for the synthesis of glycoproteins and proteoglycans~~

Synthesis of amino sugars and sialic acids, significance for the synthesis of glycoproteins and proteoglycans. Synthesis and metabolism of glucuronic acid (the uronic acid pathway).

SYNTHESIS OF AMINO SUGARS

→ They contain an amine group instead of a hydroxyl group.

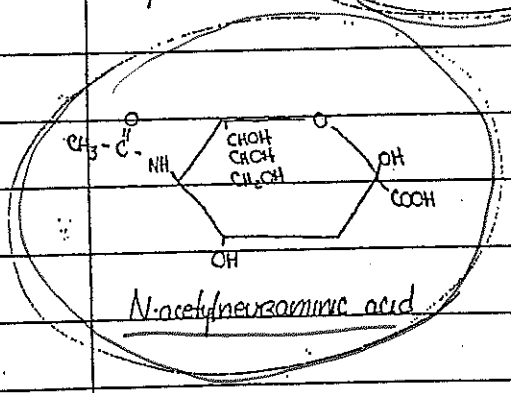


fructose-6-P

glucosamine-6-P

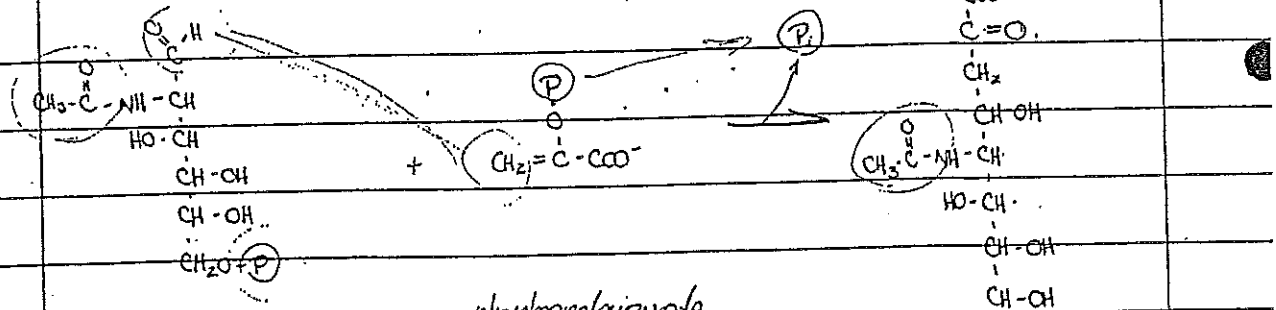
(most of times $-NH_2$ group reacts with acetylCoA to give N-acetylhexosamines)

SYNTHESIS OF SIALIC ACIDS — acetylated derivatives of N-acetylneuraminic acid



N-acetylneuraminic acid

formation:



N-acetylmannosamine-6-P

phosphoenolpyruvate

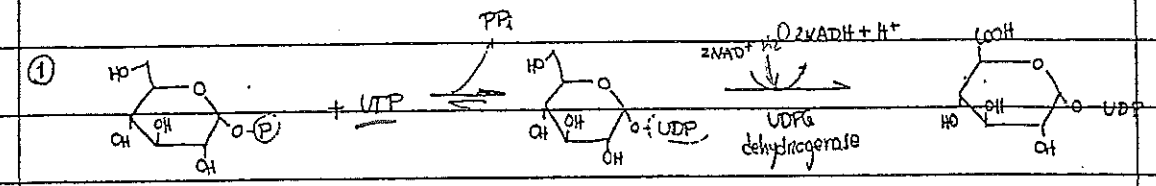
N-acetylneuraminic acid! (9-P)

URONIC PATHWAY — alternative oxidative pathway for glucose

↳ supplies glucuronic acid (and in most animals ascorbic acid)

↓

finally metabolized to pentoses, intermediates of glycolysis

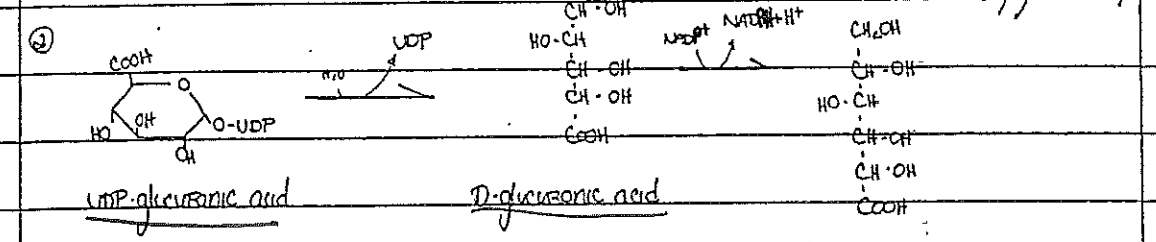


glucose-1-(P)

UDP-glucuronic acid

→ synthesis of glycosaminoglycans

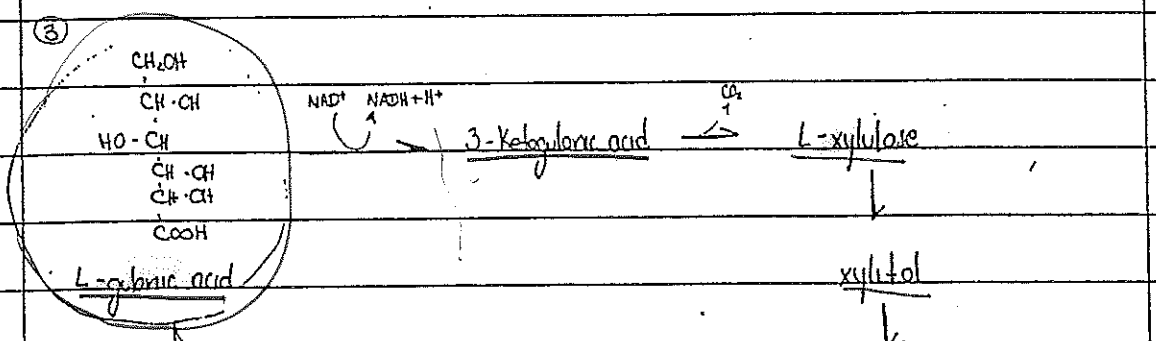
→ conjugation w/ xenobiotics



UDP-glucuronic acid

D-glucuronic acid

L-gulonic acid



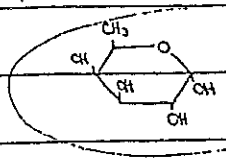
goes to pentose-P pathway, where it is converted to intermediates of glycolysis

URONIC ACIDS,

AMINOSUGARS and SIALIC ACIDS are important for synthesis of proteoglycans and glycoproteins

Saccharides found in glycoproteins glycolipids

- URONIC ACIDS: glucuronic acid ; galacturonic acid
- ACETYL HEXOSAMINES: N-acetylglucosamine ; N-acetylgalactosamine (GlcNAc ; GalNAc)
- SIALIC ACIDS: N-acetylneuraminic acid (Neu5Ac)
- Hexoses: glucose, galactose, mannose
- Deoxyhexoses: L-fucose (Fuc)
- Pentoses: xylose, arabinose



D-fucose

example: blood group A ⇒

Fuc — GalNAc
|
Gal
|
GlcNAc
|
Gal
|
ceramide/protein (sphingolipid)

Synthesis of glycoproteins

⇒ before being incorporated into the oligosaccharide chains, monosaccharides are activated by formation of nucleotide sugars (similarly to formation of UDP-glucose)

Glucose-6-P ⇌ glucose-1-P $\xrightarrow{UTP}$ UDP-glucose ⟶ UDP-galactose

UDP-glucuronic acid ⟶ UDP-xylose

Fructose-6-P ⇌ mannose-6-P ⇌ mannose-1-P $\xrightarrow{GTP}$ GDP-mannose ⟶ GDP-fucose

N-acetylglucosamine-6-P ⇌ N-acetylglucosamine-1-P $\xrightarrow{UTP}$ UDP-N-acetylglucosamine

↳ UDP-N-acetylgalactosamine

UDP-N-acetylmannosamine

CMP-N-acetylneuraminic acid $\xleftarrow{CTP}$ N-acetylneuraminic acid

33 Natural occurring tensides (structural types, micelles, biomembranes, tensides in lipid digestion)

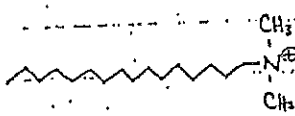
SURFACTANTS / SOAPS / TENSIDES = are agents that lower the surface tension of a liquid, allowing easier spreading and lower the interfacial tension between 2 liquids.

are amphiphilic compounds — they contain both hydrophobic groups (their "tails") and hydrophilic groups (their "heads") thus they are soluble in both organic solvents and water.

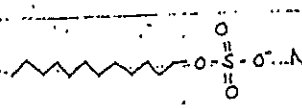
TYPES OF TENSIDES
- cationic
- anionic (see medical chemistry book)
- non-ionic
- zwitterionic (dual charge) (bipolar)

EXAMPLES:

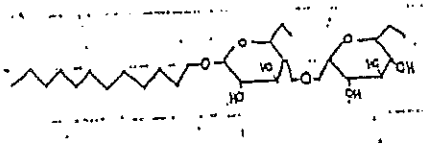
- CATIONIC = cetyl trimethylammonium bromide (CTAB) (16C)



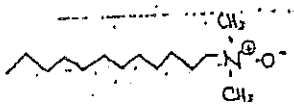
- ANIONIC = sodium dodecyl sulfate (SDS) (12C)



- NON-IONIC = β-D-dodecylmaltoside (lauroylmaltoside) (12C)



- ZWITTERIONIC = lauryldimethylamine oxide (LDAO) (dodecylamine N-oxide) (12C)



→ Surfactants find numerous applications in the chemical process industries such as foods, pharmaceuticals, household products, agricultural chemicals and mineral processing industries.

→ Natural occurring tensides in plants, animals and humans have important biological roles on physiological functions.

The most common biological example of surfactant is that coating the surfaces of the alveoli, small sacs in the lungs for gas exchange.

↳ helps in keeping tissues inside/ing collapse at expiration

BIOMEMBRANES

↳ A bilayer of amphipathic lipids has been regarded as the basic structure in biological membranes: The hydrophilic heads faced outside and the lipophilic tails inside:

MICELLES

↳ When a critical CXT of these lipids is present in aqueous medium they form micelles; the concentration at which surfactants begin to form micelles is the critical micelle concentration, c

When micelles form in water, their tails form a core that can encapsulate an oil droplet and their polar heads form an outer shell that contacts with water.

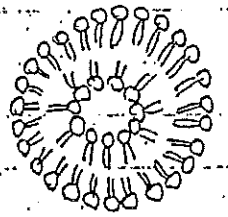
When surfactants assemble in oil they form reverse micelles (the heads in the core and the tails on the outer shell)


micelle


reverse micelle

LIPID DIGESTION

↳ Aggregations of bile salts into micelles and liposomes and the formation of mixed micelles with the products of fats digestion ARE IMPORTANT IN FACILITATING ABSORPTION OF LIPIDS FROM THE INTESTINE!


→ liposom

Liposomes are spheres of lipid bilayers that enclose part of the aq. medium They are used, combined to antibodies, as carrie of drugs into circulation, targeting to specific

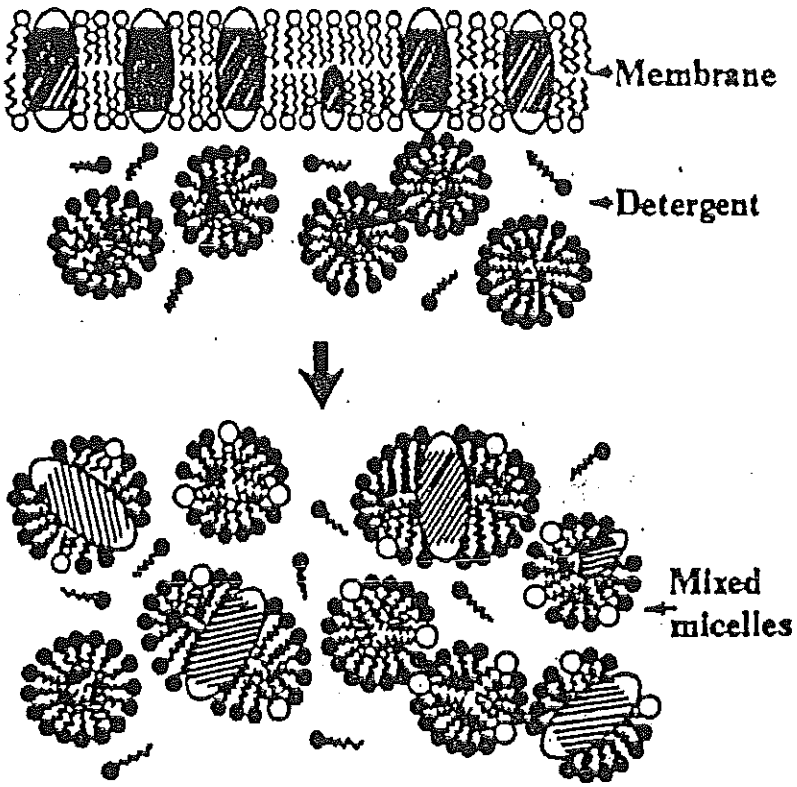


Fig. 5-1. A simple and schematic view of membrane solubilization by detergents.

CMC Critical micellar concentration

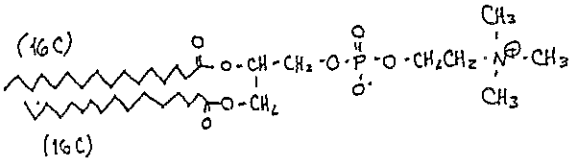
High CMC detergent forms small micelles and is easy to be removed by dialysis.

Low CMC detergent forms large micelles and is difficult to be removed by dialysis.

Detergents are classified as:

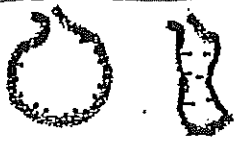
- Ionic detergents
  - Anionic
  - Cationic
  - Zwitterionic--Zwittergent
- Nonionic (uncharged) detergents

— dipalmitoyl-phosphatidylcholine



Lung surfactant

The major component of lung surfactant is dipalmitoylphosphatidylcholine. It contributes to a reduction in the surface tension within the alveoli (air spaces) of the lung, preventing their collapse in expiration. Less pressure is needed to re-inflate lung alveoli when surfactant is present.



The respiratory distress syndrome (RDS) of premature infants is caused, at least in part, by a deficiency in the synthesis of lung surfactant.

16

34. Biosynthesis of fatty acids; control mechanisms. Biosynthesis of triacylglycerols.

FATTY ACID SYNTHESIS ⇒ sequential process - by addition of 2-C units from acetyl-CoA

↳ IN CYTOSOL OF LIVER, ADIPOSE TISSUE AND MAMMARY GLANDS

important molecules:
- (ACP) (acyl carrier protein) - have phosphopantetheine that binds intermediates to -SH group
- (malonyl-CoA) - donnor of 2-C units
- (NADPH) - reductant in f.a. synthesis (NAD+, FAD: oxidant in f.a. degradation)

FAVOURABLE CONDITIONS TO FATTY ACIDS SYNTHESIS:
- fed state - high [glucose] and [acetyl-CoA]
- high [ATP] in cells

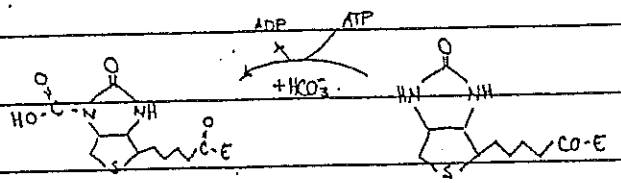
F.A. SYNTHESIS (2 STAGES):
1. SYNTHESIS OF MALONYL-CoA
2. SEQUENCE REACTIONS CATALYZED BY F.A SYNTHASE COMPLEX

→ (BUT FIRST OF ALL acetyl-CoA has to be transported to cytosol - check South next page

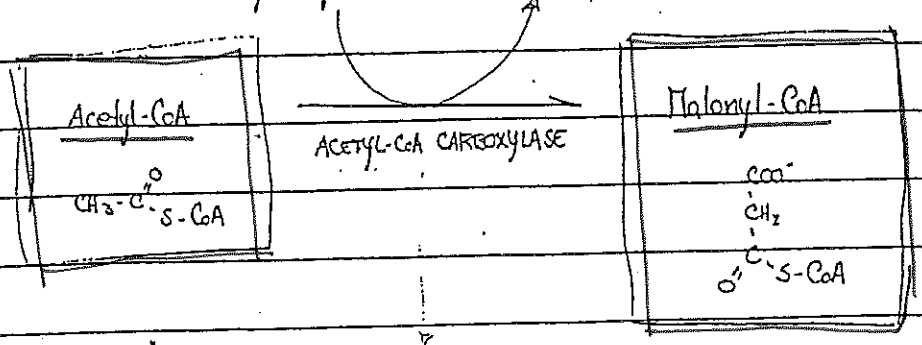
Citrate + ATP + CoA-SH + H_2O —(citrate lyase)→ acetyl CoA + ADP + Pi + OAA

(81)

1. SYNTHESIS OF MALONYL-CoA



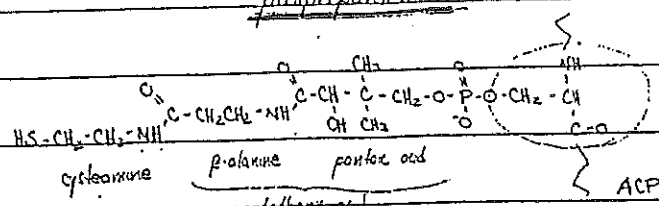
Carboxybiotinyl-E → biotinyl-E



ACTIVATED BY: citrate and dephosphorylation

INACTIVATED BY: palmitoyl-CoA and phosphorylation

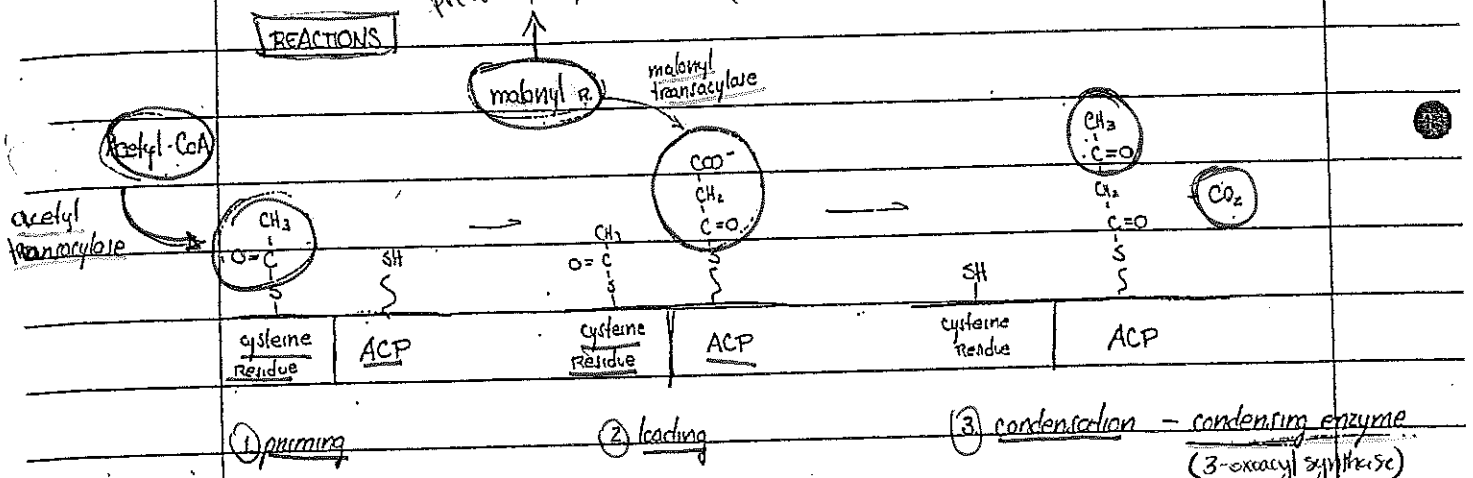
(2) FA SYNTHASE COMPLEX — homoDIMER: each monomer carries 7 enzymes and has one ACP (acyl carrier protein), to which phosphopantetheine arm is attached!



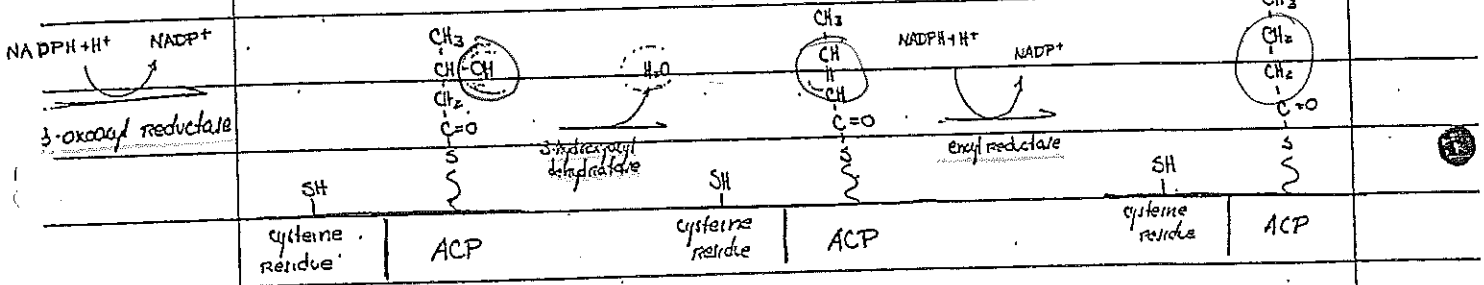
PANTETHEINE + SERINE residue

previously synthetized by acetyl CoA

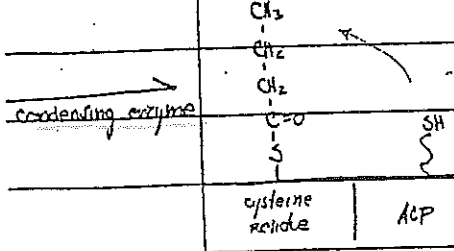
REACTIONS



(1) priming (2) loading (3) condensation – condensing enzyme (3-oxoacyl synthase)



(4) 1st Reduction (5) dehydration (6) 2nd reduction



(7) condensation

THIS IS ONLY ONE ELONGATING CYCLE

→ from here another one begins: acetyl binds to ACP and new malonyl binds to butyryl

⇒ the elongation cycles only stop when C_{16}-unit (palmitoyl) is formed

→ in mammals (16:0) PALMITATE is the major product of F.A. synthesis

→ a minor product is stearate (18:0)

⇒ CONTROL OF F.A. SYNTHESIS → regulation by REVERSIBLE PHOSPHORYLATION OF ACETYL-CoA CARBOXYLASE

this enzyme is phosphorylated by AMP-dependent protein kinase

it will be activated by dephosphorylation (stimulated by insulin)

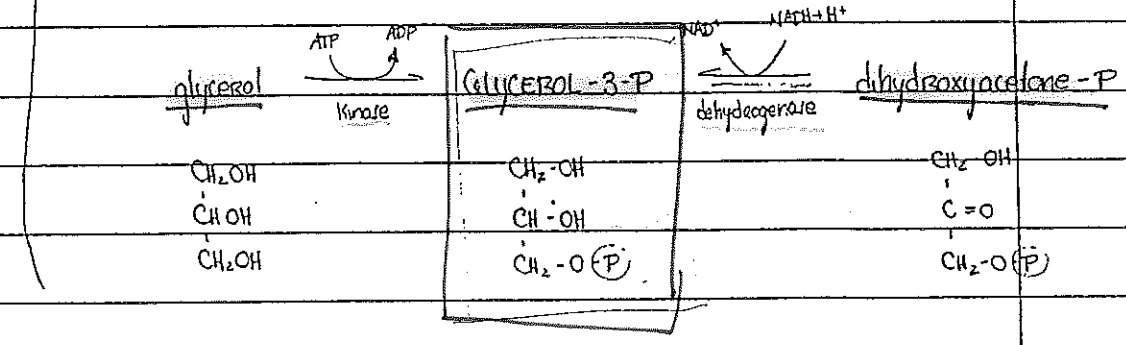
also activated by citrate

(palmitoyl-CoA inhibits it)

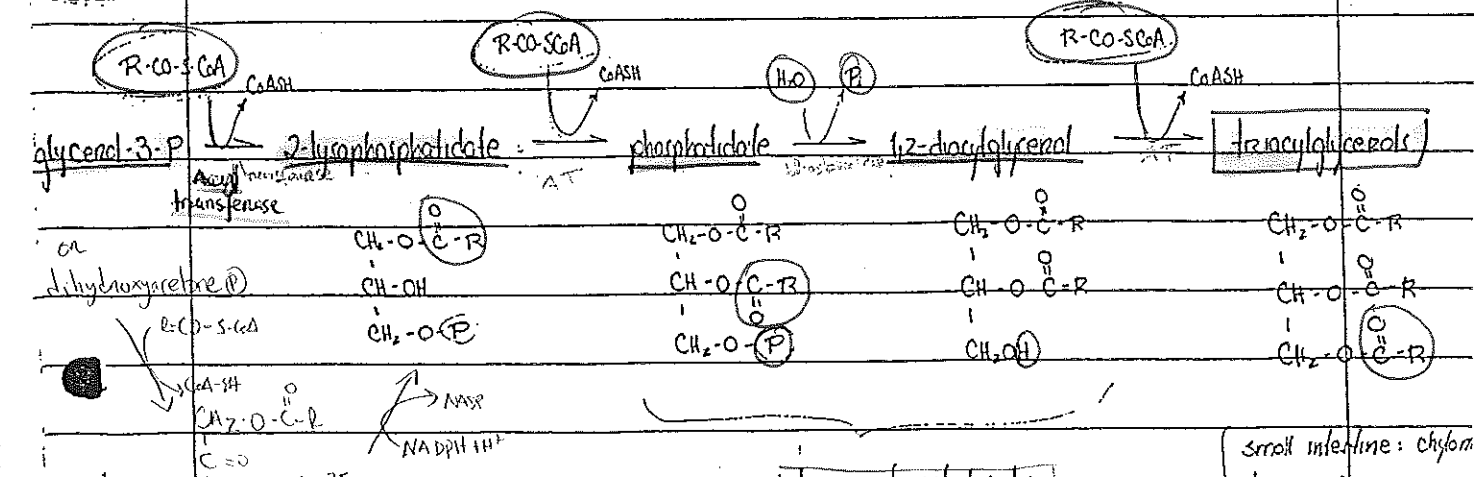
SYNTHESIS OF TRIACYLGLYCEROLS → esterification of glycerol-3-P by acyltransferases (activated y.c.)

Obtaining glycerol-3-P

- IN LIVER and SMALL INTESTINE → glycerol is phosphorylated by glycerol kinase
- IN OTHER TISSUES → Reduction of dihydroxyacetone by glycerol-P dehydrogenase



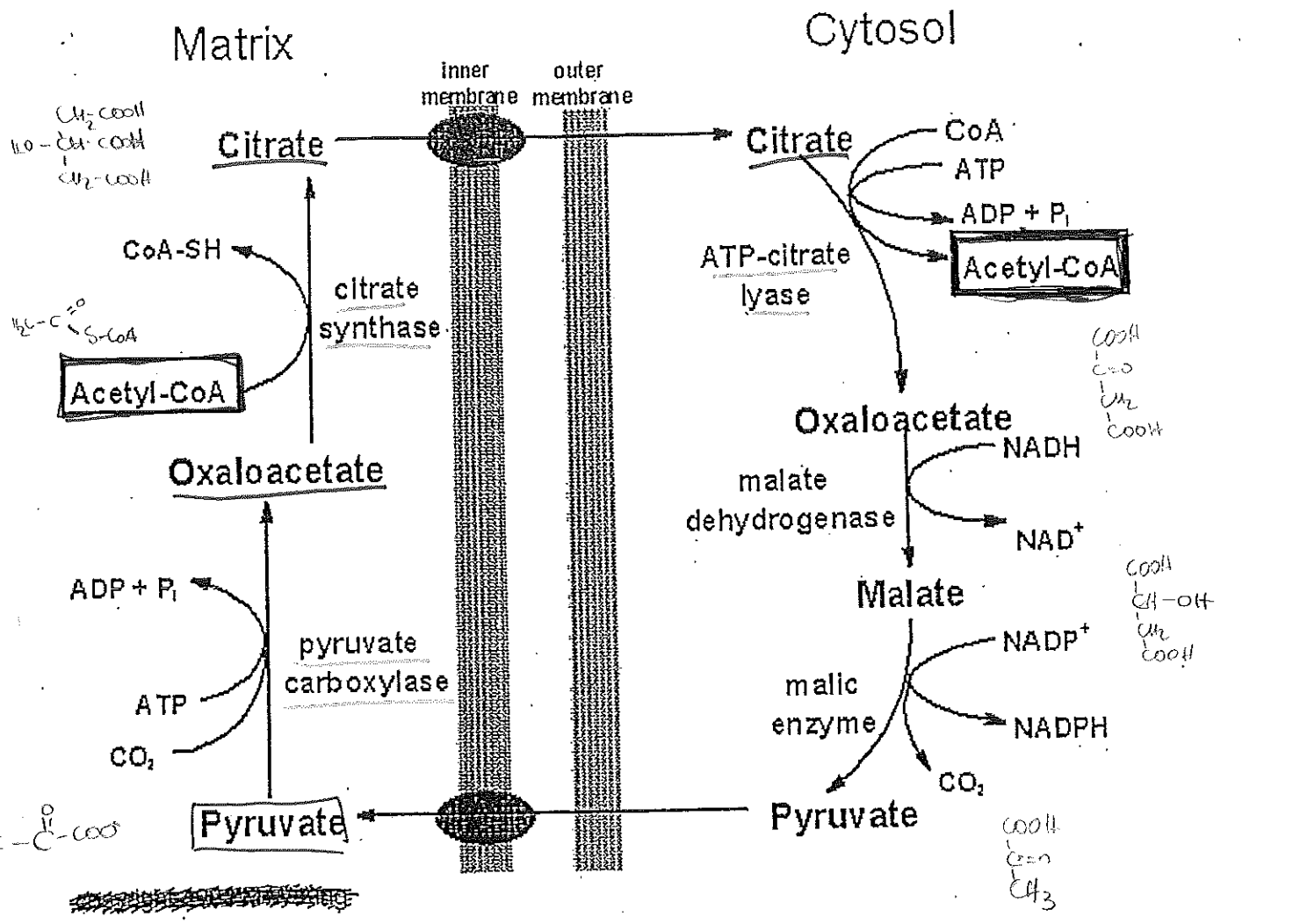
REACTIONS

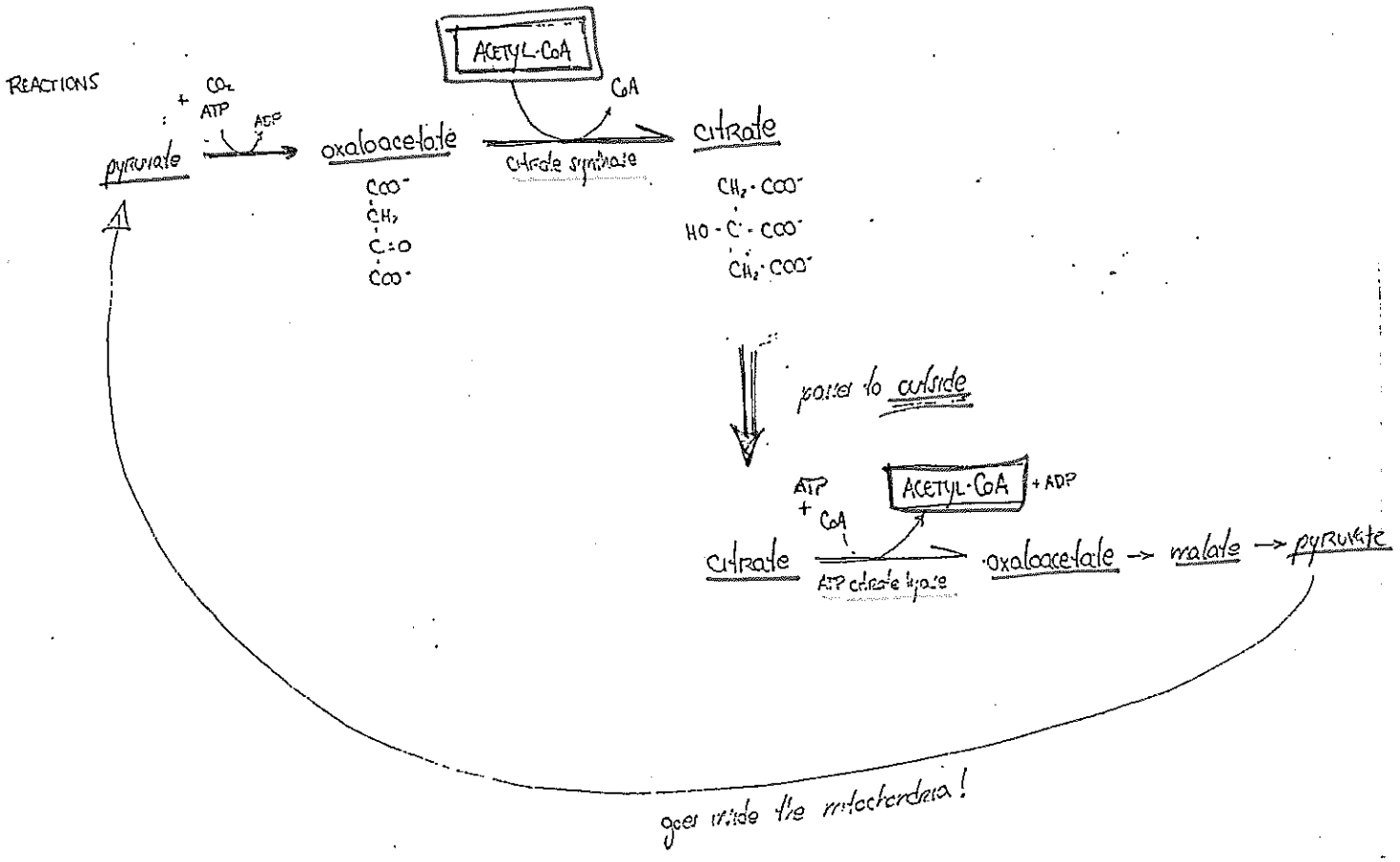


glycerophosphlipids

small intestine: chylom
liver cells: VLDL

TRANSPORT OF ACETYL-CoA TO THE CYTOSOL





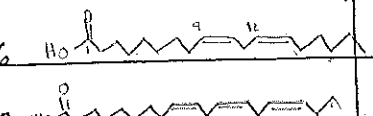
35 - Desaturation of fatty acids. Polyunsaturated f.a. (Sources and interconversions, significance)

DESATURATION OF FATTY ACIDS → occurs in ER membranes of liver cells

↳ involves 4 (in mammals) fatty acyl-CoA desaturases that insert double bonds in carbons 4, 5, 6 and 9 (f.a. containing double bonds further then C9 are synthesized by plants)

⇒ Polyunsaturated f.a. are essential for animals:

ESSENTIAL FATTY ACIDS:
- LINOLEATE - 18:2 (9,12) → n-6
- LINOLENATE - 18:3 (9,12,15) → n-3



SOURCES of PUFA (polyunsaturated fatty acids):
- n-6 series: all plant oils
- n-3 series: fish oils

ESSENTIAL n-6 and n-3 ⇒ precursors of eicosanoids (prostanoids and leukotrienes)

(n-6) Linoleate → arachidonate 20:4 (5,8,11,14)

(n-3) α-linolenate → eicosapentaenoate ~~18:5~~ ~~(5,8,11,14,17)~~ 20:5 (5,8,11,14,17)

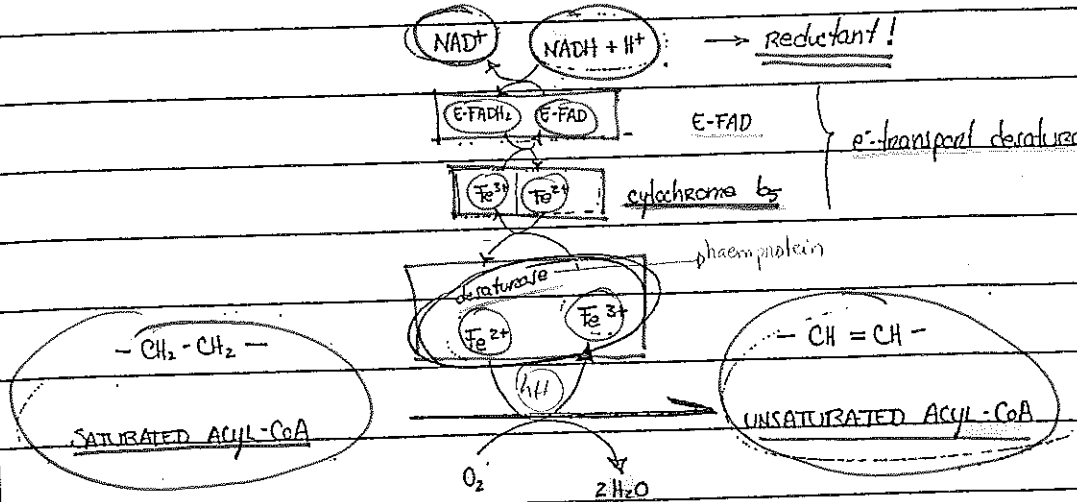
these interconversions result of many successive desaturations and elongations

MECHANISM OF DESATURATION

↳ DESATURASES are hydroxylating monooxygenases

↳ (NADH + H+) is the reductant!

↳ flavin-enzyme and cytochrome b_5 carry e⁻ to desaturases



ELONGATION → addition of 2 Carbons to the existing f.a. chain (previous of action)

36 Transport of long chain fatty acyl-CoA into mitochondria and the transport of acetyl-CoA into the cytosol (control mechanisms).

- LONG-CHAIN FATTY ACYL-CoA: cytosol → mitochondria → CARNITINE SHUTTLE (p.o. degradation)
- ACETYL-CoA: mitochondria → cytosol → CITRATE (p.o. synthesis)

→ TRANSPORT OF ACETYL-CoA INTO CYTOSOL = CITRATE

↳ CoA can't cross the membrane; only acetyl group is transported in the form of citrate

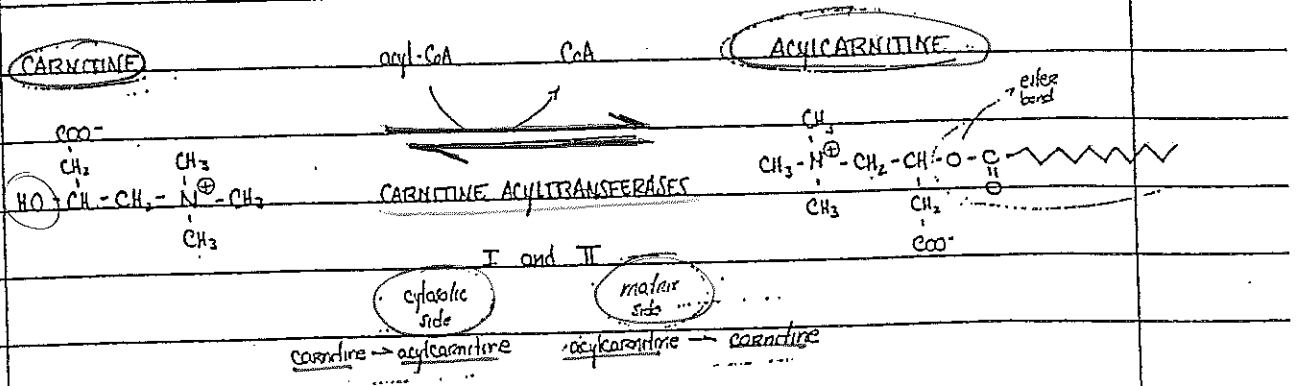
regulation by enzyme CITRATE LYASE, that catalyzes the reaction:

citrate + ATP + CoASH $\xrightarrow{+H_2O}$ acetyl-CoA + oxaloacetate + ADP + P_i

REGULATION: this pathway is enhaced when [citrate] is high, this occurres when isocitrate DH is inhibited by ATP ⇒ causing citrate and isocitrate to accumulate

→ TRANSPORT OF LONG-CHAIN ACYL-CoAs INTO MITOCHONDRIA = CARNITINE SHUTTLE

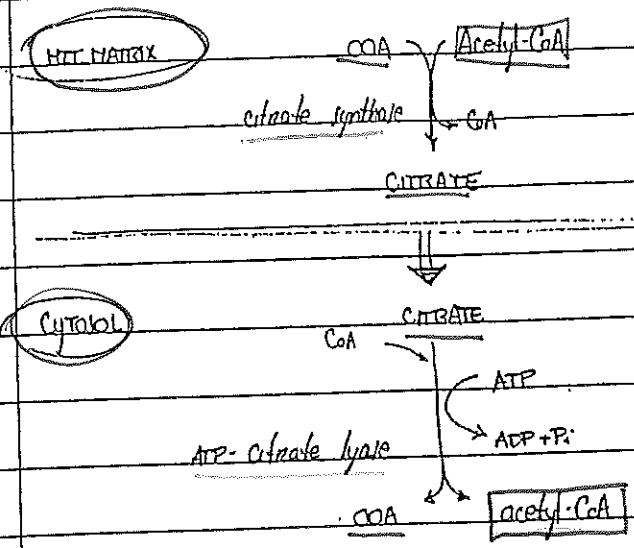
↳ CoA can't cross the membrane, acyl groups are transferred to carnitine → acylcarnitine (short-chain f.a. (4-10 C) don't need the carnitine shuttle)



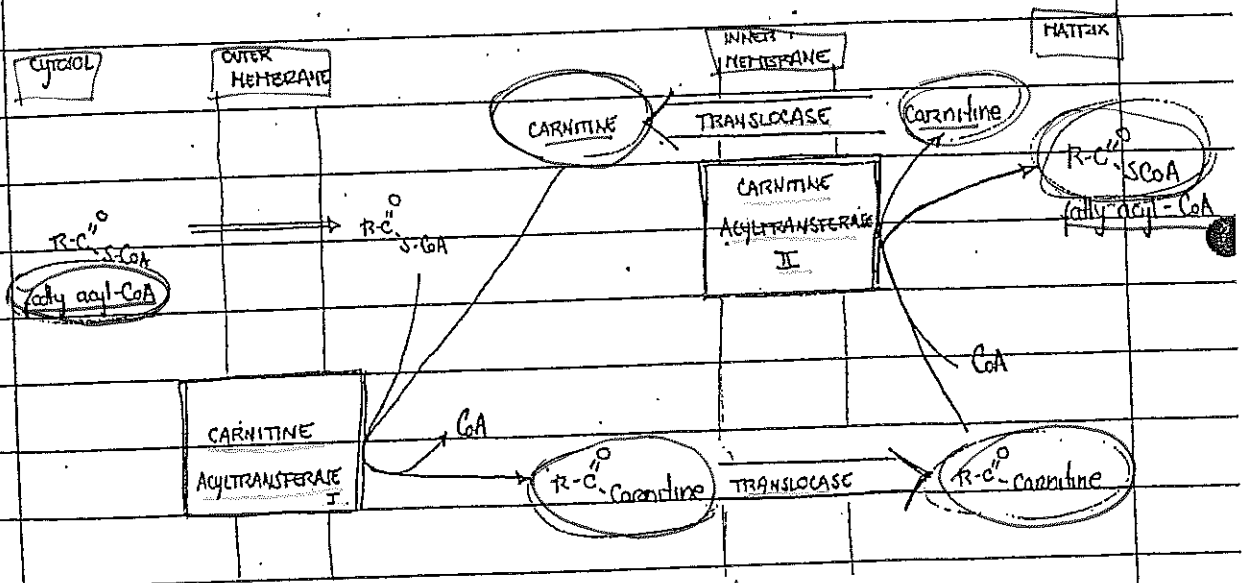
↳ enzyme carnitine-acylcarnitine translocase transports acylcarnitine into mitochondria

↳ CARNITINE ACYLTRANSFERASE I is allosterically inhibited by malonyl-CoA, intermediate of f.a. biosynthesis, in order to prevent futile

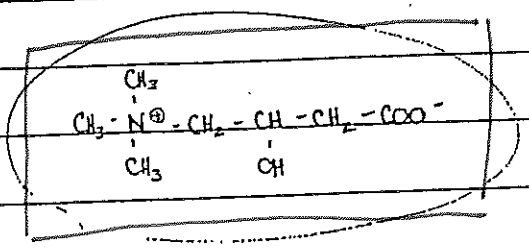
CITRATE SHUTTLE → from the mitochondrial matrix to cytosol:



CARNITINE SHUTTLE → from the cytosol into mitochondria

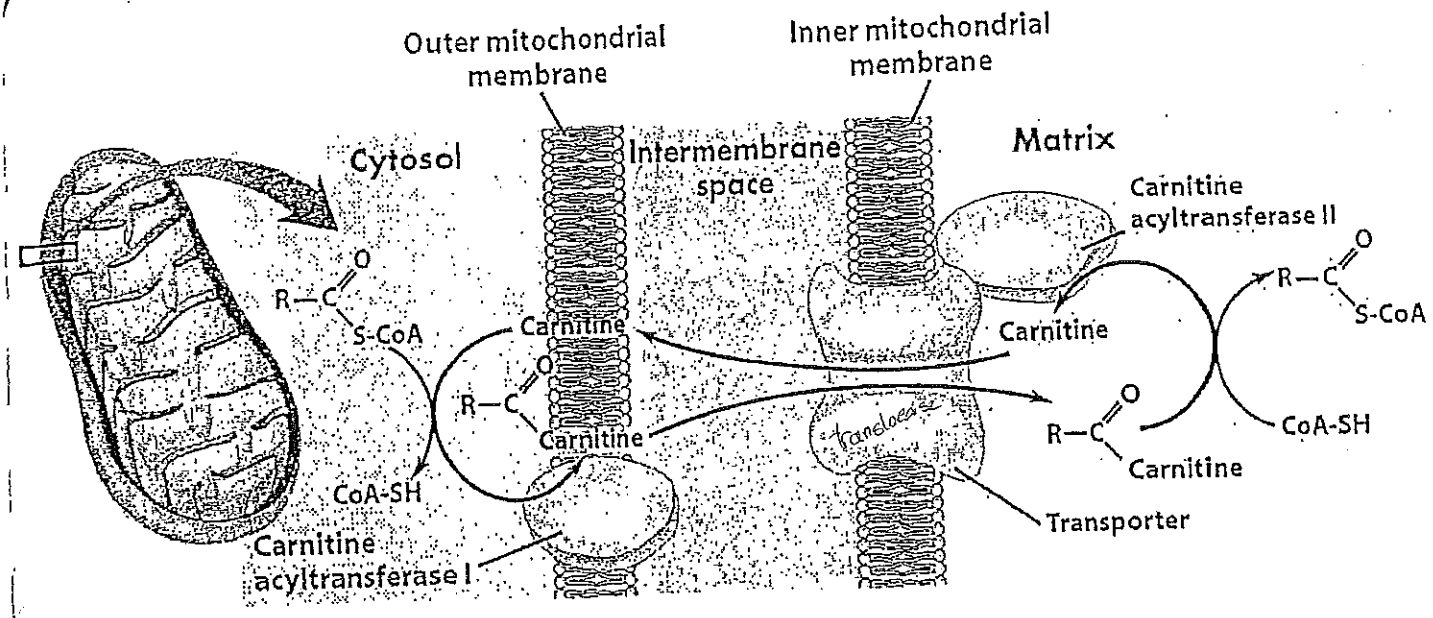


Carnitine →



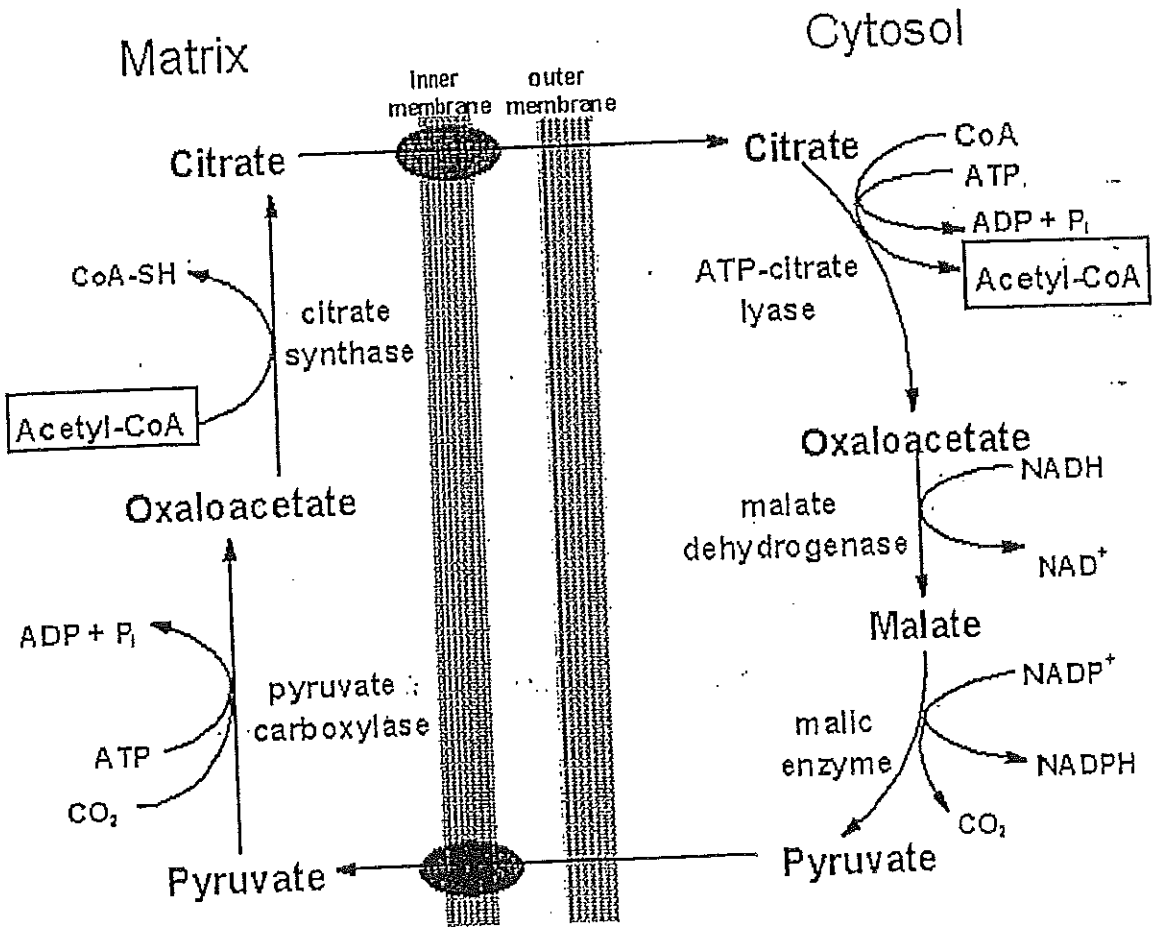
TRANSPORT CYTOSOL – MATRIX

CARNITINE



TRANSPORT MATRIX – CYTOSOL

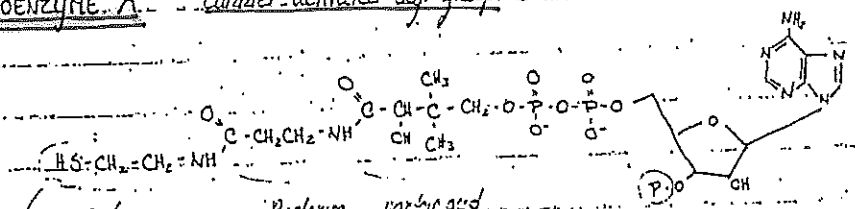
CITRATE



37. **Coenzymes of acyl transferases. Transfer of acyls (coenzyme A, phosphopantetheine; lipoamide, carnitine).**

Acyltransferases — enzymes that catalyze the transfer of an acyl group from a donor to an acceptor.

➔ **COENZYME A** = carries activated acyl groups:



cysteamine — β-alanine — pantoic acid

pantothenic acid

PANTETHEINE

the terminal sulfanyl group is the **reactive site** to which acyl groups will bind by **thioester bond**.

Eg: in the utilization of ketone bodies by extrahepatic tissues, acetoacetate is activated by transfer to CoA from succinyl-CoA, in a reaction catalyzed by succinyl-CoA transferase:

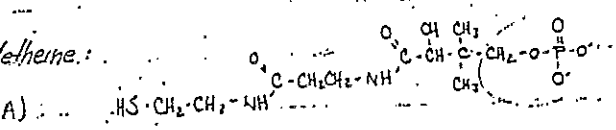
acetoacetate + succinyl-CoA ⇌ acetoacetyl-CoA + succinate

➔ **PHOSPHOPANTETHEINE** = is the prosthetic group of acyl carrier proteins (ACP) in some multienzyme complexes where it serves as a "swinging arm" for the attachment of activated fatty acid or aa groups.

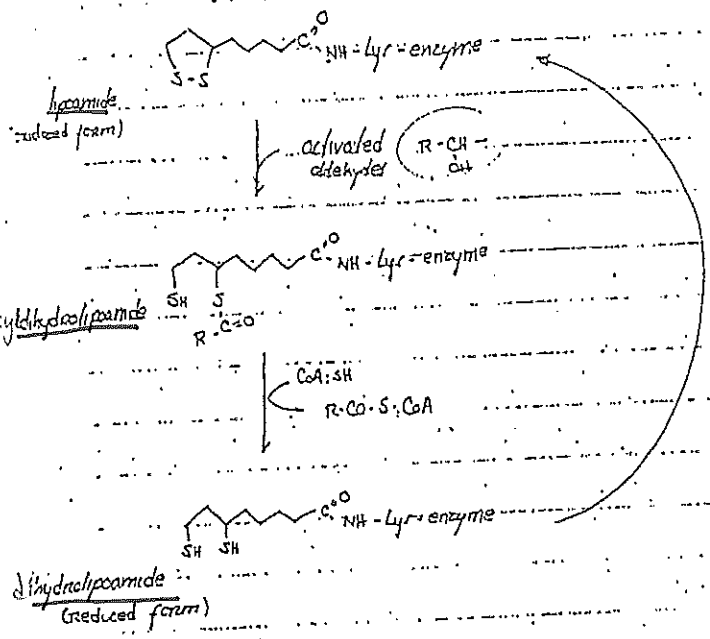
ACP = small proteins that carry the acyl intermediates bound as thioesters to the terminus of P-pantetheine

= important in biosynthesis of F.A. ⇒ the intermediates link covalently to the synthase while the flexibility and length of P-pantetheine arm allows them to have access to spatially distinct enzyme active sites. This increases the effective molarity of the intermediate and allows an assembly line like process.

(4)-phosphopantetheine: (derives from CoA):

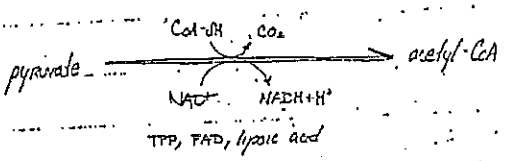


→ LIPOAMIDE = prosthetic group of oxidoreductases — cyclic disulfide attached to the transacylase subunit of the 2-oxoacid DH complex (ex. lipoamide)



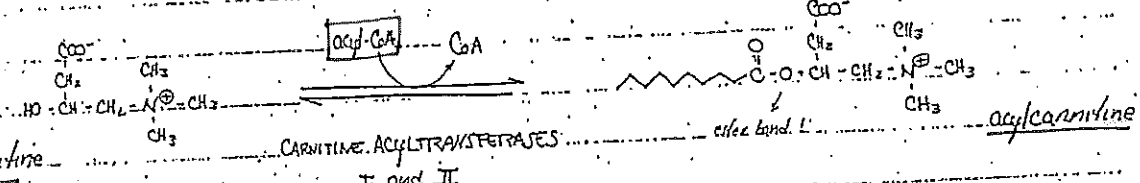
acts as an oxidant of activated aldehydes (carried by thiamine diphosphate), binds the resulting acyl as a thioester (acyldihydrolipoamide) and transfers the acyl onto coenzyme A

Review of the reaction catalyzed by pyruvate DH complex



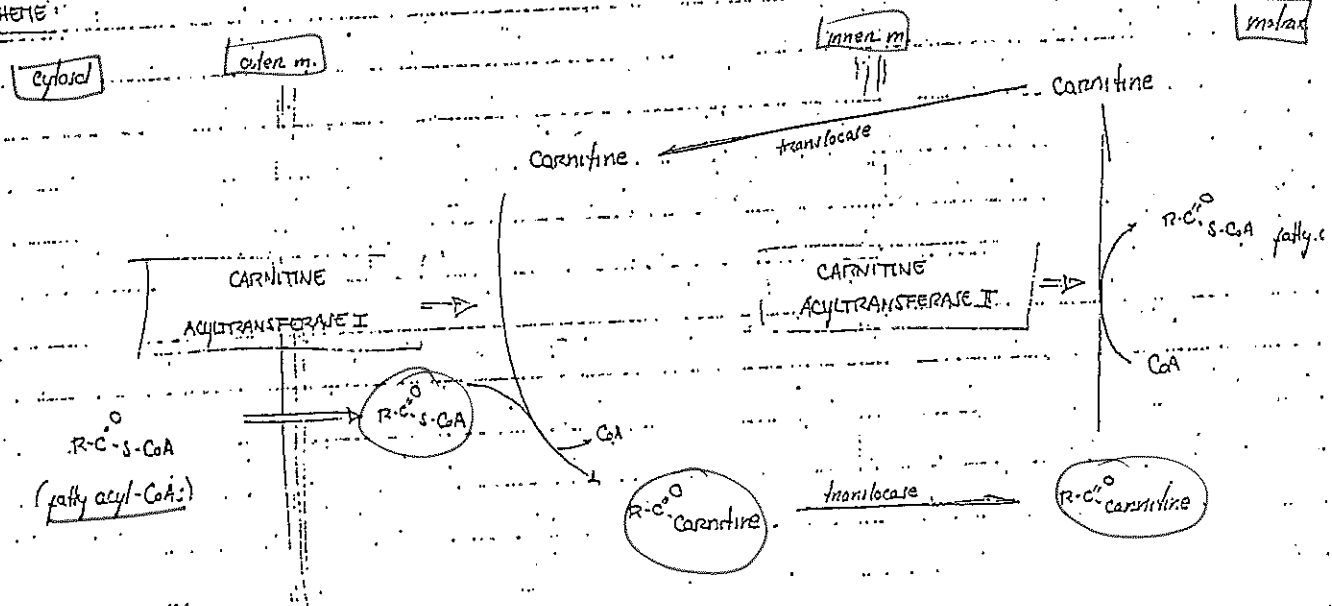
→ CARNITINE — ~~at Coenzyme A can't cross the mit membrane~~, the transport of long-chain acyls into mitochondria is made through carnitine (CARNITINE SHUTTLE) (question 34 ?)

(short f.a. : (4-10C) don't need carnitine).



carnitine → acylcarnitine — acylcarnitine → carnitine

SCHEME:



38 OXIDATIVE BREAKDOWN OF FATTY ACIDS (location, reaction sequence, energetic yield, control mechanisms)

DEGRADATION OF FATTY ACIDS

(β-OXIDATION PATHWAY)

1st STEP: ACTIVATION OF F.A → linking to CoA

2nd STEP: TRANSPORT OF CoA INTO MIT. MATRIX (conjugation w carnitine)

3rd STEP: β-OXIDATION OF ACYL-CoA → acetyl-CoA (citrate c)

- LOCATION OF FATTY ACIDS DEGRADATION/ => MITOCHONDRIA of most cells (except cns and erythrocytes)

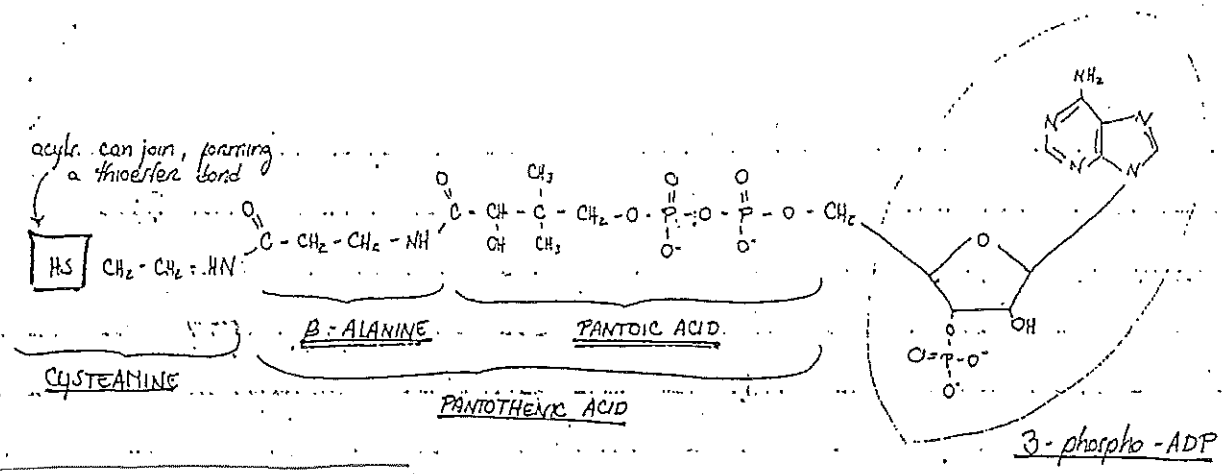
  (≠ synthesis → cytoplasm)

- COFACTORS OF F.A. DEGRADATION => FAD + NAD+

  (≠ synthesis → NADPH)

However, the essentials of the 2 processes are reversals of each other.

(both processes use acetyl-CoA, but the one in synthesis exists temporarily bound to the enzyme complex as malonyl-CoA)

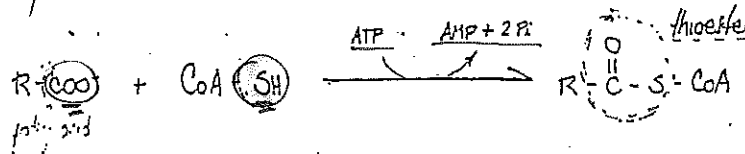


β-OXIDATION PATHWAY

1ST STEP — ACTIVATION OF FATTY ACIDS BY JOINING TO COENZYME-A

(Question 36)

↳ enzyme: acyl-CoA synthetase → located in the outer mitochondrial membrane



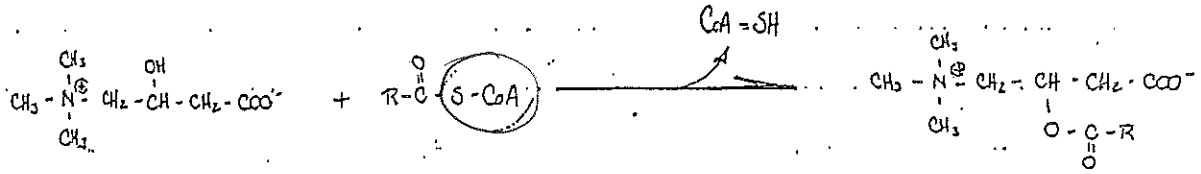
2ND STEP – TRANSPORT OF ACYL-CoA INTO THE MITOCHONDRIAL MATRIX → CARNITINE SHUTTLE

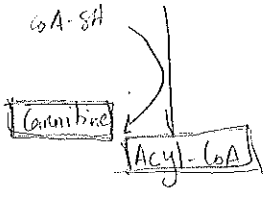
(Question 37)

Short-chain fatty acids (4-10 carbons) can cross the mit. membrane, BUT LONGER CAN'T!

These are bound to carnitine and cross the membrane as ACYLCARNITINE

↳ When they reach the inner side they are converted back (through the reverse reaction





3RD STEP – β-OXIDATION OF ACYL-CoA → ACETYL-CoA

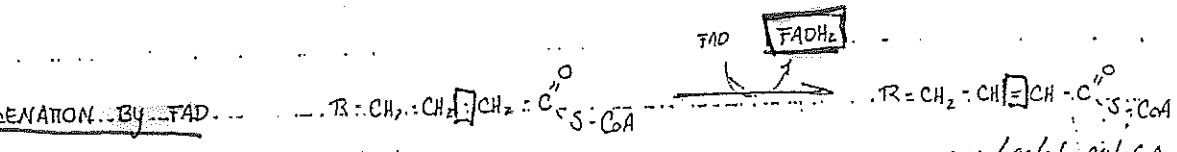
through 4 reactions:

1. dehydrogenation by FAD
2. hydration
3. (second) dehydrogenation by NAD^+
4. thiolysis by CoA

By this process $FADH_2$, $NADH + H^+$ and ACETYL-CoA are generated!

1. DEHYDROGENATION BY FAD

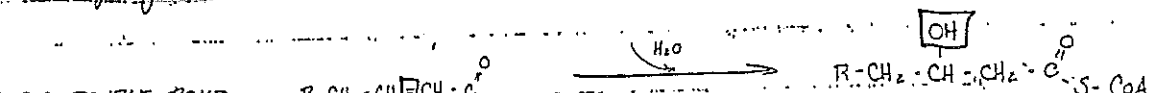
↳ acyl-CoA dehydrogenase



saturated acyl-CoA — unsaturated acyl-CoA

2. HYDRATION OF DOUBLE BOND

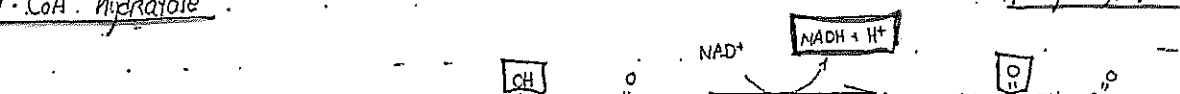
↳ enoyl-CoA hydratase



β-hydroxyacyl-CoA

3. second DEHYDROGENATION BY NAD+

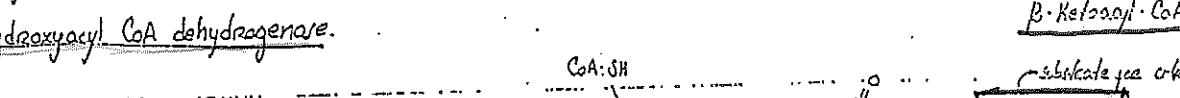
↳ 3-hydroxyacyl CoA dehydrogenase.



β-Ketoacyl-CoA

4. THIOLYSIS BY Co-A

↳ thiolase



substrate for citric acid cycle

β-OXIDATION OF PALMITATE → 8 acetyl-CoA + 8 acyl-CoA + 7 FADH2 (14 ATP) + 7 NADH (21 ATP) + 7 H+

⇒ TOTAL ENERGETIC YELD : 129 ATP

14 ATP + 21 ATP − 2 ATP (activation of palmitate) + 8 × 12 ATP by acetyl-CoA in citric acid cycle

CONTROL MECHANISMS

β-OXIDATION OCCURS WHEN CELLS REQUIRE ENERGY AND THE ACCESS TO GLUCOSE IS NOT SUFFICIENT

HORMONE-SENSITIVE LIPASE – hydrolysis ester bonds of triacylglycerols ⇒ free fatty acids + glycerol are released in the

↳ mobilizes fat stores, which are taken up by the liver.

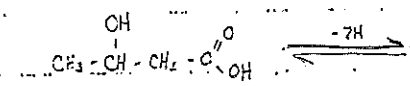
and other peripheral tissues (muscle, myocard, kidney, ...)

REGULATION
- ⇒ GLUCAGON (low glucose) and ADRENALINE / NORADRENALINE → stimulate its activity (+)
- ⇒ INSULIN – slows it down (−)

⇒ A great part of acetyl-CoA is diverted to the production of KETONE BODIES, which are released in the blood and serve as an excellent nutrient for extra-hepatic tissues.

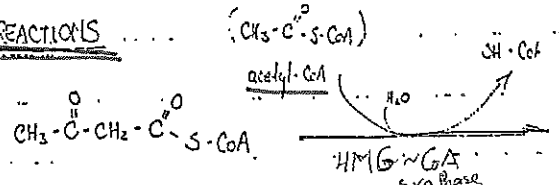
39 Ketogenesis - localization, the pathway and the control of it; the utilization of ketone bodies. The circumstances causing ketoacidosis

KETOGENESIS - formation of ketone bodies ⇒ IN LIVER MITOCHONDRIA



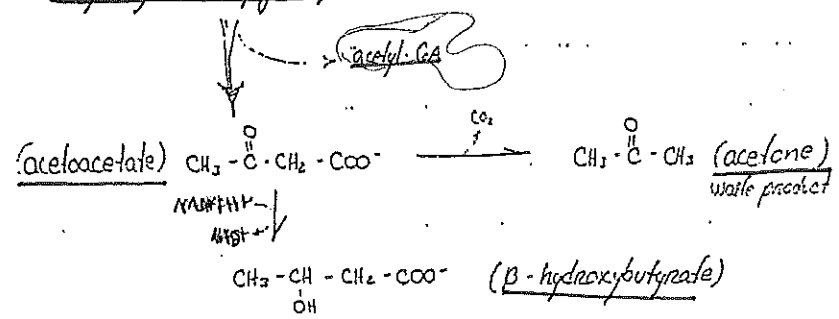
β-hydroxybutyric acid — acetoacetic acid — acetone

REACTIONS



acetoacetyl-CoA

3-hydroxy-3-methylglutaryl-CoA (HMG-CoA)



∴ **REGULATION** ⇒ production of ketone bodies is stimulated by high levels (4) of glucagon in blood, when fat stores are mobilized (starvation, diabetes mellitus, ...)

Cases of **KETOSIS** (extreme production of ketone bodies) are very dangerous because ketogen is a proton-producing process that evolves ketoacidosis → pH of blood seriously decreases

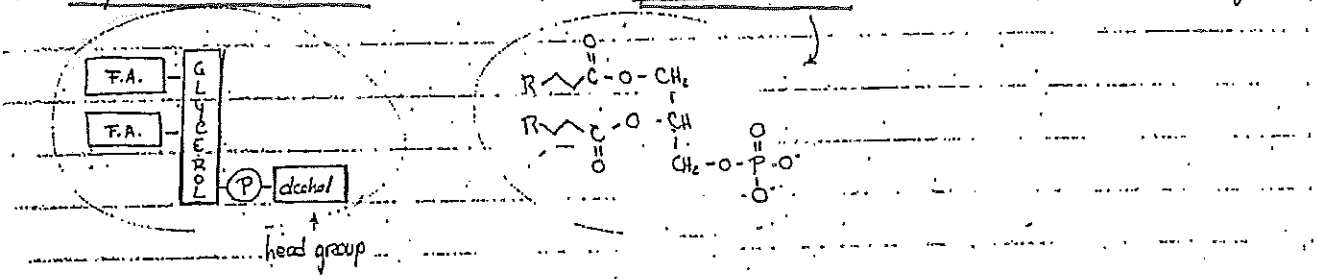
UTILIZATION OF KETONE BODIES

- β-hydroxybutyric acid ∴ provides E for peripheral tissues (extrahepatic)
- acetoacetate - reactivated to acetoacetyl-CoA (reaction with CoA from succinyl-)
- acetone - waste product

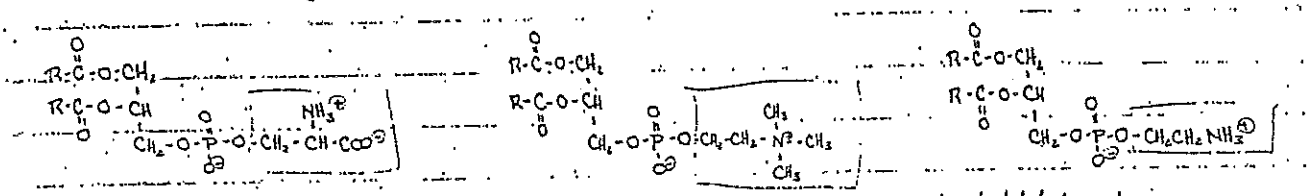
→ β-hydroxybutyric acid and acetoacetic acid can be reconverted to acetyl-CoA to produce energy, via citric acid cycle

40. Metabolism of glycerophospholipids (biosynthesis and degradation).

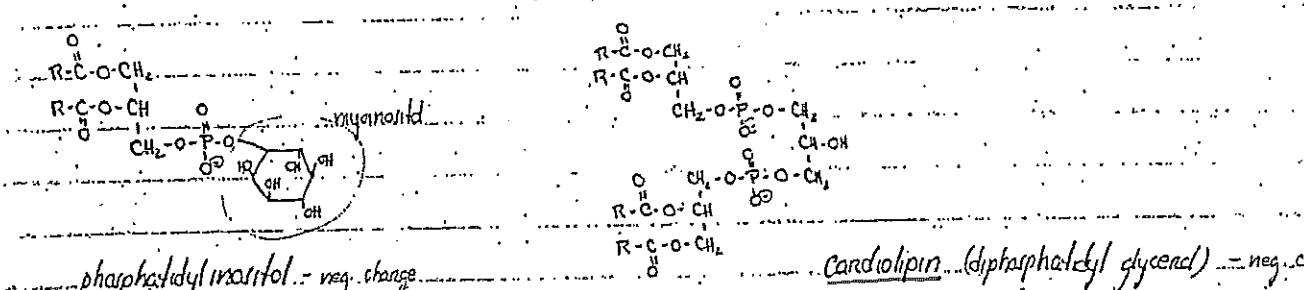
GLYCEROPHOSPHOLIPIDS → the simplest is phosphatidic acid - essential in biosynthesis of other glycero-P-lip



MAIN GLYCEROPHOSPHOLIPIDS

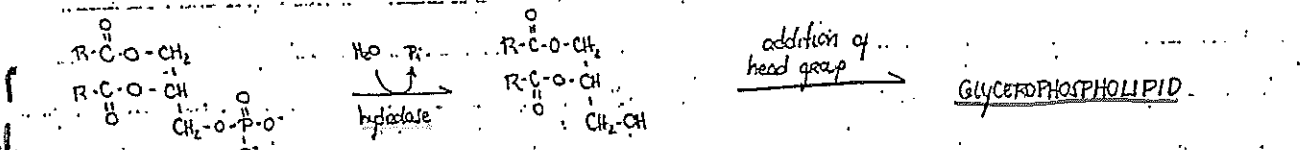


phosphatidylserine - negative charge | phosphatidylcholine - neuter | phosphatidylethanolamine - neuter



phosphatidylinositol - neg. charge | cardiolipin (diphosphatidyl glycerol) - neg. charge

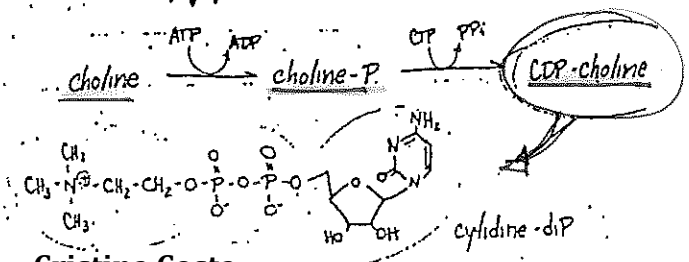
BIOSYNTHESIS OF GLYCEROPHOSPHOLIPIDS → in the membrane of ER



phosphatidate | 1,2-diacylglycerol | GLYCEROPHOSPHOLIPID

There are 2 methods of addition of the head group:

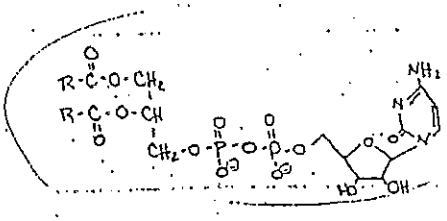
1°- Diacylglycerol accepts CDP-ACTIVATED CHOLINE OR ETHANOLAMINE - synthesis of phosphatidyl-serine, choline, ethanolamine



1. diacylglycerol + CDP-choline → CMP + phosphatidylcholine
2. diacylglycerol + CDP-ethanolamine → CMP + phosphatidylethanolamine
3. phosphatidylethanolamine —(serine, ethanolamine)→ phosphatidylserine !

3. synthesis of phosphatidylinositol and cardiolipin

phosphatidic acid —(CTP, PPi)→ CDP-diacylglycerol



1. CDP-diacylglycerol + inositol → CMP + phosphatidylinositol

2. CDP-diacylglycerol + glycerol-3-P → CMP + phosphatidyl-glycerol-3-P

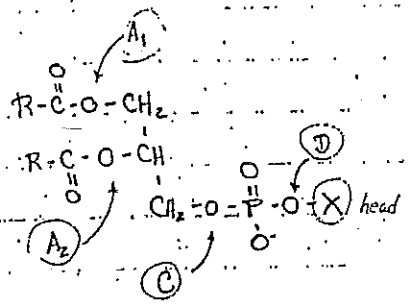
→ Pi + phosphatidyl glycerol

CDP-diacylglycerol + phosphatidyl glycerol → CMP + CARDIOLIPIN

DEGRADATION OF GLYCEROPHOSPHOLIPIDS — catalyzed by phospholipases — in cell memb. or in lysosomes

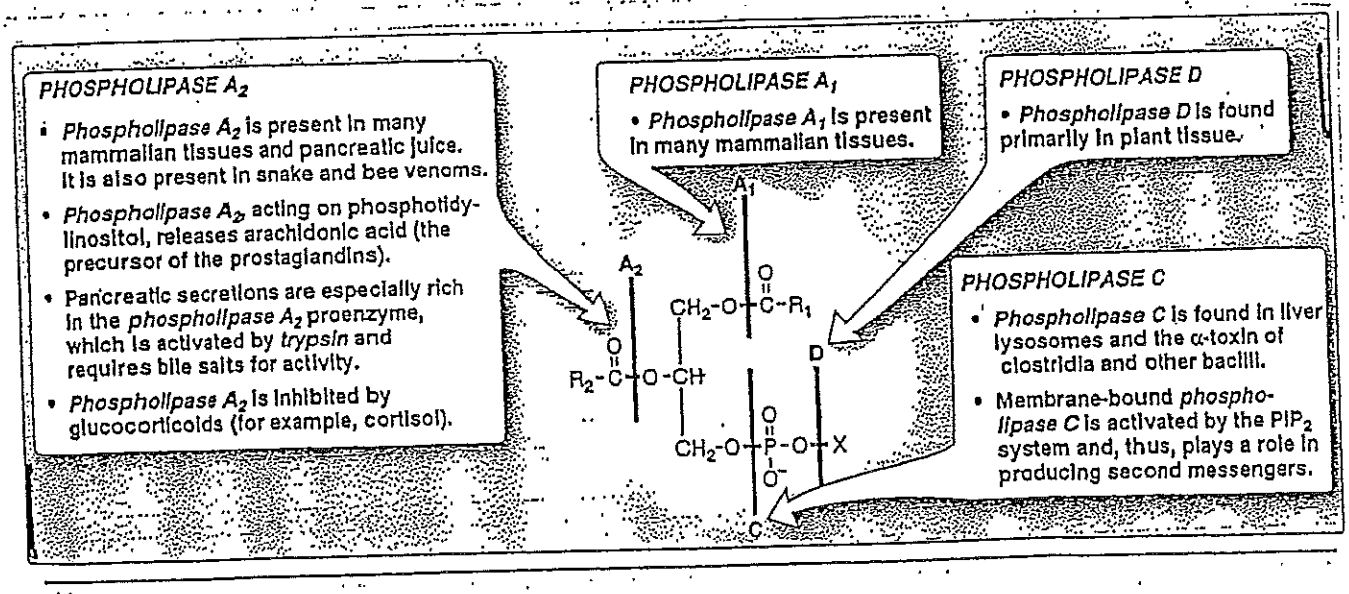
Different types of PHOSPHOLIPASES hydrolyze specific ester bonds: (A_1, A_2, C, D)

- (A_1) — prefers phosphatidylethanolamine
- (A_2) — prefers phosphatidylcholine
  important because it liberates arachidonic acid as a precursor of eicosanoids



→ A_1 and A_2 liberate ONLY ONE acyl group, the other will be removed by PHOSPHOLIPASE B (lysophospholipase = transacylase)

- C — stimulated by neurotransmitters



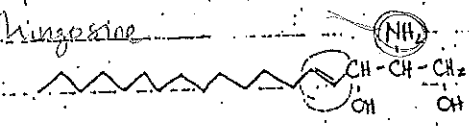
41. Metabolism of sphingolipids (biosynthesis and degradation)

SPHINGOLIPIDS → have ceramide as the lipidic part.

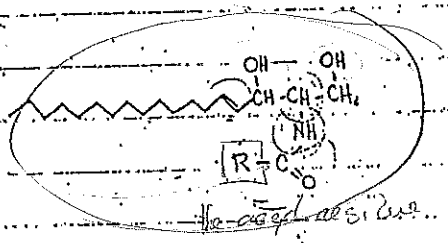
= class of lipids derived from sphingosine

= N-acetylated sphingosines

sphingosine + FA



ceramide



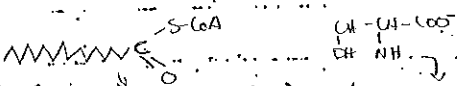
Biosynthesis on the next page

the acyl residue usually have 24 carbon

sphingomyelins !

SPHINGOLIPIDS
- sphingophospholipids: ceramide + P + ethanolamine / CHOLINE
- sphingoglycolipids: ceramide + saccharide
  - → monoglycosylceramides: cerebrosides
  - → oligo / sulpho / sialoglycosylceramides: gangliosides

BIOSYNTHESIS OF SPHINGOSINE → Condensation of acetyl-CoA (mainly PALMITOYL-CoA) and SERINE



Sphingosine + acyl-CoA → ceramide *

BIOSYNTHESIS OF SPHINGOMYELIN = CDP carries phosphoryl-choline

Ceramide + CDP-choline → CMP + sphingomyelin (ceramide-P-choline)

BIOSYNTHESIS OF GLYCOLIPIDS

Ceramide + UDP-Gal → cerebroside + UDP

(or other monosaccharide)

↳ attachment of further glycosyls occurs in a similar way.

↳ sulphate group is transferred from PAPS (3'phosphoadenosyl-5'-phosphosulfate)

↳ sialyl group (NeuAc in gangliosides) is transferred from CMP-NeuAc:

oligoglycosyl ceramide + CMP-NeuAc → ganglioside + CMP

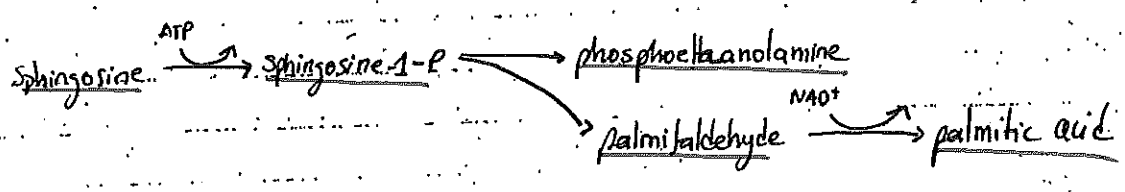
DEGRADATION OF SPHINGOLIPIDS → in lysosomes ! — enzymes hydrolyse ester and glycoside bonds

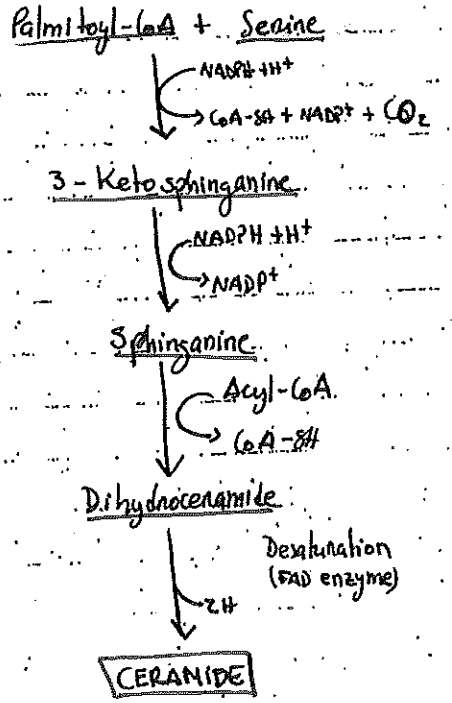
→ SPHINGOMYELIN → lose P-choline to give ceramide

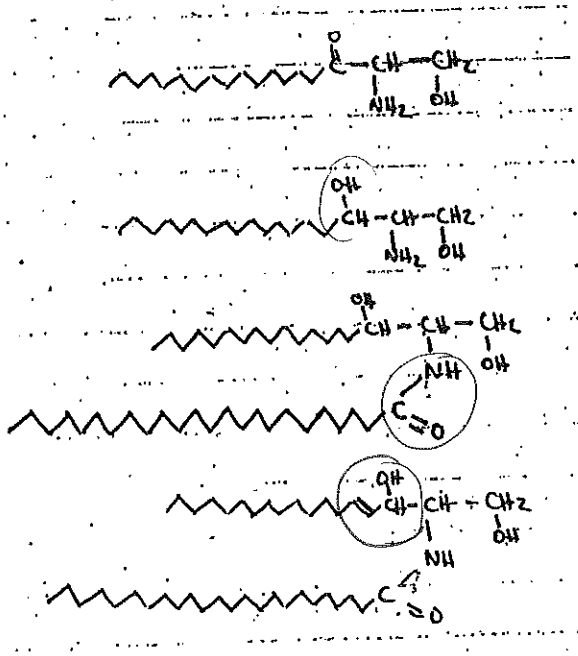
→ GLYCOLIPIDS → lose the saccharide component to give ceramide

→ CERAMIDE is hydrolysed (ceramidase) fatty acid + sphingosine

→ SPHINGOSINE - its degradation is almost the reversal of its biosynthesis: after phosphorylation, sphingosine is broken down to phosphoethanolamine (decarboxylated serine) and palmitaldehyde that is oxidized to palmitate.







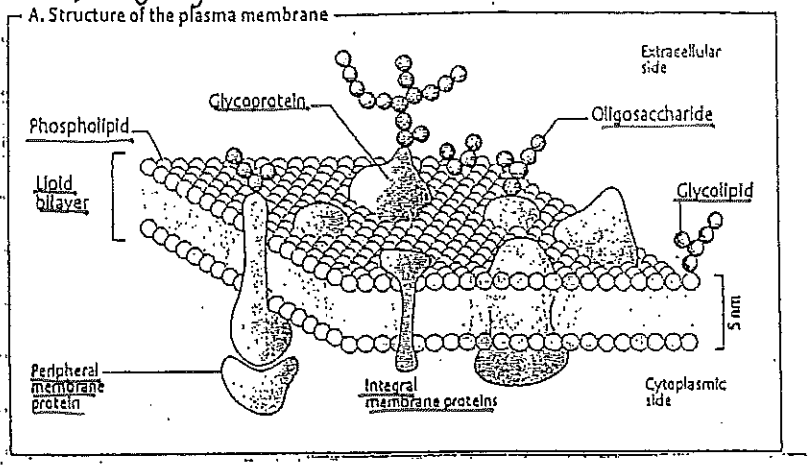
Glycocalix → exterior coating of membrane - made of glycolipids and glycoproteins

42 Membrane structure, the assembly and recycling of membranes. Specialized structures of plasma membranes - lipid rafts, caveols, tight junctions.

PLASMA MEMBRANE

↳ consist of a continuous bilayer of amphipatic lipids, approx. 5nm thick into which proteins are embedded.

↳ Some membranes also have carbohydrates bound to lipids and proteins



↳ membrane lipids are strongly ampiphatic molecules, with a polar head an apolar hydrophobic tail. They are held together by hydrophobic and Van der Waals forces, so they present some fluidity

⇓

- of membranes depend on lipidic composition and on temperature:
  - the more PUFA, the more fluid is the membrane
  - Cholesterol increases fluidity of closely packed membranes and stabilizes those with high PUFA c

↳ "FLUID MOSAIC" model → because proteins and lipids can shift easily within one layer of the membrane; switching between the 2 layers (flip-flop) is rare but possible for lipids

MEMBRANE LIPIDS → PHOSPHOLIPIDS (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol and sphingomyelin), CHOLESTEROL (in animals only), GLYCOLIPIDS (cerebrosides and gangliosides) and GLYCOPROTEINS:

MEMBRANE PROTEINS

- → Integral: 20-25 aa, mainly hydrophobic that form a right-handed α-helix
  - TYPE I and II - only one transmembrane helix
  - TYPE III - several transmembrane helices
  - TYPE IV and V - carry lipid anchors: f.a, isoprenoids or glycolipids, covalently bound.
  - PORINS → integral proteins with antiparallel β-sheet struc
- → Peripheral - associated with the head groups of phospholipids or with another integral membrane protein.

GLYCOCALIX → exterior coating of membrane - composed of glycolipids and glycoproteins

↳ FUNCTIONS: protect the cell from chemical and mechanical damage ; provide information concerning cell-cell recognition (processes like fertilization, blood-clotting, inflammatory respo

MEMBRANE ASSEMBLY — very complex process about which much is left to be learned

→ Vesicles formed from membranes of the ER and Golgi apparatus exhibit TRANSVERSE ASYMMETRIES of both lipid and protein, which are maintained during fusion of transport vesicles with the plasma membran

↳ inside of vesicle becomes the outside of plasma membranes and the cytoplasmic side of the vesicles remains the cytosolic side of the membrane.

↳ Since the transverse asymmetry already exists in the ER vesicles, the problem with membrane assembly becomes understanding how the integral proteins are inserted into the lipid bilayer of ER... ???

SYNTHESIS OF PHOSPHOLIPIDS → enzymes responsible for synthesis are located in the cytoplasmic surface of the cisternae of the ER — the phospholipids are synthesized there.

DEGRADATION → the turnover rates of lipids and proteins are independent, though turnover rate between 2 proteins / 2 lipids varies greatly.

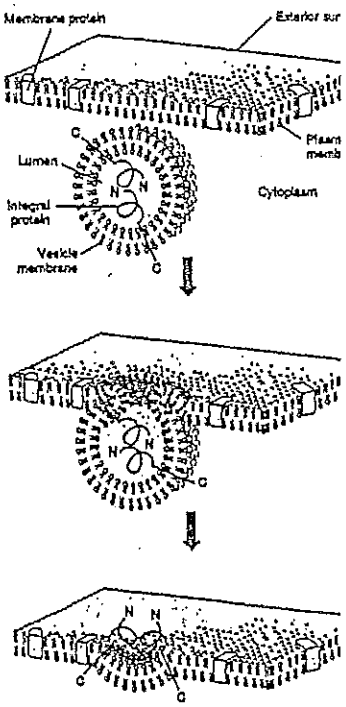


Figure 46–8. Fusion of a vesicle with the plasm membrane preserves the orientation of any integr proteins embedded in the vesicle bilayer. Initially, amino terminal of the protein faces the lumen, or cavity, of such a vesicle. After fusion, the amino te nal is on the exterior surface of the plasma membr That the orientation of the protein has not been r versed can be perceived by noting that the other of the molecule, the carboxyl terminal, is always ir mersed in the cytoplasm. The lumen of a vesicle a the outside of the cell are topologically equivalen drawn and modified, with permission, from Ledish I Rothman JE: The assembly of cell membranes. Sci A (Jan) 1979;240:43.)

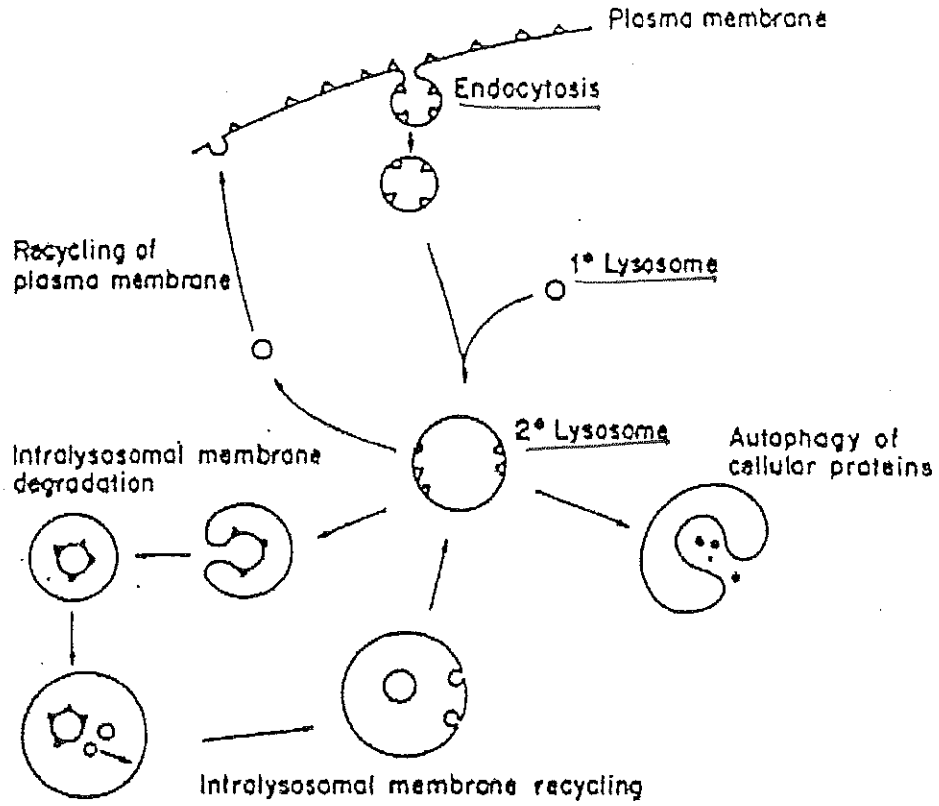
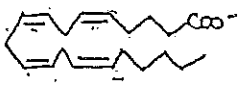


Figure 6.9 Pathways of membrane uptake into lysosomes. Primary lysosomes fuse with plasma-membrane vesicles formed by endocytosis to generate secondary lysosomes in which both the contents and some membrane components may be degraded. Soluble proteins are taken up into lysosomes by autophagy. It is also possible that membrane is selected for degradation by intralysosomal degradation or recycled to the cell surface.

43 Eicosanoids (basic structural types, the main steps of the synthesis, the basal features of their function, inhibitors of eicosanoid production as anti-inflammatory agents).

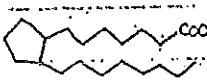
EICOSANOIDS → family of polyunsaturated C_{20} fatty acid derivatives which act as HORMONES and have a wide range of biological functions
↳ of restricted activity: they act in the close vicinity or in the own cell

their major precursors are the essential PUFA:
- → eicosatrienoic acid: 20:3 (8,11,14) → n-6 series)
- → ARACHIDONIC ACID (eicosatetraenoic a.): 20:4 (5,8,11,14) → n-6 series



- → EICOSAPENTAENOIC ACID: 20:5 (5,8,11,14,17) → n-3 series

Types of EICOSANOIDS:
- → PROSTANOIDS - derivatives of prostanoic acid:



  - prostaglandins
  - prostacyclins
  - thromboxanes
- → LEUKOTRIENES - have 3 conjug. double bonds
- → LIPOXINS - have 4 conj. double bonds and 3 -OH groups.

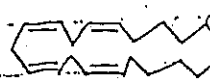
Eicosanoids synthesis:
- CYCLOOXIGENASE PATHWAY — leads to formation of PROSTAGLANDIN H, percursor of the PROSTANOIDS!
- LIPOXYGENASE PATHWAY — converts the acid percursors to acyclic HYDROPEROXIACIDS, from which LEUKOTRIENES AND LIPOXINS are formed.

(1.) CYCLOOXYGENASE PATHWAY - synthesis of cyclic eicosanoids: PROSTANOIDS.

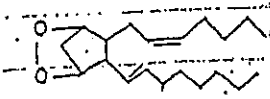
Cox (prostaglandin endoperoxide synthase) → membrane-bound enzyme — exists in 2 forms:
- Cox-1 - constitutive enzyme, present in all tissues
- Cox-2 - is inducible - its synthesis is induced by cytokines in inflammed tissue

↓ Inhibition has anti-inflammatory effect

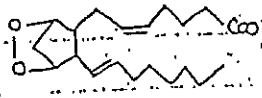
→ COX catalyzes the conversion of arachidonate to PGH_2 - common precursor of all the prostanoids of the 2-series (dene prostanoids)!


ARACHIDONATE

$+ 2O_2$ — cyclooxygenase


PROSTAGLANDIN G_2

$2GSH$ / $H_2O + GSSG$ — cyclooxygenase (peroxidase activity)


PROSTAGLANDIN H_2

PROSTAGLANDIN H_2 → PGI SYNTHASE → prostacyclin PGI_2

PROSTAGLANDIN H_2 → PGE SYNTHASE → prostaglandin PGE_2

PROSTAGLANDIN H_2 → TXA SYNTHASE → thromboxane TXA_2

PGI_2, PGE_2, TXA_2 → ALL PROSTANOID DERIVATIVES

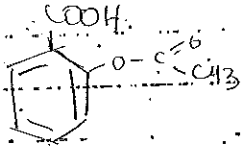
REGULATION → inhibition of Cox blocks prostanoids production → mediate the inflammatory response

when prostanoid production is inhibited we get desirable and undesirable effects:

- desirable:
  - anti-inflammatory effect
  - relief of pain and fever,
- undesirable
  - lower blood platelet aggregation
  - protection of endothelial cells,

→ SO INHIBITORS OF CYCLOOXYGENASE ACT AS NONSTEROIDAL ANTI-INFLAMMATORY DRUGS (analgetic, antipyretic, anti-inflammatories)

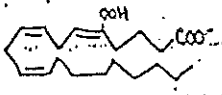
- ACETYLSALICYLIC ACID → inhibits both COX-1 and COX-2 irreversibly (Aspirin)
- ACETAMINOPHEN and IBUPROFEN → reversible COX inhibitors

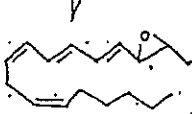


② LIPOXYGENASE PATHWAY


ARACHIDONATE

O_2 — 5-lipoxygenase

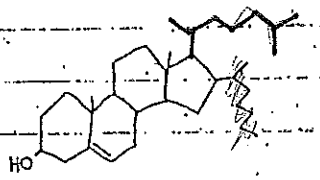

5-HYDROPEROXYEICOSATETRAENOIC


LEUKOTRIENE LTA_4 → precursor of leukotrienes of the 4-series

- Leukotrienes are produced primarily in leukocytes and mast cells and all of them have 3 conj. double bonds (trienes)
- The classes of LTs are designated by letters, the subscript denotes the no. of double bonds
- Leukotrienes are the most effective eicosanoids eg. their vasodilator effect is about 5000x than that of histamine.

44. Biosynthesis of cholesterol (the most important reactions and stages), regulation. Excretion of cholesterol and the cholesterol balance in the body.

CHOLESTEROL (27C)

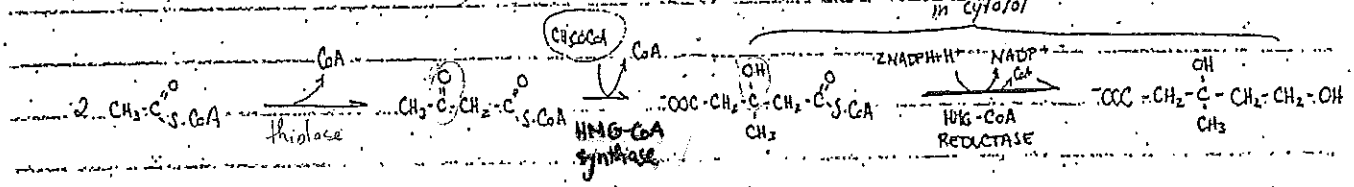


- constituent of all animal membranes, modulates their fluidity
- cholesterol is synthesized in all nucleated cells
- necessary precursor for the biosynthesis of bile acids, steroid hormones and calcidiol (vit. D)

BIOSYNTHESIS OF CHOLESTEROL ⇒ from acetyl-CoA
↳ in the cytosol and in membranes of ER ⇒ mainly in liver (1/3 total)

4 stages:
1. synthesis of mevalonate from acetyl-CoA;
2. conversion of 2 mevalonates to 2 activated isoprene units;
3. condensation of 6 mol. of activated isoprenes ⇒ squalene
4. the cyclization of squalene and its conversion into cholesterol

① SYNTHESIS OF MEVALONATE FROM ACETYL-CoA - ER membrane ⇒ cytosol



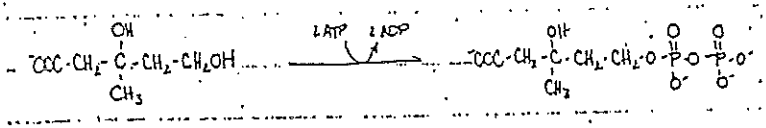
Acetyl-CoA — acetoacetyl-CoA — HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) — MEVALONATE

→ THE CONTROL OF CHOLESTEROL BIOSYNTHESIS IS DONE BY REGULATION OF HMG-CoA REDUCTASE

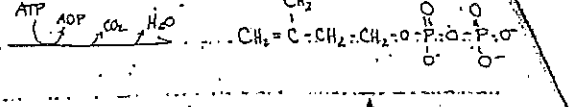
inhibition of HMG-CoA REDUCTASE:
- cytosolic free cholesterol (feed-back control)
- reversible phosphorylation of the enzyme
- drugs called statins
  ↳ competitive inhibitors of HMG-CoA reductase, either fungal products or synthetic compounds

↑ glucagon ⇒ ↑ cAMP ⇒ activates protein kinase A → inactivates the enzyme!
Thus cholesterol synthesis is decreased when ATP availability is low!

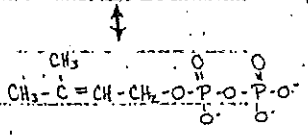
(2) CONVERSION OF MEVALONATE TO ACTIVATED ISOPRENE UNITS



mevalonate → mevalonate-5-diphosphate



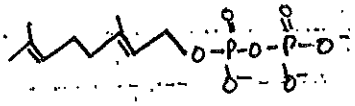
ISOPENTENYL DIPHOSPHATE



3,3-DIMETHYLALLYL DIPHOSPHATE

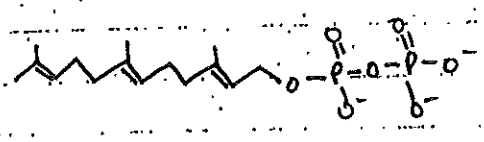
(3) CONDENSATION OF MOLECULES OF ACTIVATED ISOPRENES (⇒ SQUALENE)

isopentenyl diphosphate (5C) + 3,3-dimethylallyl diphosphate (5C)

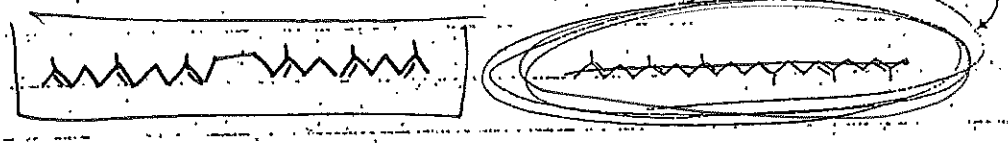


geranyl diphosphate (10C) + isopentenyl-diP (5C)

farnesyl diphosphate (15C) + farnesyl diphosphate (15C)

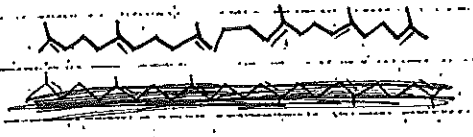


SQUALENE (30C)

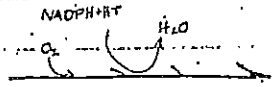


3 × C + 6 double bond

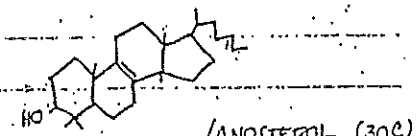
(4) CYCLIZATION OF SQUALENE AND CONVERSION INTO CHOLESTEROL



SQUALENE (30C)

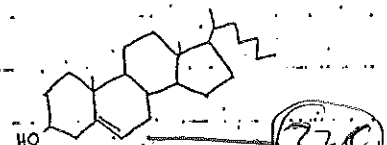


MONOOXYGENASE
2,3-OXIDOSQUALENE CYCLASE



LANOSTEROL (30C)

- removal of 3 $-CH_3$ groups
- rearrangement of double bond
- saturation of 1 double bond



CHOLESTEROL (27C)

taurine → $NH_2-CH_2CH_2-SO_3^-$

45 Synthesis of bile acids. (location, main steps of the synthesis, secretion and elimination from the body)

BILE ACIDS

- PRIMARY
  - CHOLATE + taurine/glycine
  - CHENODEOXYCHOLATE + taurine/glycine
- SECONDARY (reabsorbed by intestine)
  - DEOXYCHOLATE
  - LITHOCHOLATE

glycine: $CH_2(NH_2)-COOH$ taurine: $CH_2(NH_2)-CH_2-SO_3^-$

⇒ The primary bile acids (cholate and chenodeoxycholate) are CONJUGATED WITH GLYCINE OR TAURINE, WITHIN THE ER OF LIVER CELLS

↳ these CONJUGATED BILE ACIDS are then secreted into bile tubules.

BIOSYNTHESIS OF BILE ACIDS – ONLY IN LIVER CELLS

(1°) hydroxylation of cholesterol at C-7 → catalyzed by 7α-HIDROXYLASE (monooxygenase of the cytochrome P450 class)

cholesterol → (NADPH+H⁺, O_2, H_2O; NADP⁺; cytP450 (monooxygenase)) → 7α-hydroxycholesterol

(ER membrane)

5β-cholestane-3α,7α,12α-triol

(mitochondria) CoA + ATP → propionyl-CoA + ADP

Cholate

CoA + ATP → AOP

Choloyl-CoA

(IN ER) glycine/taurine → CoA

GLYCOCHOLATE / TAUROCHOLATE

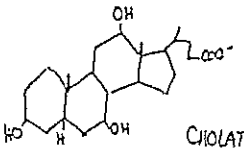
⇒ the primary bile acids are direct products of cholesterol degradation in liver.

↳ in the intestine they may suffer bacterial action and give secondary bile acids

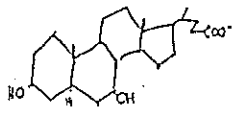
BILE ACIDS UNDERGO THE ENTEROHEPATIC CIRCULATION

↳ A mixture of primary, secondary and bile salts is absorbed in the ileum. They are transported by albumin into the liver, where the primary and secondary bile acids are converted into bile salts by conjugation with taurine/glycine. These are released in the bile → cyclic process

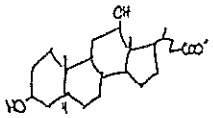
⇒ Approx. 0,5 g of bile salts are lost in the feces in one day. Synthesis of bile acids in liver replaces the lost ones.



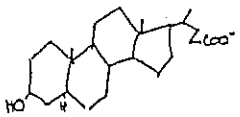
CHOLATE



CHENODEOXYCHOLATE ⇒ primary



DEOXYCHOLATE



LITHOCHOLATE ⇒ secondary

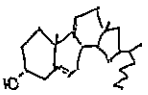
Primary bile acids → from cholesterol in liver !

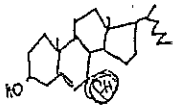
Secondary " " → primary bile acids that suffer bactericidal action in the intestine

ENTEROHEPATIC CIRCULATION → bile acids are absorbed in ileum and transported by albumin to the liver, where they are converted into bile salts by conjugation with glycine/taurine.

↳ released again in bile

cholesterol —(HYDROXYLATION, 7-α-HIDROXYCASE)→ 7-α-hydroxycholesterol → CHOLATE → (Gl. / Taur.) glycocholate / taurocholate





~~CHOLESTEROL → 7-α-hydroxycholesterol → 5β-cholestene-3α,7α,12α-diol~~

~~↓~~

~~cholate~~

46 Intracellular degradation of proteins

INTRACELLULAR DEGRADATION OF PROTEINS

↳ eliminates abnormal proteins, allowing the regulation of cellular metabolism

1. PROTEIN DEGRADATION IN LYSOSOMES → ATP INDEPENDENT!
   ↳ contain many proteolytic enzymes, - CATHEPSINS (about 2

PROTEINS DEGRADED:
- 1.1 - EXTRACELLULAR PROTEINS (that enter the cell via endocytosis) — are hydrolysed in phagolysosomes (invaginated vesicles fuse with... lysosomes)
- 1.2 - MEMBRANE-BONDED PROTEINS
- 1.3 - LONG-LIVED INTRACELLULAR PROTEINS — autophagy: digestion of cytoplasmic proteins by the cell's own lysosomes

2. CYTOSOLIC UBIQUITIN SYSTEM → ATP DEPENDENT!

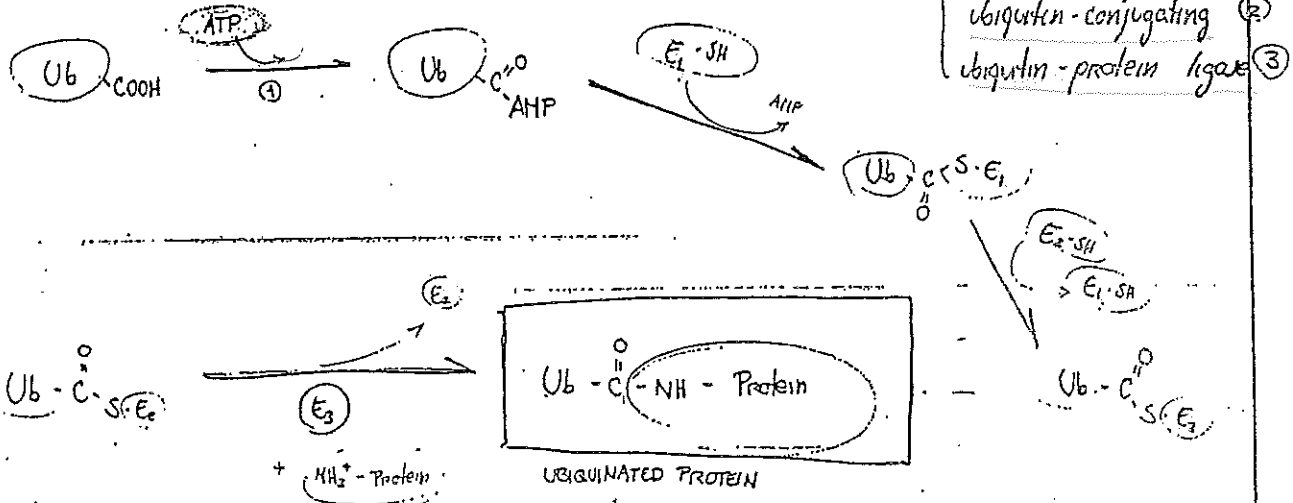
small protein present in all eukaryotic cells — TAGS PROTEINS FOR DESTRUCTION (MARCA)

ACTION OF UBIQUITIN — its glycine residue attaches covalently to the amino groups of several lysine residues (isopeptide bonds) on proteins destined to be degraded.

PROTEINS DEGRADED:
- 2.1 - INTRACELLULAR UNDER STRESS CONDITIONS
- 2.2 - " NON-STRESS "

} incorrectly folded or old proteins

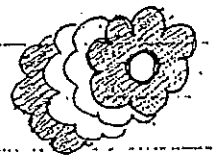
ACTIVATION OF TARGET PROTEIN BY UBIQUITIN — 3 enzymes needed
- ubiquitin-activating (1)
- ubiquitin-conjugating (2)
- ubiquitin-protein ligase (3)



11

THE UBIQUITIN-TAGGED PROTEIN HEADS TOWARDS PROTEASOME

PROTEASOME → digests ubiquitin-tagged proteins in an ATP-DRIVEN PROCESS, from which ubiquitin is spared and then recycled



→ Consists of 28 subunits arranged in 4 rings
⇒ THE ACTIVE SITES ARE INSIDE!

→ inside the proteasomes the ubiquinated proteins suffer proteolysis (enzymes: proteases) and are reduced to peptides (7-9 amino acyl residues)

↓

GO TO CYTOPLASM where they are cleaved to aa by peptidases

//

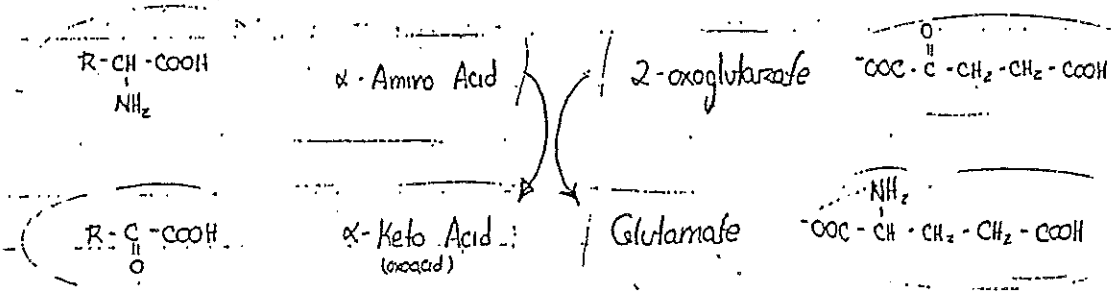
47

ELIMINATION OF α-AMINO NITROGEN FROM AMINO ACIDS → next page

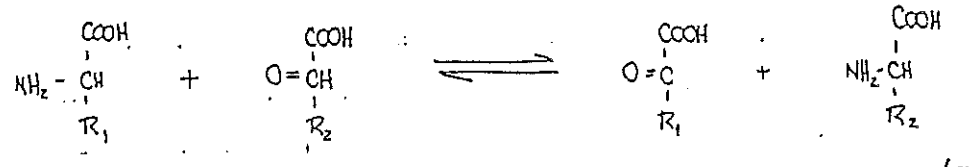
1) TRANSAMINATION → most aa except Arg, Lys, Met, Thr, Trp, Pro, His
→ aa that cannot be deaminated directly

↓

the α-amino group of the aa is transferred to 2-oxoglutarate (α-Ketoglutarate) TO FORM GLUTAMATE:



GENERAL EQUATION OF TRANSAMINATION



aminoacid 1 — Keto-acid 1 — Keto acid 2 — aminoacid 2

47) Deamination of aminoacids and transamination (deamination types, reaction course, coenzymes, consequence of reactions in removal of amino groups from aas).

TRANSAMINATIONS = catalyzed by transaminases → both in anabolism and catabolism of aa:
↳ the amino group is transferred to a 2-oxoacid! The NH_2-group is temporarily taken by pyridoxal phosphate: (which becomes pyridoxamine-P)

If NH_2 is released as ammonia the process is referred to as DEAMINATION!
↳ there are many ways of releasing ammonia - some types of deamination:

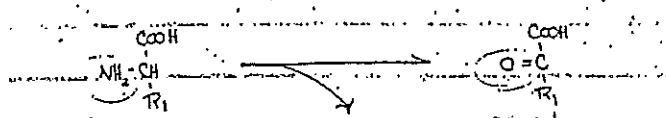
see other side

• Change of amino group with the one of the ketoacid

↳ TYPES OF DEAMINATION:

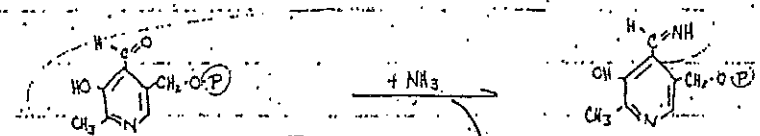
most aa except: Proline Trp Lysine (and) Arg (in mammals) His
Three Methods (7 aa)

(1) TRANSAMINATION = catalyzed by transaminases, that have a lysine residue bound to PYRIDOXAL-P



the α-aminogroup of many aa that cannot be deamined directly is transferred to 2-oxoglutarate to form glutamate

glutamate is then oxidatively deaminated by glutamate DH to yield ammonium ion NH_4^+



pyridoxal-P → aldimine (Schiff's base)

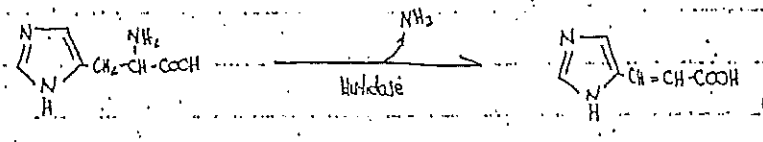
↳ 2 important AMINOTRANSFERASES: (1.) aspartate aminotransferase (AST) (2.) alanine aminotransferase (ALT)
and the respective reactions: Aspartate —(2-oxoglut. / Glu)→ oxaloacetate Alanine —(2-oxoglut. / Glu)→ pyruvate

(2) ELIMINATING DEAMINATION (DESATURATION) = degradation of His and Ser: H_2O is firstly eliminated, yielding an unsaturated intermediate. This compound will next take up H_2O and give NH_3 and an oxoac

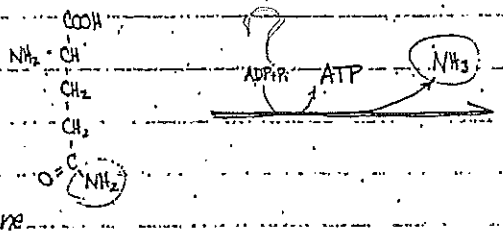
Serine —(H_2O)→ iminocompound —(H_2O / NH_3)→ pyruvate (oxoacid)

Histidine → Urocanic acid

(next page)

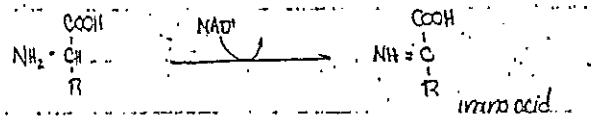


3. HYDROLYTIC DEAMINATION — Asparagine (Asn) and Glutamine (Gln) have amide groups in the side chains from which NH_3 can be released by hydrolysis



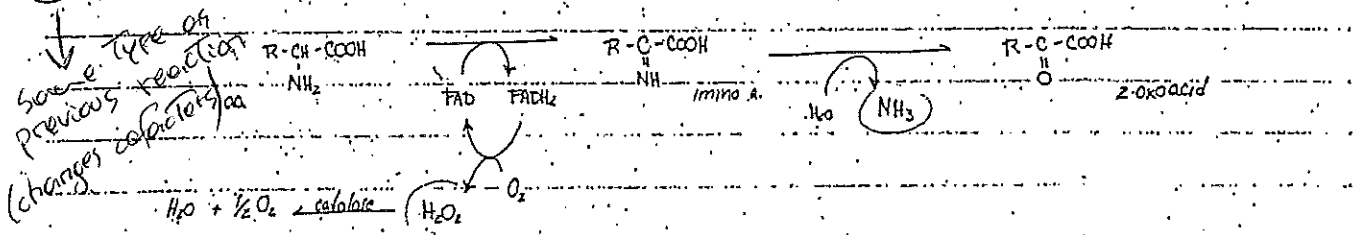
4. OXIDATIVE DEAMINATION: 1. the amino group is oxidized to an imino group and the reducing equivalents transferred to NAD^+ or $NADP^+$ — BY DEHYDROGENATION

2. the amino group is cleaved by hydrolyzes → this produces a 2-oxoacid + ammonia



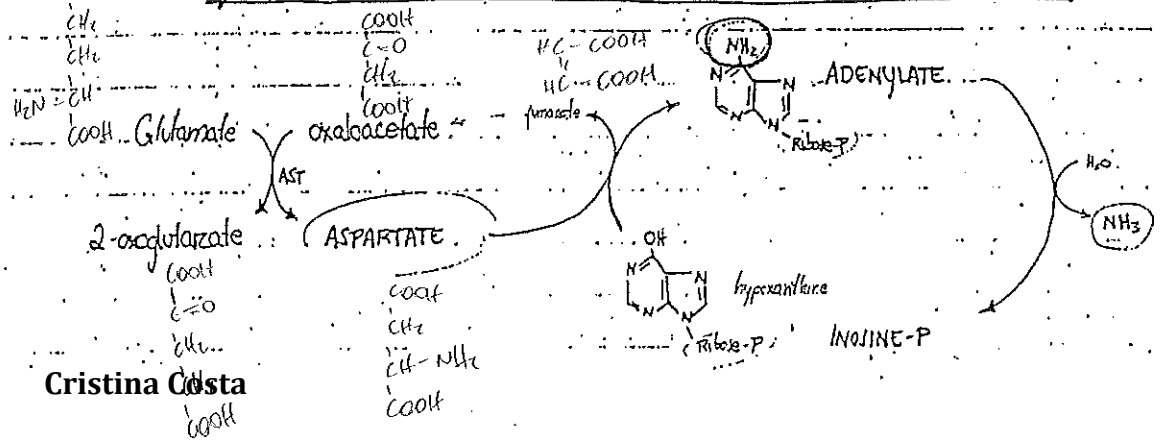
↳ by this mechanism, glutamate is converted to 2-oxoglutarate, reaction catalyzed by the mitochondrial enzyme glutamate DH (GLD)

5. OXIDATIVE DEAMINATION IN PEROXISOMES: catalyzed by L-amino acid oxidases (flavoproteins!)



6. INDIRECT DEAMINATION OF GLUTAMATE = "purine nucleotide cycle"

↳ IN SOME TISSUES (skeletal muscle eg) Glu suffers transamination with oxaloacetate to give Aspartate and 2-oxoglutarate. THE AMINO GROUP OF ASPARTATE IS then transferred to HYPOXANTHINE, giving ADENYLATE, which will be deaminated by adenylate deaminase



45 TH

48) Detoxification of ammonia (the ureosynthetic cycle, glutamine, glutamate).

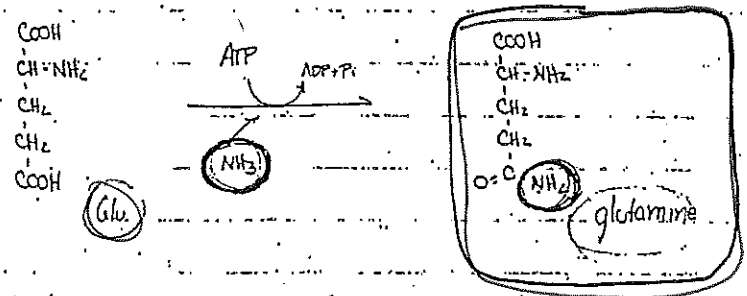
TRANSPORT OF AMMONIA IN THE BLOOD

↳ refers to both NH_4^+ and NH_3

AMMONIA IS TOXIC, so it is transported between tissues in the form of glutamine !

[glutamine] in blood plasma = 400-700 μmol/L

SYNTHESIS OF GLUTAMINE (catalyzed by glutamine synthase)



↳ Glutamine is transported in blood to the liver, where it is cleaved into glutamate + ammonia (by glutamin

(ANOTHER TRANSPORT mechanism is ALANINE → pyruvate + ammonia ⇌ alanine (ALT))

300 μmol 400 μmol

↳ THE UREOSYNTHETIC CYCLE = liver cells detoxify NH_4^+ by the synthesis of urea $NH_2-\overset{O}{\overset{\|}{C}}-NH_2$

↓

they take up glutamine and ammonium ions from the blood

$NH_2-\overset{O}{\overset{\|}{C}}-NH_2$ → the C atom comes from HCO_3^- anion, one N from ammonium and another from aspartate (hydrogen carbonate)

UREOSYNTHESIS

synthesis is an (ENDERGONIC) process = 3 ATP are hydrolyzed for the synthesis of 1 mol of urea

↳ consume energy

UREA IS SYNTHESIZED IN 5 STEPS:

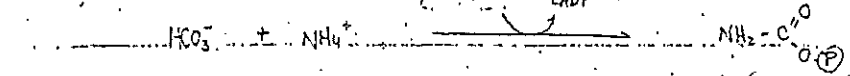
IN MITOCHONDRIAL MATRIX:
1. Synthesis of carbamoyl-P
2. transfer of carbamoyl to ornithine

IN CYTOPLASM:
3. transport of citrulline to cytoplasma and condensation with Asp
4. cleavage of arginosuccinate into arginine and fumarate
5. hydrolysis of arginine generates urea and ornithine

OVERALL STOICHIOMETRY: $Asp + NH_3 + CO_2 + (3ATP) \longrightarrow$ urea + fumarate + $3H_2O$ + 2ADP + 1AMP + 2Pi + PPi

mitochondrial form

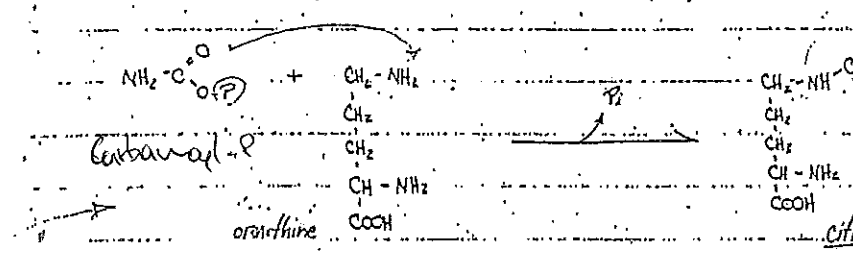
1. Synthesis of carbamoyl-P (in mitochondrial matrix) = catalyzed by carbamoyl-P synthase I, which Requires an allosteric activator:



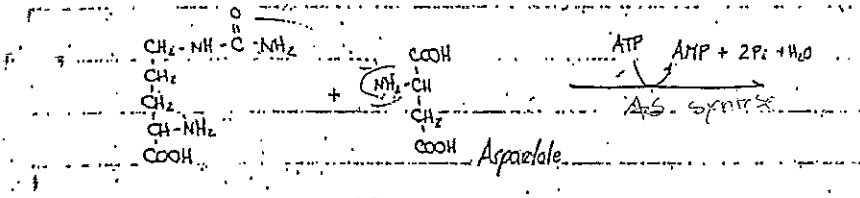
N-acetylglutamate

Carbamoyl-P

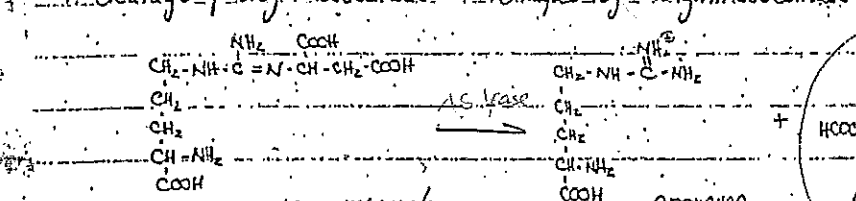
2. Transfer of carbamoyl to ornithine = catalyzed by ornithine transcarbamoylase



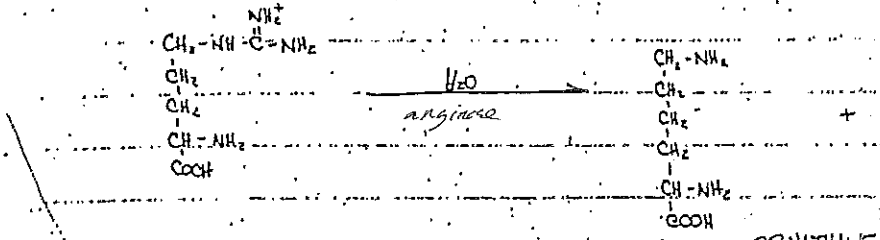
3. TRANSPORT OF CITRULLINE TO CYTOPLASM and condensation with aspartate — cat. by argininosuccinate synthase



4. Cleavage of argininosuccinate = catalyzed by argininosuccinase



5. Hydrolysis of arginine = catalyzed by arginase



will be used again in step 2.

49 Glucogenic and Ketogenic amino acids ("families" according to the resulting amphibolic intermediates, reversible interconversions of amino acids, essential amino acids)

- Degradation of the 20 proteinogenic aa produces 7 degradation products, five of those (2-OXOGLUTARATE; SUCCINYL-CoA; FUMARATE, OXALOACETATE, PYRUVATE) are precursors for gluconeogenesis, so they can be converted to glucose by the liver and kidneys!

GLUCOGENIC AMINOACIDS are the aa whose degradation supplies one of those 5 metabolites.

→ all proteinogenic aa except for Lysine and Leucine are glucogenic

- proteinogenic aa. { = Glucogenic a.a. / = Ketogenic a.a.

- The other 2 degradation products are ACETOACETATE and ACETYL-CoA, these cannot be channeled into gluconeogenesis (no means of converting them into precursors). Instead, they are used to produce Ketone bodies, fatty acids and isoprenoids

KETOGENIC AMINOACIDS are those which supply acetoacetate or acetyl-CoA in their degradation.

→ Lysine and Leucine (PURELY KETOGENIC)

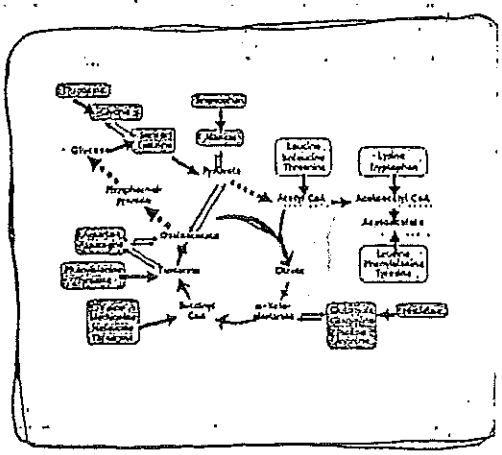
Many aa yield both glucogenic and ketogenic degradation products — they are both GLUCOGENIC and KETOGENIC:

→ Phenylalanine, Tyrosine, Tryptophan, Isoleucine + Threonine

- IRREVERSIBLE CONVERSIONS in the aa metabolism show which proteinogenic aa are essential

Non-essential aa and their reversible conversions:

- Serine ⇌ glycine ← THREONINE (essential aa)
- Cysteine
- Alanine ⇌ PYRUVATE
- Aspartate ⇌ OXALOACETATE / FUMARATE
- Asparagine
- Glutamate
- Glutamine ⇌ α-KETOGLUTARATE
- Proline
- Arginine
- Tyrosine — formed by hydroxylation of phenylalanine

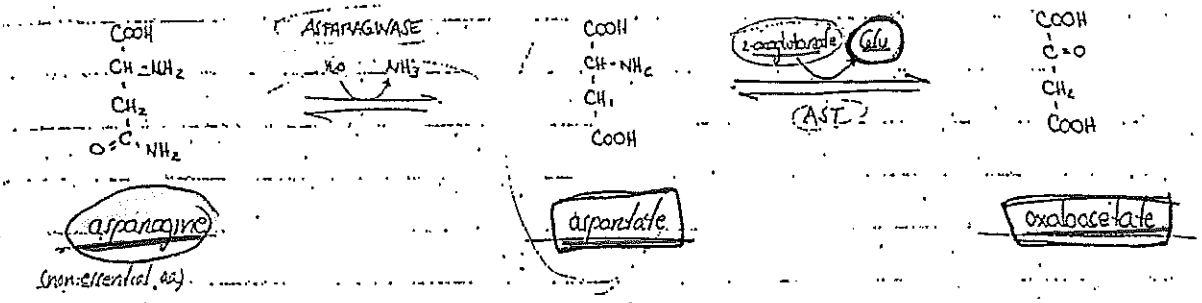


ESSENTIAL

1. Threonine
2. Methionine
3. Lysine
4. Valine
5. Leucine
6. Isoleucine
7. Histidine
8. Phenylalanine
9. Tryptophan

50 Metabolism of dicarboxylic amino acids → ASPARTATE → Glutamate

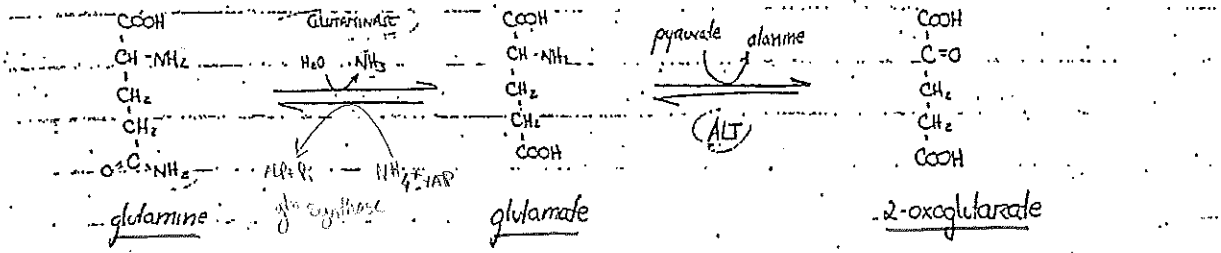
Aspartate → non essential and glucogenic aa
gives oxaloacetate by transamination



TRANSAMINATION

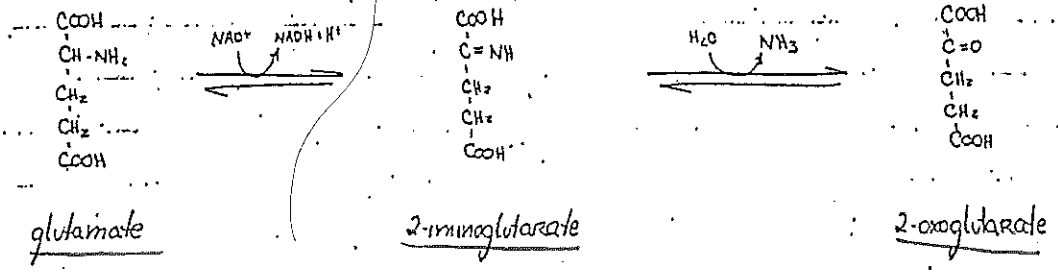
Utilization of ASPARTATE
- gives: NH3 for the synthesis of urea, AMP and purine incorporated into pyrimidine bases
- gives: β-alanine

2. Glutamate → non essential and glucogenic
gives 2-oxoglutarate readily by oxidative deamination or transamination

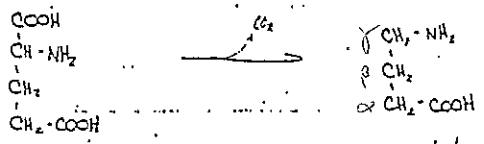


DEAMINATION

DIRECT OXIDATIVE DEAMINATION OF GLUTAMATE BY DEHYDROGENATION - catalyzed by mitochondrial enzyme GLUTAMATE DH (GLD) — requires NAD+ or NADP+ as coenzyme.

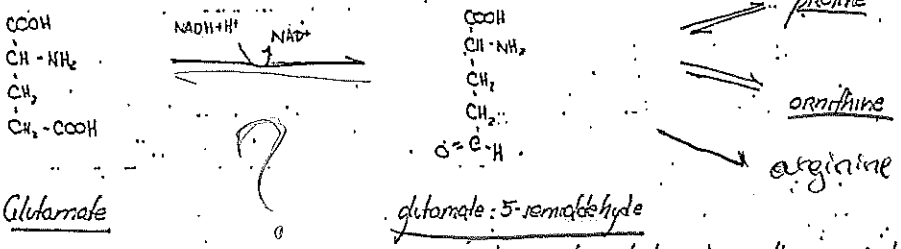


- Decarboxylation of glutamate - very active form in brain.



glutamate → γ-aminobutyric acid (GABA) → an inhibitory neurotransmitter in CNS

- Reversible reaction of glutamate



Glutamate → glutamate-5-semialdehyde → proline / ornithine / arginine

↳ intermediate in the synthesis and degradation of proline and arginine

"Utilization" of GLUTAMATE → can originate His, Pro, Gln, Arg and ornithine

- is a excitatory neurotransmitter
- can give γ-aminobutyrate, an inhibitory neurotransmitter
- intermediate of folate and glutathione.

51. Conversions of Glycine and Serine, the utilization in anabolic pathways (one-carbon units), (aminolevulinate, purine, creatine, conjugation to aromatic acid)

- **Glycine** = a nonessential and glucogenic aa (may accept 1C to form serine → pyruvate)
  (NH₂-CH₂-COOH)
  ↳ originates from serine or from CO_2, NH_3 and C_1 group

Secondary degradation pathways

REVERSIBLE REACTION → (unavailable)

Gly + CH_2OH-FH_4 ⇌ Ser + FH_4 → important source of 1C groups!

Gly: NH_2-CH_2-COOH

Ser: $CH_2(OH)$-$CH(NH_2)$-COOH

TRANSAMINATION

NH_2-CH_2-COOH + CH_3-C(=O)-COO⁻ ⇌ NH_2-$CH(CH_3)$-COOH + $O{=}C(H)$-COO⁻

Gly + pyruvate → Ala + glyoxylate

OXIDATIVE DEAMINATION

NH_2-CH_2-COOH —(FAD → $FADH_2$, NH_3)→ $O{=}C(H)$-COO⁻

Gly → glyoxylate

limited processes, the enzymes needed for them only act in peroxisomes!

- **MAJOR DEGRADATION PATHWAY** (in mitochondria): NH_2-CH_2-COOH + FH_4 ⇌ CO_2 + NH_3 + CH_2OH-FH_4 — catalyzed by GLYCINE SYNTHASE
  ↳ synthesis of 1 mol. glycine requires the cleavage of 3 mol. ATP

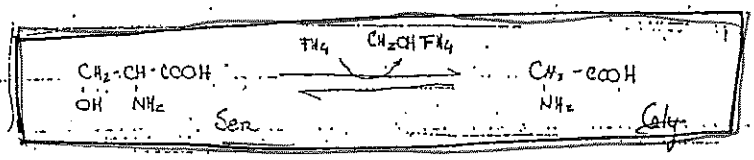
- **UTILIZATION OF GLYCINE** — synthesis of:
  - creatine (guanidino group (Arg) + Gly —(kidney)→ guanidinoacetate —(liver, SAM → SAH)→ creatine)
  - glutathione GSH
  - porphyrin — Aminolevulinic acid, the key precursor to porphyrins is biosynthesized from glycine and Succinoyl-CoA.
  - purine — Glycine provides the central C_2N subunit of all purines
  - glycine conjugates — of bile acids, aromatic acids (hippuric acids)

- **SERINE** = nonessential and glucogenic → DIRECT DEAMINATION TO PYRUVATE
  ↳ originates from 3-P-glycerate

CH_2-CH(OH)-COOH (Ser) —(H_2O)→ CH_2=C(NH₂)-COOH ↔ CH_3-C(=NH)-COOH —(H_2O, NH_3)→ CH_3-C(=O)-COO⁻ (pyruvate)

enamine ↔ imine

SERINE IS A GOOD SOURCE OF ONE-CARBON UNITS !



(the reverse reaction is not favorable)

→ constituent of phospholip.

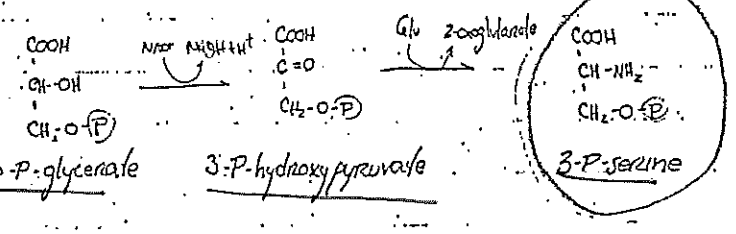
→ Decarboxylation of Ser gives ethanolamine, which will give choline by methylation

HO-CH2-CH2-NH2 ethanolamine

HO-CH2-CH2-N(+)(CH3)3 choline

Methylation

SYNTHESIS OF SERINE FROM GLUCOSE



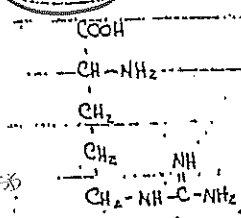
Utilization of Serine = synthesis of:

- phosphatidylserine
- ethanolamine → phosphatidylethanolamine
- choline → phosphatidyl-choline
- sphingosine → sphingomyelins

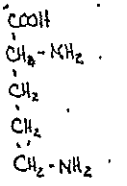
52 Conversions of arginine, utilization of the guanidine part. (biosynthesis of creatine, nitroxide formation)

ARGININE — but essential during tissue growths — nonessential and glucogenic → degraded to 2-oxoglutarate

IN THE LIVER → in the ureosynthesis cycle



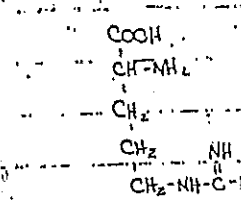
arginine



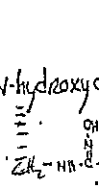
ornithine

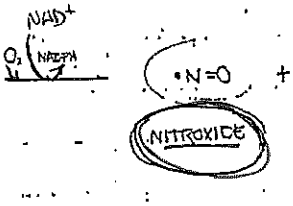
→ then ornithine is degraded by transamination of the 5-amino group to glutamate-5-semialdehyde → glutamate → 2-oxoglutarate

NITROXIDE (NO) FORMATION



(N-hydroxyarginine)





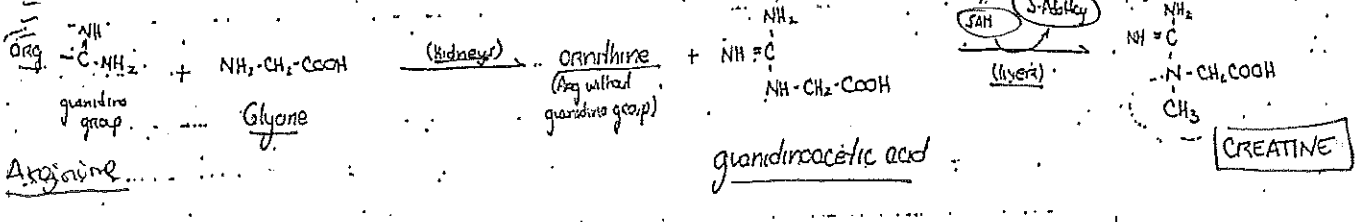
CITRULLINE

Arg

↳ this reaction is catalyzed by NITROXIDE SYNTHASE (NOS), employing 5 REDOX COFACTORS (NADPH, FAD, FMN, Cytochrome, BH_4)?

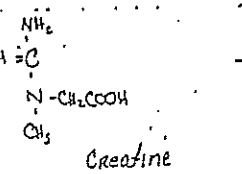
↳ NOS has 3 isoenzymes: endothelial NOS (responsible for vasodilation and inhibition of platelet aggregation), neuronal NOS (modulates events on synapses) and NOS in phagocytes.

SYNTHESIS OF CREATINE → Arginine is the donnor of AMIDINO GROUP (guanidine)

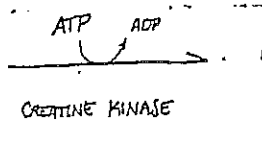


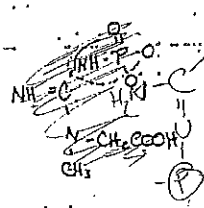
Arginine

Form of creatine in muscles:



Creatine

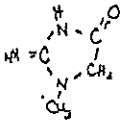




(muscle phosphogen)

non-enzymatic dehydration

H_2O

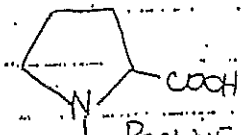


CREATININE

(excreted into urine)

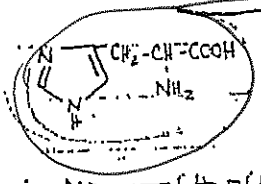
53. Metabolites and specialized products of proline, histidine, and tryptophan significant in metabolism.

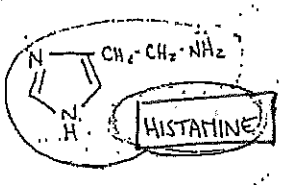
- **Proline** = nonessential and glucogenic aa
  - from glutamate — gives glutamate — 2-oxoglutarate
  - hydroxylation → 4-HYDROXYPROLINE — occures only in collagen; it's formed by hydroxylation of prolyl residues in procollagen.



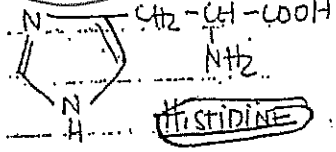
PROLINE

- **Histidine** = nonessential and glucogenic (but essential for children = in tissue growth)
  - gives glutamate and 2-oxoglutarate
  - does not undergo transamination; it is deamined by deaturation (conversion from saturated to unsaturated) → urocanic acid





HISTAMINE — is a biogenic amine stored within granules of basophils and mast cells and within synaptosomes of certain CNS neurons.

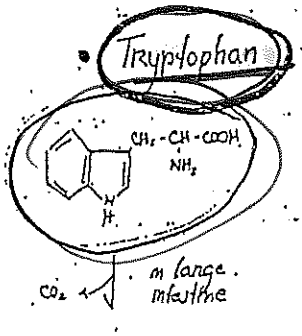


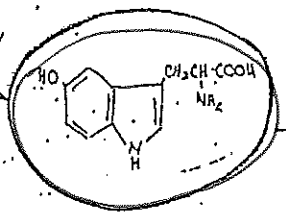
HISTIDINE

When released, histamine induces complex physiological and pathological effects, including IMMUNOLOGICAL REACTIONS (symptoms of allergies affecting skin and airways), GASTRIC ACID secretion, smoth muscle contractions ... (e.g. BRONCHOCONSTRICTION), and profound VASODILATION.

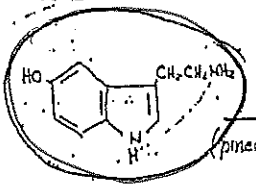
ANTIHISTAMINICS - drugs which antagonize the effects of histamine.

- **Tryptophan** — essential and glucogenic and ketogenic aa
  - degradation gives alanine (→ pyruvate) and acetoacetate

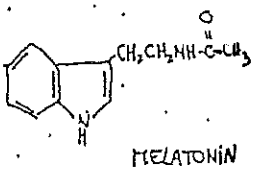




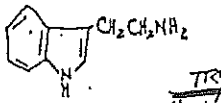
5-HYDROXYTRYPTOPHAN



SEROTONIN (pineal gland)



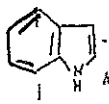
MELATONIN



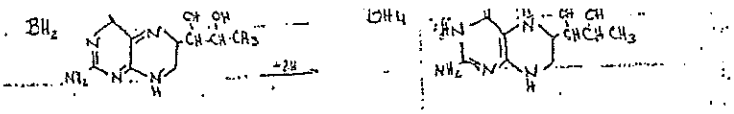
TRYPTAMINE (biogenic amine)

→ Serotonin is a neurotransmitter in CNS and a local hormone of enterochromaffin cells (intestinal mucosa)

→ secretion of Melatonin from the pineal gland is increased in darkness. Its physiological roles are not yet clear but they involve CHRONOBIOLOGIC RHYTHMS.



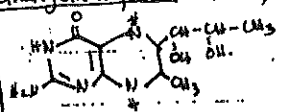
INDOLE

Coenzyme: 

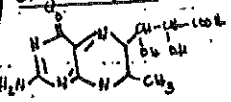
(54) Hydroxylation of phenylalanine, tyrosine and tryptophan. (Coenzyme, phenylketonuria, DOPA, serotonin).

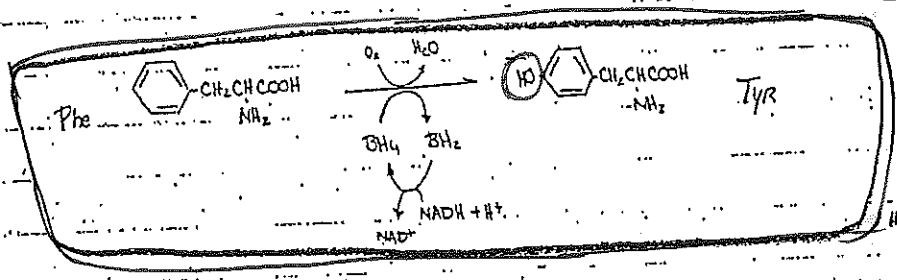
- HYDROXYLATION OF PHENYLALANINE TO TYROSINE – catalyzed by a monooxygenase: PHENYLALANINE HYDROXYLASE, for which the coenzyme is BH_4.

tetrahydrobiopterin: (BH_4)



dihydrobiopterin:



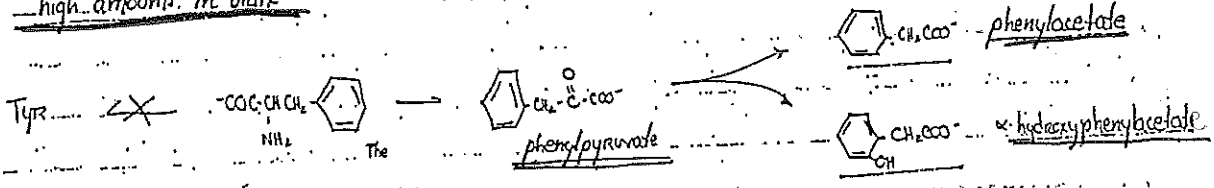


PHENYLKETONURIA → defect in PHENYLALANINE HYDROXYLASE (I blocks the reaction above.)

↳ has to be recognized through compulsory screening in newborns and treated with a low-Phe diet till the age of 8-10.

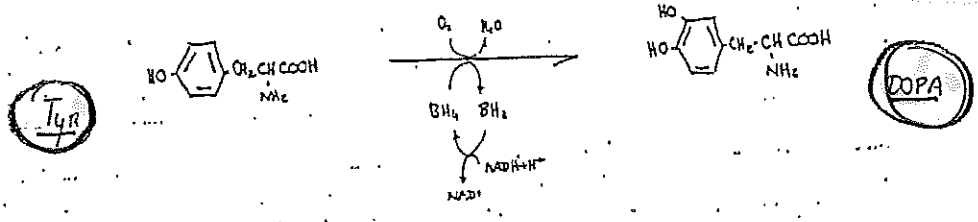
↳ Untreated PKU causes mental retardation!

Besides high levels of Phe in the blood, other metabolites are produced and excreted in high amounts in urine:

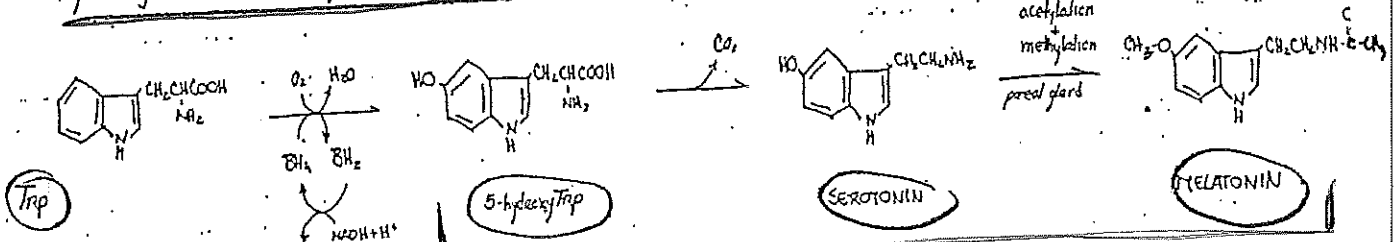


- MALIGNANT HYPERPHENYLALANINAEMIAS (III and IV) – lack of BH_4 or impedive reduction of BH_2 to BH_4.
  ↳ due to defective BH_4 synthesis from guanylate

- HYDROXYLATION OF TYROSINE TO DOPA (dihydroxyphenylalanine) – by TYROSINE 3-HYDROXYLASE



- HYDROXYLATION OF TRYPTOPHAN TO 5-HYDROXYTRYPTOPHAN – by TRYPTOPHAN 5-HYDROXYLASE



equal to ~~phenylalanine~~ Transformations previous page

SEROTONIN = neurotransmitter in CNS and local hormone of argentaffin cells of the intestine.

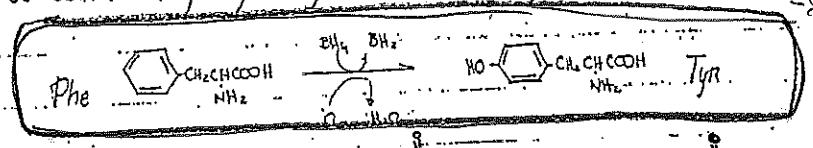
MELATONIN – its secretion, by the pineal gland, is increased in darkness. Its physiological roles are not yet clear, but they involve CHRONOBIOLOGIC RHYTHMS.

55 Catabolism of tyrosine; metabolic disorders of tyrosine catabolism.

Tyrosine → nonessential and glucogenic and ketogenic → gives fumarate and acetoacetate

↳ it can be obtained by HYDROXYLATION OF PHENYLALANINE:

CATABOLISM



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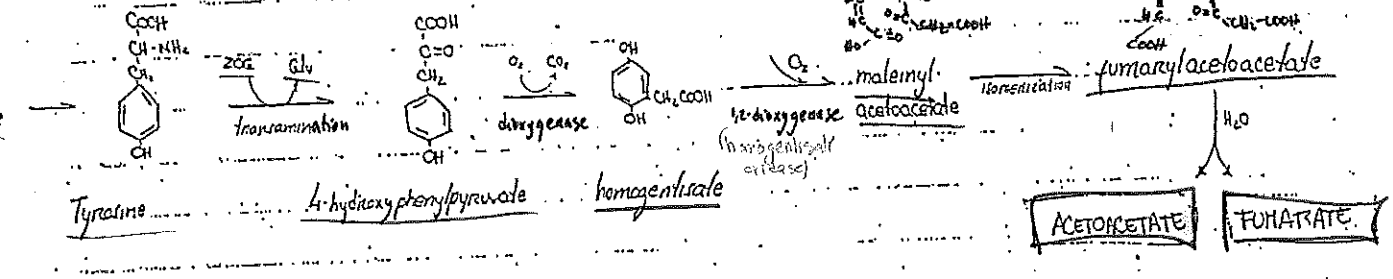
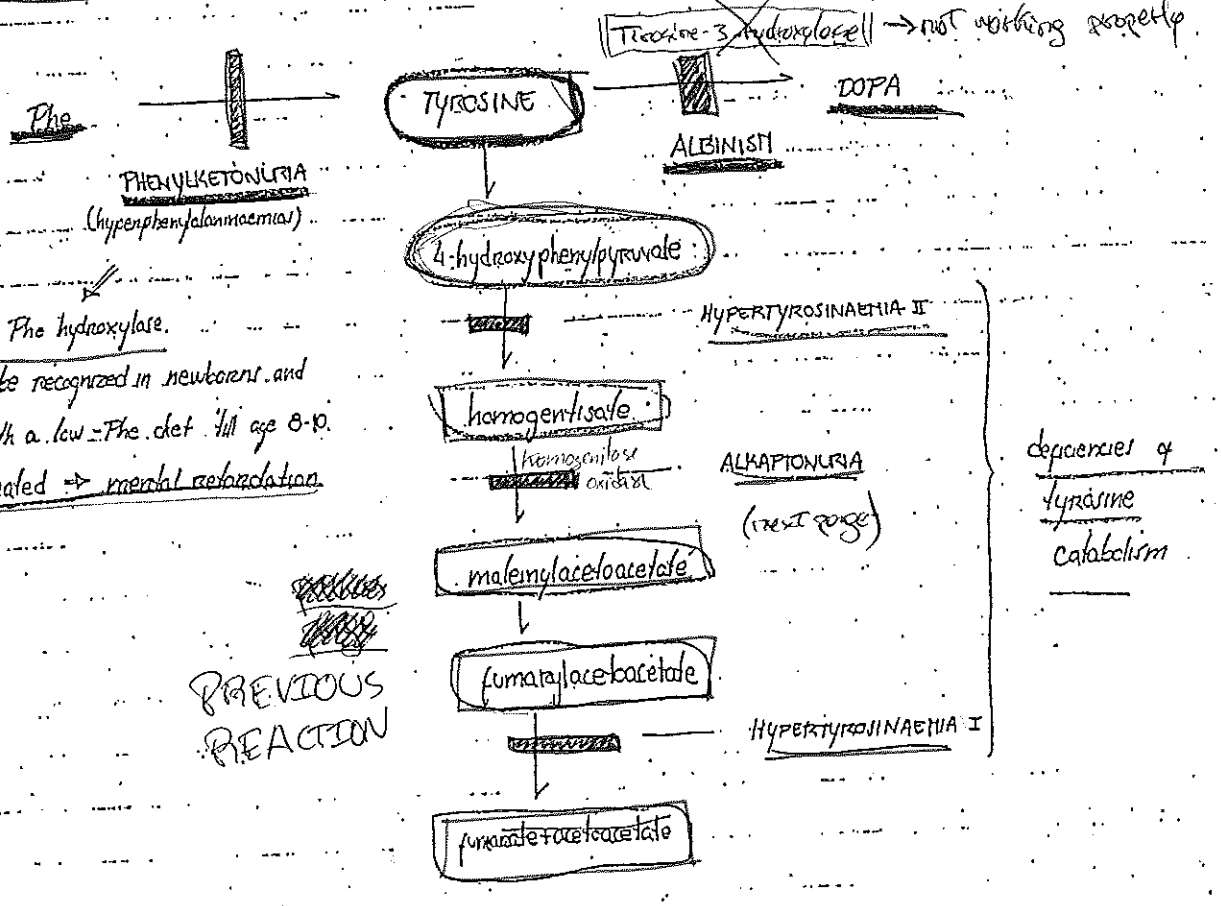


Tyrosine → 4-hydroxyphenylpyruvate → homogentisate

ACETOACETATE + FUMARATE

INBORN METABOLIC DISORDERS OF PHENYLALANINE CATABOLISM



deficiencies of tyrosine catabolism

defect in Phe hydroxylase.

PKU has to be recognized in newborns and treated with a low-Phe diet till age 8-10.

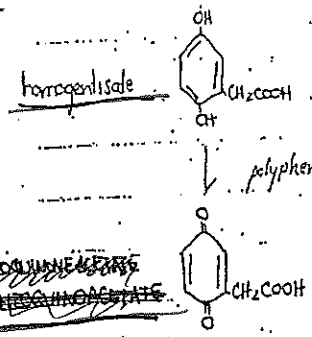
↳ if untreated → mental retardation

HYPERTYROSINAEMIAS → in many forms. Caused by a deficit of enzymes which catalyze either: the transamination of Tyr; oxidation of p-hydroxyphenylpyruvate, or hydrolysis of fumarylacetoacetate.

A low Tyr-diet may be v. useful!

ALKAPTONURIA — inborn deficit of HOMOGENTISATE OXIDASE; characterized by homogentisate excretion in urine.

→ THE ONLY manifestation in youth: darkening of urine on the air. Later (20-30 yrs), deposits of pigments in the connective tissue begin to appear (OCHRONOSIS = bluish colouring of the sclerae, the ears and nasal cartilages, etc.) which are the cause of deforming arthritis.



↓ polyphenol oxidase or O_2 in the air

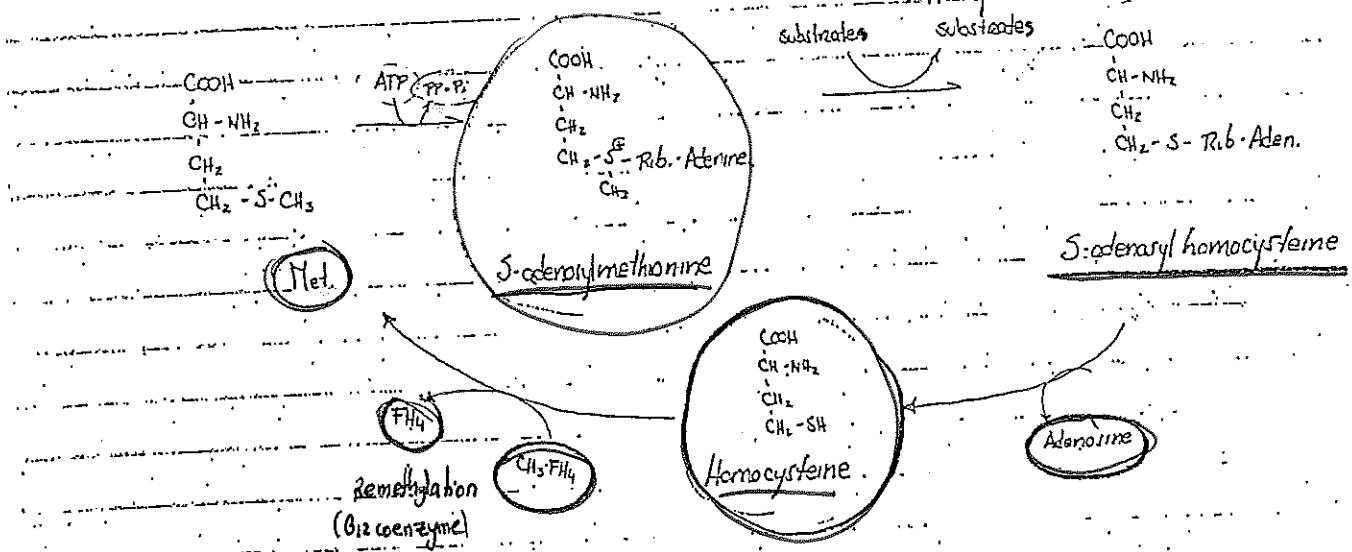
maleylacetoacetate

↓

polymerization to black-brown pigments

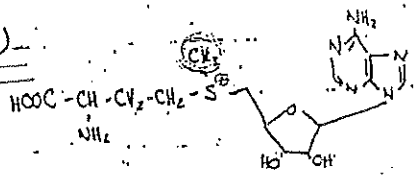
56 Metabolism of sulphur-containing amino acids (Met, Cys) Selenocysteine

- METHIONINE → essential and glucogenic → yields succinyl-CoA

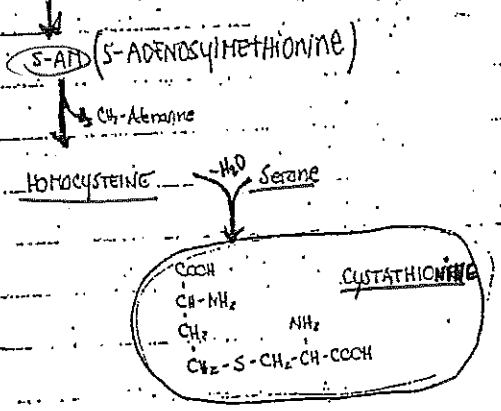


METHIONINE IS THE COMMON METHYL DONOR IN THE CELL!

↳ activated methionine: S-ADENOSYLMETHIONINE (S-AM)

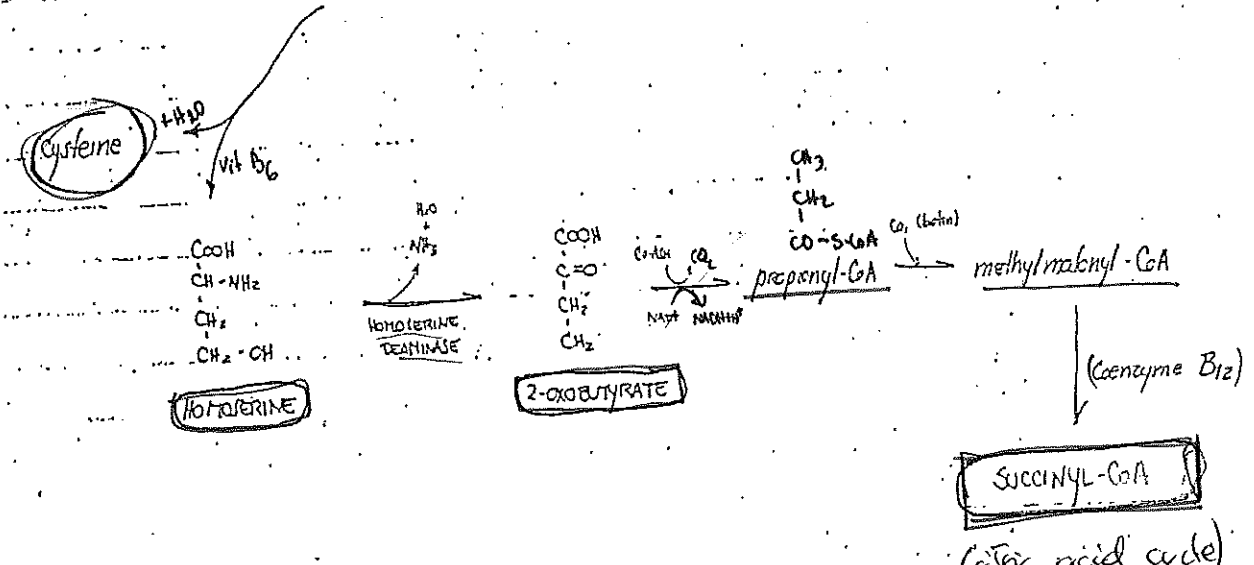


CATABOLISM OF METHIONINE



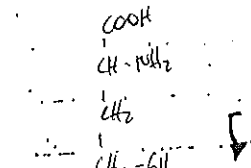
STEPS OF CATABOLISM:

1. demethylation to homocysteine
2. transsulfuration with serine to homoser. and cysteine
3. conversion to 2-oxobutyrate, propionyl-CoA and succinyl-CoA



HOMOCYSTEINE → important intermediate of methionine metabolism.

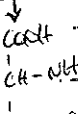
↳ excess of homocysteine (failure in its metabolism to methionine or homoserine) results in injury of endothelial cells (high production of ROS, lipoperoxidation) and decreased vitality of blood platelets...



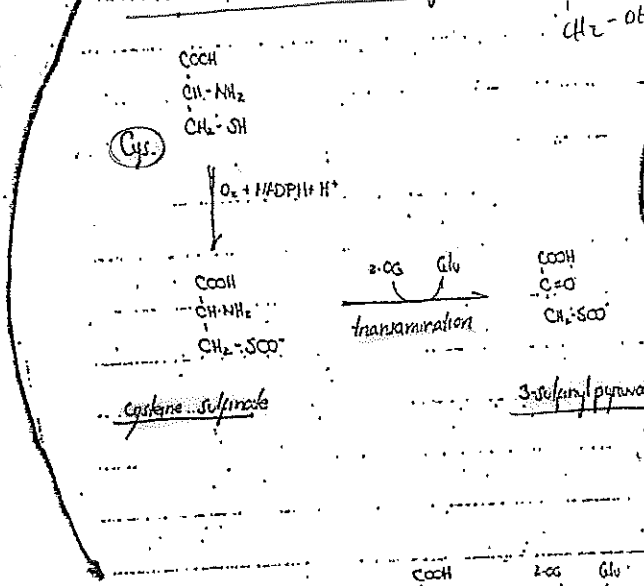
• high [homocysteine] in blood plasma is a marker of cardiovascular disease — a risk factor for atherosclerosis (which is quite independent on the [x] of cholesterol)

CYSTEINE — nonessential and glucogenic → converted to pyruvate.

↳ synthesis from Ser ; (+ alternative from Met)

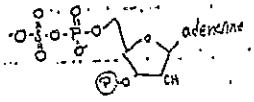


MAJOR CATABOLIC PATHWAY:

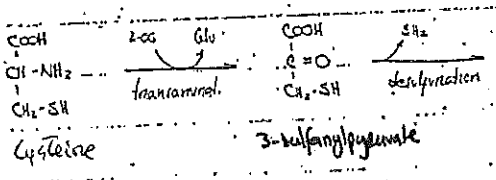


PROTON-PRODUCING PROCESS - the catabolism of sulfur-containing aa slightly acidifies the body.

will either be excreted in urine or used as sulfate donors after activation to PAPS (3-P-adenosyl-5-P-sulfate)



An ALTERNATIVE catabolic pathway:

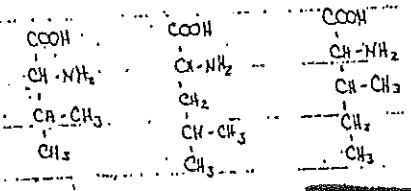


Utilization of METHIONINE → as S-AM (S-ADENOSYLMETHIONINE) for methylations

Utilization of CYSTEINE - synthesis of...
- cysteamine (constituent of CoA)
- glutathione → mercapturic acids
- taurine (conjugation with bile acids)
- PAPS - sulfate donor for sulfate esters

(57) Significance and the basic features of the branched-chain amino acids catabolism

BRANCHED-CHAIN AMINO ACIDS → ALL ARE ESSENTIAL



VALINE (glucogenic) | LEUCINE (Ketogenic) | ISOLEUCINE (both gluco- and Ketogenic)

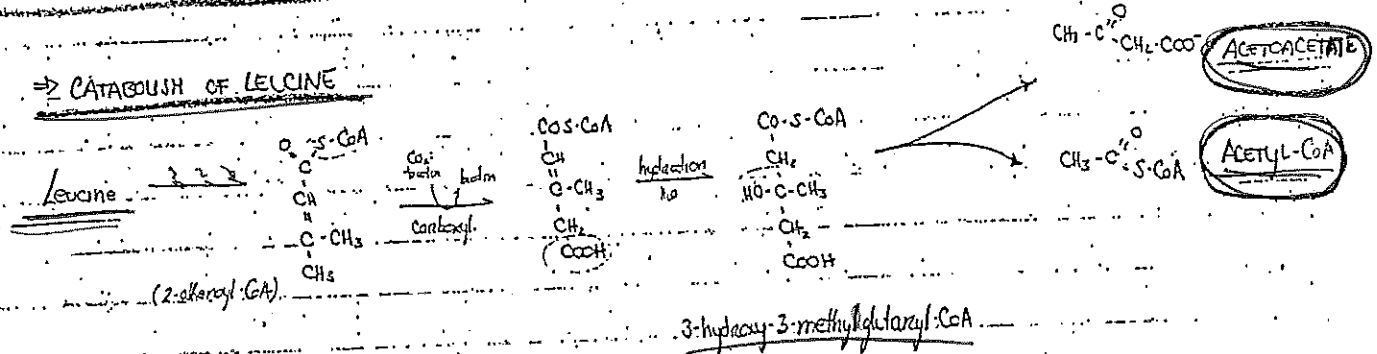
CATABOLISM (in the muscles):

3-INITIAL COMMON CATABOLIC REACTIONS

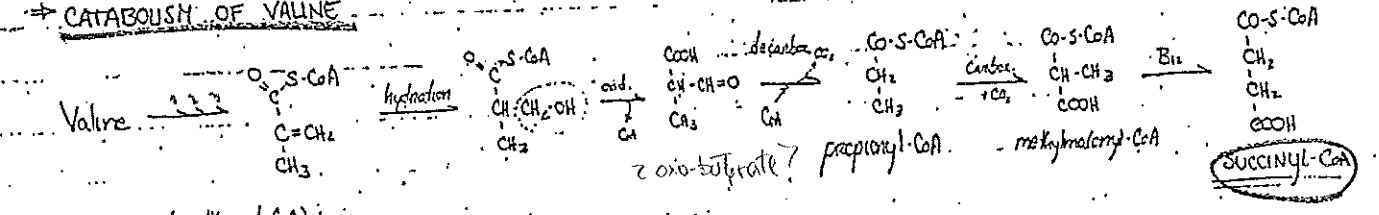
1. TRANSAMINATION – to corresponding 2-oxoacids.
2. OXIDATIVE DECARBOXYLATION – catalyzed by 2-oxoacid DH, producing acyl-CoA thioesters
3. SECOND DEHYDROGENATION – between carbons α and β, catalyzed by flavin DH, producing 2-alkenoyl-CoA thioesters

... AFTER THESE 3 REACTIONS, THE CATABOLISMS DIFFER ...

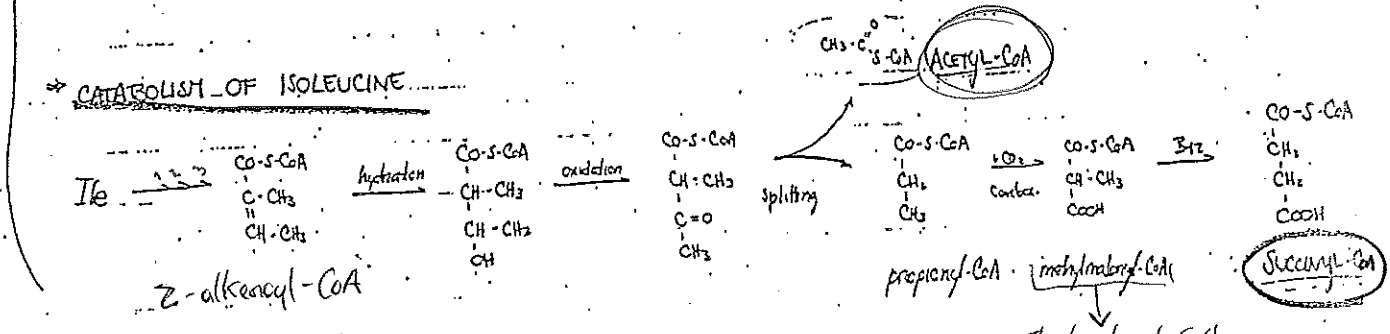
⇒ CATABOLISM OF LEUCINE



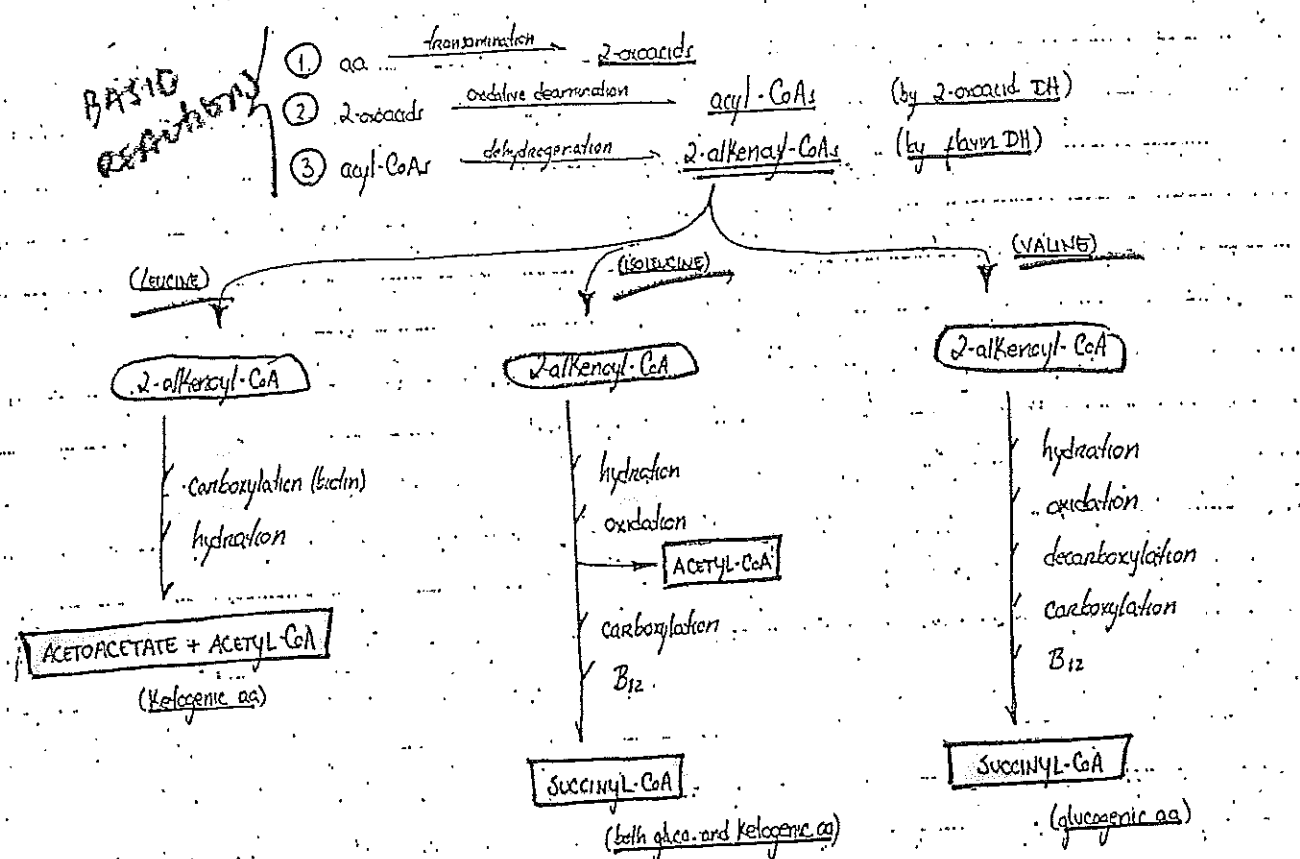
⇒ CATABOLISM OF VALINE



⇒ CATABOLISM OF ISOLEUCINE



SUMMARY - basic features of catabolism



BRANCHED-CHAIN aa → are primarily metabolized by skeletal muscles, rather than by the liver.

→ after ingesting a meal (well-fed state) we call it the absorptive state. In this state there is an increased uptake of branched-chain aa by the muscles, which will be used for protein synthesis and as sources of energy!

non-essential, but in periods of tissue growth they must be supplemented in diet

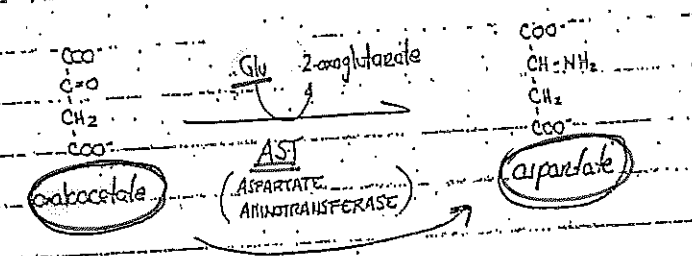
58. Nutritionally essential amino acids. Biosynthesis of non-essential aa: (Asp, Glu, Ser, Pro, Cys, Tyr)

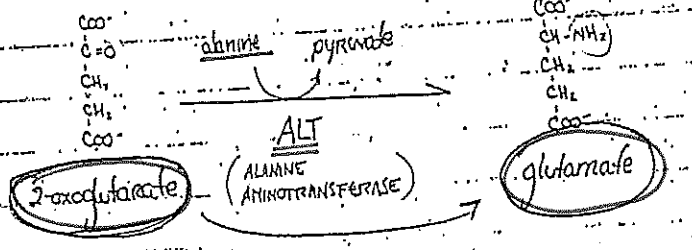
ESSENTIAL aa:
1. Threonine
2. Methionine
3. Lysine
4. Valine
5. Leucine
6. Isoleucine
7. Histidine (for children)
8. Phenylalanine
9. Tryptophan

Help In Learning These Little Molecules Proves Truly Valuable
1 2 3 4 5 6 7 8 9

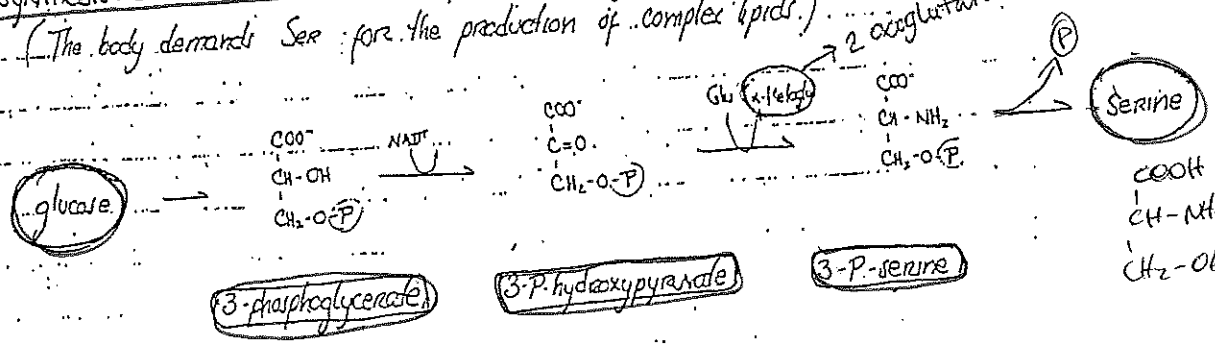
NON-ESSENTIAL aas: are synthesized from intermediates of metabolism or from essential aas (Tyr and Cys)
- ALANINE – formed by transamination of pyruvate
- ASPARTATE, ASPARAGINE – from oxaloacetate
- GLUTAMATE, GLUTAMINE, PROLINE, ARGININE – from 2-oxoglutarate
- SERINE, GLYCINE, CYSTEINE, HISTIDINE – from glycolysis

1. BIOSYNTHESIS OF ASPARTATE AND GLUTAMATE → synthesized by transfer of an amino group to the α-Keto acids oxaloacetate and α-ketoglutarate, respectively:

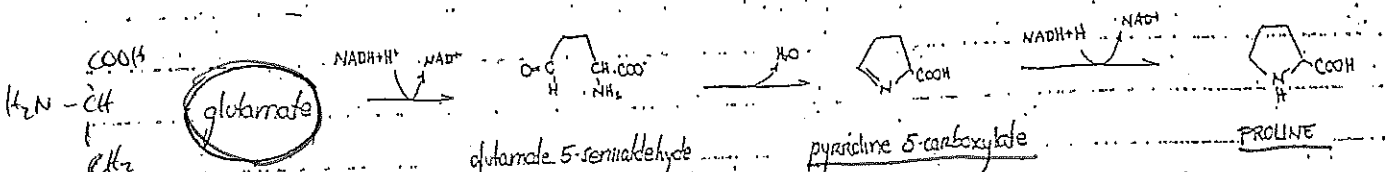




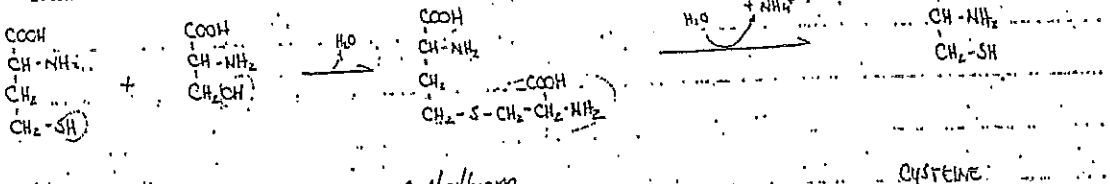
2. BIOSYNTHESIS OF SERINE → arises from 3-P-glycerate, an intermediate of glycolysis: (The body demands Ser for the production of complex lipids.)



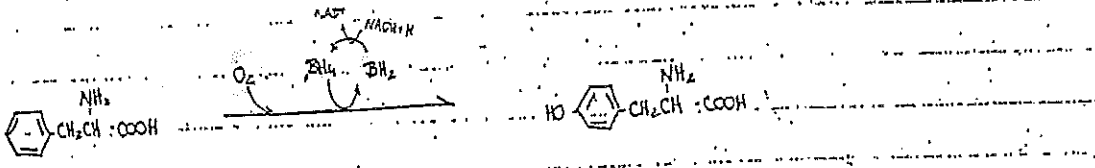
③ BIOSYNTHESIS OF PROLINE: glutamate is converted to proline by cyclization and reduction reactions.



④ BIOSYNTHESIS OF CYSTEINE: Homocysteine (which derives from methionine) combines with serine, and the product will be hydrolyzed to give cysteine

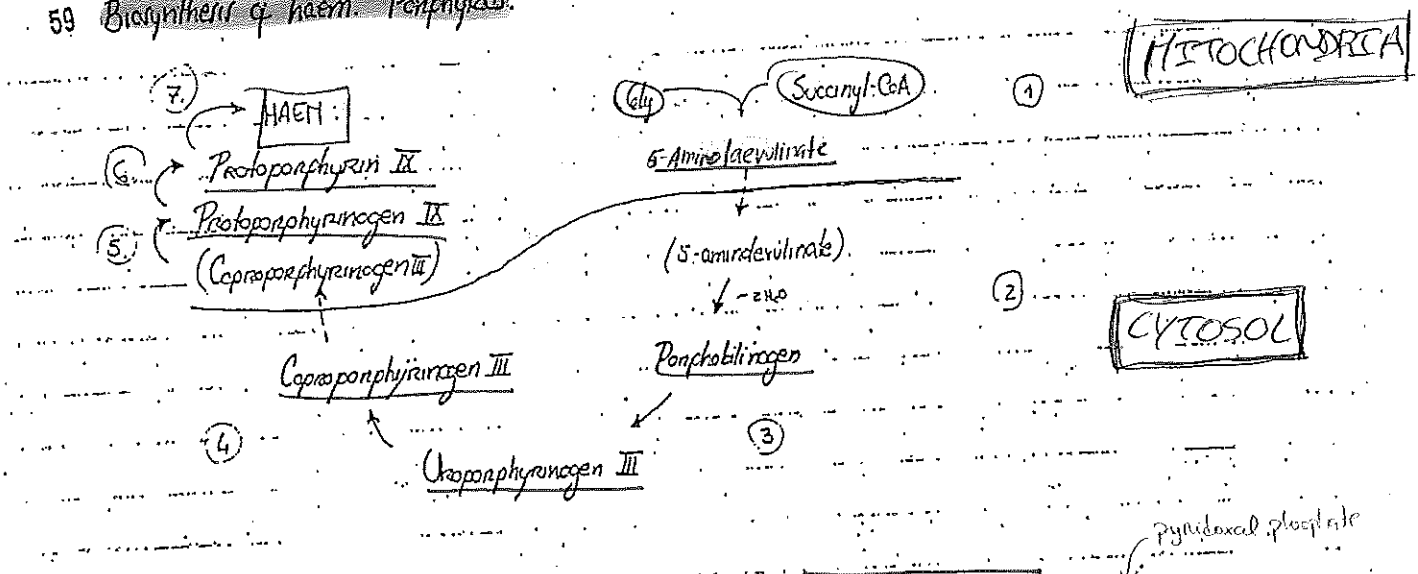


⑤ BIOSYNTHESIS OF TYROSINE - from phenylalanine by phenylalanine hydroxylase. This reaction requires the coenzyme BH_4, which will be oxidized to BH_2 (dihydrobiopterin)



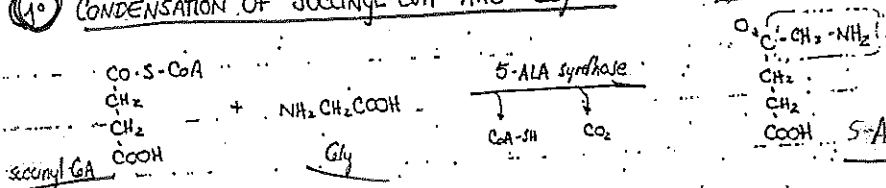
(Question 55)

Biosynthesis of haem. Porphyrias.



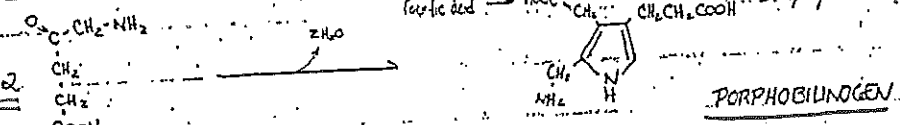
1° CONDENSATION OF SUCCINYL-CoA AND GLYCINE = IN MITOCHONDRIA (PLP as cofactor)

Pyridoxal phosphate



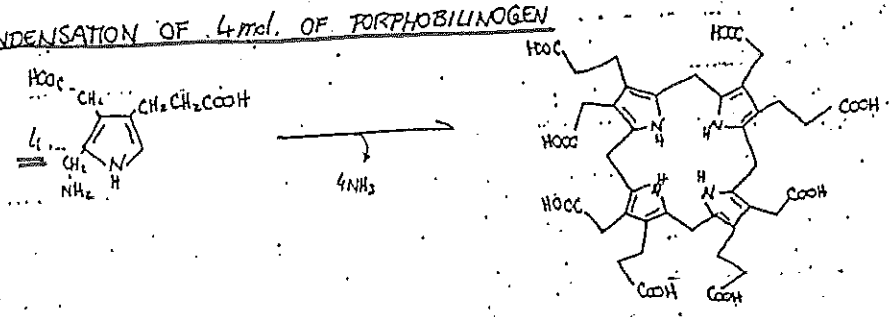
⇒ transported to cytosol

2° CONDENSATION OF 2 MOL. OF 5-Aminolaevulinate — IN CYTOSOL



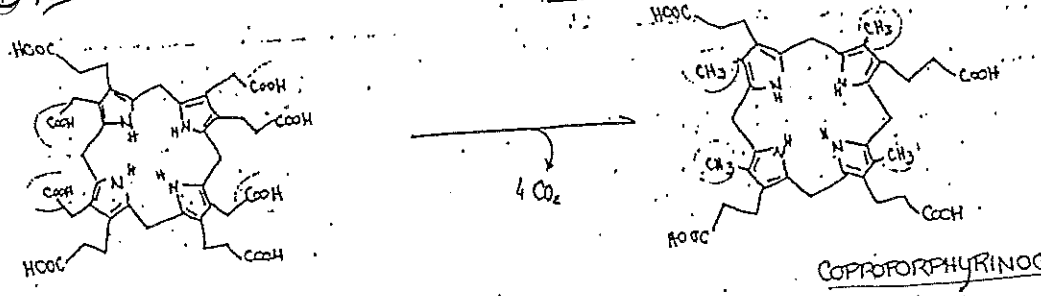
PORPHOBILINOGEN

3° CONDENSATION OF 4 mol. OF PORPHOBILINOGEN



UROPORPHYRINOGEN III

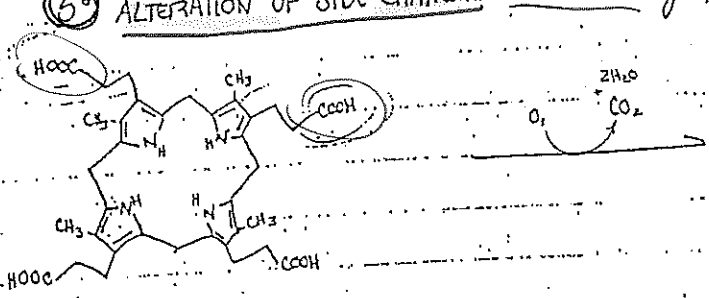
4° ALTERATIONS IN THE SIDE CHAINS: 4 -CH2-COOH groups are substituted by 4 -CH3 groups

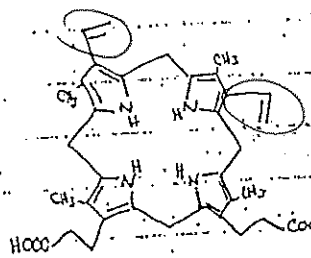


COPROPORPHYRINOGEN III

↳ will be transported into mitochondria

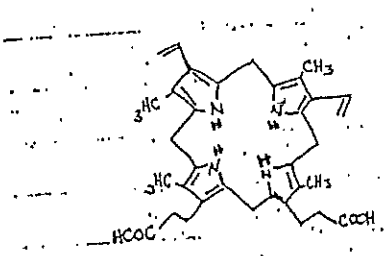
5° ALTERATION OF SIDE CHAINS :- 2 $-CH_2CH_2COOH$ groups are substituted by 2 vinyl groups

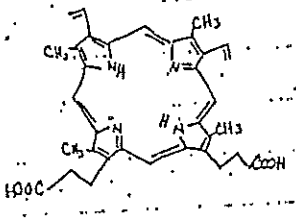




PROTOPORPHYRINOGEN IX (colourless).

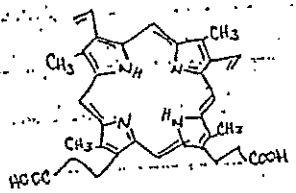
6° DESATURATION OF THE PORPHYRIN RING (protoporphyrinogen IX → protoporphyrin IX)

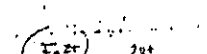


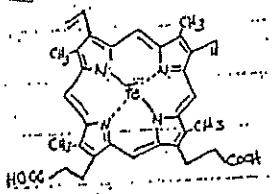


PROTOPORPHYRIN IX (colored)

7° CHELATION OF Fe^{2+} ION → HAEM







HAEM = prosthetic group of Hb, myoglobin, cytochromes, catalase and peroxidase

→ proteosynthesis of globin α and β-chains

→ myoglobin, cytochromes, catalase

→ HAEMOGLOBIN MONOMERS (1 Fe)

→ HAEMOGLOBIN (4 Fe) $\alpha_2\beta_2$

HAEM is synthesized:
- 15% in the liver
- 85% in erythroid cells

PORPHYRIAS - genetic diseases characterized by defects in the Haem synthesis

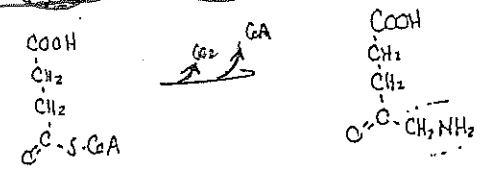
→ there is often overproduction of porphyrins and their percursors :— increased excretion of porphyrins in urine and feces.

SOME SYMPTOMS:
- skin lesions in exposure to sunlight
- disturbances of erythropoiesis
- disturbance of liver functions
- neurophsychiatric disturbances

SUMMARY

MITOCHONDRIA

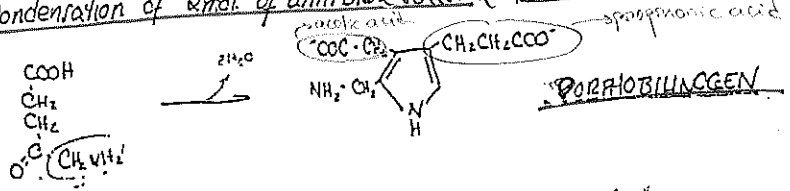
1. Condensation of Glycine and Succinyl-CoA — IN MITOCHONDRIA



5-AMINOlevulinate → transported into cytosol

CYTOSOL

2. Condensation of 2mol of aminolaevulinate — IN CYTOSOL



PORPHOBILINOGEN

3. condensation of 4 mol. of phorphobilinogen $\xrightarrow{4NH_3}$ UROPORPHYRINOGEN III (4 acetic acid R-) (4 propionic acid R-)

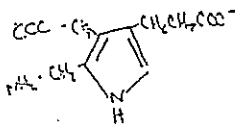
4. Side chain alterations $\xrightarrow{CO_2}$ COPROPORPHYRINOGEN III (4 methyl R-) (4 propionic acid R-)

(coproporphyrinogen is transported into mitochondria)

5. side chain alterations $\xrightarrow[2H_2O+CO_2]{O_2}$ PROTOPORPHYRINOGEN IV (4 methyl R-) (2 propionic R- + 2 vinyl R-) — IN MITOCHONDRIA

6. deraturation of the porphyrin ring $\xrightarrow[3H_2O]{3O_2}$ PROTOPORPHYRIN IX

7. Chelation of the Fe ion $\xrightarrow[2H^+]{Fe^{2+}}$ HAEM



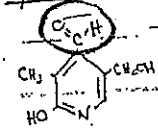
(60) Decarboxylation of amino acids (coenzyme, some physiologically important reaction products and significance of them).

Several aa are broken down by decarboxylation, this reaction gives rise to BIOGENIC AMINES, which have various functions (components of biomolecules, signaling substances,

examples:
- ethanolamine – component of phospholipids
- cysteamine and β-alanine – components of Coenzyme A and pantetheine.
- γ-aminobutyrate – important neurotransmitter derived from Glu.
- dopamine – neurotransmitter and percursor of adrenaline and noradrenaline (CATECHOL AMINES)
- Serotonin – synthesized from Trp, it's a neurotransmitter in CNS and a local hormone of argentaffin cells of the intestine.
- Tyramine
- HISTAMINE: causes profound VASODILATATION. (Histamine for example)

→ Monoamines are inactivated into aldehydes by AMINE-OXIDASE (MAO). MAO inhibitors thus play an important role in pharmacological interventions in neurotransmitter metabolism.

PYRIDOXAL-PHOSPHATE – coenzyme involved in amino acid decarboxylations (also transaminations and dehydratations)



→ the aldehyde group is rarely found free; it is generally bound to a lysine residue as an aldimine.

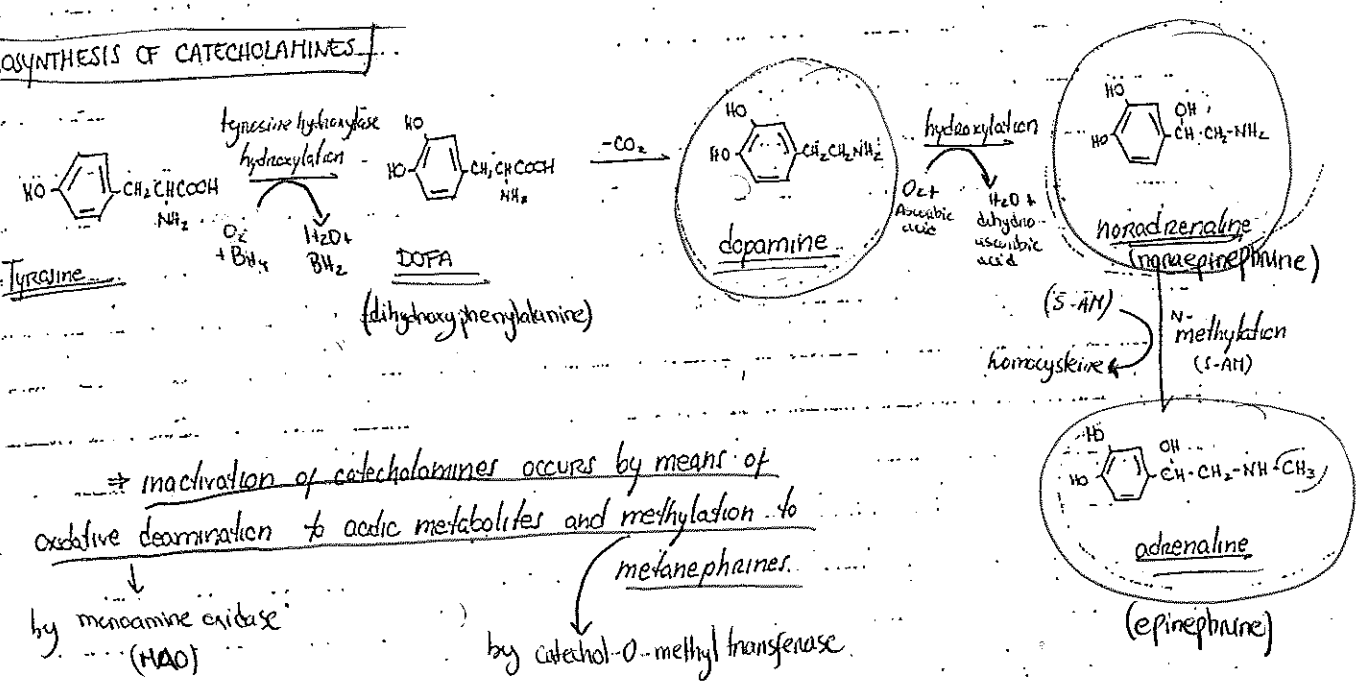
CATECHOLAMINES { DOPAMINE, NORADRENALINE, ADRENALINE

61. Biosynthesis of catecholamines

CATECHOLAMINES ⇒ DOPAMINE, NORADRENALINE and ADRENALINE (epinephrine) are biologically active amin synthesized by adrenal medulla

functions:
- Dopamine and noradrenaline are neurotransmitters in the brain and the autonomic nervous syste
- Outside the nervous system, adrenaline and noradrenaline act as regulators of lipid and carbohydrates metabolism.
- Adrenaline and noradrenaline are released from the adrenal medulla in response to fright, exercise, cold, and low levels of blood glucose. They increase the degradation of glycogen and triacylglycerol, as well as an increase in blood pressure and output of the heart.
  ↳ these effects are part of a COORDINATED RESPONSE to prepare the individual for emergencies ⇒ so-called "fight-or-flight" reactions.

BIOSYNTHESIS OF CATECHOLAMINES



Tyrosine — DOPA (dihydroxyphenylalanine) — dopamine — noradrenaline (norepinephrine) — adrenaline (epinephrine)

⇒ inactivation of catecholamines occurs by means of oxidative deamination to acidic metabolites and methylation to metanephrines.
- by monoamine oxidase (MAO)
- by catechol-O-methyl transferase

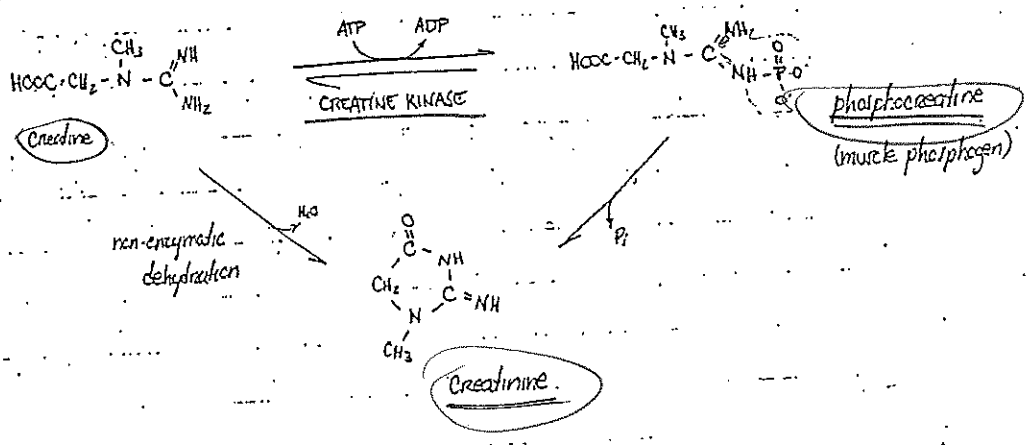
62. Biosynthesis of creatine, function in muscles, conversion and excretion.

CREATINE PHOSPHATE (phosphocreatine) – phosphorylated derivative of creatine found in muscles

↓

high-energy compound that reversibly donates a (P) group to ADP to form ATP; the way it maintains the intracellular level of ATP during the first few minutes of intense muscular contraction!.

Creatine in skeletal muscle:

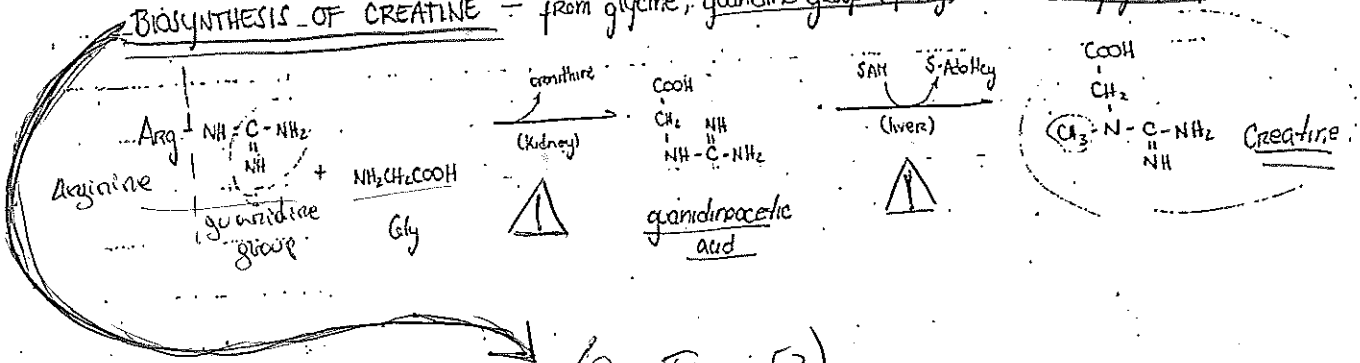


DEGRADATION OF CREATINE: Creatine and creatine-P form Creatinine, which is excreted in the urine.

(the amount of creatinine excreted gives us the proportion of creatine-P → estimation of muscle mass

∴ Also, any rise in blood creatinine indicates a kidney malfunction.

BIOSYNTHESIS OF CREATINE – from glycine, guanidine group (of Arg) and a methyl group of SAM:



(Question 52)

thioredoxin → small protein that supplies $2H^+$ for the deoxygenation of ribosyl

63. Basic steps in purine and pyrimidine nucleotide synthesis (the compounds donating the nitrogen and carbon atoms of the heterocyclic ring) and regulation.

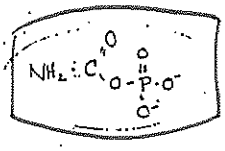
→ PURINE AND PYRIMIDINE nucleotides are SYNTHESIZED DE NOVO and used in biosynthesis of nucleic acids.

→ The allosteric feedback control mechanisms balance the synthesis of all purine and pyrimidine ribonucleotides and deoxyribonucleotides

→ Glu and Asp are donors of amino groups for the both syntheses. Asp also supplies 3C for pyrimidine ring.

BIOSYNTHESIS OF PYRIMIDINE NUCLEOTIDES de novo — Uridine, cytidine, thymidine

→ the heterocycle ring of pyrimidine bases is synthesized from: carbamoyl-P and aspartate. Orotic acid is then attached to the ribosyl 5-P

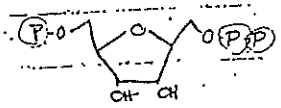


formed in cytosol:

Glutamine + HCO_3^- —(2ATP, 2ADP, Pi)→ carbamoyl-P + Glt

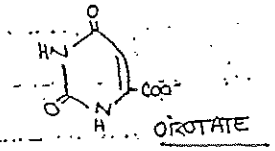
catalyzed by carbamoyl-P synthase II, one of the 3 activities of the protein DIHYDROOROTATE SYNTHASE that catalyzes the next 2 reactions:

supplied by PHOSPHORIBOSYL DIPHOSPHATE (PRPP)

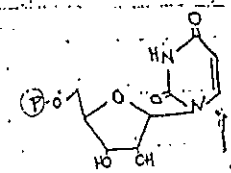


Ribose-5-P + ATP —(PRPP synthase)→ PRPP + AMP

carbamoyl-P + aspartate —(Pi)→ carbamoyl aspartate —(H_2O)→ dihydroorotate —(dihydroorotate DH; NAD+ → NADH+H+)→ OROTATE



OROTATE —(PRPP, −Pi; uridine phosphate synthase)→ uridine-5-P —(CO_2)→ URIDINE 5-PHOSPHATE (UMP)

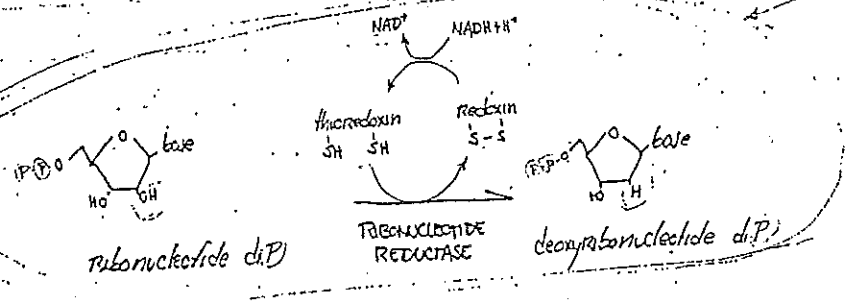


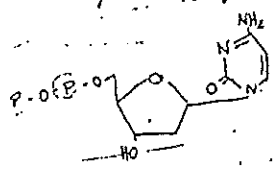
will be converted to other pyridine nucleoti

CONVERSION OF URIDINE TO CYTIDINE

UMP —(ATP)→ UDP —(ATP)→ UTP —(glutamine-dependent reaction; 4-amination; ATP)→ CTP —(ATP)→ CDP —(thioredoxin reduced; 2-reduction)→ dCDP —(ATP)→ dCTP

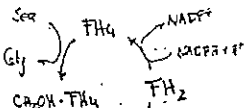
(Uridine monoP) (U-diP) (U-triP) (cytidine ribonucleosides) (cytidine 2-deoxyribonucleotides)



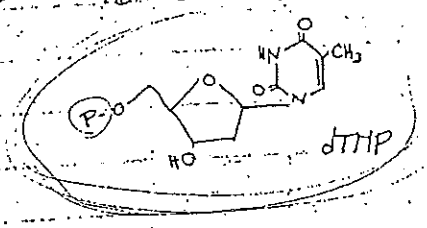


2-deoxycytidine diphosphate = dCDP

CONVERSION OF URIDINE TO THYMIDINE



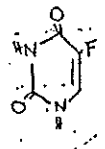
UMP $\overset{ATP}{\rightleftharpoons}$ UDP $\xrightarrow{2\text{-reduction}}$ dUDP $\overset{ATP}{\rightleftharpoons}$ dUMP $\xrightarrow{\text{THYMIDYLATE SYNTHASE}}$ TMP $\rightleftharpoons$ TDP $\rightleftharpoons$ TTP



INHIBITORS — of THYMIDYLATE SYNTHASE or FH_2

They stop division in rapidly dividing cells so they are effective ANTI-TUMOR DRUGS AND ANTISUPRESSANTS:

- AMINOPTERIN and METHOTEXATE = anti-folate drugs - competitive inhibitors of FH_2 reductase
- TRIMETHOPRIM - another folate analog - inhibits FH_2 reductase. Has antibacterial and antiprotozoal activity
- FLUOROURACIL - converted to fluorodeoxyuridylate that inhibits irreversibly thymidylate synthase, acting as normal substr



⇒ REGULATION OF PYRIMIDINE N. SYNTHESIS - regulated by feedback inhibition, UCT and CTP act as allosteric inhibitors! ATP and PRPP stimulate the biosynthetic pathway

↳ dATP or ATP signal an abundance of deoxyribonucleotides to RIBONUCLEOTIDE REDUCTASE, diminishing its activity.... When deoxyribonucl. are missing, it triggers the reduction of UDP and C

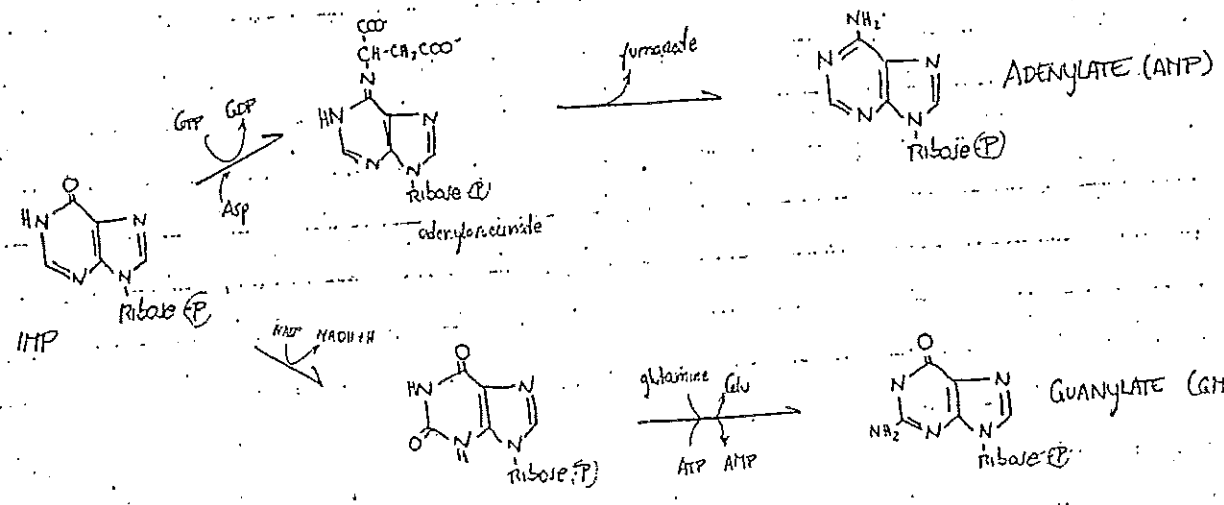
↳ This pattern of regulation supplies the appropriate balance of deoxyribonucleotides for DNA synth

BIOSYNTHESIS OF PURINE NUCLEOTIDES - de novo:

Starting from PRPP, and with the addition of 2 glutamine, glycine, Methenyl-FH_4, CO_2, aspartate, formyl-FH_4 in a sequence of 10 REACTIONS catalyzed by 6 enzymes, we obtain.... INOSINE 5-PHOSPHATE (IMP)

↓

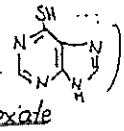
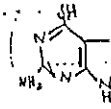
... IMP will be transformed to adenosine 5-P (AMP) or guanosine 5-P



SCAVENGER PATHWAYS – Reactions through which extrahepatic cells reconvert a part of free purine bases released from nucleotides (mostly hypoxanthine and guanine) again to nucleotides, so the bases don't have to suffer further degradation.

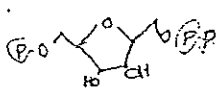
enzymes: 5-nucleotidases and nucleoside phosphorylases

INHIBITORS OF PURINE NUCLEOTIDES SYNTHESIS – can be also of limited use in cancer chemotherapy and as immunosuppressants.

- Glutamine analogs
- Purine analogs – 6-mercaptopurine  , 6-thioguanine  , azathioprine
- Anti-folate drug (folate analog) – methotrexate

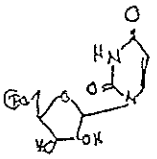
REGULATION OF THE PURINE NUCLEOTIDE BIOSYNTHESIS – by positive and negative feedback

The committed step in the purine synthesis is the conversion of PRPP into phosphoribosylamine the enzyme (glutamine phosphoribosyl aminotransferase) is feedback-INHIBITED by synergistic action of AMP and GMP.



SYNTHESIS OF PYRIMIDINE NUCLEOTIDES – Uridine, Cytidine, Thymidine

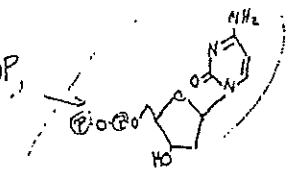
Carbamoyl-P + aspartate → orotic acid $\xrightarrow[\text{Uridine-P synthase}]{PRPP,\ P_i}$ URIDINE 5-P (UMP)



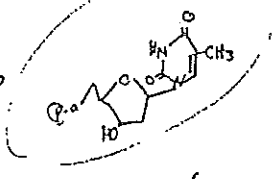
↓

will be converted into cytidine and thymidine

- UTP $\xrightarrow{\text{Amination}}$ CTP → CDP $\xrightarrow[\text{reduction}]{\text{Thioredoxin reductase}}$ dCDP

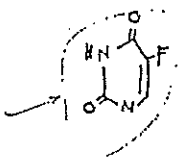


- UDP $\xrightarrow{\text{Reduction}}$ dUDP → dUMP $\xrightarrow[\text{THYMIDILATE SYNTHASE}]{CH_2H_4F \rightarrow H_2F,\ NADPH}$ dTMP



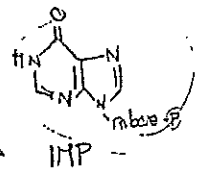
↓ inhibitors – act as anti-tumour drugs and anti-suppressants:

- aminopterin
- trimethoprim
- fluorouracil

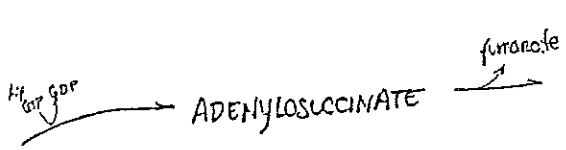


REGULATION – feedback inhibition by UDT and CTP
– ATP and PRPP stimulate the biosynthesis...

SYNTHESIS OF PURINE NUCLEOTIDES



PRPP → IMP – (Inosine 5-P)



ADENYLOSUCCINATE → (fumarate) ADENYLATE (AMP)

committed step of regulation;
enzyme inhibited by AMP and GMP
(feedback control)

XANTHOSINE-5-P → (Gln, Glu; ATP, AMP) GUANYLATE (GMP)

synthesis of 1 mol of AMP or GMP → 7 and 8 mol ATP, resp.

64 Catabolism of purine and pyrimidine nucleotides and elimination of the end-products

CATABOLISM OF NUCLEOTIDES → nucleotides are hydrolytically DEGRADED TO NUCLEOSIDES by 5-nucleotidase:

nucleotide + H_2O → nucleoside + P_i

the glycoside bond in nucleosides is cleft in phosphorolytic reactions catalyzed by nucleoside phosphorylases

nucleotide + $H_2PO_4^-$ → free base + (d)ribose 1-phosphate

Ribose 1-P is isomerized to Ribose 5-P → substrate in biosynthesis of PRPP

→ SOME OF THE BASES ARE RECYCLED to form nucleotides by SCAVENGER PATHWAYS (salvage reactions)

- pyridine bases are rarely recycled – they are mostly degraded and excreted
- purine bases are recycled in great extent, this is very important in CNS, bone marrow and blood cells.

CATABOLISM OF PYRIMIDINE BASES – it essentially the reverse of the synthesis.

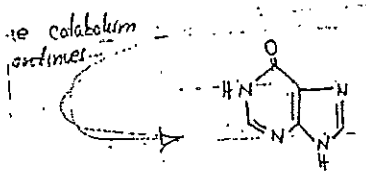
- CYTOSINE — deamination (H_2O, NH_3) → uracil — reduction ($NADH + H^+$, NAD^+) → dihydrouracil — amide hydrolysis (H_2O) → β-ALANINE → will be deaminated to malonic semialdehyde (→ acetyl-CoA)
- THYMIDINE → Thymine → β-AMINOISOBUTYRATE → will be deaminated to methylmalonic semialdehyde (→ succinyl-CoA)

CATABOLISM OF PURINE BASES – adenine and guanine are firstly deaminated : USUAL PATHWAY :

- AMP → Adenosine —(ADENOSINE DEAMINASE)→ Inosine → HYPOXANTHINE
- GMP → Guanosine → Guanine —(GUANINE DEAMINASE)→ XANTHINE

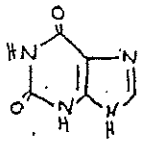
(Xanthine oxidase)

the catabolism continues...



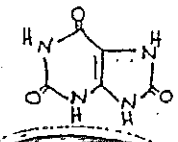
HYPOXANTHINE

—(XANTHINE OXIDASE)→



XANTHINE

—(XANTHINE OXIDASE)→



URIC ACID

final product of purine catabolism in h and uricolelic animals; excreted in urine

→ XANTHINE OXIDASE (XO) is a molybdenum and iron containing flavoprotein, which exists in 2 forms : • D-form • O-form

D-form → catalyzes oxidation as a DH
→ the acceptor of e⁻ is NAD^+

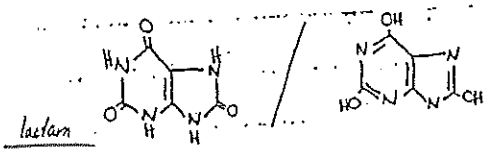
(HYPO) XANTHINE → (NAD+ / NADH+H+) → URATE

O-form → catalyzes oxygenation as an OXYGENASE
→ the acceptor of e⁻ is O_2

(HYPO) XANTHINE → (H_2O, O_2, O_2^- → H_2O_2) → URATE

(↳ this reaction produces H peroxide (ROS))

URIC ACID



lactam / lactim — $pK_{a_1} = 5,75$ (weak acid)

∴ the predominant form of uric acid in the body is the HYDROGEN URATE ANION.

↳ it has low solubility in water, above the solubility limit begins precipitation of monosodium urate crystals

Excess of these crystals deposits in soft tissues (specially in interstitium of kidney and joints)

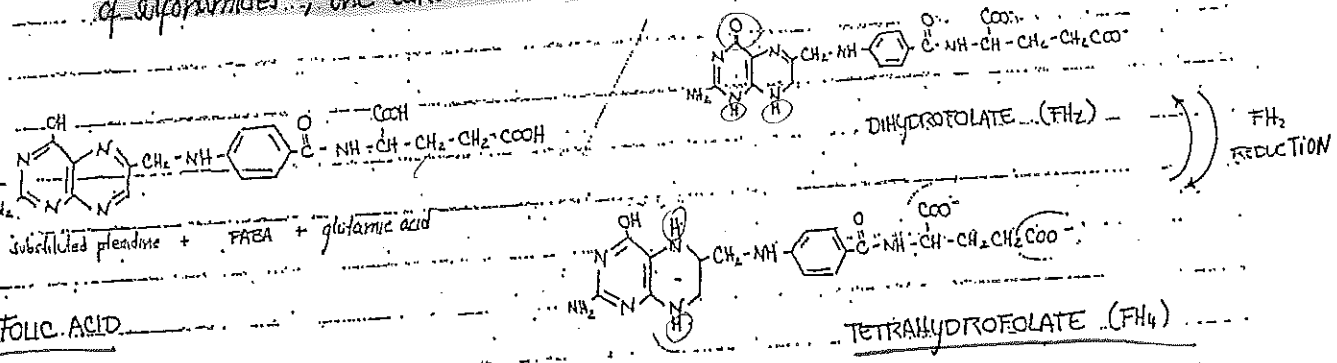
ON THE OTHER HAND, INCREASE OF [URATE] CAN BE BENEFICIAL: urate is a highly effective antioxidant

Nucleotides:

Nucleosides:

bases:

65 Folate and tetrahydrofolate (FH_4) – structures, relations to 4-aminobenzoate and action of sulfonamides; one-carbon units: sources, transfer and interconversions, the utilization.



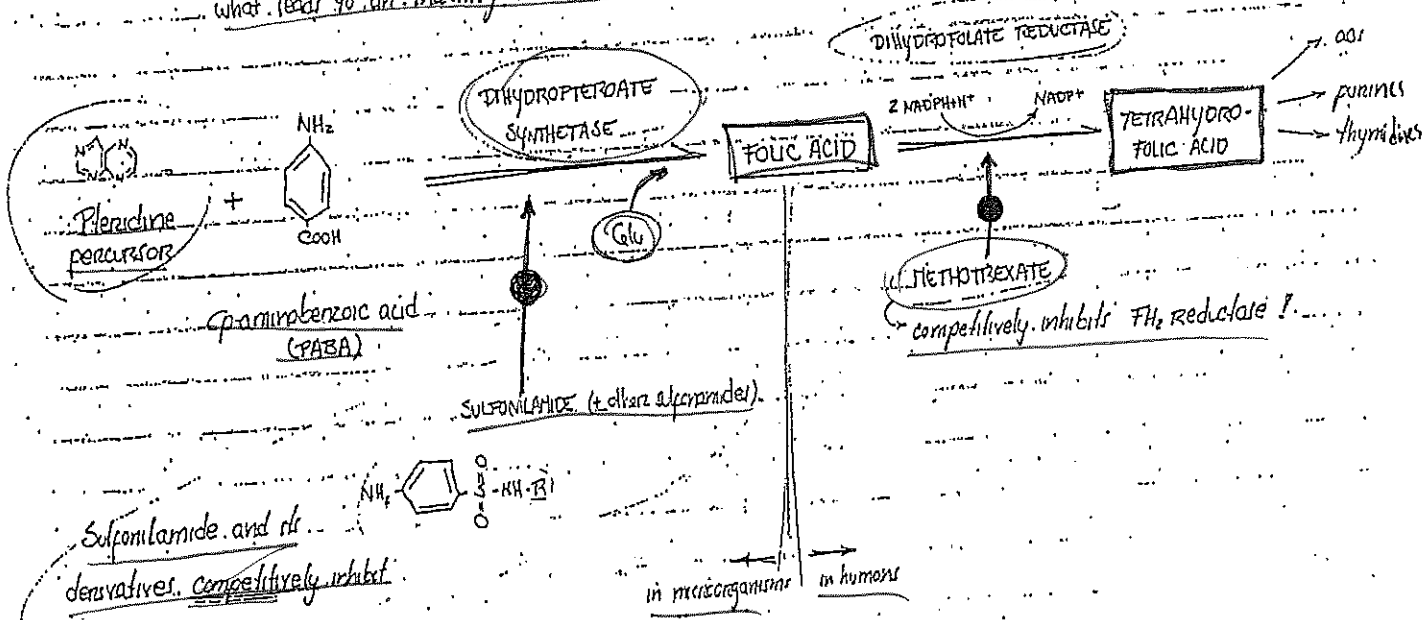
FOLIC ACID

plays a key role in one-carbon metabolism and it is essential for the biosynthesis of many compounds

FUNCTION OF FOLIC ACID: FH_4 receives 1-C fragments from donors such as serine, glycine and histidine, and transfers them to intermediates in the synthesis of aa, purines and thymine (DNA pyrim

Folic acid deficiency can cause MEGALOBLASTIC ANEMIA, caused by diminished synthesis of purines and thymidines (cells accumulation of megaloblasts (red cell precursors) in bone marrow)

what leads to an inability to make DNA, so cells cannot divide



Sulfonilamide and its derivatives competitively inhibit folic acid synthesis

ROLE OF FOLIC ACID IN AMINO ACID METABOLISM: some synthetic pathways require the addition of single carbon groups. These one-C groups exist in a variety of oxidation states, eg:

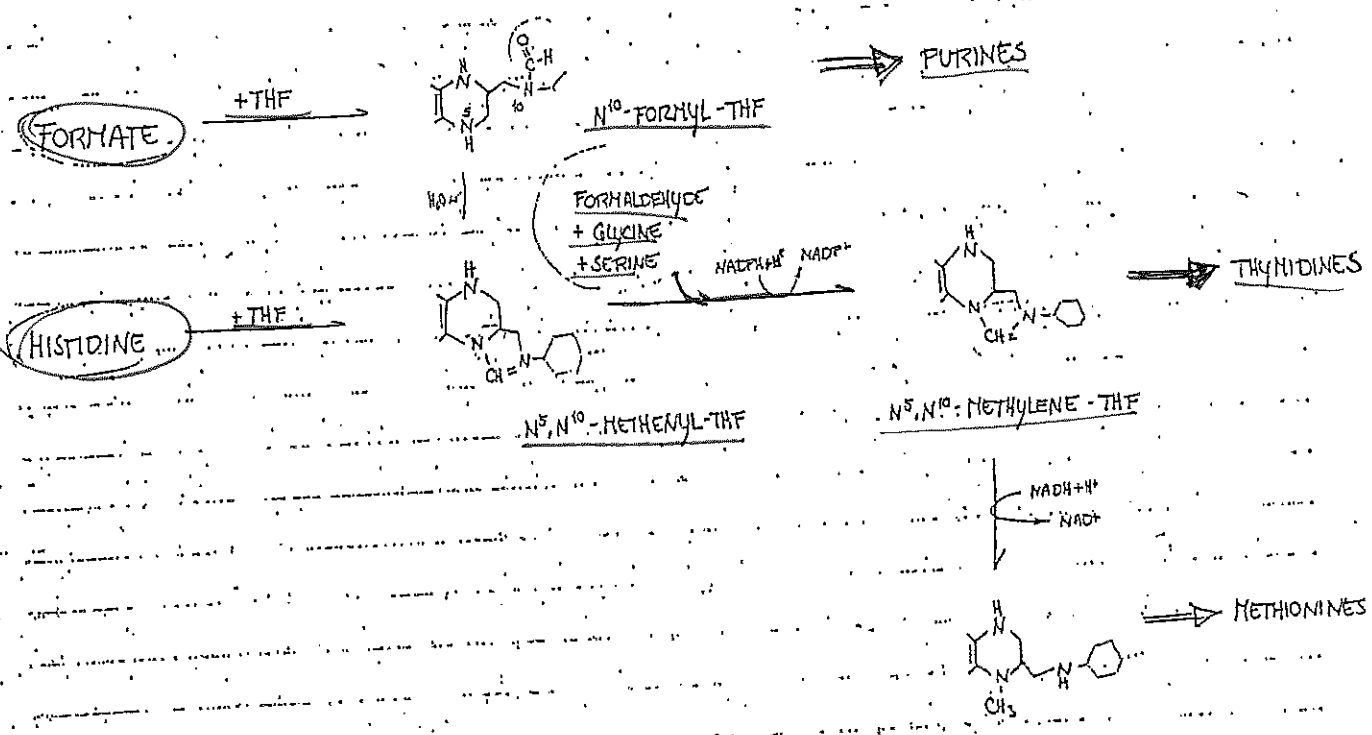
- methane
- methanol
- formaldehyde
- formic acid
- carbonic acid (sp-CH_3-OH) → carried by biotin

It is possible to incorporate 1-C units at each of these oxidation states (except methane) into other organic compounds

→ These single-C units can be transferred from carrier compounds (FH_4, SAM) to specific structures that are being synthesized or modified.

FH_4 → The active form of folic acid = tetrahydrofolic acid (THF) = is produced from folate by DIHYDROFOLATE REDUCTASE in a 2-step reaction, requiring 2 mol of $NADPH+H^+$

→ THF allows 1-C compounds to be recognized and manipulated by biosynthetic enzymes; they bind to N atom no. (5) and/or (10).

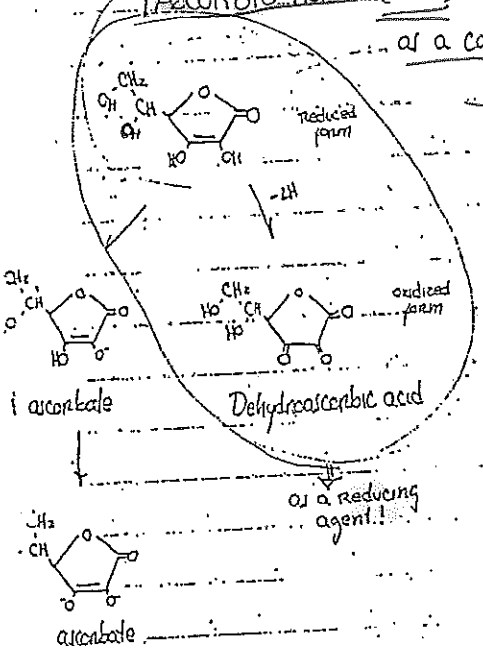


Sources of 1-C units:
- Purines: formic acid
- Thymidines, Methionines: Histidine, formaldehyde, glycine, serine ...

66 L-Ascorbate - sources, utilization in biochemical redox reactions (examples)

ASCORBIC ACID (vit. C) → acts as an e⁻ donor for 8 enzymes (catalyzing redox reactions)

→ it's a reducing agent in many different reactions: it participates as a coenzyme in hydroxylation reactions (eg. hydroxylation of prolyl- and lysyl- residues of collagen) → Therefore vit. C is required for maintaining normal connective tissue, and also for wound healing.



→ OTHER REDOX PROCESSES IN WHICH ASCORBATE PARTICIPATES: (as an e⁻ donor)

- biosynthesis of carnitine
- " of norepinephrine from dopamine
- modulation of tyrosine metabolism
- (...)

→ Vit. C facilitates the absorption of iron from the intestine.

→ Vit. C, as well as vit. E and β-carotene, is an ANTIOXIDANT that reduces oxidative stress, avoiding some chronic diseases and cancer.

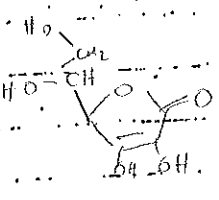
SOURCES OF ASCORBATE → the richest natural sources are fruits and vegetables (eg. Red pepper, Kiwi, broccoli, orange, ...). It is also present in some cuts of meat, especially liver.

Lack of ascorbate can cause SCURVY → a deficiency disease that results from insufficient intake of vit. C, which is required for correct collagen synthesis

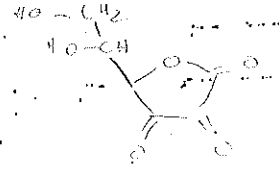
(ESCORBUTO ☠)

originates problems with hydroxylation reactions → in scurvy, the prolyl and lysyl hydroxylases cannot hydroxylate proline and lysine, due to the lack of ascorbate

ascorbic acid: reduced form

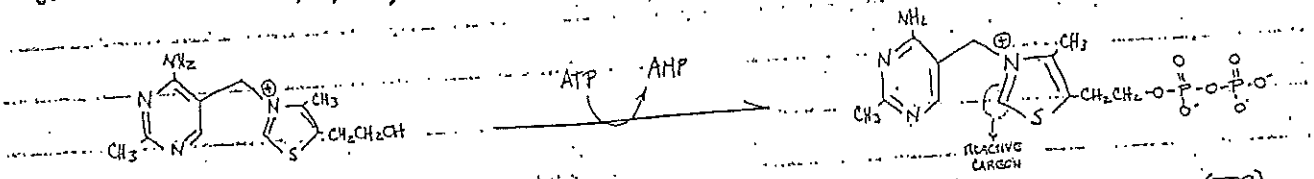


dehydroascorbic acid



TDP = thiamine pyruvate dehydrogenase

67 Thiamine – the physiological role of TDP (examples of reactions demanding TDP)

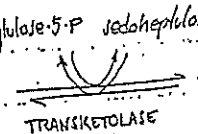


THIAMINE — THIAMINE PYROPHOSPHATE (TPP)

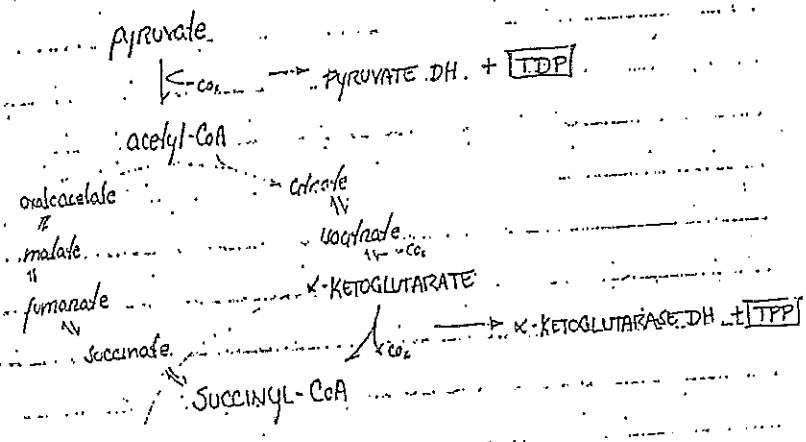
TPP → biologically active form of the vitamin B1, formed by transfer of a pyro(P) group from ATP to thiamin
↳ serves as a coenzyme in the synthesis or degradation of α-ketols by transketolase... and in the oxidative decarboxylation of α-ketoacids

examples of reactions demanding TPP as a cofactor:

- synthesis / degradation of α-ketols by transketolase



- oxidative decarboxylation of α-ketoacids



IN THIAMINE DEFICIENCY: these 2 DH reactions have reduced activity
↳ reduced ATP production
↳ impaired cellular function!

→ Thiamine occurs in liver, kidney and eggs.
↳ thiamine deficiency can cause:
- BERI-BERI: in areas where polished rice is the main diet component
- WERNICKE-KORSAKOFF SYNDROME: insufficient or impaired intestin absorption of the vit.

apoenzyme → enzyme without the necessary cofactor...

carboxylase (apoenzyme) + biotin (biocytin residue) → holoenzyme (biocytin).

68 Methylation and carboxylation – reaction sequences, enzymes and coenzymes, the roles in metabolism

CARBOXYLATION → reaction in which a carboxylic acid group is introduced in a substrate

γ-carboxylation → in post-translational modification of glutamate residues in proteins – it is necessary for proteins (mostly blood clotting-related and bone proteins) to function

Carboxylation occurs in the liver and is performed by γ-glutamyl carboxylase

Other carboxylation reactions:

- → carboxylation of pyruvate to oxaloacetate (gluconeogenesis)
- → " of acetyl-CoA to malonyl-CoA (synthesis of f.a.)
- → " of propionyl-CoA to methylmalonyl-CoA
- → carboxylations in the branched-chain aa breakdown

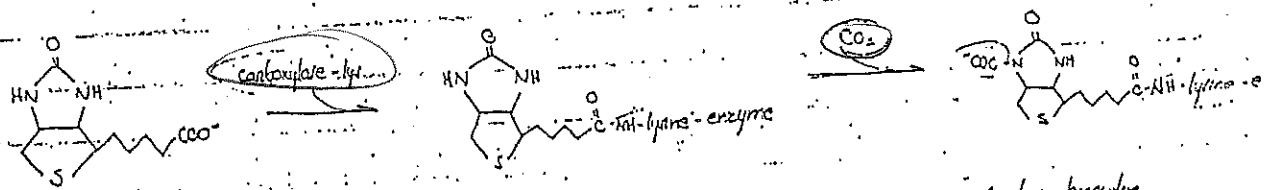
} require BIOTIN as a coenzyme of carboxylases!

BIOTIN → transfers CO_2 in a small no. of carboxylation reactions

→ a HOLOCARBOXYLASE SYNTHETASE acts on a lysine residue of the apoenzymes of... to react with free biotin to form the BIOCYTIN residue of the holoenzyme

} acetyl-CoA carboxylase, pyruvate ..." , propionyl-CoA ..."

the reactive intermediate is 1-N-carboxybiocytin, formed from bicarbonate with ATP consumption



biotin — biocytin (biotinyl-lysine) — carboxybiocytin

↓

the carboxyl group is then transferred to the substrate → carboxylation

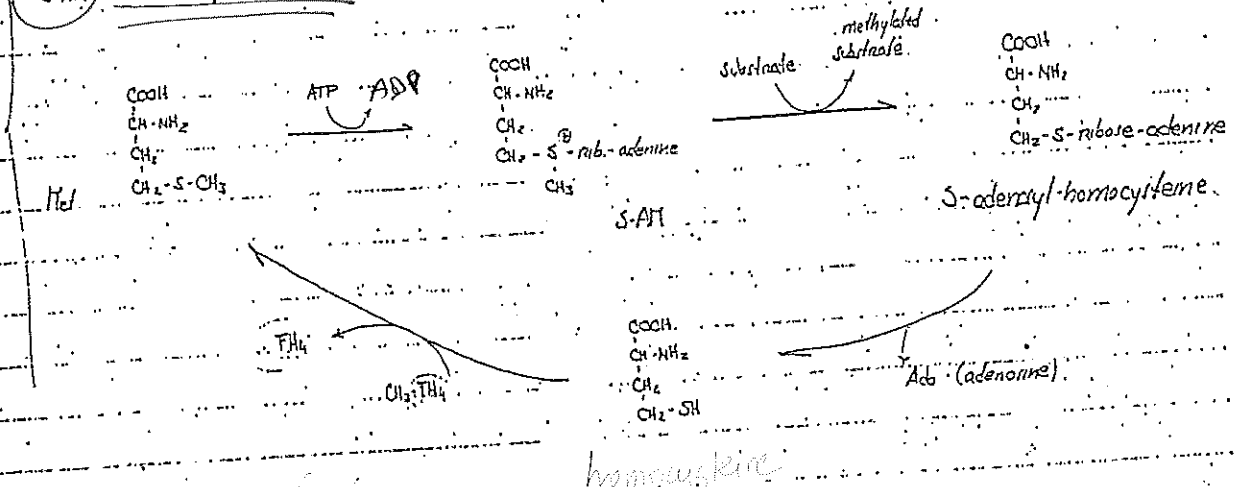
METHYLATION – attachment or substitution of a methyl group on various substrates.
(Replacement of an H atom with a methyl group)

DNA methylation – often at CpG sites (cytosine-guanine), which results in conversion of the cytosine to 5-methylcytosine – reaction catalyzed by DNA methyltransferase.
These sites are often found near promoters and can have a great impact in gene expression.

PROTEIN methylation – usually on Arginine or Lysine residues in the aa sequence:
- Arginine can be methylated once or twice by peptidylarginine methyltransferase
- Lysine " " once, twice or 3x by lysine methyltransferases.

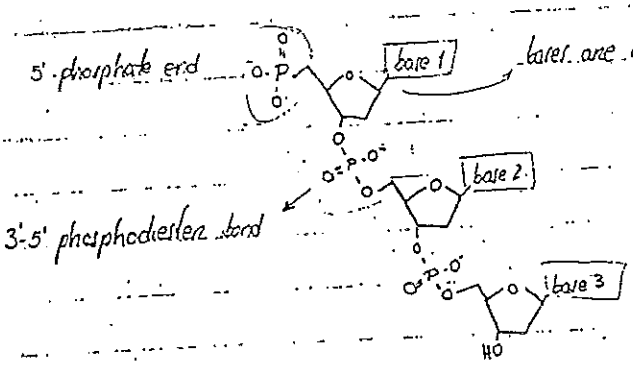
- mostly studied on HISTONES – the transfer of methyl groups from SAM (S-adenosylmethionine) to histones can activate or repress expression of a certain gene.
- Protein methylation is one type of post-translational modifications.

S-AM – S-adenosylmethionine = activated Met – main donor of methyl groups!



69 DNA organization and replication in eukaryotes (topoisomerases and other factors involved in replication, particular steps and polarity of replication).

Basal facts on the DNA structure



⇒ by convention, the direction of reading is 5' → 3'

IN EUKARYOTES — NUCLEAR DNA is linear and double-stranded : 70% of nucleotide sequences are unique, but only 3% code for proteins

— MITOCHONDRIAL DNA is double-stranded and circular.

SECONDARY DOUBLE HELICAL STRUCTURE OF DNA – 2 polynucleotide chains wind about a common axis with a right-handed twist. The 2 strands are antiparallel – they run in opposite directo

⇒ the negatively charged phosphate groups bind positively charged groups of proteins and simple c

⇒ the bases fill the inner of the helix as complementary base pairs (linked by H bonds)

dA = dT ; (RNA: A = U) ; dG ≡ dT (RNA: G ≡ C)

HIGHER LEVELS OF DNA ORGANIZATION – Chromatin

→ Human genome = 3×10^9 bp. There are 23 chromosome pairs in diploid cells.

3 higher levels of DNA organization into Chromatin (important in metaphase)

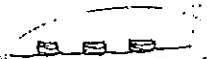
1st LEVEL: fibrils of nucleosomes

2nd LEVEL: superhelix of nucleosome fibrils, solenoid

3rd LEVEL: Radial loops of solenoids surrounding a central nuclear prot scaffold form the fibres of interphasic chromatin.

(In mitosis: METAPHASIC CHROMOSOMES originate by condensation of interphasic chromatin fibres!)

double-stranded DNA ⟹ 10 bp per turn ; 2nm diameter

1. FIBRILS OF NUCLEOSOMES — "beads on a thread"  ↕ 10 nm
   ↳ 2 turns of DNA duplex (140bp) wound around the cluster of histones (octamer)
    contains 2 of each type of histones H2A, H2B, H3
   (HISTONES — proteins comprising about 100 aminoacyl residues)

2. SOLENOID = fibrils of nucleosomes coiled in a superhelix  ↕ 30nm

3. FIBRES OF INTERMITOTIC CHROMATIN - radial loops of sclereids anchored to the nuclear protein scaffold. (300 nm diameter)

EUKARYOTIC DNA REPLICATION — nuclear DNA is replicated in the S phase of the cell cycle

INTERPHASE
G_2 - preparation for mitosis → MITOSIS - cell division
S-PHASE
G_1 growing

Each of the 2 strands of the ds DNA serves as a template for the replication of new complementa. strands. The replication is semiconservative

→ Unwinding of the double helix in replicated segments causes overwinding (supercoiling) at other parts of the molecule. (positive supercoiling = twisting of the helix in the same direction as the original)
(↳ this strain is then removed by topoisomerases)
↳ Helicases then separate single strands of the double helix and single-strand binding proteins stabilize the single strands

TOPOISOMERASES ⟹ repair overwound segments.

TOPOISOMERASES TYPE I - exhibit endonuclease activity : they uncoil DNA by temporary breaking a single strand of the double helix. After rotation of the stand on each side of the nick, they catalyze resealing of the strand !

TOPOISOMERASES TYPE II - make 2 temporary breaks in both strands of the DNA helix. After another DNA helix has passed through the intervening space, the breaks are resealed.

INHIBITORS OF TOPOISOMERASES = make replication impossible... (Some serve as anti-cancer drugs)

- TOPOTECAN = inhibits type I topoisomerases.
- DAUNORUBICIN } inhibit type II topoisomerases
- ETOPOSIDE }

OTHER FACTORS INVOLVED IN THE SYNTHESIS OF THE NEW STRAND → DNA POLYMERASES recognize the nucleotide sequences in the template strands and catalyze formation of 3'-5' phosphodiester bonds in the new strands. They elongate existing oligo- or polynucleotide chains IN THE 5'→3' DIRECTION.

→ Nucleases catalyze hydrolytic splitting of phosphodiester bonds.

A polym.

→ 3'-5' exonuclease activity cleaves phosphodiester bonds from the 3'end to the 5'end WHEN ANY MISMATCH OCCURS: if DNA polymerase mispairs a nucleotide with the template, the 3'-5'-exonuclease activity of the DNA polymerase δ and ε is used to excise the mismatched nucleotide.

→ Endonucleases: cleave bonds within the chains, producing single-stranded nicks.

→ DNA HELICASE = unwinding protein that uses ATP.

DNA POLYMERASES CAN'T START THE SYNTHESIS OF DNA de novo, they are only able to elongate existing chains! (because they can only add nucleotides to 3'-ends)

↳ So, before replication begins, a short RNA sequence, complementary to the template, RNA prime is synthesized = has about 10-20 nucleotides.

↳ PRIMASE catalyzes the synthesis of DNA de novo - in eukaryotes, DNA POLYMERASE α HAS PRIMASE ACTIVITY, used to synthesize RNA PRIMERS.

↳ after a sequence of some deoxyribonucleotides has been added to the primer, DNA POL α is disp

→ REPLICATION IS INITIATED AT MULTIPLE ORIGINS IN BOTH DIRECTIONS!

In each replicon (replication bubble) there are 2 replication forks, which move in opposite directions:

- The strand along which the fork slides is called LEADING STRAND.
- The antiparallel strand of the fork is synthesized from short (100-200 nucleotides) fragments called OKAZAKI FRAGMENTS, which are joined after removal of primers and filling in the gaps. THIS STRAND IS CALLED LAGGING STRAND.

SYNTHESIS OF LEADING STRAND = continuous:

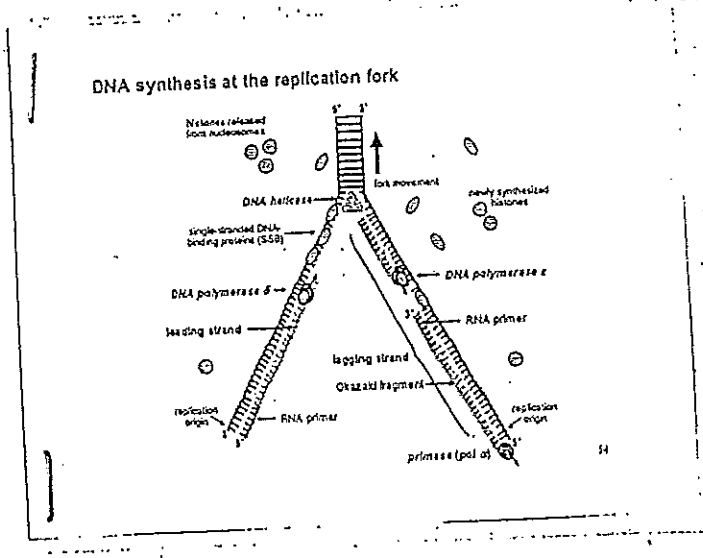
1. "Preprimming" proteins and primase bind onto a single-strand and displace SSB-proteins
2. A short RNA primer is synthesized and elongated by DNA polymerase, synthesizing the new DNA strand continously until reaching adjacent replicon.

5'→3'

SYNTHESIS OF LAGGING STRAND = discontinuous:

1. Synthesis of new RNA primers, which are elongated by DNA polymerase, synthesizing short DNA strands
2. RNA primer is removed by the 5'-3'-exonuclease activity of polymerase β. This enzyme also replaces primer with DNA by elongating the Okazaki fragment, filling in the gap. DNA ligase joins the fragments.
3. DNA polymerase moves back to initiate a new Okazaki fragment.

3'→5'



SUMMARY OF THE ENZYMES INVOLVED IN DNA REPLICATION:

- TOPOISOMERASES and HELICASES – unwinding of dsDNA
- PRIMASE ACTIVITY, exhibited by DNA polymerase α, catalyzing formation of RNA primers. (α has exonuclease activity)
- DNA POLYMERASE δ and ε synthesizing leading DNA strand (δ) and Okazaki fragments in the lagging strand (ε). Both enzymes have 3-5'-exonuclease activity. (δ and ε are optional)
- 5'-3' EXONUCLEASE ACTIVITY (excision of primers) and DNA POLYMERASE ACTIVITY (filling the gaps), exhibited by DNA POLYMERASE β.
- DNA LIGASE (ATPase activity) Removes nicks by joining the fragments through phosphodiester bonds.
- TELOMERASE ACTIVITY (absent in some cells) allow replication of 3'-ends of linear chromosomes

Major type	Function	Exonuclease activity	Relative activity
Polymerase α	primase activity (synthesis of the RNA primer)	none	80 %
Polymerase δ	elongates the leading strand	3' - 5'	
Polymerase ε	elongates the lagging strand	3' - 5'	

TELOMERES AND TELOMERASES

At the end of linear eukaryotic chromosomes there are DNA sequences called telomeres. The telomeric DNA is unusual; it contains up to 1000 tandem repeats of a G-rich sequence (TTAGGG, in humans) (hexanucleotide)

Telomeres protect the ends of chromosomes against nuclease activities.

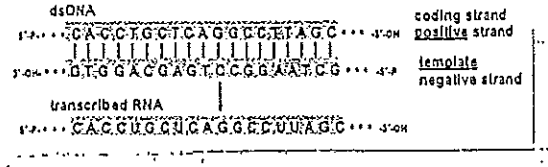
TELOMERASE → elongate the telomeres by attaching the newly synthesized telomeric hexanucleotide repeat

→ is a specialized reverse transcriptase that carries its own RNA template

→ it's a nucleoprotein whose RNA component contains a segment that is complementary to the telomeric tandem repeats.

70 RNA synthesis. (RNA polymerases, transcription signals in eukaryotic cells).

One of the dDNA strands (the negative one) serves as template for the synthesis of RNA. The sequence of the transcribed RNA corresponds to that of the POSITIVE (CODING) STRAND, only with U instead of T



THE NEW RNA CHAIN GROWS ALWAYS IN THE 5'→3' DIRECTION

RNA SYNTHESIS - ribonucleotides (P) are the substrates for the synthesis
= RNA POLYMERASES - recognize the nucleotide sequence in the template, initiates the synthesis of new chains of RNA (without a primer!), and catalyzes the formation of 3'-5'-phosphodiester bonds

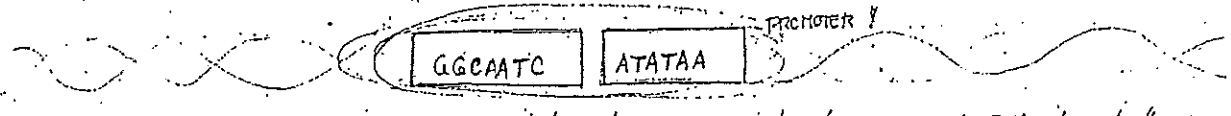
In eukaryotes, the nucleus contains 3 types of RNA polymerases: (there is a fourth type in mitochondria)
their mechanism of action is the same but they differ in binding onto different promoters, location in the nucleus and susceptibility to inhibitor α-AMANITIN (blocks elongation phase of RNA synthesis):

RNA polymerase		primary transcripts	
pol I	nucleolus	pre-rRNA 45S	susceptible to α-amanitin
pol II	nucleoplasm	pre-mRNAs, some snRNA	
pol III	nucleoplasm	pre-tRNAs, rRNA 5S, some snRNAs	

TRANSCRIPTION OF DNA ⟹ initiation, elongation and termination

→ STARTS AT PROMOTERS on the DNA template
→ sequences of DNA that direct the RNA polymerase to the proper initiation site!
→ The effectiveness of promoters can be regulated by specific DNA sequences ENHANCERS or SILENCERS also called cis-acting elements; these sequences are binding sites for TRANSCRIPTION FACTORS

EUKARYOTIC PROMOTER SITE → for binding of RNA pol. II



CAAT BOX: specifies the frequency of initiation // TATA BOX: directs TFII D and RNA pol. II to the correct site

⇒ Polymerase II and transcription factors bound onto the promoter form a complex called the BASAL TRANSCRIPTION APPARATUS - it regulates basal gene expression.

CONSTITUTIVELY EXPRESSED GENES → genes that are regulated wholly in this way.

✳ Regulated expression of numerous genes is mediated by GENE SPECIFIC TRANSCRIPTION FACTORS. These proteins bind to regulatory DNA sequences distant from promoters (they also regulate the basal transcription apparatus).

TRANSCRIPTION – 3 PHASES:

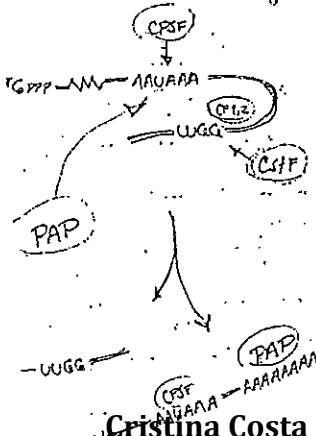
1. **INITIATION** – begins with binding of **TF II D** (transcription factor D for pol. II) to the TATA BOX. This will enable binding of other transcription factors, such as HELICASE that separates the DNA duplex (so that polymerase II can bind).
   (Pol II contains an unphosphorylated carboxyl-terminal domain – CTD)
   ↳ pol. II with its CTD initiates transcription, producing short transcripts of 20-25 nucleotides
   often transcription is initiated, most transcription factors are released
   ↳ CTD is then phosphorylated → change in conformation of pol II: enables binding of CAPPING ENZYME (CE) and METHYLTRANSFERASE (MT).
   These 2 enzymes MODIFY THE 5'-END OF THE TRANSCRIPT (from 5'-PPP to 5'-m^{7}GPPP), so that transcription can go on!

SWITCH TO

2. **ELONGATION**
   ↳ pol II then uses ribonucleotides triphosphates and releases pyrophosphate each time a nucleotide is added to the growing chain.

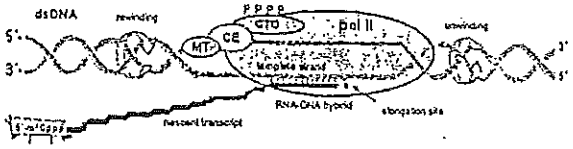
3. **TERMINATION**
   ↳ Transcripts produced by DNA polymerase II are released from the transcription apparatus AFTER THE POLYADENYLATION SIGNAL (AAUAAA) and the GU- or U-rich sequence
   The terminal sequences of the transcripts are decompared in 3'-POLYADENYLATION.

POLYADENYLATION OF TRANSCRIPTS:



1. Cleavage and Polyadenylation Specificity Factor (CPSF) binds to polyadenylation signal AAUAAA
2. A GU- or U-rich sequence binds the Cleavage Stimulation Factor (CStF) and Cleavage Factors (CF I,2) → a loop is formed.
3. Binding of Poly(A) Polymerase (PAP) stimulates cleavage 20 nucleotides downstream the poly(A) signal. (the cleavage factors are released)
4. PAP adds 12 adenylate residues, provided by (poly(A)-binding protein

Elongation phase



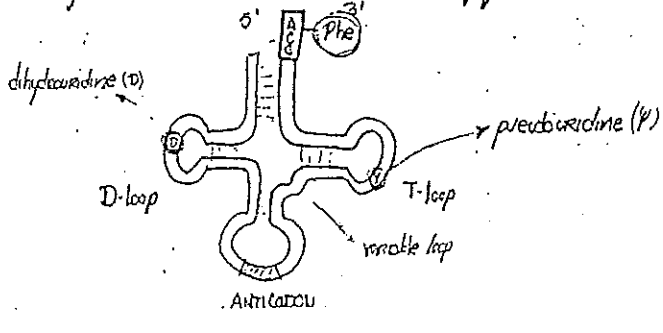
71 Species of RNA and the functions of them, processing of the primary transcripts generating the functional RNA types.

RNAs = ribonucleic acids = polymers consisting of nucleoside-(P) components, linked with diester bonds.
↳ bases: uracil, cytosine, adenine and guanine

⇒ rRNA - ribosomal RNA = the majority of cellular RNAs (80%); structural and functional component of ribosomes
↳ produced from DNA by transcription in the nucleolus and assembled with proteins there to form ribosomes subunits.
↳ It has a long life span and its function is translation.

⇒ mRNA - messenger RNA = transfer genetic information from the nucleus to the cytoplasm.
↳ still in the nucleus, the primary transcripts are modified (RNA maturation), they must not form stable tertiary structures so that they can be read codon by codon in the ribosome
↳ This is ensured by attachment of RNA-binding proteins, which prevents base pairing.
↳ Their lengths vary depending on how much information they carry, and their lifespan is short

⇒ tRNA = transfer RNA = function as links the nucleic acids and proteins during translation.
↳ small RNA molecules consisting of 70-90 nucleotides, which recognize specific mRNA codons with their anticodons through base pairing. At the same time they carry, in their 3' end (...CCA-3') the aa that is assigned to the relevant mRNA codon.
↳ tRNA has a specific tertiary structure, resembling a cloverleaf, containing some unusual and modified components - eg. pseudouridine (Ψ), dihydrouridine (D), thymidine (T) and some methylated nucleotides such as 7-methylguanidine.

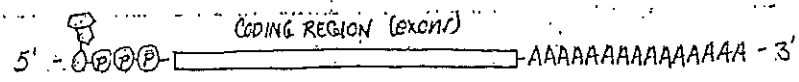


PROCESSING OF THE PRIMARY TRANSCRIPTS

→ primary transcripts of genes transcribed by RNA polymerase I (precursor of mRNAs) undergo processing, mostly before their transcription is over.

→ the transcripts of non-coding sequences of the gene (introns) are cut off and the transcripts of coding sequences (exons) spliced — the process is called splicing.

Transcription ends with the addition of a POLYADENYLATE chain to the 3'-end:



5'end triphosphate + 7-methylguanosine "cap" — polyadenylate tail

↳ prevents mRNA against 5'-endonucleases and it's the marker recognized in protein synthesis!

→ Some mRNAs also suffer alteration of base sequence by so-called RNA editing processes.

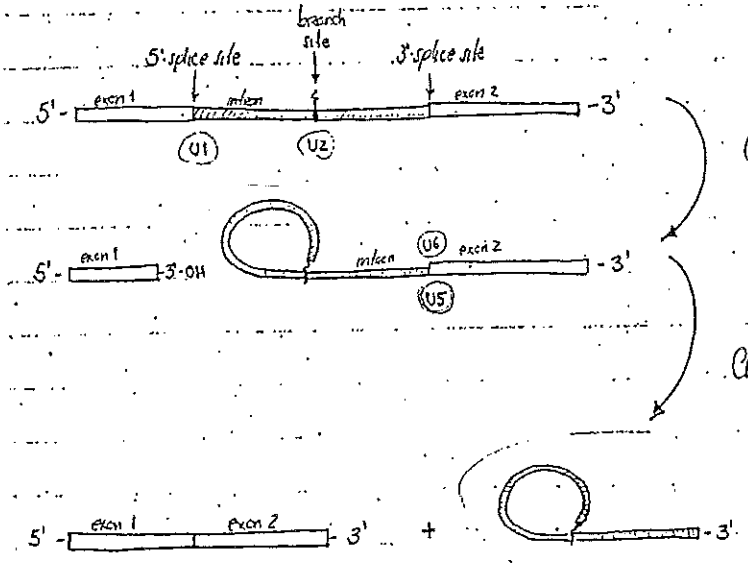
⇒ Some nuclear RNAs with less than 300 nucleotides (Small Nuclear RNAs – snRNAs) are needed for splicing pre-mRNA. — they associate with proteins to form SMALL NUCLEAR RIBONUCLEOPROTEIN PARTICLES (snRNP, "snurps"): U1 – U6:

snRNPs 1,2,4,5,6 and other proteins form large assemblies (about 60s) called SPLICEOSOMES

- U1 – binds to 5'-splice site and 3'-splice site
- U2 – binds to branch site (part of catalytic center)
- U5 – binds to 5'-splice site
- U6 – catalyzes splicing
- U4 – masks the catalytic activity of U6

snRNP's = nuclear RNA's + proteins

SPLICING SCHEME



looping molecule formed of introns

Cleavage on 5'-splice site and formation of a LARIAT: phosphodiester bond connects 5'-end of intron and A do of the branch site.

Cleavage on 3'-splice site and joining of 5'-end and 3'-end of the 2 exon sequences

→ excised intron sequence will be degraded in the nucleus

12 · Regulation of eukaryotic gene expression. — mainly in transcription.

Regulation at the level of:
1. Chromatin and DNA
2. transcription
3. processing of primary transcripts
4. translation and post-translational process

1. REGULATION AT THE LEVEL OF CHROMATIN AND DNA

1.1 - Control of gene accessibility for transcription — chromatin occurs in 2 kinds, either as condensed heterochromatin (with genes transcriptionally inactive) or diffused euchromatin.

Chromatin: heterochromatin / diffused

Each cell has the same complement of genes; however the changes in chromatin structure (occurring in development and differentiation of tissues) results in differential gene expression.

Transcriptionally active genes

1.2 - Chromatin remodelation → mechanisms that change the organization of ds DNA in chromatin fibers that are required for initiation of transcription.

Eg unwinding of ds DNA segments from nucleosomes depends on both hydrolysis of ATP and covalent modification of histones.

1.3 - Methylation of DNA — methylation of cytosine to 5-methylcytosine occurs often in the GC-rich sequences near promoters (catalyzed by methylase).

Genes containing 5-methylcytosine are transcribed less easily than those non-methylate

1.4 - Selective gene rearrangements — the coding segments of DNA can recombine with the particu gene or may associate with other genes within the genome.

Eg: this is why there is a vast diversity of specific antibodies.

1.5 - Amplification of genes — during development or in response to drugs.

Certain parts of chromosomes are repeatedly replicated during particular cell cycle. are excised in the form of small unstable chromosomes (called double minutes) that are incorporated into other chromosomes. Those extra rounds may become frozen in the g

Eg: methotrexate causes patients to develop drug resistance by increasing number of FH_2 reductase genes by gene amplification.

(FH_2 reductase inhibitor)

(Gene amplification is usually due to mistakes - or drugs)

→ transcription factors often attach to the DNA major groove

② REGULATION AT THE LEVEL OF TRANSCRIPTION

REGULATION OF TRANSCRIPTION BY STEROID AND THYROID HORMONES (IODOTHYRONINES)

↓

they diffuse through the plasma membrane into cells and bind to intracellular receptors, forming complexes called SPECIFIC TRANSCRIPTION FACTORS

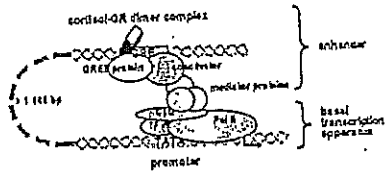
They bind onto regulatory DNA sequences called Hormone Response Elements (HRE)

then they interact with co-activators / co-repressors and then with mediator proteins, which will interact with the basal transcription apparatus, initiating or inhibiting the transcription of a particular gene

example:

Example: Initiation of transcription by cortisol

Active complex cortisol-receptor binds onto DNA at the specific sequence GRE (glucocorticoid response element, one of the HRE – hormone response elements).
The coactivator and specific hormone response element-binding proteins (HREB-proteins) are also attached. This complex acquires the ability to act as enhancer that supports initiation of transcription on the promoter by means of mediator proteins.



GR dimer – intracellular glucocorticoid receptor (dimer)
GRE – glucocorticoid response element
GREB protein – GRE binding protein (a specific transcription factor)

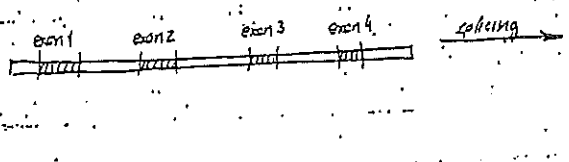
35

TRANSCRIPTION FACTORS that bind onto regulatory DNA sequences:
- helix-turn-helix
- zinc-fingers
- leucine zipper

③ REGULATION AT THE LEVEL OF PROCESSING OF PRIMARY TRANSCRIPTS = 2 processes:

- ALTERNATIVE SPLICING = can cause a single gene to produce various proteins

Eg:



- RNA EDITING = in some mRNAs, the base sequence is altered by processes other than RNA splicing; these are called RNA EDITING and are not very rare.

Eg: cytidine residue may be deaminated to uridine; adenosine to inosine.

4. REGULATION AT THE LEVEL OF TRANSLATION → mediated mostly through changes in activities of eukaryotic initiation factors (eIFs)

Eg: The synthesis of globin in reticulocytes is controlled by phosphorylation of the (eIF 2), which is active in dephosphorylated form.

Haem prevents eIF 2 from phosphorylation, so when Haem is present, (eIF 2 is active: the translation occurs → globin chains are synthesized

2. REGULATION AT THE LEVEL OF TRANSCRIPTION

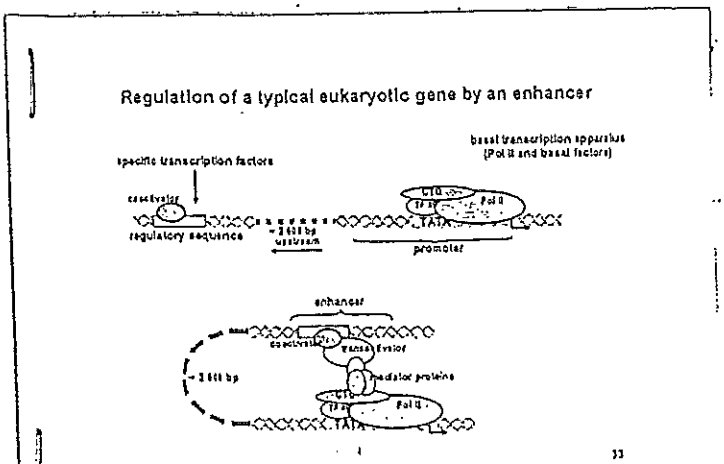
Basal control of transcription – common to all genes: includes binding of basal transcription factors to the promoter or adjacent sites (GC and CAAT boxes eg)

SPECIFIC CONTROL OF GENE EXPRESSION

- REGULATORY DNA SEQUENCES within the same DNA molecule, which can influence transcription – they can act as:
  - ENHANCERS: increase the rate of transcription; bind coactivators
  - SILENCERS: decrease " " " " ; bind corepressors
  - HORMONE-RESPONSE ELEMENTS: act as enhancers or silencers; bind complexes of hydrophobic hormones (steroid and thyroid hormones) with their intracellular receptors.
- SPECIFIC TRANSCRIPTION FACTORS – proteins originating from genes presumably located on different chromosomes (trans-acting elements) which bind to regulatory DNA sequences remote from the promoter.
  - they can act as activators (coactivators) or repressors (corepressors) of transcription
  - they mediate the effects of enhancers, silencers and hormone-response elements THROUGH INTERACTIONS OF OTHER MEDIATOR PROTEINS that interact directly with basal transcription factors and support or disable transcription of particular genes

REGULATION OF TRANSCRIPTION FACTORS (basal and specific)

1. synthesis (down- or up-regulation)
2. binding of stimulatory or inhibitory ligands (and also by cooperation of transcription f
3. phosphorylation or dephosphorylation, due to various extracellular signals (hormo ...growth factors, cytokines, etc.)



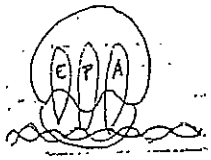
AUG → initiation codon

73 Proteosynthesis (ribosome components, formation of the initiation complex, peptide elongation cycle and termination of protein synthesis)

Components required for TRANSLATION:
1. aa
2. tRNA
3. aminoacyl-tRNA synthetases = enzymes needed to attach aa to the corresponding tRn
4. mRNA
5. Protein factors - catalytic or stabilizing function
6. ATP and GTP = as E sources
7. RIBOSOMES

RIBOSOMES - large complexes of 4mol. RNA (in eukaryotes) and ribosomial proteins
- made up of 2 subunits: one large (5S, 58S - or 28S-) and one small (18S-)
= its function is protein synthesis.

The ribosomes have 3 binding sites for tRNA molecules (A, P and E), they bind:
- A site: binds incoming aminoacyl-tRNA (according to specified by the codon).
- P site: occupied by peptidyl-tRNA (which carries the aa that have already been synthesized).
- E site: occupied by the empty tRNA as it is about to exit the ribosome.

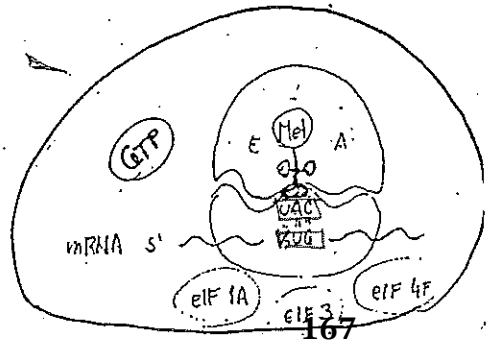


LOCATION OF RIBOSOMES → either free in cytosol or in close association with the rough ER; the RER associated ribosomes are responsible for synthesizing proteins to be exported from the cell.

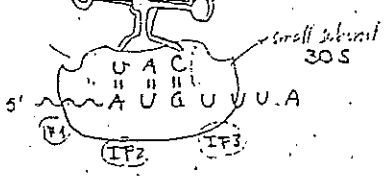
DNA TRANSLATION → in ribosomes; DNA is translated from its 5'end to its 3'end

Beggining of translation: assembly of the components of the TRANSLATION SYSTEM: 2 ribosomal subunit, the mRNA to be translated, the aminoacyl-tRNA specified by the first codon, GTP and initiation factors that faciliate the assembly of this INITIATION COMPLEX!
(in eukaryotes there are 10 initiation factors = eIFs)

→ Formation of the 80S initiation complex:
- small ribosomal subunit (40S) + large rib. subunit (60S)
- Met-tRNA (initiation codon)
- eIF 1A, 3, 4F
- mRNA

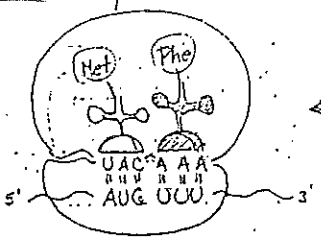


TRANSLATION



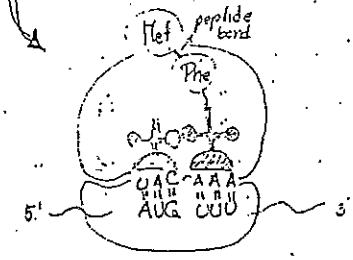
INITIATION

1. Initiation factors aid in the formation of the 30S INITIATION COMPLEX.
2. GTP is cleaved and initiation factors are released when the large subunit is added to form the 70S INITIATION COMPLEX

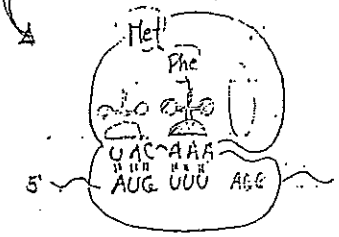


ELONGATION CYCLE

3. Elongation factors direct the binding of the appropriate tRNA to the codon in the empty A-site.



4. Peptidyltransferase (component of the large subunit) transfers the aa (or peptide chain) from the P-site onto the aa on the A-site, and catalyzes peptide bond formation



5. The ribosome moves a distance of 3 nucleotides along the mRNA in the 5' → 3' direction

6. Steps 3, 4 and 5 are repeated until the peptide is complete

TERMINATION

7. A termination codon is recognized by a release factor (RF), which activates the release of the newly synthesized peptide and dissolution of the synthesizing complex → recycling!

TERMINATION OF PROTEIN SYNTHESIS

- Elongation continues until the A-site reaches a stop codon.
- Releasing factors (eRFs) bind to the A-site.
- Peptidyltransferase catalyzes hydrolysis of the ester bond between the polypeptide and tRNA
- Ribosomal subunits dissociate, mRNA is released.

74. Posttranslational processing of proteins (various types of covalent modification), Golgi complex and glycosylation of proteins.

POSTTRANSLATIONAL MODIFICATION OF PROTEINS = may include removal of part of the translated sequence, or the covalent addition of one or more chemical groups required for protein activity.

COVALENT ALTERATIONS = proteins may be activated or inactivated by covalent attachment of a variety of chemical groups:

1. PHOSPHORYLATION = occurs on thy -OH groups of (serine) (threonine) or (tyrosine) residues in a protein. The phosphorylations are catalyzed by protein kinases and may be reversed by phosphatases. The phosphorylation may decrease or increase the protein activity.

2. HYDROXYLATION = Proline and (lysine) residues of the x-chains of (collagen) are extensively hydroxylated in the ER.

3. GLYCOSYLATION = many of the proteins that are destined to become part of a plasma membrane or lysosome or to be secreted from the cell have carbohydrate chains attached to (serine) or (threonine) -OH groups (O-linked) or to the amide N of asparagine (N-linked). The stepwise addition of sugars occurs in the ER and Golgi apparatus:

GLYCOPROTEIN SYNTHESIS

- before being incorporated into the oligosaccharide chain, monosaccharides are activated by formation of nucleotide sugars. The glycosyls of these sugars can be transferred to suitable acceptors provided appropriate transferases are available.

eg:

Glucose-6-P → Fructose-6-P → N-acetylglucosamine 6-P ⇌ (...)-1-P $\xrightarrow{UTP}$ UDP-N-Acetylglucosamine

UDP-N-acetylgalactosamine

Sometimes glycosylation is used to target proteins to specific organelles – eg. enzymes destined to be incorporated into lysosomes are modified by addition of mannose-6-P residues

OTHER COVALENT MODIFICATIONS: may be required for functional activity of a protein.

eg: additional (-COOH) carboxyl groups can be added to glutamate residues by vitamin K-dependent carboxylation. The resulting γ-carboxyglutamate is essential for many blood-clotting proteins.

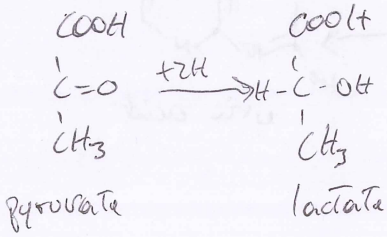
GOLGI APPARATUS - complex network of flattened sacs that sorts and packs mature proteins.

↳ there is a region called trans Golgi network (TGN). The post-translational modification of proteins, which started in the ER, continues in these sections.

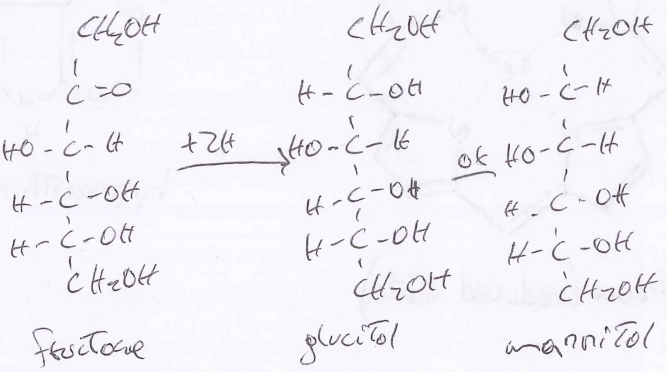
→ From Golgi Apparatus, the proteins are transported by vesicles to lysosomes, plasma membrane and secretory vesicles that release their contents into the extracellular space by exocytosis.

→ Protein transport can either proceed continuously (constitutive) or regulated by chemical signals. – it all depends on the signal sequences or signal structures that proteins carry with them, like address labels.

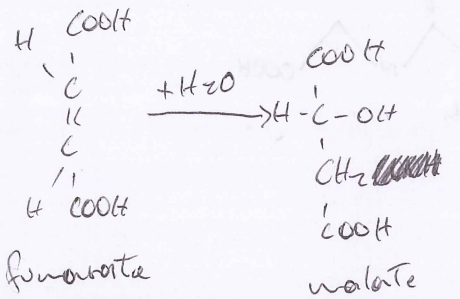
FORMULAS – Esz. II (diap. 27)



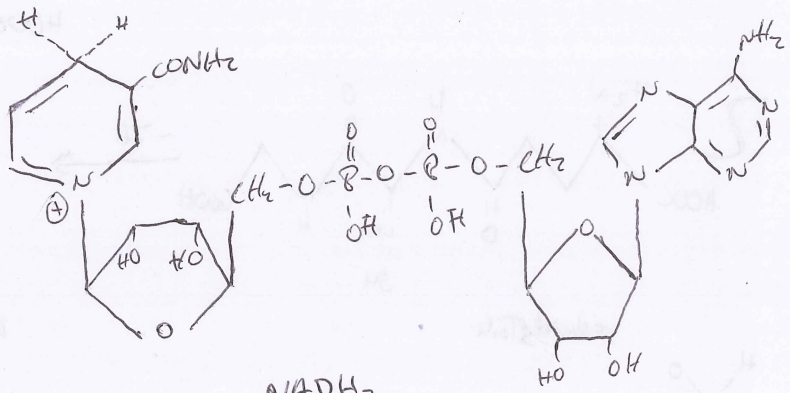
pyruvate lactate



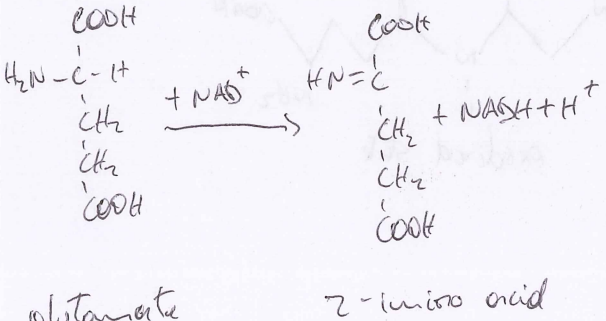
fructose glucitol mannitol



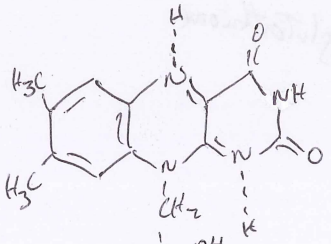
fumarate malate

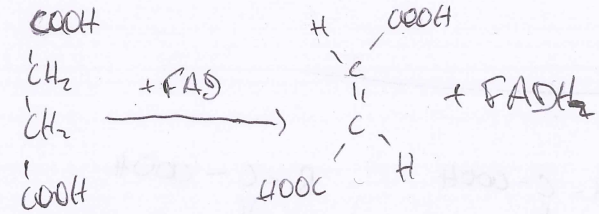


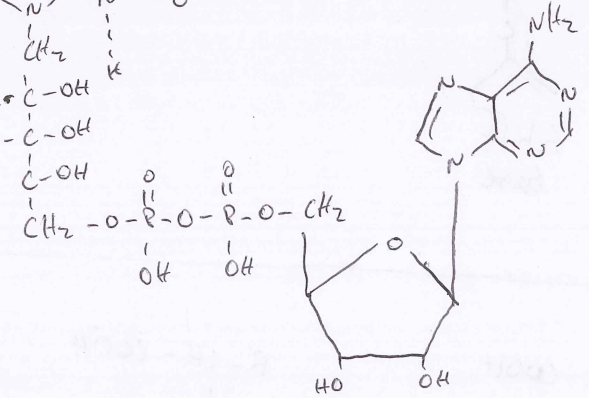
$NADH_2$



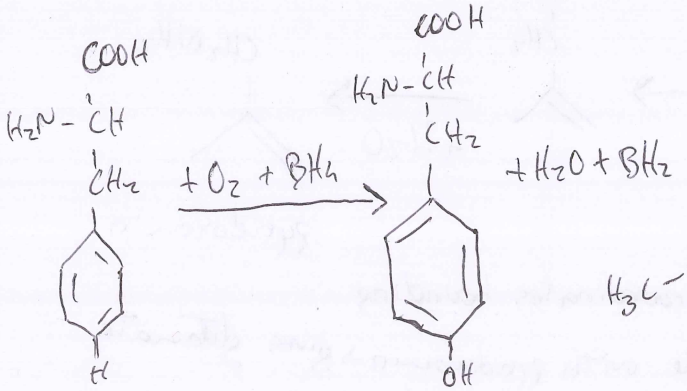
glutamate 2-imino acid

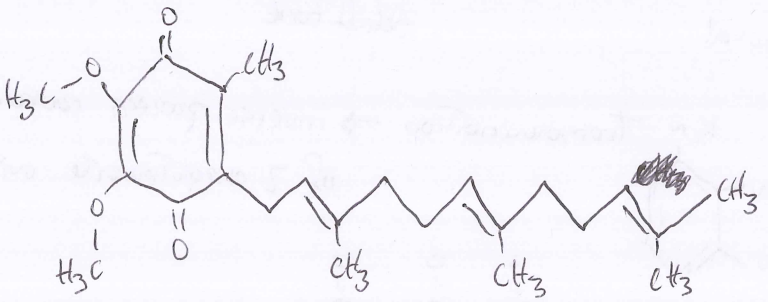


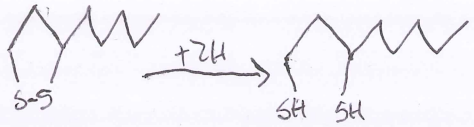




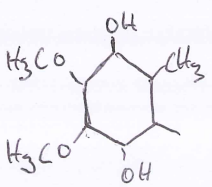
$FADH_2$





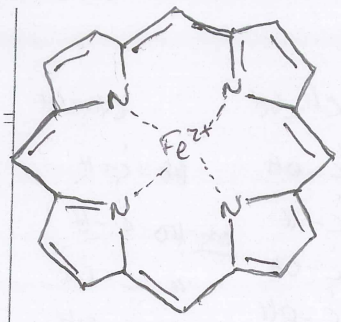


lipoate dihydrolipoate

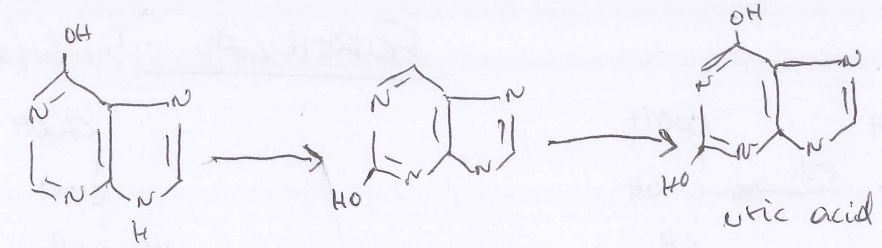


ubiquinone

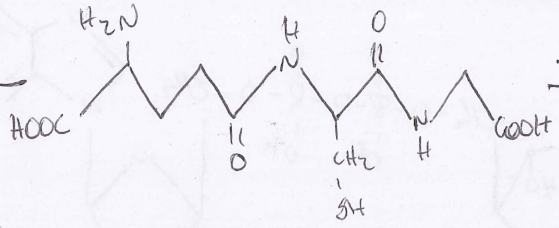
fully reduced – ubiquinol



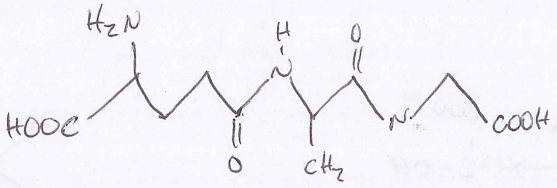
Heme (reduced state)

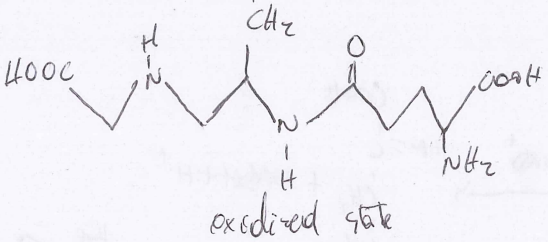


hypoxanthine → xanthine → uric acid



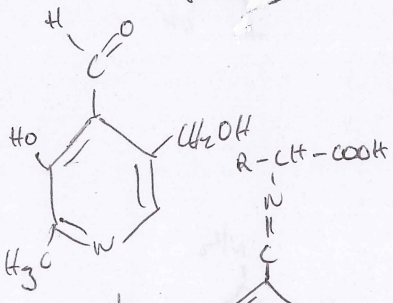
reduced state





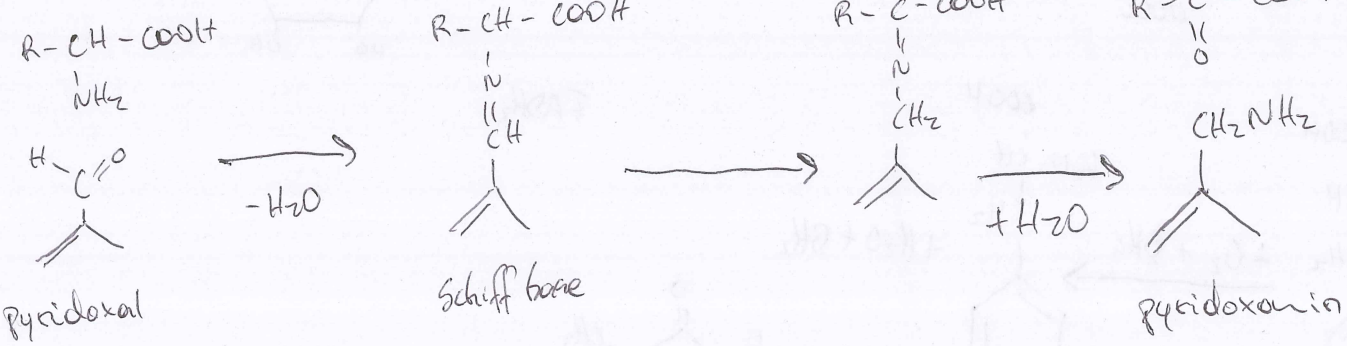
glutathione

oxidized state



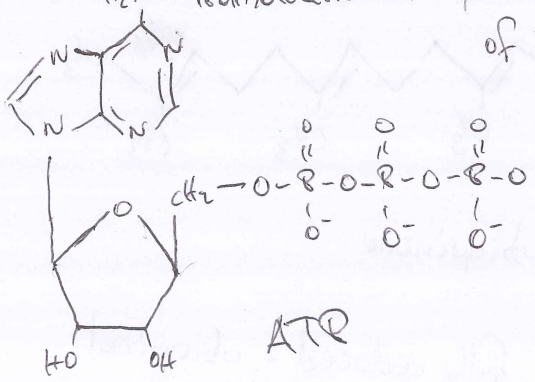
Pyridine

Schiff base

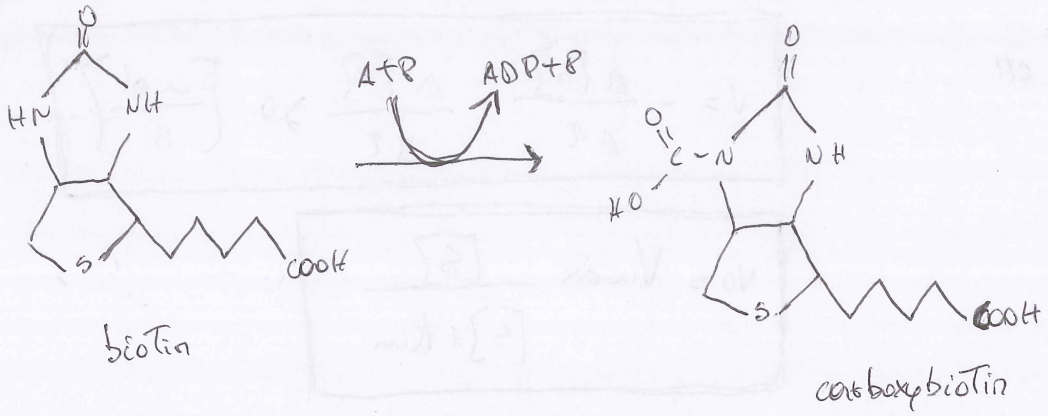


Pyridoxal → Schiff base → Pyridoxamin

H_2N Transamination → inverse process coordinates bounding of 2-oxoglutarate with pyridoxamin → gives glutamate

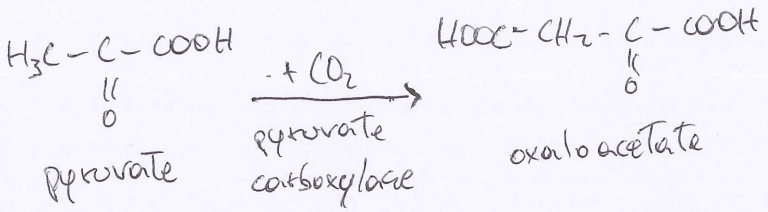


ATP



biotin

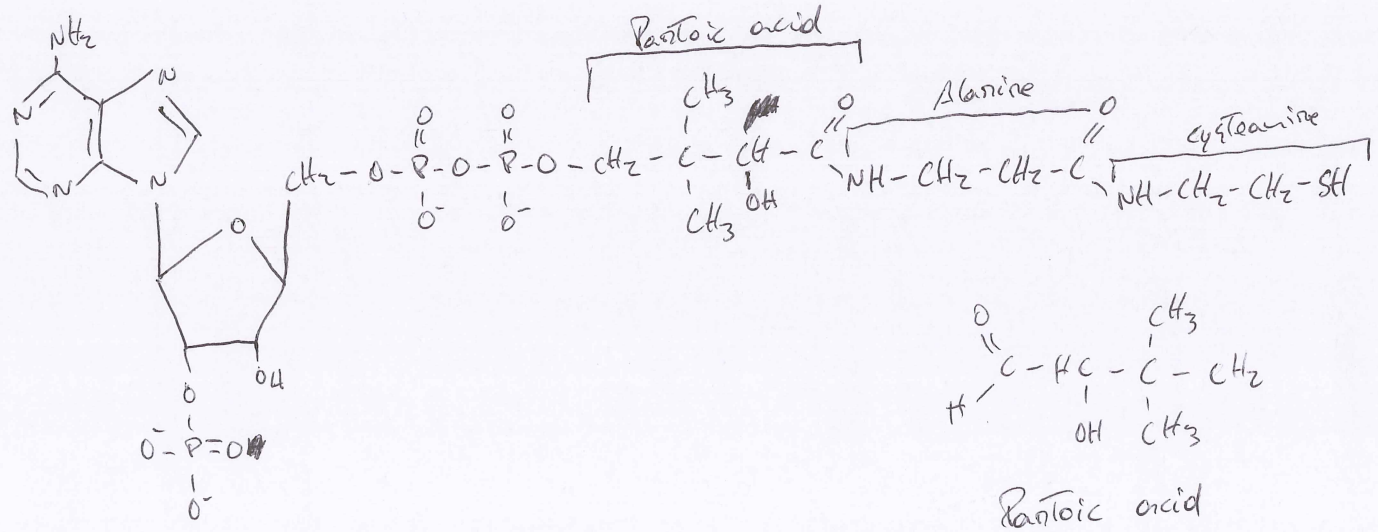
carboxybiotin



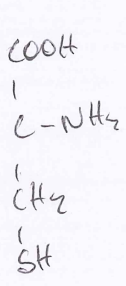
pyruvate

pyruvate carboxylase

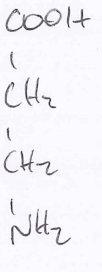
oxaloacetate



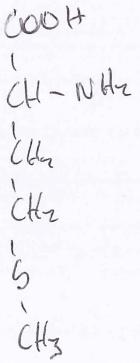
Pantoic acid



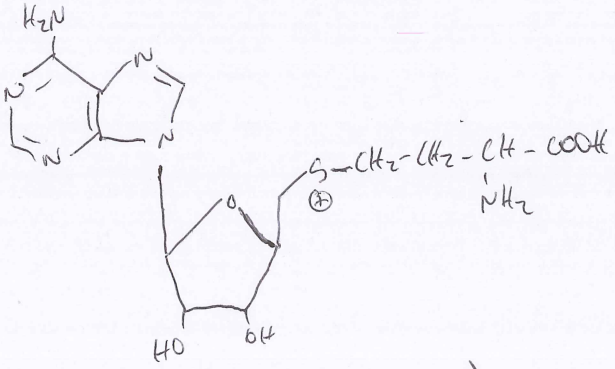
Cysteine



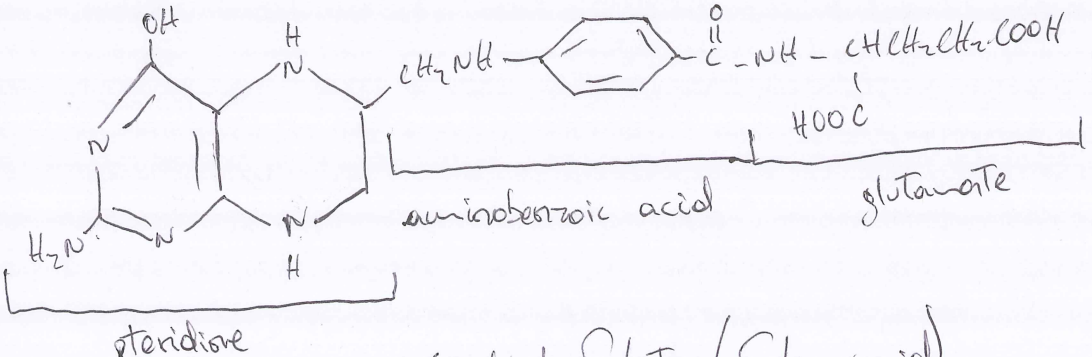
Alanine



Methionine



SAM (S-Adenosylmethionine)

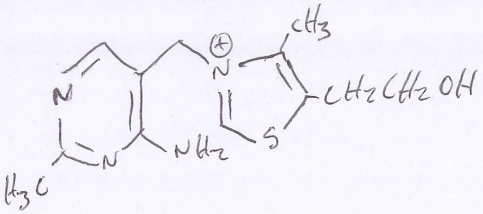


pteridine

aminobenzoic acid

glutamate

tetrahydrofolate (folic acid)

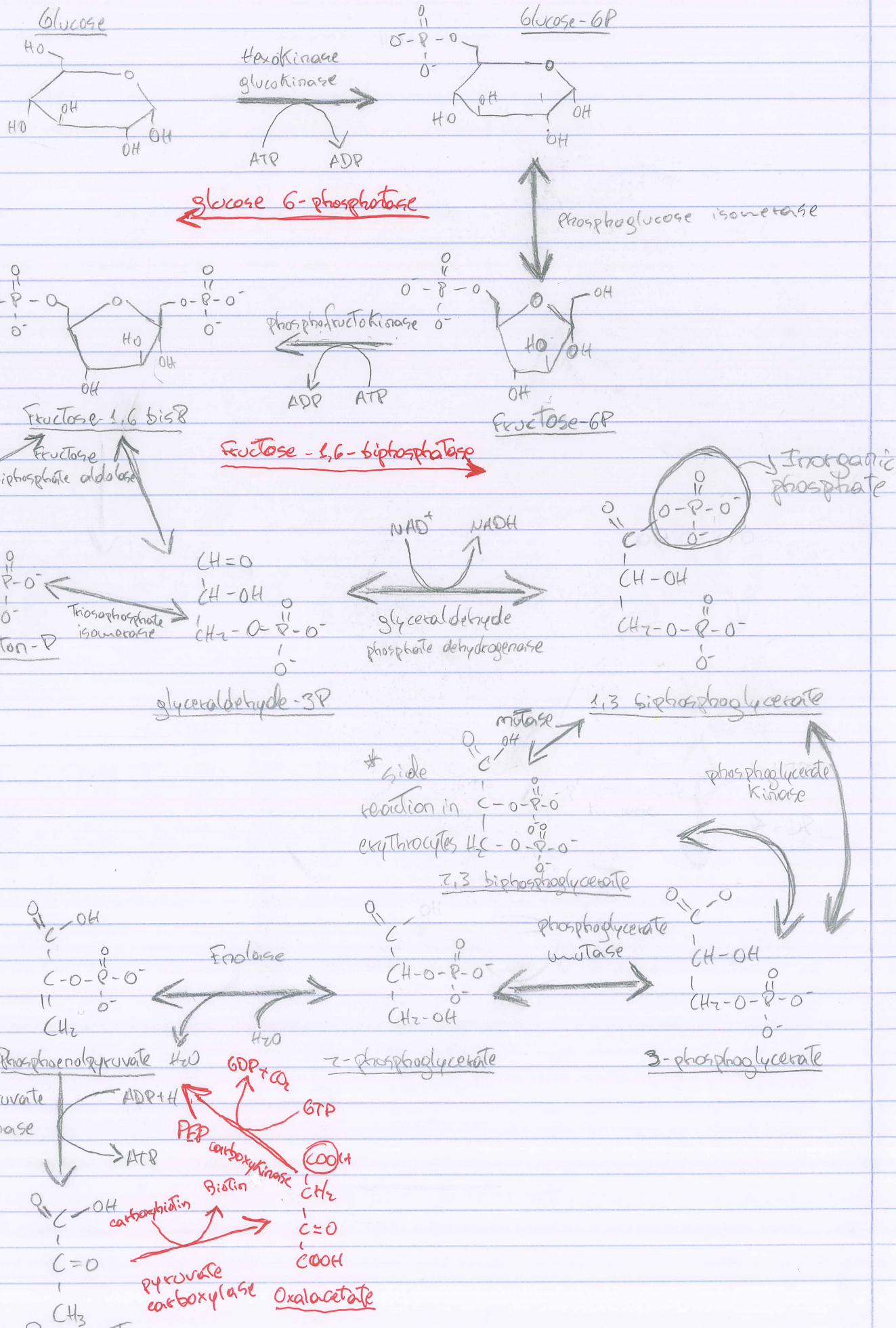


Thionin

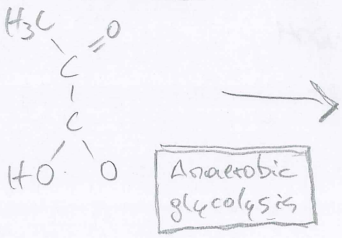
$$v = -\frac{\Delta[S]}{\Delta t} = \frac{\Delta[P]}{\Delta t} > 0 \quad \left[\frac{mol}{s}\right]$$

$$v_0 = V_{máx}\,\frac{[S]}{[S] + K_m}$$

GLYCOLYSIS


Glucose
Hexokinase
glucokinase
ATP
ADP
Glucose-6P
glucose 6-phosphatase
Phosphoglucose isomerase
Phosphofructokinase
ADP
ATP
Fructose-1,6 bisP
Fructose-6P
Fructose - 1,6 - biphosphatase
Fructose biphosphate aldolase
Inorganic phosphate
NAD+
NADH
Triosephosphate isomerase
dihydroxyacetone-P
glyceraldehyde-3P
glyceraldehyde phosphate dehydrogenase
1,3 biphosphoglycerate
mutase
* Side reaction in erythrocytes
2,3 biphosphoglycerate
phosphoglycerate Kinase
Enolase
H2O
phosphoglycerate mutase
Phosphoenolpyruvate
2-phosphoglycerate
3-phosphoglycerate
Pyruvate Kinase
ADP+H
ATP
GDP+CO2
GTP
PEP carboxykinase
Biotin
carbonbiotin
pyruvate carboxylase
Oxalacetate
Pyruvate

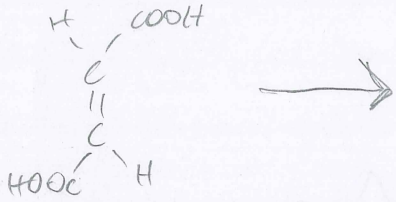
Pyruvate → Lactate



Anaerobic glycolysis

Oxidoreductase
- dehydrogenase
- oxygenase
- oxidase
- peroxidase

Fumarate → Malate



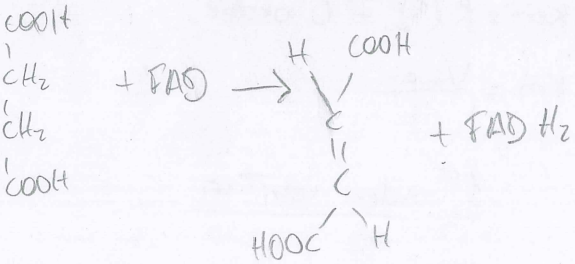
Transferase
- kinase
- phosphomutase
- amino/methyl/glucosyl Transferases

ethanol + NAD^+ → acetaldehyde + NADH + H^+

Σ aminoacid + NAD^+ → Σ iminoacid + NADH + H^+

Hydrolase
- esterase
- glycosidase
- proteinase
- amidase
- ATPase

Succinate → Fumarate

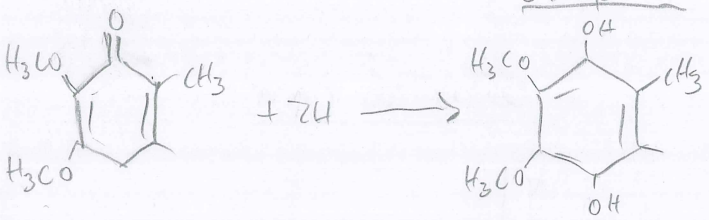


Lyase
- ammonia lyase
- decarboxylase
- aldolase
- dehydratase
- synthase

Phenylalanine + BH_4 → Tyrosine + BH_2

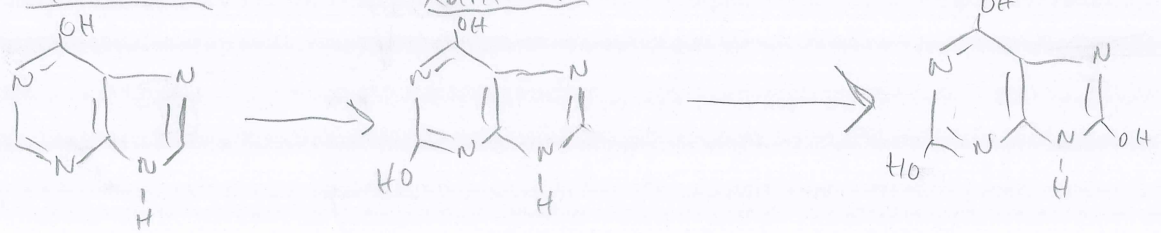
Isomerase
- epimerase
- racemase
- mutase

Ubiquinone → Ubiquinol

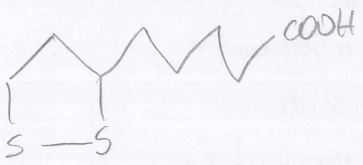


Ligase
- carboxylase
- synthetase

Hypoxanthine → Xanthine → Uric acid



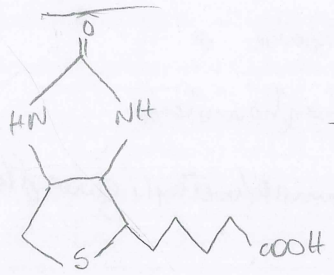
Lipoate



+ 2H ⟶

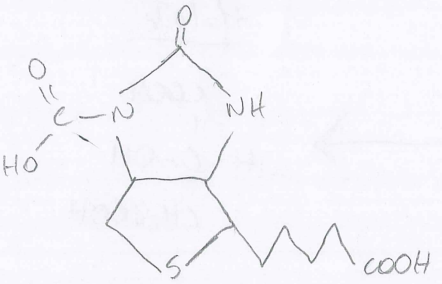
Dihydrolipoate

Biotin

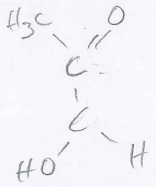


A+P → ADP+P

Carboxybiotin

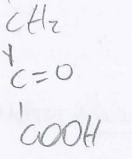


Pyruvate



Pyruvate carboxylase
+ CO_2 ⟶

Oxacelate



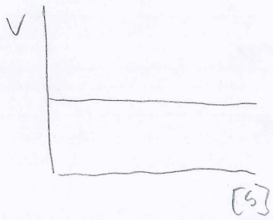
$$V_0 = V_{max} \times \frac{[S]}{[S] + K_m}$$

⟶ If $[S] \ll K_m = k[S]$ - 1ST order

⟶ If $[S] \gg K_m = k[S]^0$ - 0 order

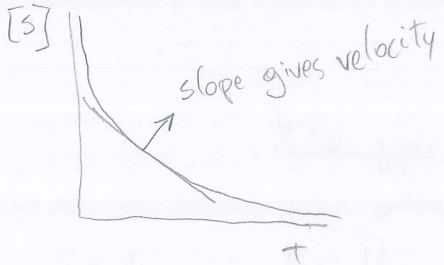
⟶ If $[S] = K_m = \frac{V_{max}}{2}$

0 order reaction

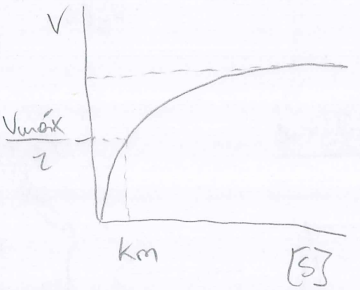


1ST order reaction

Kinetic curve

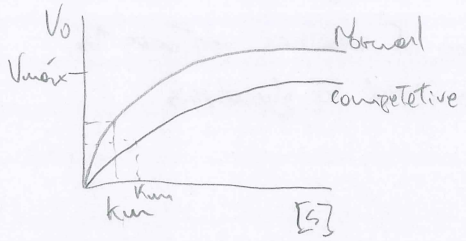


Saturation curve



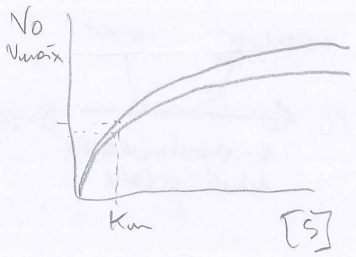
$\frac{\Delta A}{E \times l \times \Delta t}$ → Catalytic concentration of the enzyme (mol/l/s)

Competetive inhibition



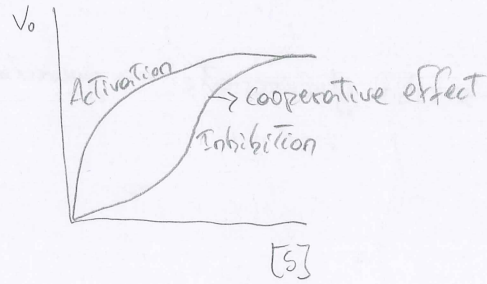
Vmáx is also reached but at much higher [S]

Non-competetive inhibition



Decrease of Vmáx but whithout changing Km

Allosteric enzymes



Curve is sigmoidal
they alter their conformation (conformation changes)
Isoenzymes → catalyze the same reaction

Enzymes
- indicators of pathologies
- analytic reagents
- medication

$glucose + O_2 \xrightarrow[\text{oxidase}]{\text{glucose}} gluconolactone + H_2O_2$

$H_2O_2 + H_2A \xrightarrow[\text{peroxidase}]{} 2H_2O + (A)$

[glucose] depends on colour

Gibbs energy

$$\Delta G = \Delta G^\circ + R \times T \times \ln \frac{[C]^c \times [D]^d}{[A]^a \times [B]^b}$$

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

Glycolysis

Gluconeogenesis

Glycogen synthesis (glycogenesis) → primer is needed (pre-existing fragment); formation of α-1,4 glycosidic bonds (iniciation, elongation); branching

Degradation of glycogen (phosphorolysis) →
→ debranching enzyme converts structure into a linear one; phosphorylase attacks remaining α-1,4-linked chain

Pentose phosphate pathway

Oxidative phase

glucose-6-P —(NADP+ → NADPH+H+; glucose-6P dehydrogenase)→ 6-phosphoglucono lactone —(lactonase)→ 6-phosphogluconate —(NADPH+H+ → NADP+; 6-phosphogluconate dehydrogenase)→ Ribulose-5-P + CO_2

Non-oxidative phase

3 ribulose-5-P ⇌ 2 fructose-6-P + glyceraldehyde 3P

* conversion of useless pentoses to products used in glycolysis

Fructose metabolism

- mainly in liver
- metabolized faster than glucose
- don't stimulate insulin release

Uronic acid pathway → supplies glucuronic acid

glucose-6P ⇌ glucose-1P —(UTP)→ UDP glucose —(H_2O, NAD^+)→ UDP glucuronate → glucuronate

Synthesis of amino sugars

Fructose 6-P → Glucosamine 6-P (Glutamine; Glutamic acid)

Proteasomes → degrade regulatory proteins (short half-life) and abnormal/unfolded proteins

↓ inhibited by bortezomib

Essential amino acids: valine, leucine, isoleucine, threonine

Semiessential amino acids: phenylalanine, tryptophan, lysine, methionine

Catabolic pathway of amino acids

Proteins —(proteolysis)→ amino acids —(transamination)→ glutamate —(dehydrogenation + deamination)→ NH_3 —(detoxication in liver)→ urea

NH_3 —(detoxication)→ glutamine

glutamine —(deamination in kidney)→ glutamate + NH_4^+

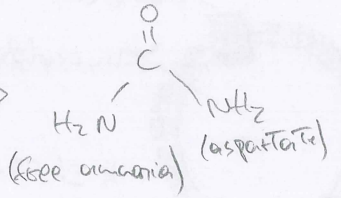
glutamate + NH_4^+ —(excretion by urine)→ NH_4^+

glutamate + NH_4^+ ← 2-oxoglutarate + NH_4^+

Sources of ammonia in the organism
- deamination of glutamate in tissues
- bacterial putrefaction in large intestine

Ureasynthesis in liver

1) Carbamoyl phosphate synthetase (matrix)
2) Citrulline formation
3) Citrulline + NH_2 ⟶ argininosuccinate
   ↓
   from aspartate
4) Cleavage of argininosuccinate giving arginine + fumarate
5) Hydrolysis of arginine gives ornithine - urea ⟶



Synthesis of non-essential amino acids

→ glycine from serine
→ serine from glycolysis intermediate (3P-hydroxypyruvate)
→ alanine from pyruvate
→ aspartate from oxaloacetate and glutamate
→ glutamate from 2-oxoglutarate
→ proline from glutamate
→ Tyrosine from phenylalanine
→ glutamine from glutamate and ammonia
→ cysteine from methionine

Intermediates of amino acid catabolism

- oxaloacetate: aspartate, asparagine
- fumarate: phenylalanine, tyrosine, Aspartate
- pyruvate: serine, glycine, threonine, alanine, cysteine, Tryptophan
- 2-oxoglutarate: arginine, glutamic acid, histidine, proline, glutamine
- Succinyl-CoA: valine, isoleucine, methionine
- acetyl-CoA: isoleucine, leucine, lysine, threonine
- acetoacetate: leucine, lysine, phenylalanine, tryptophan, tyrosine

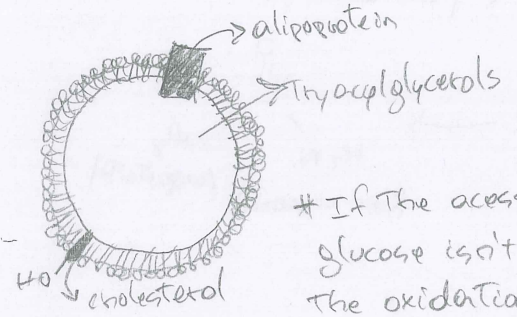
Simple lipids → Triacylglycerols

Complex lipids | phospholipids
glycolipids

Derived lipids | cholesterol and other steroids
eicosanoids
carotenoids

In the intestine → fat droplets are emulsified with bile salts and form mixed micelles

In extracellular fluids → Transported as lipoprotein particles (chylomicrons)



* If the access to glucose isn't enought the oxidation begins

Insulin initiates the synthesis of Triacylglycerols

Fatty acids act as energy source of most of cells

→ activation by linking to CoA (by citrite ↑)
→ Transport of acyl CoA to mitochondrial matrix
→ β- oxidation of acyl CoA

129 ATP formed

Fatty acid synthesis

1) Transfer of acetyl group of acetyl CoA to the sulfur of a cystein residue
2) Malonyl group is transferred to the sulphur of phosphopantetheine
3) joining acetyl unit — formation of acetoacetyl
4) reduction resulting in 3-hydroxyacyl unit
5) dehydration to Trans-2-enoyl
6) reduction resulting in saturated acyl
7) Transfer of saturated acyl to cysteine sulfur
8) Palmitate is the main result of FA synthesis

Essential fatty acids | linoleate
α-linolenate
arachidonate
eicosapentaenoate

Glycerophospholipids

Activation
- diacylglycerol + activated head group
- head group + activated phosphatidate

Catabolism → hydrolysis of glycerophospholipids is made by phospholipases