PART III. ENZYMOLOGY: Herminia González Navarro

LESSON 6. REACTIONS CATALYZED BY ENZYMES
LESSON 7. ENZYMATIC KINETICS AND REACTION VELOCITY
LESSON 8. REGULATION OF ENZYMATIC ACTIVITY

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LESSON 6. REACTIONS CATALYZED BY ENZYMES

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Herminia González Navarro 2021

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FUNCTIONS AND PROPERTIES OF ENZYMES

Biological functions require **chemical reactions at high velocity to provide cellular needs.**

ENZYMES are biological molecule that catalyze chemical reactions.

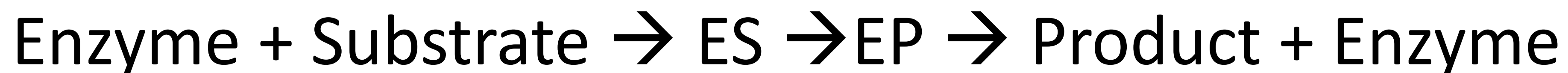
They are proteins that facilitate biochemical reactions by increasing their velocity by between 10^3 and 10^8 times.

Among all biochemical reactions that are energetically possible, enzymes channel and modify the chemistry to achieve greater efficiency in terms of timing.

Enzymes perform all metabolic processes.

ENZYMES IN THE CHEMICAL REACTION

The enzymes bind substrates in the active site and transform them into new molecules called products.

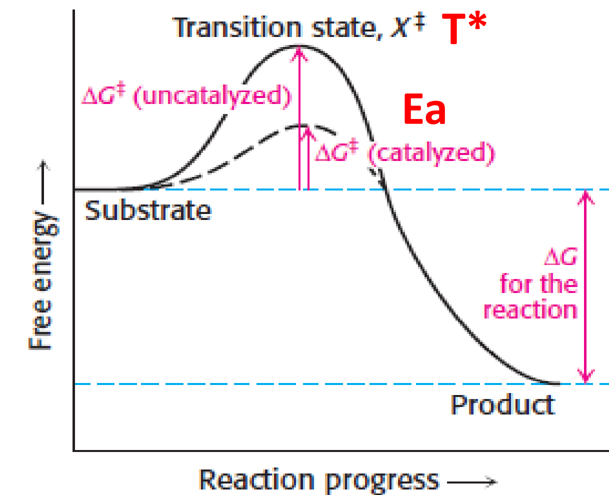


CHEMICAL REACTIONS: ENERGY OF ACTIVATION AND TRANSITION STATE

All chemical reactions have an **energy barrier** between substrates and products.

ACTIVATION ENERGY (Ea):

This is the difference in energy between the substrates and the **reaction intermediate** that has the highest energy content. This intermediate with the highest energy is called the **Transition state (T*)** because it is unstable and because its high energy content will allow the reaction to resume.



The most useful energy magnitude to determine whether a chemical reaction will occur is the **change in Gibbs free energy (ΔG).**

A **Gibbs free energy ΔG<0** indicates a favorable reaction.
A Gibbs free energy ΔG>0 indicates a non-favorable reaction and if the system requires this reaction an input of energy is needed.
When the chemical reactions achieve a dynamic equilibrium state (products and substrate are in equilibrium), they are defined by the Keq. The free energy of the reaction is **ΔG=0.**

THE DIFFERENCE IN ENERGY BETWEEN THE PRODUCTS AND THE SUBSTRATES DOES NOT DEFINE OR DETERMINE THE REACTION VELOCITY.

GENERAL PROPERTIES OF THE ENZYMES

THE ENZYME AS A BIOLOGIC CATALYZER

1. **Diminishes the activation energy needed to achieve the transition state and favors the transition state.**

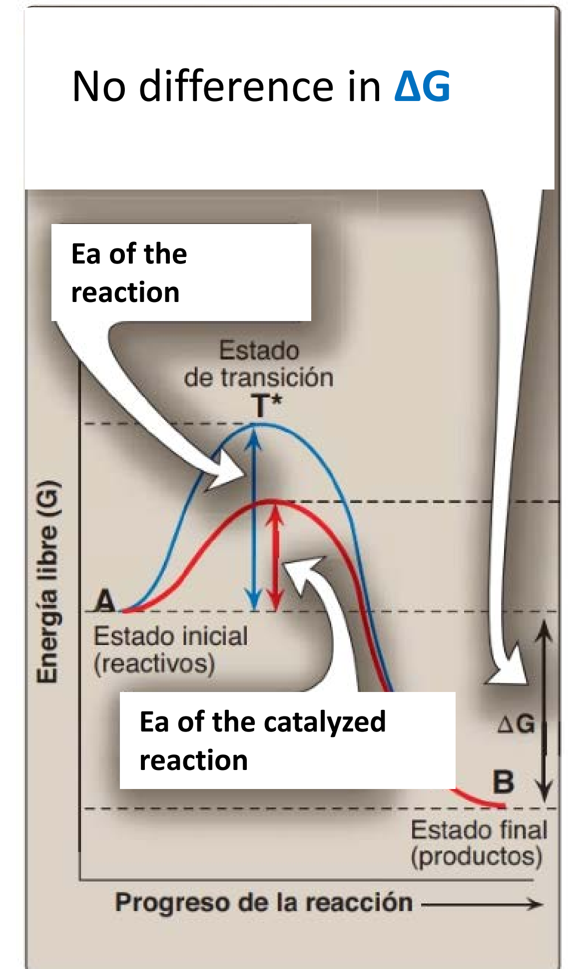
2. **Accelerates the chemical reactions. The enzyme is not altered during the reaction.**

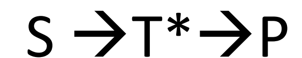
3. **Does not enable reactions that are not thermodynamically favorable.**

4. **Does not modify the reaction’s final equilibrium.**

5. **Does not modify the reaction’s energetic balance.**

6. **Is needed in small quantities.**





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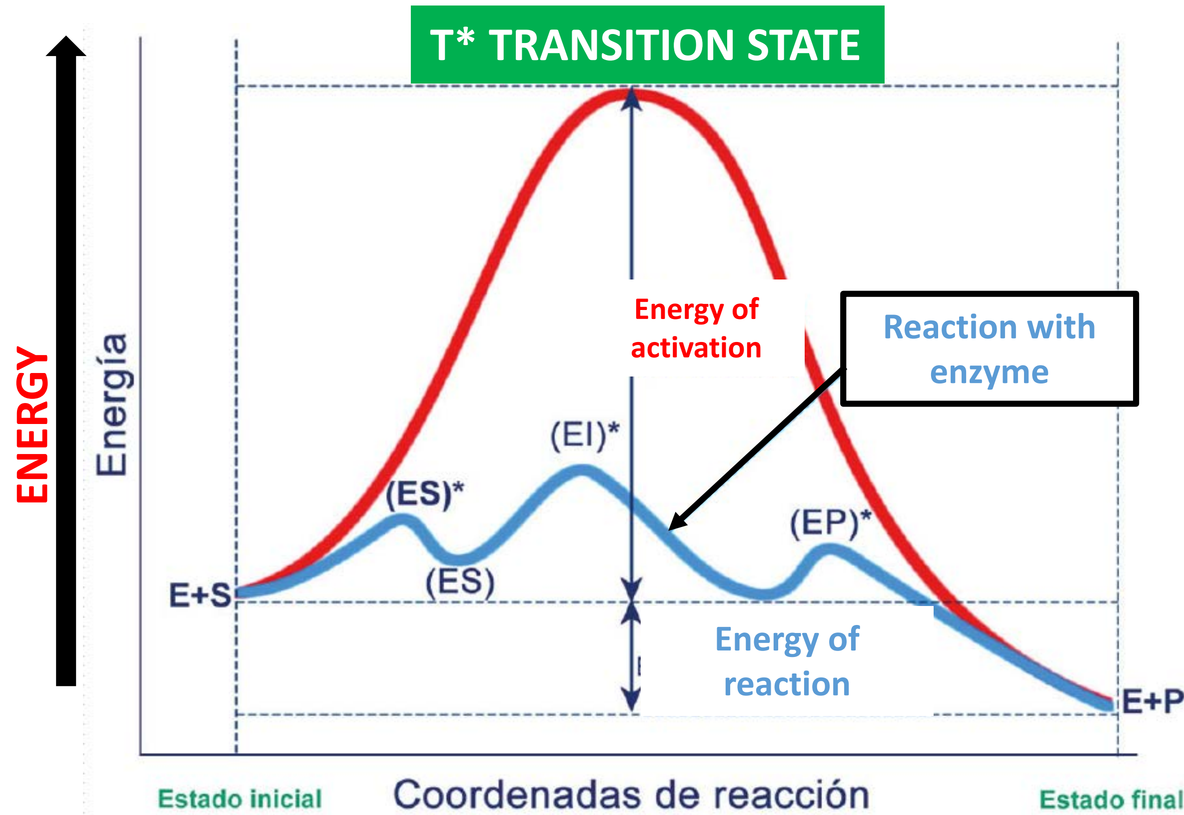
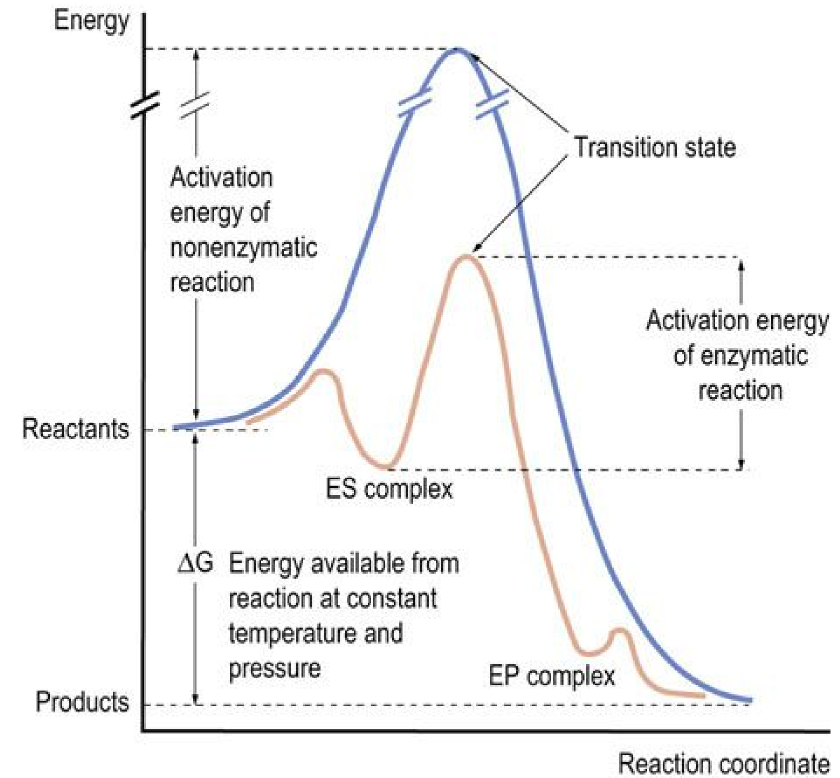
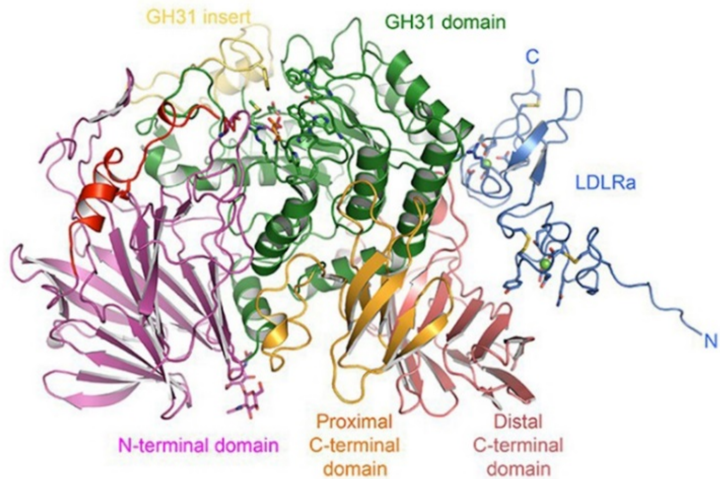
ENZYMATIC MECHANISM OF ACTION: ENERGETIC CHANGES DURING THE CHEMICAL REACTION





REACTION PROGRESS

ENZYMES ARE CATALYZERS THAT ARE CHEMICAL PROTEINS. THEY ARE GLOBULAR PROTEINS.

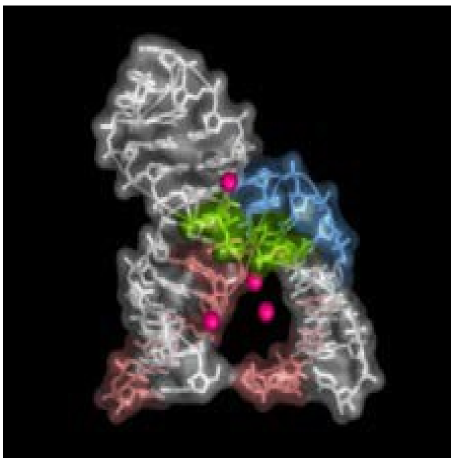


GLOBULAR STRUCTURE OF A PROTEIN WITH MULTIPLE DOMAINS

PNAS July 26, 2016, doi:10.1073/pnas.1604463113

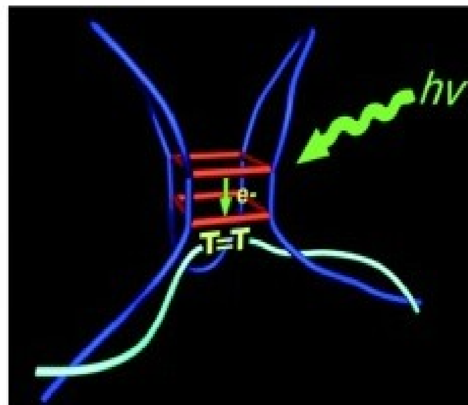
OTHER CATALYZERS THAT ARE NOT PROTEINS ALSO EXIST

RIBOZYMES: RNA

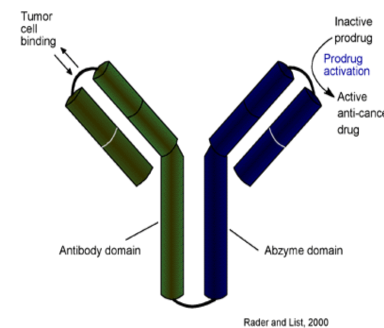


Ribosomic RNA

Deoxyribozymes: DNA

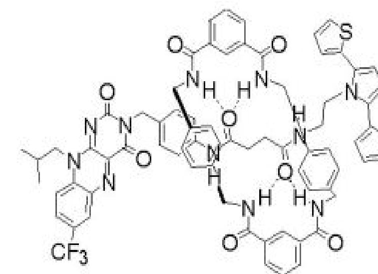


Abzymes



Antibodies with enzymatic activity

Synthetic enzymes



PROPERTIES OF ENZYMES IN THE CHEMICAL REACTIONS OF LIVING BEINGS

1. **EFFICIENCY:** they favor **the positioning of substrates** through interactions (hydrophobic, electrostatic, and even covalent unions) that favor collision between substrates or strain a substrate in such a way that it breaks or splits.
Efficiency increases the reaction velocity by 10^3 -10^8 times.

2. **SPECIFICITY:** the complex globular structure provides the enzyme with the capability to create catalytic sites that can accommodate different substrates differently. They may even discriminate between enantiomers L and D.

3. **FUNCTIONING IN BIOLOGICAL CONDITIONS**:
Temperature, physiologic pH.

4. **VARIABLE ACTIVITY AND REGULATION CAPACITY:**
Activators, inhibitors, etc.

5. **CELLULAR LOCALIZATION:**
Depending on their function, the needs of the products and the location of the substrates, enzymes are located in organelles such as mitochondria, ER, nucleus, lysosomes, etc.

PROPERTIES OF THE ENZYMES AS PROTEINS

LABILITY

Enzymes are sensitive to temperature and pH. They can undergo denaturalization and loss of protein globular structure.

ABILITY TO FORM NON-COVALENT BONDS

The non-covalent bonds will be characterized by having specific chemical groups that are needed in the chemical reaction.

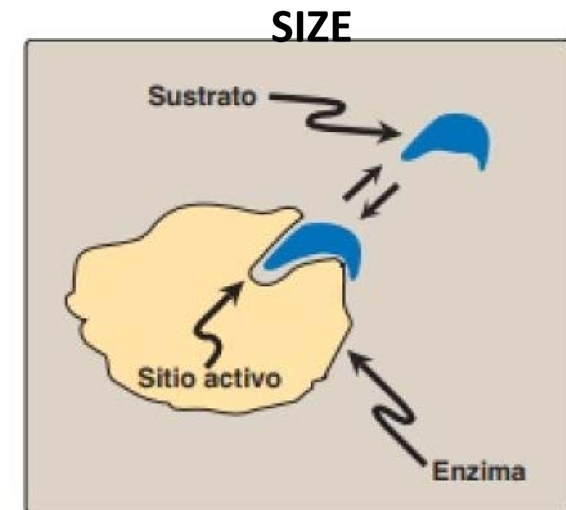
REQUIREMENTS: COFACTORS AND COENZYMES

They require non-proteic molecules that participate and help in the enzymatic reaction (Zn, Fe, FADH, NADH).

SIZE

Enzymes are much bigger than the substrates and products.

SIZE



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SPECIFICITY OF THE ENZYMES

SPECIFICTY OF THE SUBSTRATE

Enzymes differentiate between substrates with similar characteristics at different levels.

- Group specificity: enzymes that catalyze chemical reactions in a group of substrates that **share a chemical group** (PHOSPHATASES AND KINASES).

- Class specificity: enzymes that catalyze the **transformation** of substances that have a **specific type of bond** (PEPTIDASES, TRANSAMINASES).

- **Sterospecificity:** Enzymes that catalyze chemical reactions that differentiate **between D and L isomers**.

SPECIFICTY OF ACTION

Enzymes that perform reactions in the same substrate but differ in the kind of transformation they perform in that substrate.

PYRUVATE: pyruvate dehydrogenase, pyruvate kinase, pyruvate carboxylase and pyruvate decarboxylase.

NOMENCLATURE AND CLASSIFICATION OF ENZYMES

Nomenclature

CLASSIC NOMENCLATURE: -ase is added to the catalyzed substrate, the type of reaction, or the organ that provides the enzyme.

Organ: *Pancrease*

Substrate: *Protease*

Type of reaction *Hydrolase*

CURRENT NOMENCLATURE:

Current nomenclature follows the rules of the **Enzyme Commission** [E.C.] of the **IUBMB** (*International Union of Biochemistry and Molecular Biology).*

Assigned code: **E.C.: 1.1.1.1** class (transferase); subclass (chemical group); sub-subclass (type of transferase); the final four number identify the enzyme.

Systematic name:

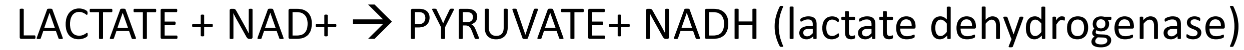
Substrate: Co-substrate and type of reaction –ase

Ethanol: NAD^+ Oxidoreductase

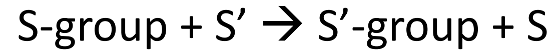
ENZYME CLASSIFICATION

The classification of enzymes follows the ***International Union of Biochemistry and Molecular Biology [IUBMB]***.
Enzymes are classified according to the general type of reaction they catalyze.

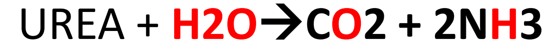
1. OXIDOREDUCTASES: REDOX reactions. An element is reduced, and another is oxidized. **ELECTRON TRANSFERENCES.**



2. TRANSFERASES: Reactions that catalyze the **transfer of chemical groups** usually containing C, N or P.
Transference of chemical groups: AMINOTRANSFERASES



3. HYDROLASES: These break bonds using water (i.e. by adding water).



4. LIASES: These break covalent bonds such as C-C C-S y C-N.

5. ISOMERASES: These reorganize chemical groups inside a molecule.

6. LIGASES: These enzymes fusionate substrates by making covalent bonds between C and O, S, N. They usually have a high energy demand and need an ATP molecule.

MECHANISM OF ACTION OF ENZYMES IN CHEMICAL REACTIONS

THE ACTIVE SITE OF THE ENZYME

The active site is a pocket or split formed by protein folding. It is usually hydrophobic in nature and **contains different Aas with lateral residues (chains)** that orientate and bind the substrate.

1. **AMINOACIDS OF BINDING:** these residues bind the substrate in a non-covalent way but orientate and position it to facilitate the chemical attack or collision. They determine substrate specificity.

2. **AMINOACIDS OF CATALYSIS:** these directly participate in the reaction and provide the specificity of the reaction (type).

The active site environment: chemical groups that support the reaction by interacting with the substrate.

Specificity is determined by the shape and size of the active site

RESIDUES or UNION AAS:
BINDING SITE

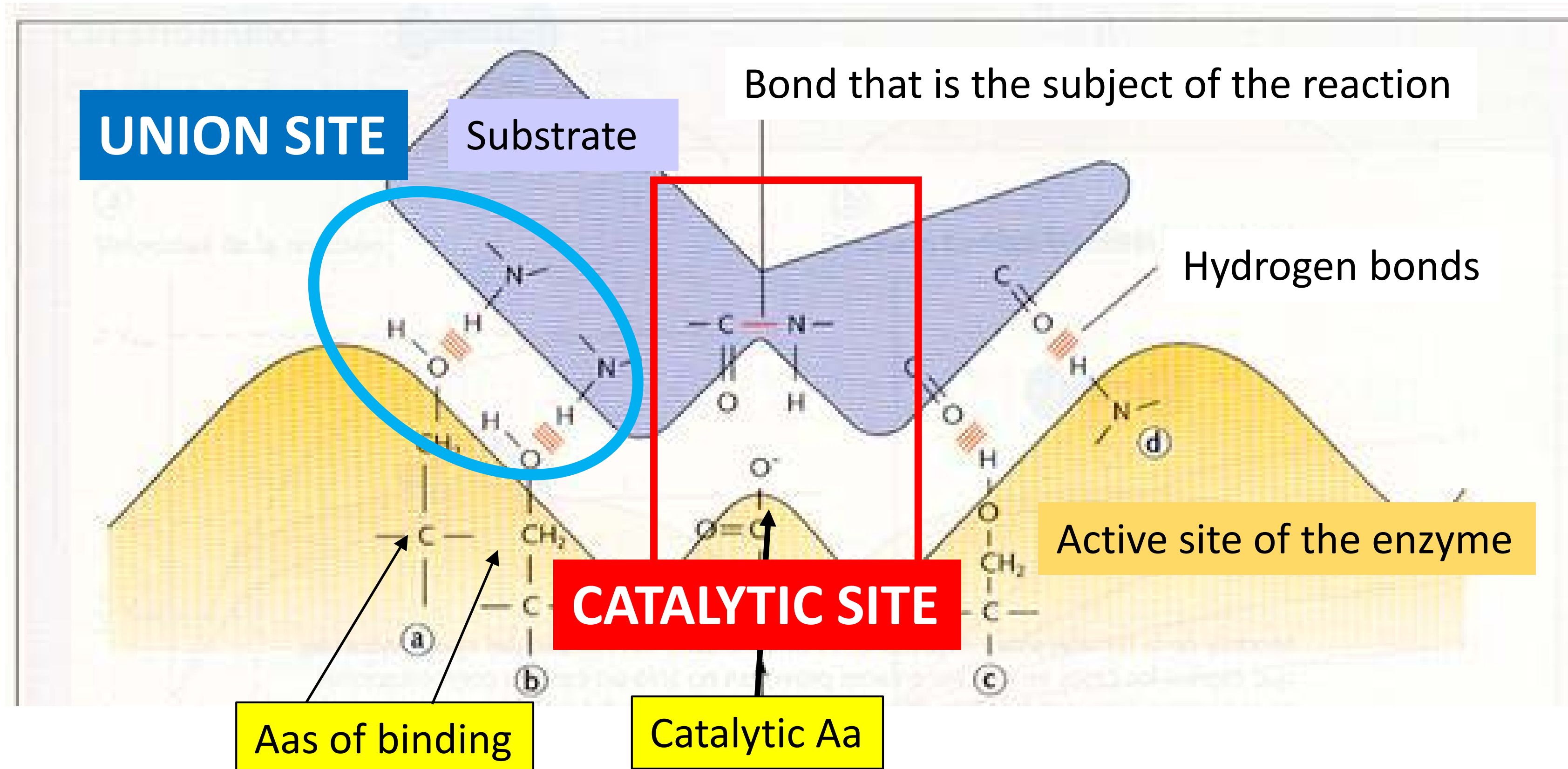
Stabilize

Orientate

Recognize

RESIDUES or CATALYTIC AAS:
CATALYTIC SITE

involved in the chemical reaction



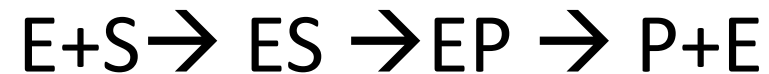
STAGES OF ENZYME AND SUBSTRATE INTERACTION. EXAMPLE OF HYDROLYSIS.

O-glycosidic bond

Asp52: covalent bond with C1

Glu35: with (-) charge will polarize the H2O; O2 will attack C1.

The water will resume the hydrolysis

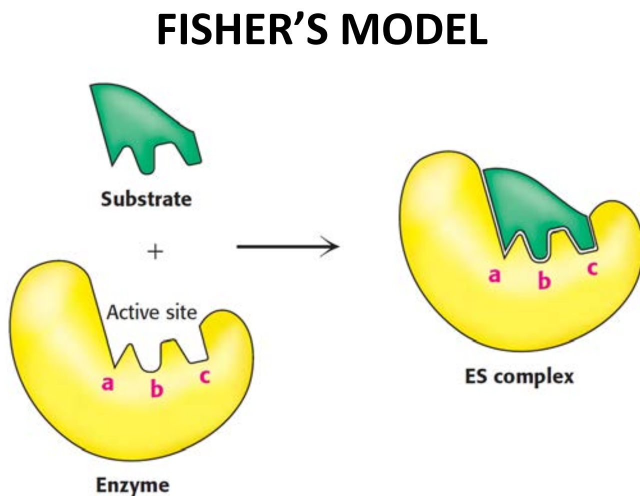


ENZYME AND SUBSTRATE INTERACTION MODELS

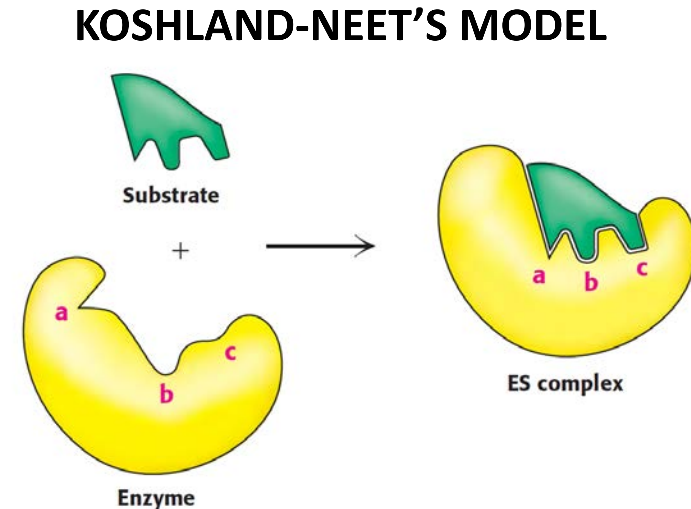
1. FISHER'S MODEL (1890): LOCK AND KEY MODEL. The substrate perfectly fits into the split of the active site.

2. KOSHLAND-NEET'S MODEL (1968):
INDUCED-FIT MODEL
The coupling occurs like a hand in a glove.
The binding of the substrate into the active site induces a conformational change.

FISHER'S MODEL



KOSHLAND-NEET'S MODEL



TYPES OF ENZYMATIC REACTION: GENERAL MECHANISMS

The binding of E+S facilitates catalysis via several mechanisms. Various mechanisms facilitate the formation of T* in the catalyzed chemical reaction:

a) **Covalent catalysis**: enzymes that bind to the substrate, form an ES complex with covalent interactions, and form an unstable intermediate.

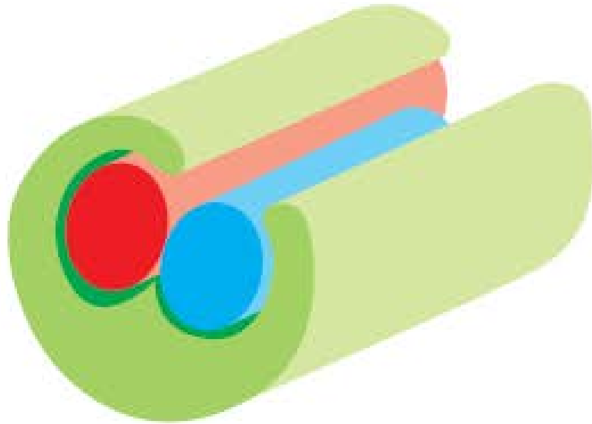
b) **Acid-base catalysis**: Aas in the catalytic site are H+ donors or acceptors (carboxyl, amine) for certain types of reactions.

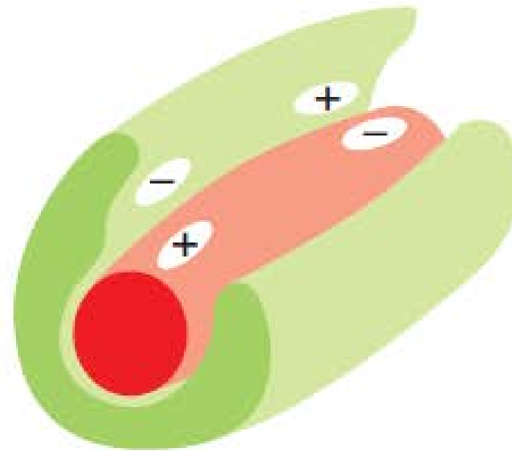
c) **Electrostatic catalysis**: electrostatic unions between the enzyme and the substrate that will generate an ES complex.

d) **Destabilization of substrate bonds.**

e) **Catalysis** produced by the **proximity and orientation of chemical groups:** the enzyme-substrate binding induces conformational changes that favor the reaction between the chemical groups that undergo the catalytic process.

ENZYME AND SUBSTRATE INTERACTION: GENERAL MECHANISMS





1. PROXIMITY AND ORIENTATION

2. ELECTROSTATIC INTERACTIONS FACILITATE THE REORGANIZATION OF THE SUBSTRATE FOR CATALYSIS

3. THE ENZYME-SUSTRATE INTERACTION INDUCES A STRAIN AND FORMS AN UNSTABLE T*.

ENZYMATIC COFACTORS: GENERAL CHARACTERISTICS, PROPERTIES AND TYPES

1. Enzymatic cofactors are **not protein molecules; they have a low molecular weight and are thermostable.**
2. They are found at **low concentrations** in cells.
3. They can be shared by **several enzymes.**
4. They cooperate with enzymes, **can be altered** during catalysis, and are not recovered after the reaction.

These types of cofactors are called **co-substrates.**

5. Some of them have the chemical structure of a heterocyclic/cyclic with **highly reactive electrons.**
6. They are **highly reactive.**
7. Most of them are **vitamin-derived.**

Enzyme that require a cofactor and are inactive without it are is called APOENZYMES.

HOLOENZYME = APOENZYME + COFACTOR

TYPES OF ENZYMATIC COFACTORS

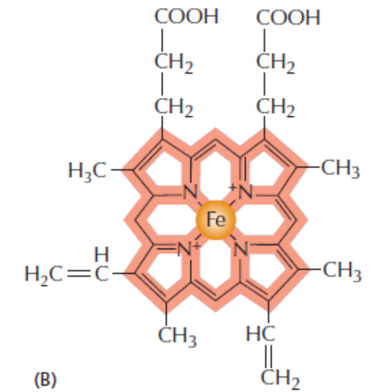
ENZYMATIC COFACTORS

1. **Metallic ions** (Fe, Cu, Mn2+, Mg2+).

2. **Coenzymes,** which have a more complex structure.

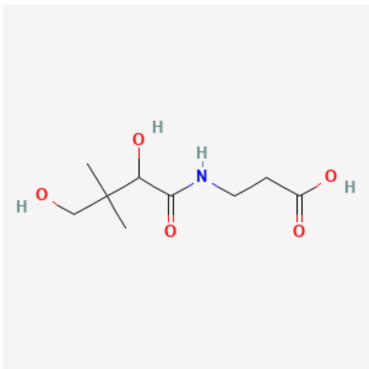
3. The group of coenzymes that are **covalently bound** to the enzyme are called **PROSTHETICS**.

Heme: prosthetic group

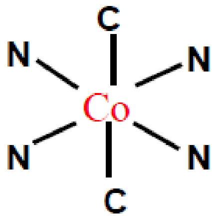


SOME COENZYMES

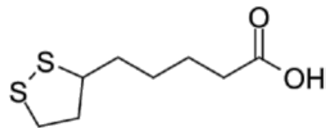
Pantothenic acid



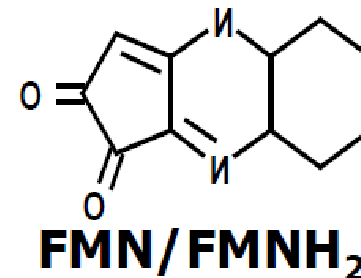
Cobalamin



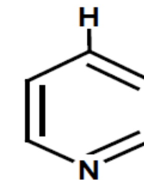
Lipoic acid



Flavin nucleotides



Nicotinamide nucleotide



COENZYME CLASSIFICATION AND MOST IMPORTANT COENZYMES

1 Nutritional origin: vitamin or not derived.

2 Functional criteria: transport of **electrons.**

3 Enzymological criteria: **the type of reaction** in which they participate.

VITAMINIC ENZYMES

TRANSFER OF CHEMICAL GROUPS

Acyl acid: Coenzyme A-pantothenic

Carboxyl: Biotin - **Vit H**

Aldehyde: **Thiamine pyrophosphate- Vit B1**

Alkyl: Cobalamin

Amine: pyridoxal phosphate **Vit B6**

Monocarbonates: Tetrahydrofolate acid

TRANSFER OF ELECTRONS

Nicotinamine nucleotides: NAD+/NADH; NADP+/NADPH VitB3

Flavin nucleotides: $FMN/FMNH_2$; $FAD+/FADH_2$ vit B2

Sugar-derived **Ascorbate VitC**

NON-VITAMINIC ENZYMES

TRANSFER OF CHEMICAL GROUPS

Phosphoryl: nucleotide-derived XTP

Adenylate: Adenilate ATP

Aminoalcohols and diacylglycerols bound to CDP –

Sugar Phosphate: XDP (ADP, CDP, **UDP**, GDP, dTDP)

Methyl groups: S-Adenosyl-Methionine

Acyl groups: Dihydrolipidic

TRANSFER OF ELECTRONS

Coenzyme Q (Ubiquinone)

Tetrahydrobiopterin

Methoxantin Factor 420

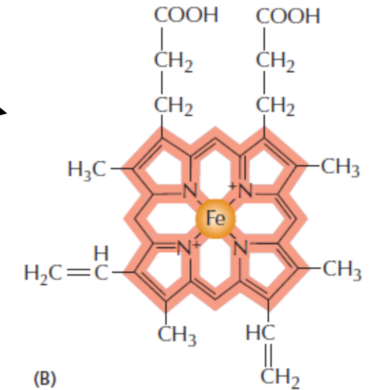
THE MOST IMPORTANT COFACTORS

1. Have a greater concentration of **positive charge**. Their ions are bound to enzymes.

2. They have **directed valences, which** allow interaction with several ligands.

3. They can exist in **more than one oxidation state** (Fe^{+2} Fe^{+3}).

4. The most important cofactors are **transition metals,** e.g. Mn, Fe, Cu, Co, Mo.



Cytochrome Oxidase Cu^{+2}
Catalase, Peroxidase: Fe^{+2} Fe^{+3}
Pyruvate kinase: K+
Hexokinase, Glucose-6-phosphatase : Mg^{+2}
Arginase, Ribonucleotide Reductase: Mn^{+2}
Alcohol Dehydrogenase Carboxypeptidases A y B: Zn^{+2}

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LESSON 7
ENZYMATIC KINETICS AND REACTION VELOCITY

Herminia González Navarro 2021

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ENZYMATIC KINETICS AND REACTION VELOCITY

ENZYMATIC KINETICS

Enzymatic kinetics is the study of the **reaction velocity** in a biochemical reaction catalyzed by an enzyme.

It is useful to know the **enzyme efficiency** and to study the **enzyme concentration** in a solution or biological sample.

REACTION VELOCITY

Reaction velocity is determined by **the change in the substrate or product concentration** per time unit.

Determining the enzyme velocity in a catalyzed reaction provides **information about**:

THE PRESENCE OF INHIBITORS OR ACTIVATORS, TISSULAR DAMAGE, etc.

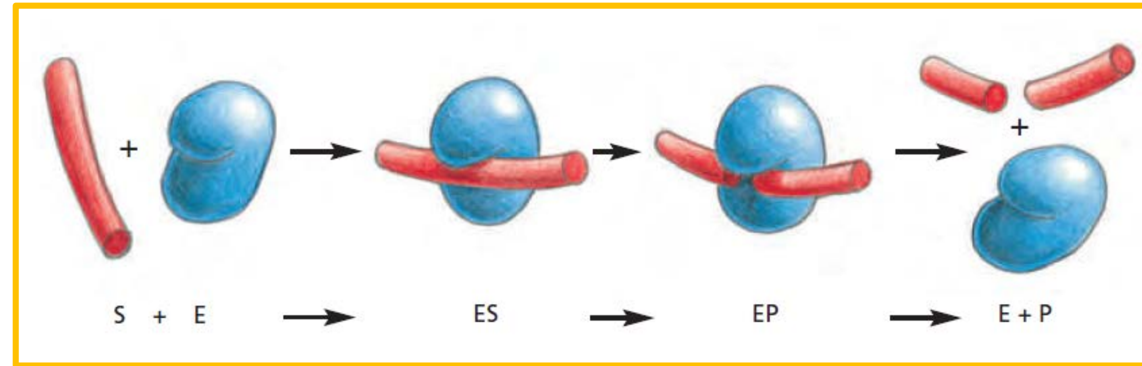
Study of the experimental parameters will be **used to:**

DESIGN INHIBITORS OR ACTIVATORS AND MODULATE ACTIVITY

ENZYMATIC MEASUREMENTS ARE IMPORTANT IN CLINICAL ENZYMOLOGY/CLINICAL BIOCHEMISTRY

The best way to study an enzyme's mechanism of action is to determine the rate and how it changes in response to experimental parameters: ENZYMATIC KINETICS

MEASURING VELOCITY IN AN ENZYMATIC REACTION



How is enzymatic activity measured?

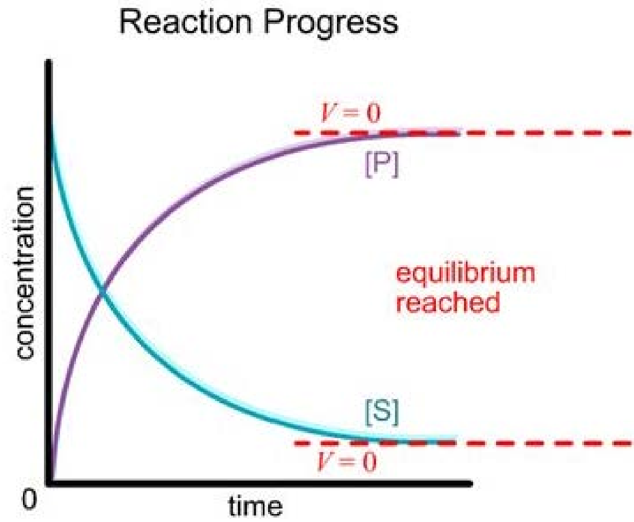
$$V = -d[S]/dt = d[P]/dT$$

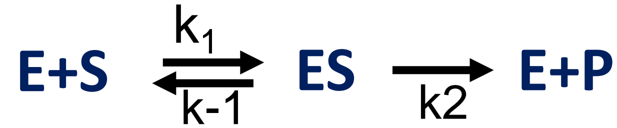
1. Measurement of **substrate disappearance or product appearance.**

2. Reaction takes place in the presence very small quantities of enzyme 10^{-8} and 10^{-12} M, catalytic quantities.

3. The velocity determined is the **initial velocity or V_0**

MATHEMATICAL EXPRESSION OF ENZYMATIC KINETICS

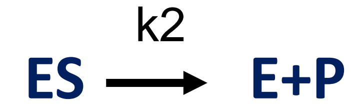




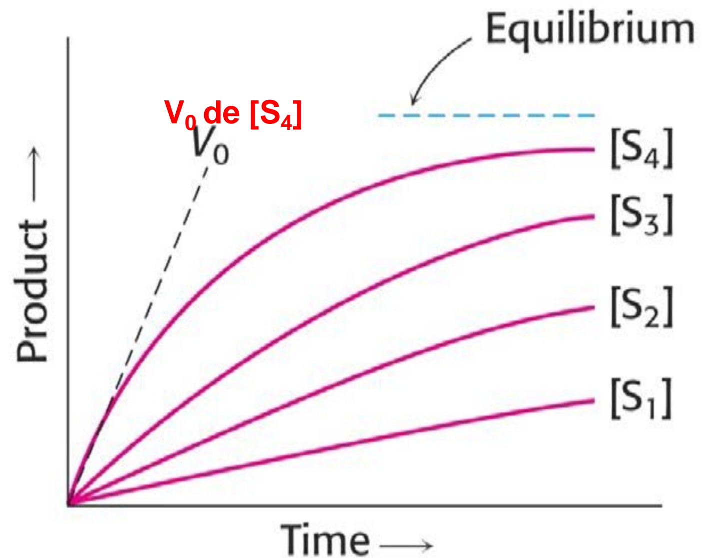
$$V = \frac{-d[S]}{dt} = \frac{d[P]}{dt}$$

At the beginning of the enzymatic reaction:

Stationary state of the enzymatic reaction:



Maximum velocity that can be reached



THE INITIAL VELOCITY IS DIFFERENT FOR EACH [S]

THE ENZYMES THAT FOLLOW THIS BEHAVIOR ARE ***MICHAELIS-MENTEN ENZYMES***

THE MICHAELIS-MENTEN MODEL

Leonor Michaelis, 1875–1949

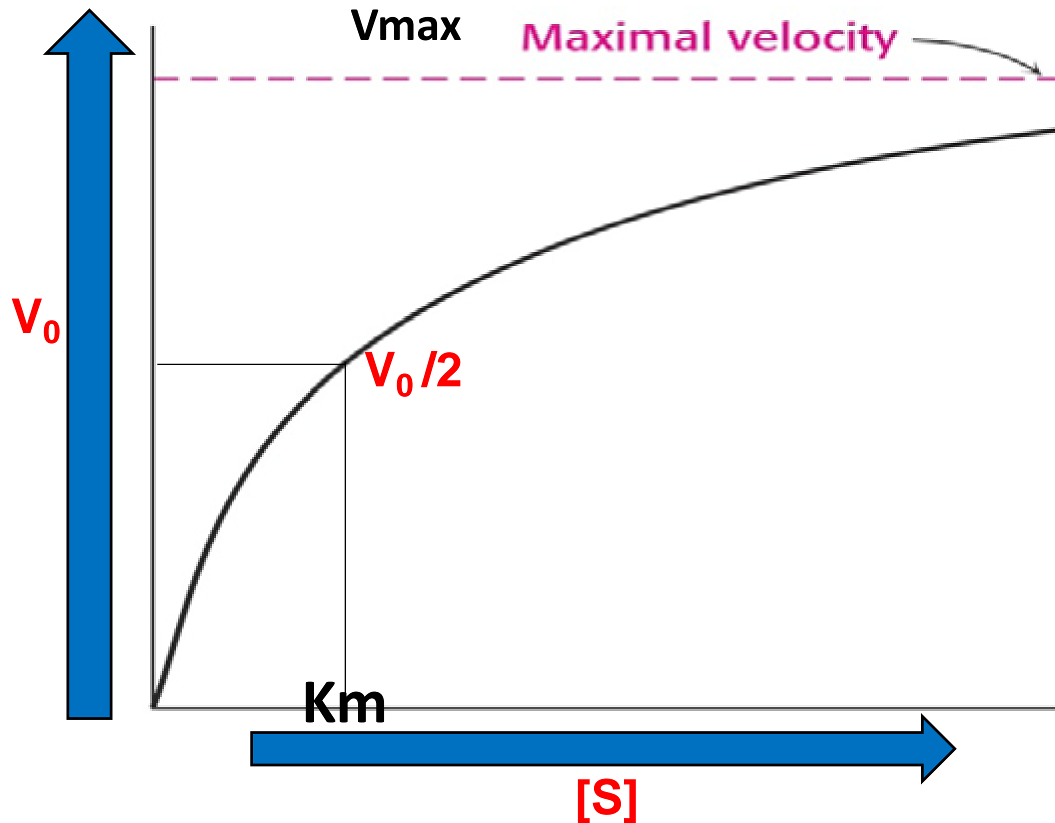
Maud Menten, 1879–1960

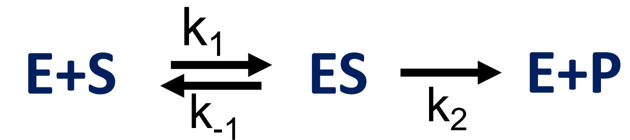
The Michaelis-Menten equation (HYPERBOLIC CURVE): dictates the behavior of the enzymatic reaction.

Most of the enzymes follow the Michaelis-Menten **mechanism of action** model.
The graph of initial velocity (V_0) vs [S] is hyperbolic.

MICHAELIS-MENTEN EQUATION

$$V_0 = \frac{Vmax \times [S]}{Km + [S]}$$





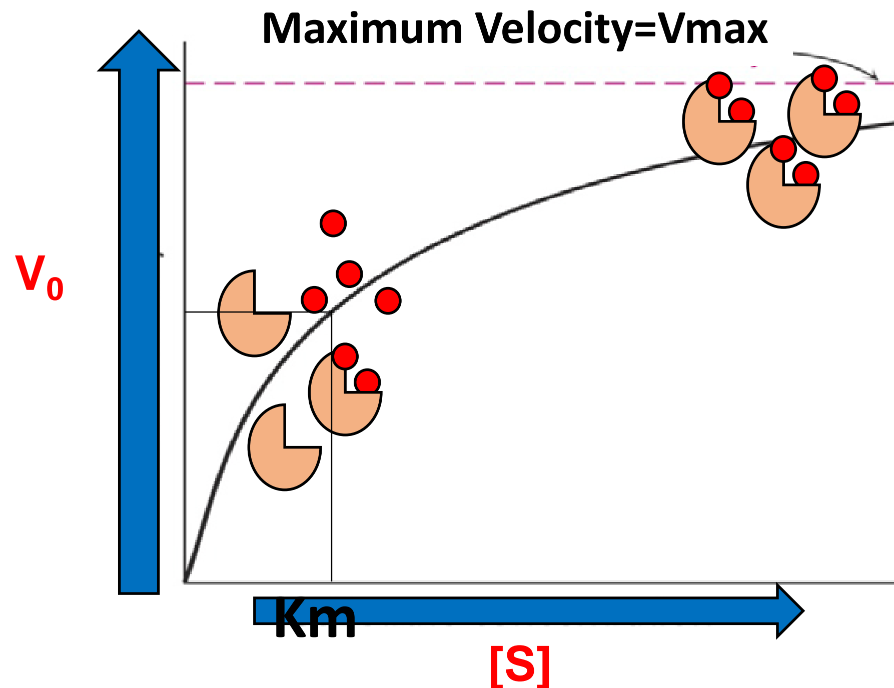
$$\mathbf{Vmax = k_2\,[ES]}$$

The enzyme is saturated by the substrate.

$$\mathbf{Km = \frac{k\text{-}1 + k2}{k1}}$$

STUDY OF ENZYMATIC BEHAVIOR DURING CATALYSIS: THE MICHAELIS-MENTEN MODEL OF ENZYMES

1. Representation of the initial velocity (V_0) for the various concentrations of substrates: $[S]_1$, $[S]_2$, etc.
2. V_0 increases as [S] increases until the enzyme is saturated and all the enzyme is bound to the substrate, [E]=[ES].
3. K_M is a constant equal to [S] when $V_0 = V_{max}/2$.
4. V_{max} is a theoretical value reached when [S] = ∞, i.e. when all the enzyme is bound to the substrate.



Km: the Michaelis-Menten constant

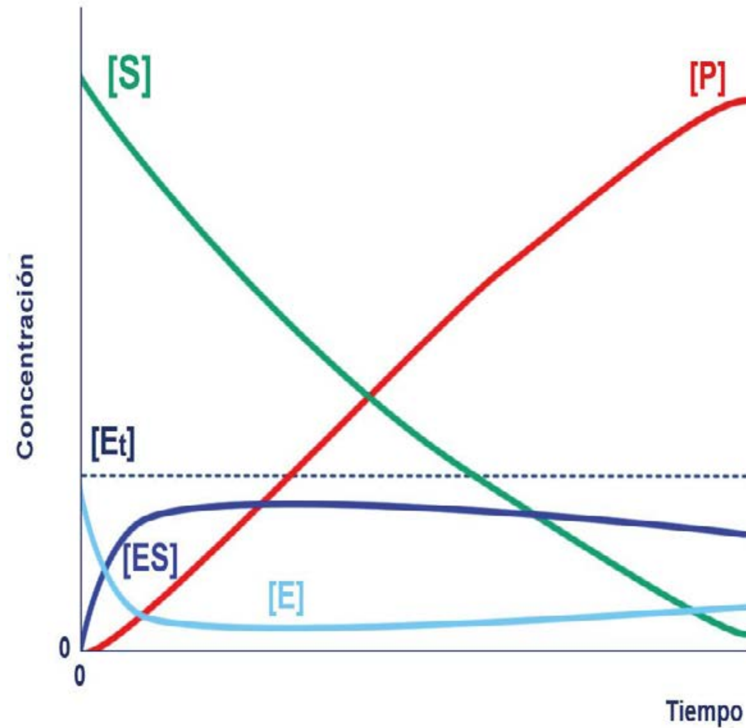
MICHAELIS-MENTEN EQUATION

$$V_0 = \frac{Vmax \times [S]}{Km + [S]}$$

When all the enzyme is bound to the substrate [ES] ≈ [E], the initial velocity is $V_0 = V_{max}$

THE STATIONARY STATE IN ENZYMATIC KINETICS

When all the enzyme is bound to the substrate, $[E_T] \approx [ES]$.
The initial velocity is $V_0 = V_{max.}$
The formation of P is maximum.

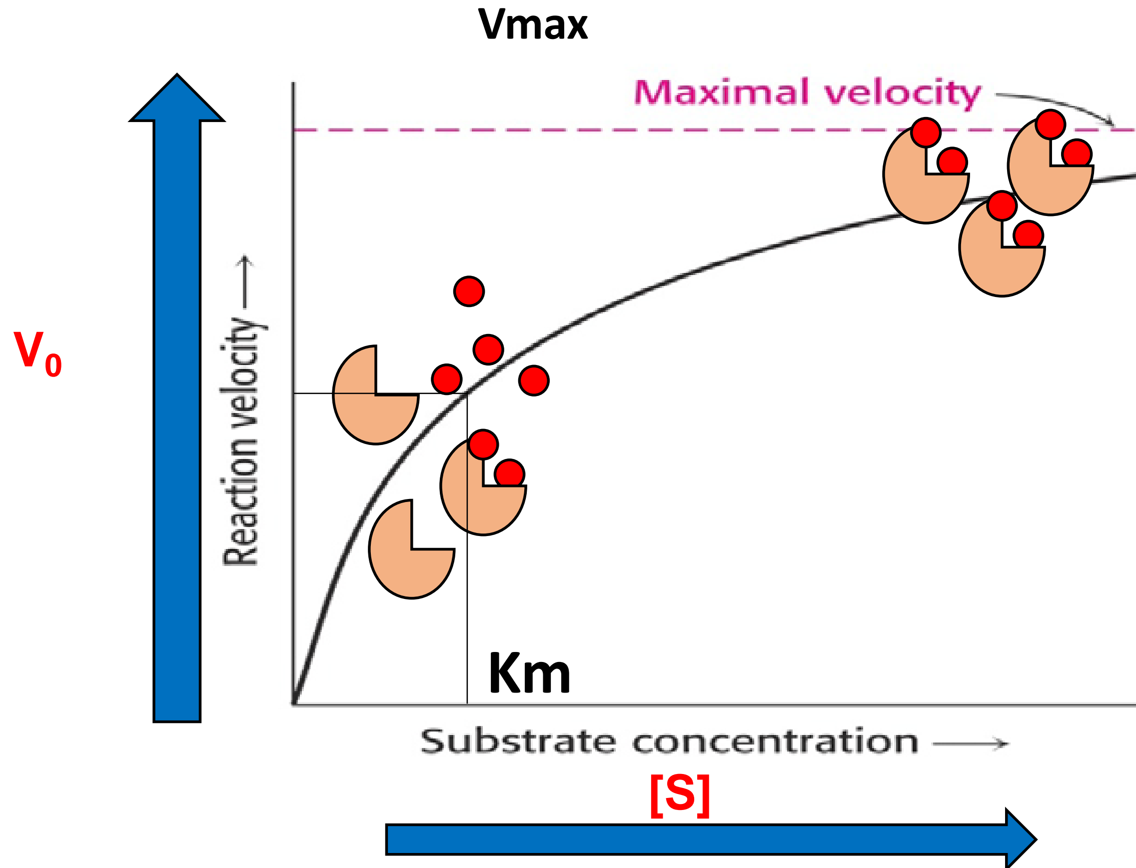


STATIONARY STATE

The concentration of ES is constant.

V formation of the ES complex = **V** destruction of the ES complex.

SUMMARY: ENZYMES THAT FOLLOW THE MICHAELIS-MENTEN MODEL



$$V_0 = \frac{Vmax \cdot [S]}{Km + [S]}$$

HYPERBOLIC CURVE

SATURATION KINETICS

Michaelis-Menten KINETICS

THE CURVE APPROXIMATES TO THE **Vmax ASYMPTOTICALLY**

$[S] = K_M$ → $V = V_{max}/2$

$[S] >> K_M$ → $V = V_{max}$

MEANING OF V_{max} IN THE MICHAELIS-MENTEN EQUATION AND CURVE

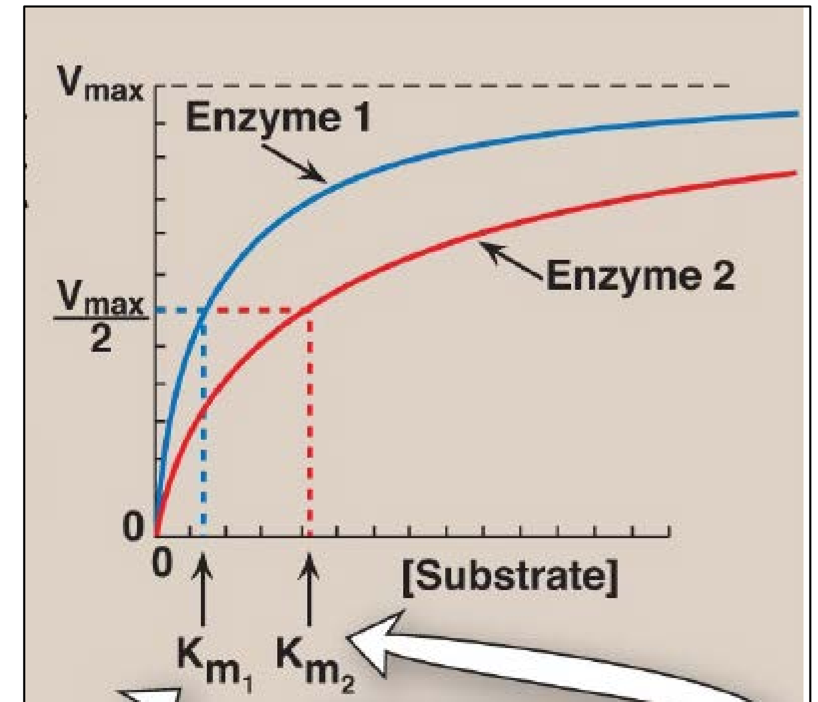
It represents the theoretical maximum velocity.
At very high concentrations of the substrate, all the enzyme molecules are bound to the substrate.
In the representation of the Michaelis-Menten equation, the hyperbolic curve V_0 versus [S] is asymptotic to the Vmax line.

MEANING OF K_M IN THE MICHAELIS-MENTEN EQUATION AND CURVE

It represents the concentration of substrate [S] at which the V_0 is ½ that of the Vmax.
It indicates **the affinity of an enzyme for a substrate.** It can be used to compare related enzymes.

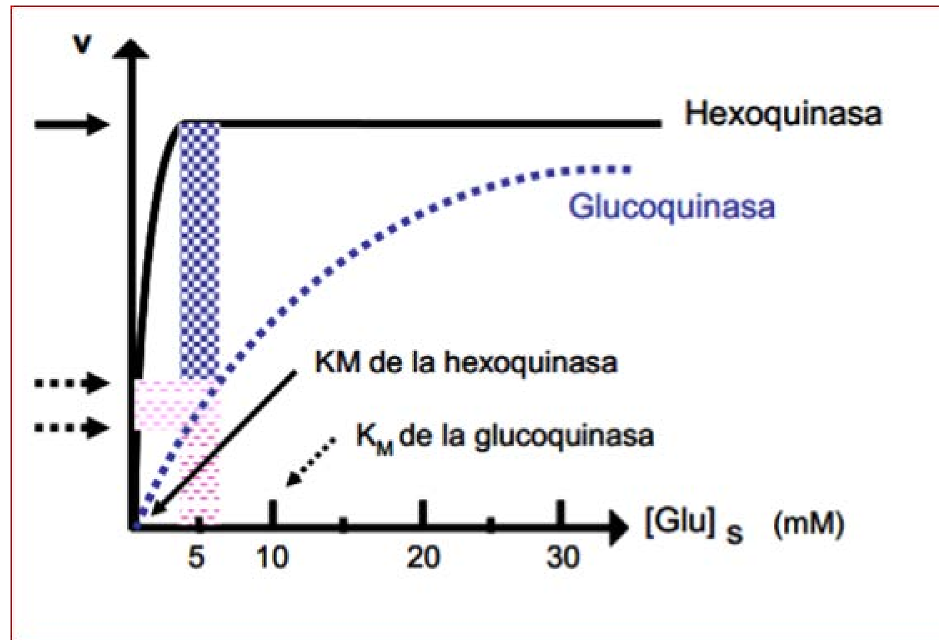
It can be **determined easily** in the laboratory.

The **reaction velocity is highly sensitive when around the [S]** at the Km point.



LIVER: GLUCOSE HOMEOSTASIS

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

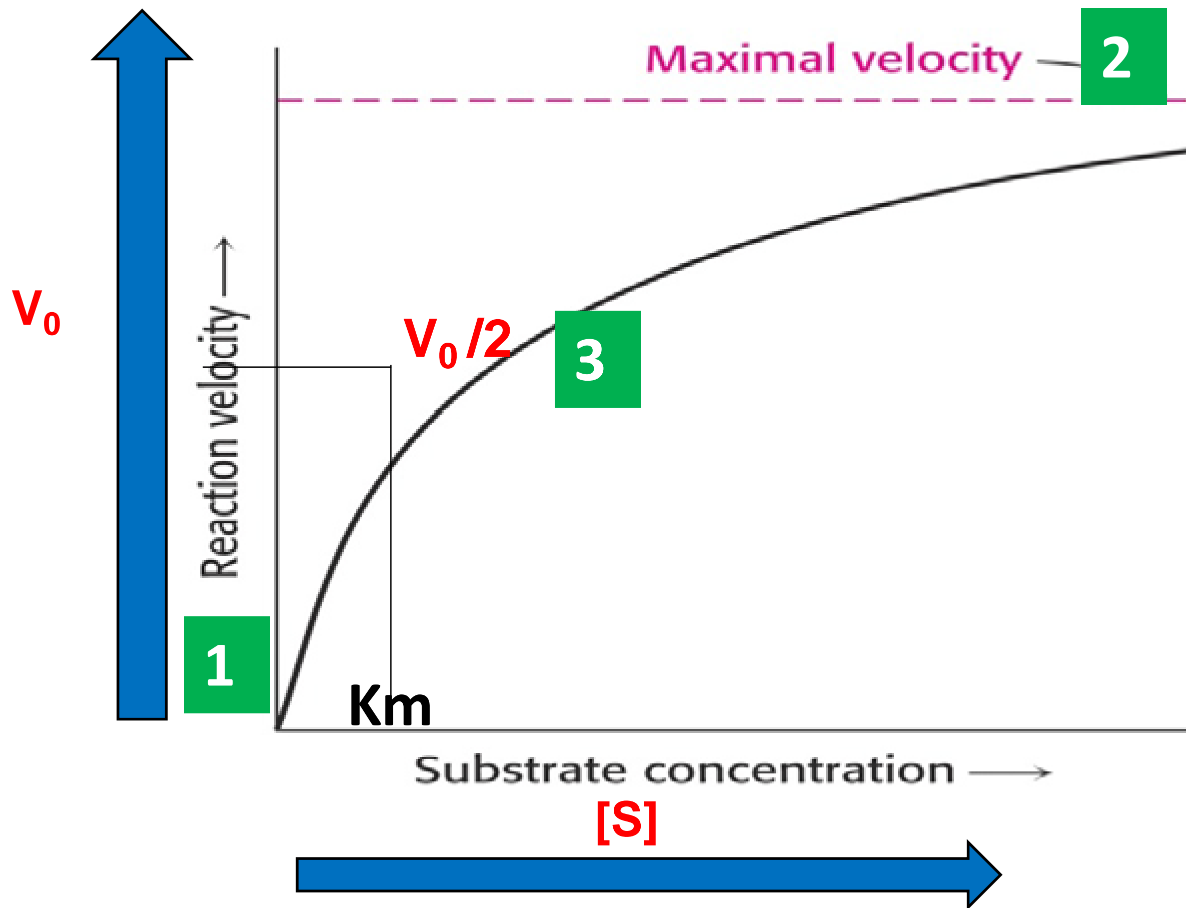


The Km of the **HEXOKINASE** is very low (0.1 mM): GLUCOSE at low concentrations is used for **GLYCOLYSIS** and energy.

The Km of the **GLUCOKINASE** is 5 mM: Glucokinase it will work on high glucose levels and the phosphorylated glucose will be used for glucose reservoir (**GLUCOGENESIS).**

MICHAELIS-MENTEN EQUATION

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



EXPERIMENTAL EXPLANATION OF THE M-M MODEL:

1. When [S] is **very low** and below Km:

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

[S] is insignificant against Km and V_0 increases with [S]:

$$V_0 = constant(Vmax/Km)x[S]$$

2. At very high [S] concentrations, the Km is very small, therefore:

$$V_0 = \frac{V_{max}\ x\ [S]}{Km + [S]} \rightarrow V_0 = Vmax$$

3. When V_0= Vmax/2, then:

$$V_0 = \frac{V_{max}}{2} = \frac{V_{max} x [S]}{Km + [S]} \rightarrow Km=[S]$$

ENZYMATIC ACTIVITY UNITS

These are used to determine the amount of an enzyme.

STANDARD UNITS OF ENZYMATIC ACTIVITY (IU):

These are the amounts of enzymes that catalyze the formation of 1 µmol of product per minute at 37ºC and at saturating [S].

Katal:

This is the amount of enzyme that catalyzes the formation of of 1 Mol of product per **at 37ºC and at saturating [S].**

1 Katal= IU/60x10^{-3}

KCAT= TURNOVER NUMBER

This is the number of substrate molecules that are transformed into product per molecule of enzyme per time in conditions of enzyme saturation.

$$Kcat=Vmax/[E]_T$$

The inverse number defines the time needed for the enzyme to transform one molecule of substrate.

THE CONCENTRATION OF ENZYME IS EXPRESSED PER VOLUME:

Enzymatic activity units per volume (IU/ml).

SPECIFIC ACTIVITY

Enzymatic activity per mg of total protein in a given sample.

LINEAL REPRESENTATIONS OF THE MICHAELIS-MENTEN EQUATIONS

THE LINEWEAVER-BURK GRAPH

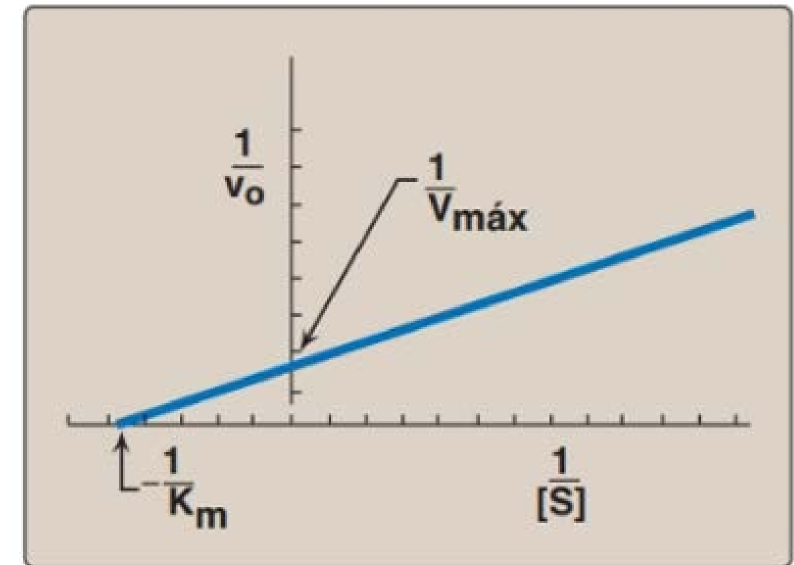
This is a linear representation of V_0 vs [S]: $V_0 = \frac{Vmax \times [S]}{Km + [S]}$

$$\frac{1}{V_0} = \frac{K_m}{V_{máx}[S]} + \frac{1}{V_{máx}}$$

A double inverse representation of $1/V_0$ vs 1/[S], it is a straight line.

It is useful for determining **Km and Vmax** with greater precision.

It is also useful for determining the parameters for finding inhibitors in the enzyme’s microenvironment.



The interception of the line with the **X axis = −1/Km.**
The interception of the line with the **Y axis = 1/Vmax.**
The SLOPE = Km/Vmax

WHICH IS THE PURPOSE OF THIS REPRESENTATION?

A direct plot of V_0 versus [S] does not always determine Vmax, basically because the plot is asymptotic. The linear representation easily depicts Vmax when 1/[S] is 0.

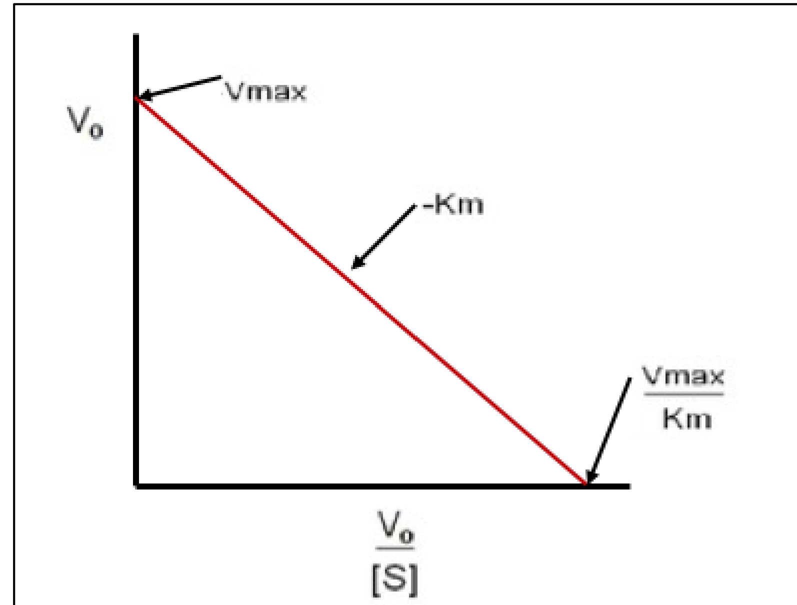
THE EADIE-HOFSTEE GRAPH

This is the linear representation of the Michaelis-Menten equation.
It is obtained after multiplying Vmax by the equation of Lineweaver-Burk and making some approximations.

$$\frac{1}{V_o} = \frac{K_m}{V_{máx}\,[S]} + \frac{1}{V_{máx}}$$

$$Vo = V_{max} - K_M \frac{Vo}{[S]}$$

$$V_0 = \frac{Vmax \times [S]}{Km + [S]}$$



Eadie-Hofstee

V_0 versus V/[S]

The graph is a straight line representing V_0 versus V/[S]
SLOPE= -Km
The intersection on the Y axis = Vmax
The intersection on the X axis = Vmax/Km

ENZYMES THAT DO NOT FOLLOW THE MICHAELIS-MENTEN MODEL: COOPERATIVISM

ALLOSTERIC ENZYMES

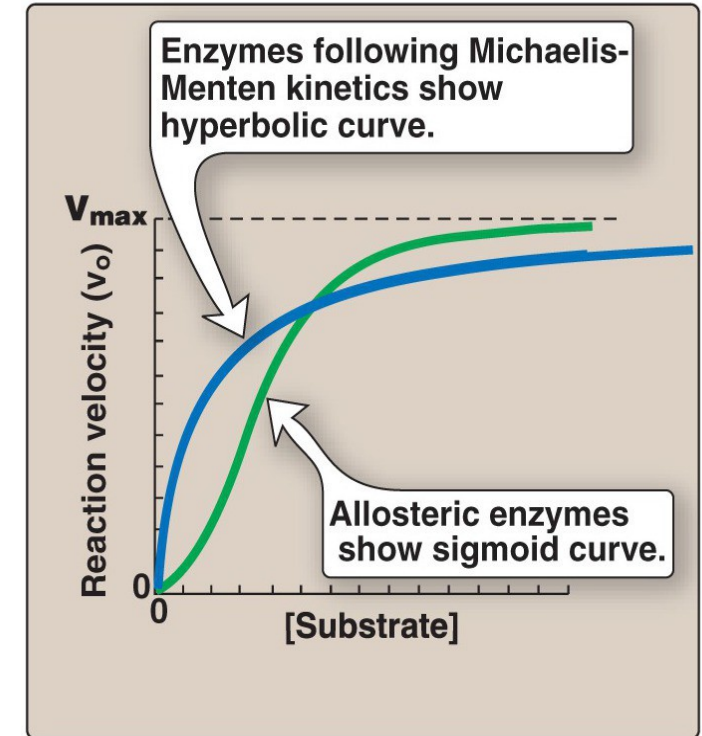
1. Some enzymes do not follow a hyperbolic curve when V is plotted against [S]. Instead, the curve is sigmoid or sigmoid kinetic: COOPERATIVISM.

2. **COOPERATIVISM** occurs when the union of the substrate to the enzyme alters the union of more substrate molecules.

3. **COOPERATIVISM is a frequent behavior of** multimeric enzymes with many binding/catalytic sites.

Positive cooperation: the union of the substrate **increases the affinity** of the subsequent molecules to the active sites.

Negative cooperation: the union of the substrate **decreases the affinity** of the subsequent molecules to the active sites.



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The equivalent of Km is $K_{0.5}$

The enzyme activity of these enzymes can be modulated by the binding of ligands.

GENERAL CHARACTERISTICS AND CONCEPTS OF LIGAND-PROTEIN INTERACTIONS

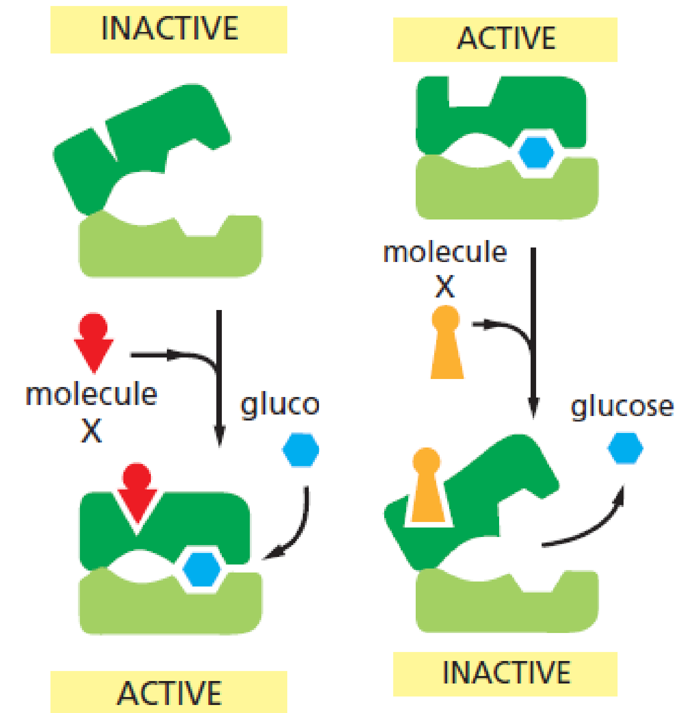
EFFECTS ON FUNCTIONALITY AND PROTEIN DYNAMICS

The **functionality** of proteins depends on the following **dynamic properties:**

1. Flexibility, which involves the many possibilities for a protein to engage different numbers of weak bonds.

2. Conformational changes, which are small changes to the tertiary structure that affect protein activities.

3. Interactions, which are reversible interactions with ligands (inhibitors, activators, substrates) or protein-protein interactions.



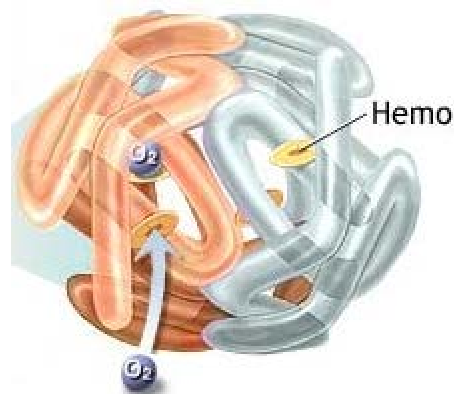
The binding of different ligands due to the dynamic properties of the proteins changes the functionality.

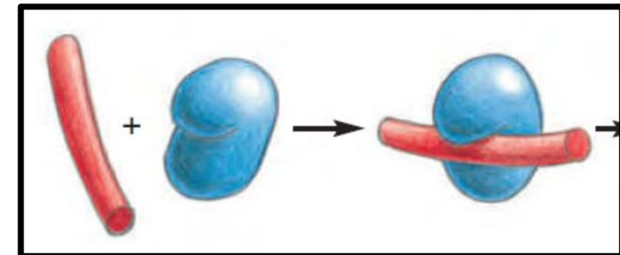
LIGANDS THAT BIND TO PROTEINS: LIGAND-PROTEIN INTERACTIONS

Ligand-protein interactions are:
- **Specific**
- **Weak bonds:** Van der Waals, hydrogen bonds, hydrophobic and ionic bonds.

Enzymatic catalysis:
P = enzyme
L = substrate, activator, inhibitors, (even a product)

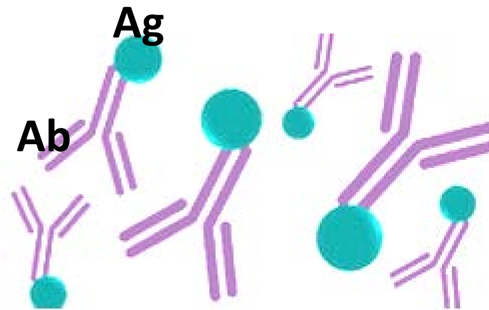
HEMOGLOBIN





In transport:
P = transporter
L = O2, lipid, sugar,

In signaling:
P = receptor
L = hormone



The immune system:
P = antibody
L = antigen

CHARACTERISTICS OF THE BINDING SITES IN LIGAND-PROTEIN INTERACTIONS

Equivalent sites: all binding sites are equal and are equally likely to be occupied by a ligand.

Independent sites: when these site are occupied, they do not affect the other biding sites.

ENZYME WITH M-M KINETICS: with several catalytic sites (binding sites)

1. If the binding sites are **equivalent and independent**, the graph representing binding kinetics versus [L] is a HYPERBOLIC CURVE.

ENZYME WITH COOPERATIVITY: with several binding sites that are not independent

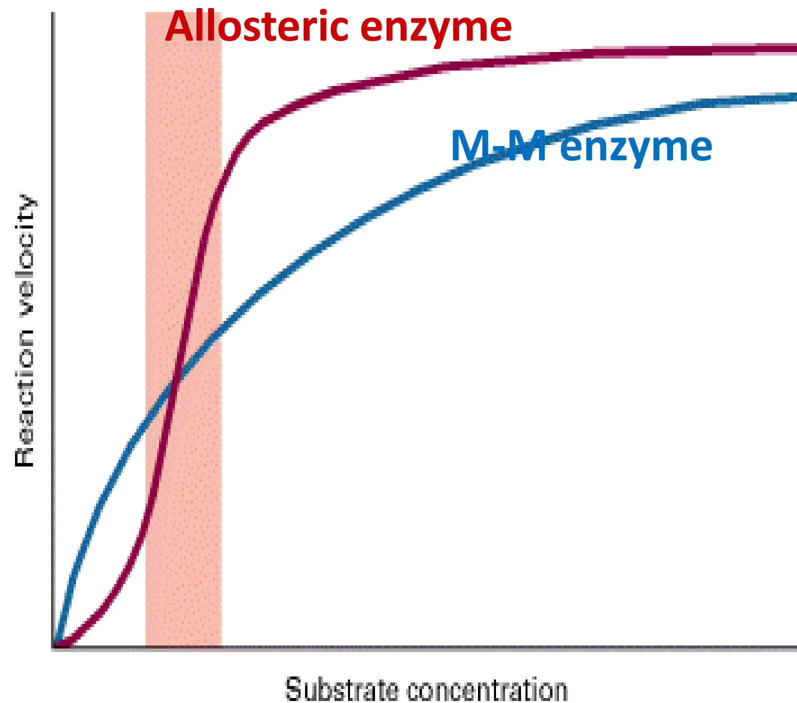
2. If the binding sites are **equivalent but not independent**, the graph representing binding kinetics versus [L] is a SIGMOID CURVE.

COOPERATIVITY PHENOMENA IN THE ENZYMES AND SIGMOID KINETICS

COOPERATIVITY AND SIGMOID CURVES: ALLOSTERIC ENZYMES.

The binding sites for the substrate are equivalent BUT NOT INDEPENDENT.

ALLOSTERIC ENZYME: affinity for the substrate increases as the binding sites/catalytic sites are occupied.



V_0 vs [S]: **Sigmoid curve**

the Vmax/2 is called $K_{0.5}$

IN ADDITION

Allosteric enzymes usually have additional binding sites for **ligands that MODULATE THE ACTIVITY.**
The sites are not equivalent and produce
HOMOTROPIC AND HETEROTROPIC EFFECTS

HOMOTROPIC AND HETEROTROPIC EFFECTS

Homotropic effects of the substrate

– the same ligand is at several binding sites (equivalent sites).

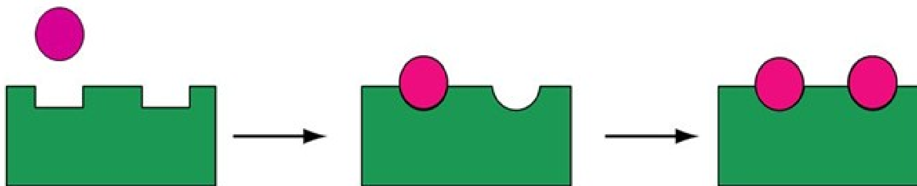
– the ligand binding affects the union of the other ligands (dependent sites).

Positive cooperativity facilitates the binding of subsequent molecules.

Negative cooperativity impairs the binding of subsequent molecules.

This is a typical organization in multimeric enzymes.

HOMOTROPIC EFFECTS AT EQUIVALENT SITES



O_2 binding favors the union of other molecules to the other sites.

A conformational change that facilitates the union of a second molecule.

HOMOTROPIC AND HETEROTROPIC EFFECTS IN ALLOSTERIC ENZYMES

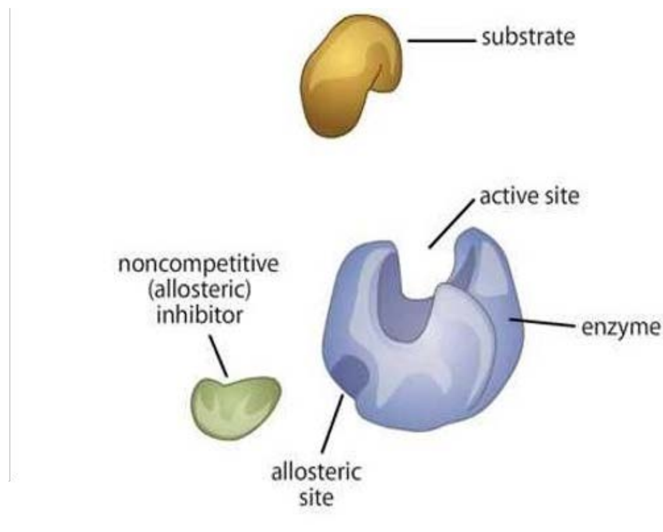
Heterotopic effects:

The effects are caused by a different molecule, not the substrate.
The molecule alters ENZYME-SUBSTRATE affinity positively or negatively.

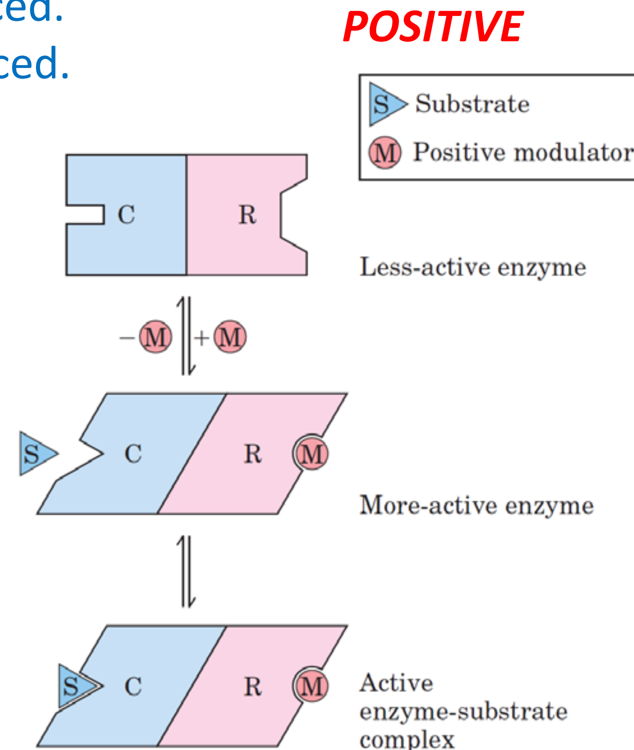
The non-substrate ligand binds to the regulator site.

ALLOSTERISM

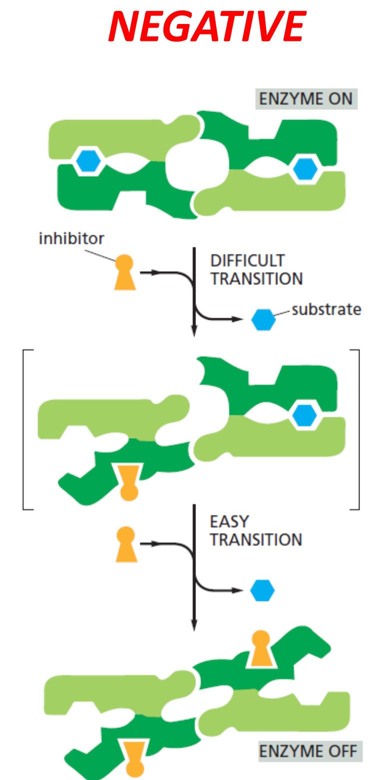
The sites are dependent.
If the ligand facilitates the catalysis, positive cooperativism is produced.
If the ligand impedes the catalysis : negative cooperativism is produced.



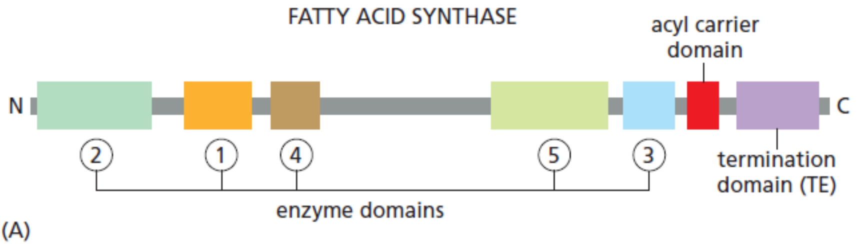
POSITIVE

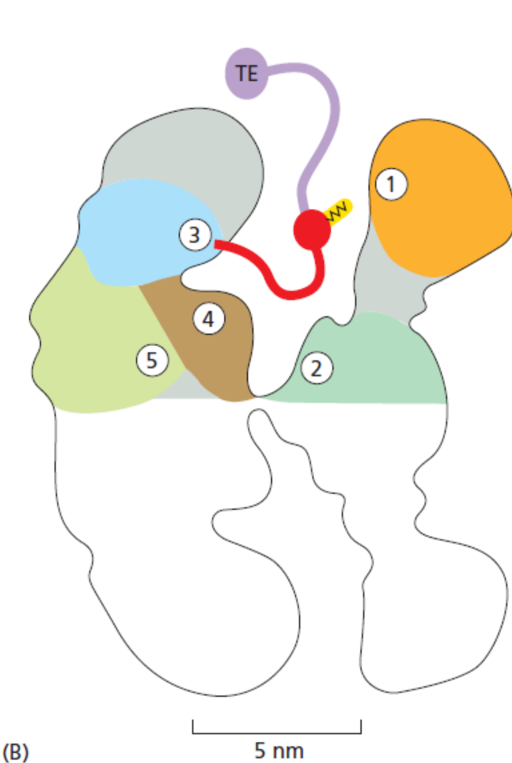


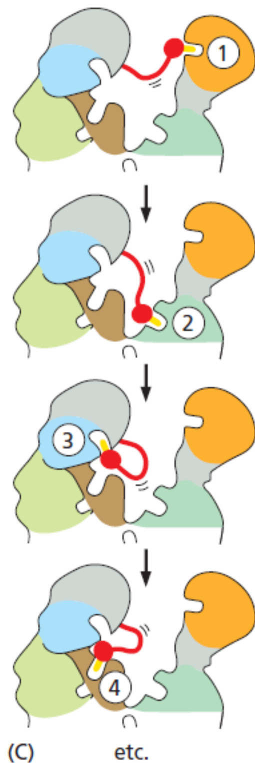
NEGATIVE

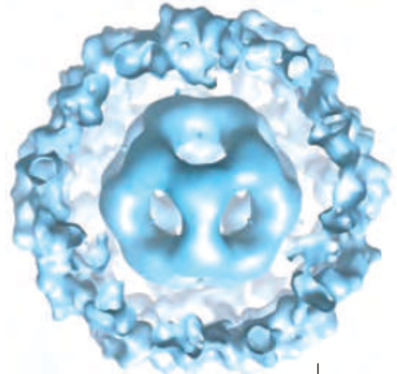


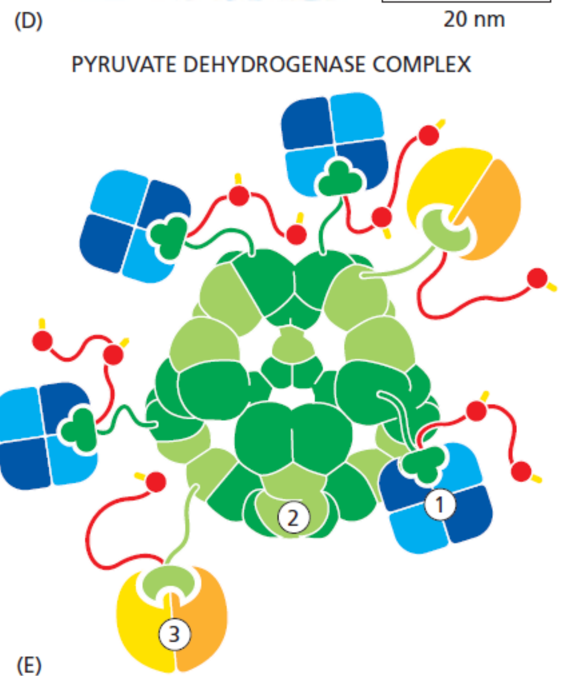
ENZYMATIC MULTICOMPLEX





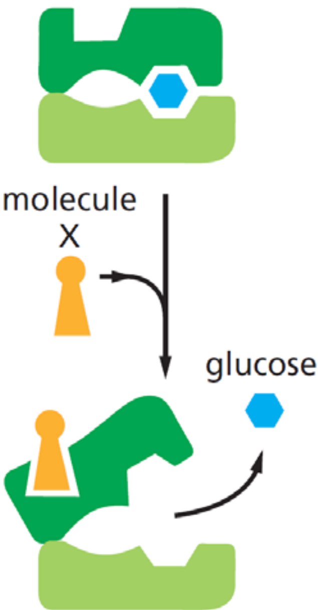


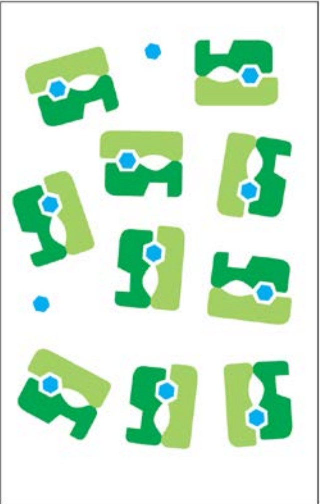




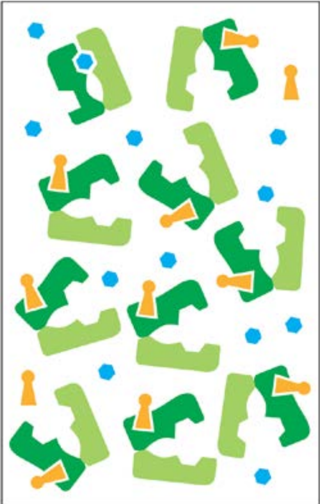
CONFORMATIONAL CHANGES TO THE ACTIVE SITE INDUCED BY THE BINDING OF ALLOSTERIC MODULATORS: HETEROTROPIC EFFECTS

HETEROTROPIC NEGATIVE EFFECT

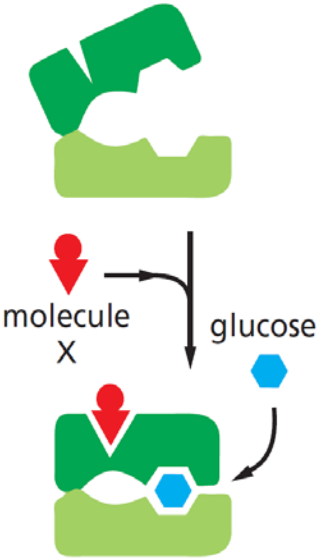


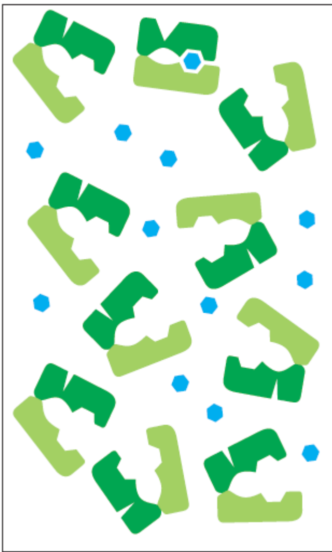


molecule
X
negative
regulation

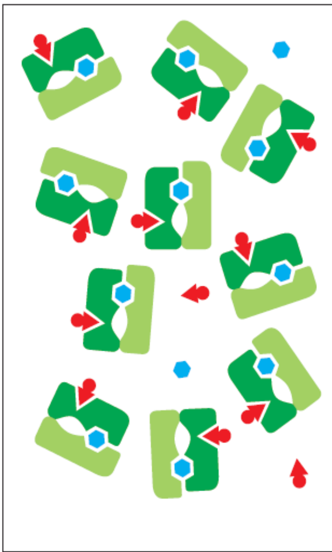


HETEROTROPIC POSITIVE EFFECT





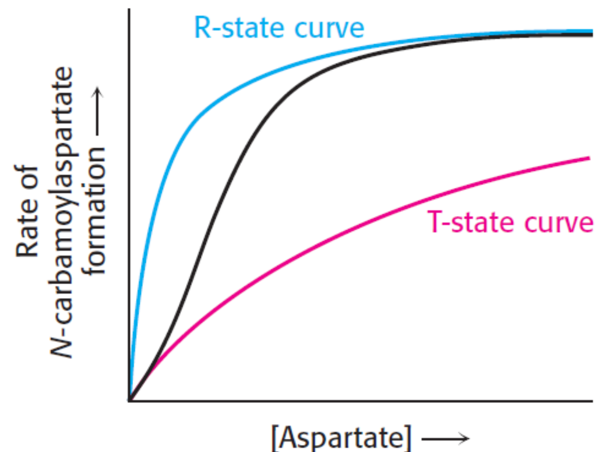
molecule
X
positive
regulation



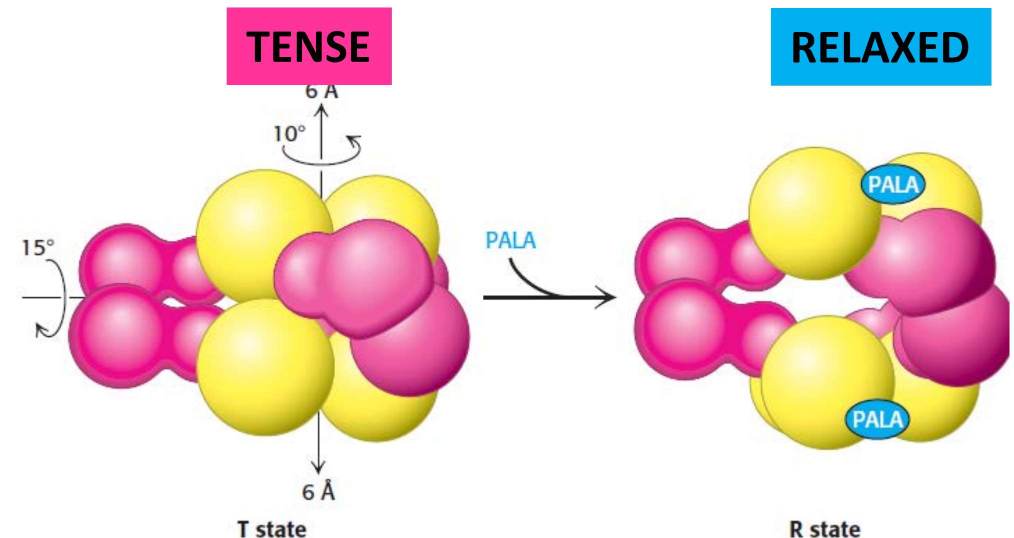
COOPERATIVITY MODELS

Cooperativity and allosterism can be explained using two models:
The concertated model: Monod, Wyman and Changeux.
The sequential model: Koshland, Nemethy and Filmer.
Both postulate that:
The subunits of the enzyme have two conformations:
Tense conformation (T): affinity to the substrate is lower.
Relaxed conformation (R): affinity to the substrate is higher.

Without ligands, both conformations are in equilibrium.



The models explain the kinetics.

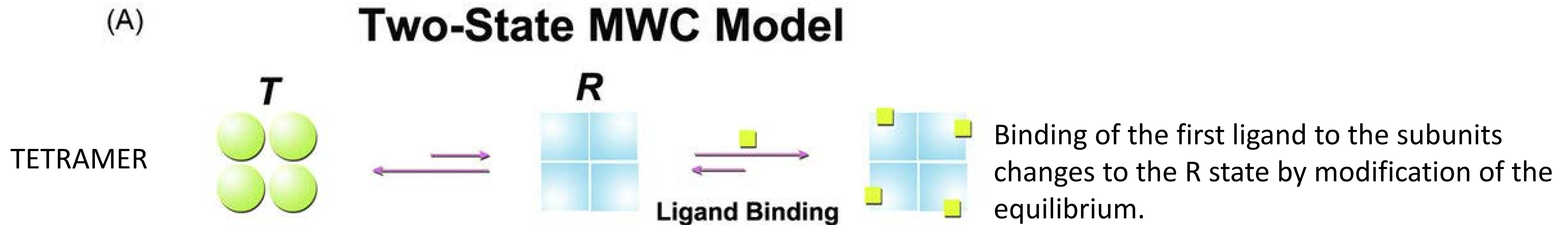


Enzyme: Asp Transcarbamylase.
PALA: *N*-(Phosphonacetyl)-L-aspartate (analogue of the intermediary of the reaction).

COOPERATIVE MODELS THAT EXPLAIN SIGMOID CURVES IN ALLOSTERISM

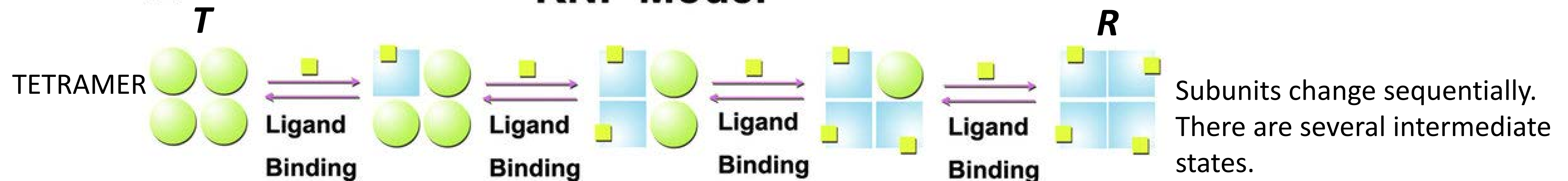
Tense conformation (T): low affinity
Relaxed conformation (R): high affinity

CONCERTATED MODEL **Monod, Wyman and Changeux**



Binding of the first ligand to the subunits changes to the R state by modification of the equilibrium.

SEQUENTIAL MODEL **Koshland, Nemethy and Filmer**



Subunits change sequentially. There are several intermediate states.

S.L.J.Zhang *Comprehensive Medicinal Chemistry III*
2017, Pages 276-296

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ANNEX

Origin of the Michaelis-Menten equation

In the stationary state:

$$k_1[E][S]=k_{-1}[ES]+k_2[ES]$$

$$[ES]=\frac{k_1}{(k_{-1}+k_2)}[E][S]$$

The Michaelis constant

$$K_M=\frac{(k_{-1}+k_2)}{k_1}$$

$$[ES]=[E]_t\frac{[S]}{(K_M+[S])}$$

V_0 is maximum when $[ES]=[E_t]$

$$[E]=[E]_t-[ES]$$

Initial velocity: $V_0=k_2[ES]$

$$V_0=k_2[ES]=k_2[E]_t\frac{[S]}{(K_M+[S])}$$

$$k_2[E]_t=V_{maxima}$$

Michaelis-Menten equation (hyperbolic curve):

$$V_0=V_{max}\frac{[S]}{(K_M+[S])}$$

LESSON 8. REGULATION OF ENZYMATIC ACTIVITY

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Herminia González Navarro 2021

INDEX

1. General mechanisms of enzymatic regulation.
2. Effects of physical and chemical variations of the media in enzymatic activity:
    - 2.1. Temperature effects
    - 2.2. pH effects
    - 2.3. Compartmentalization
3. Effects of modulators in enzymatic activity:
    - 3.1. Inhibitors and inhibition types: reversible and irreversible Inhibitors. Competitive, non-competitive and uncompetitive inhibition.
    - 3.2. Mathematics and plots representing kinetic inhibition.
    - 3.3. Regulation by allosteric modulation and related kinetics. Other mechanisms of enzyme activity.
4. Integration and mechanisms of metabolic regulation.
    - 4.1. Temporal regulation: slow, fast, reversible and irreversible.
    - 4.2. Spatial regulation.

GENERAL MECHANISMS OF ENZYMATIC REGULATION

1) Effects of physical and chemical variations

Variations enable stability and enzymatic activity in certain environments.
Effect of pH.
Effect of temperature.
Compartmentalization.

2) Effect of inhibitors: metabolic reactions and pharmacology

Reversible inhibitors.
Irreversible inhibitors.
Allosteric inhibitors.

3) Effect of activators: metabolic reactions

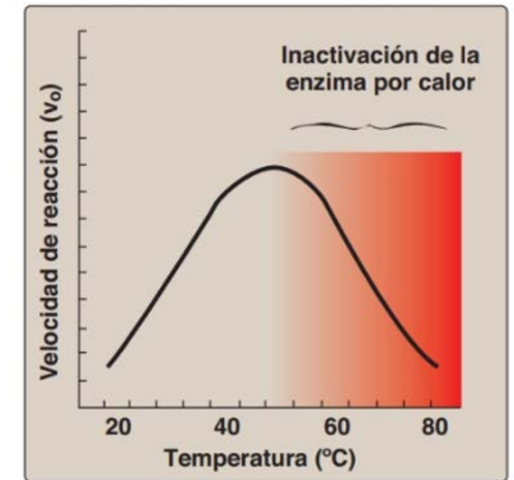
Low-molecular-weight ligands.
Protein-protein interaction.

4) Regulation by covalent modification: metabolic reactions

Irreversible modifications (proteolysis).
Reversible modifications (phosphorylation, acetylation).

Temperature

Speed increases with temperature: between 0º and 40ºC, the V0 increases x2 every 10 ºC.
The optimal temperature for most human enzymes is between 35 and 40ºC above V_0 reduction and denaturation.



Clinical example in a G6PDH mutation: Thermal instability of Glucose-6-Phosphate dehydrogenase results in anemia and loss of red blood cells (shorter half-life).

G6PDH: This important enzyme in the phosphate pentose pathway maintains oxidative homeostasis. If it is defective, erythrocytes can be lysed by oxidative stress.

ENVIRONMENTAL VARIATIONS: THE EFFECT OF pH

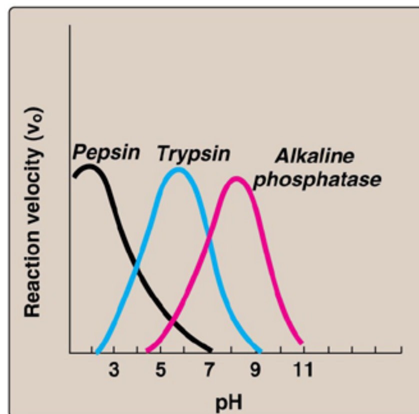
Enzymes have **optimal pHs** (pH=7.4); some (e.g. digestive enzymes) have highly acidic or basic pHs.

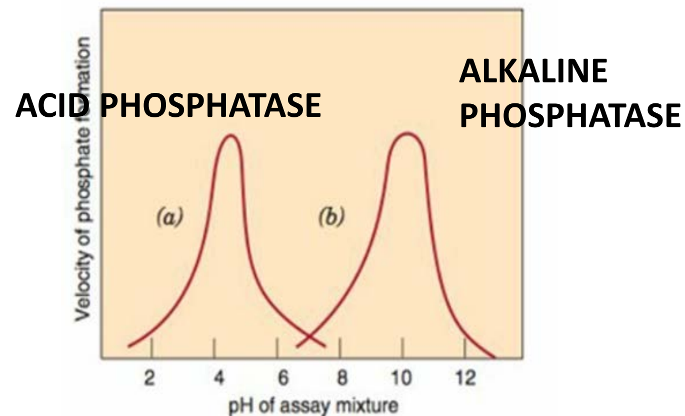
Small pH changes are cushioned by the physiological buffers of the organism. Changes have great adverse effects.

1. Effect of pH on the **ionization of the active site**: in order to interact, the catalytic process usually requires the enzyme and substrate to have specific chemical groups either in an ionized or non-ionized state.

2. Effect of pH on **enzyme conformation**: the **optimal pH** is the one at which the **conformation is best suited to catalytic activity.** Conformation is maintained by electrostatic charge of the Aa interactions in the protein structure.

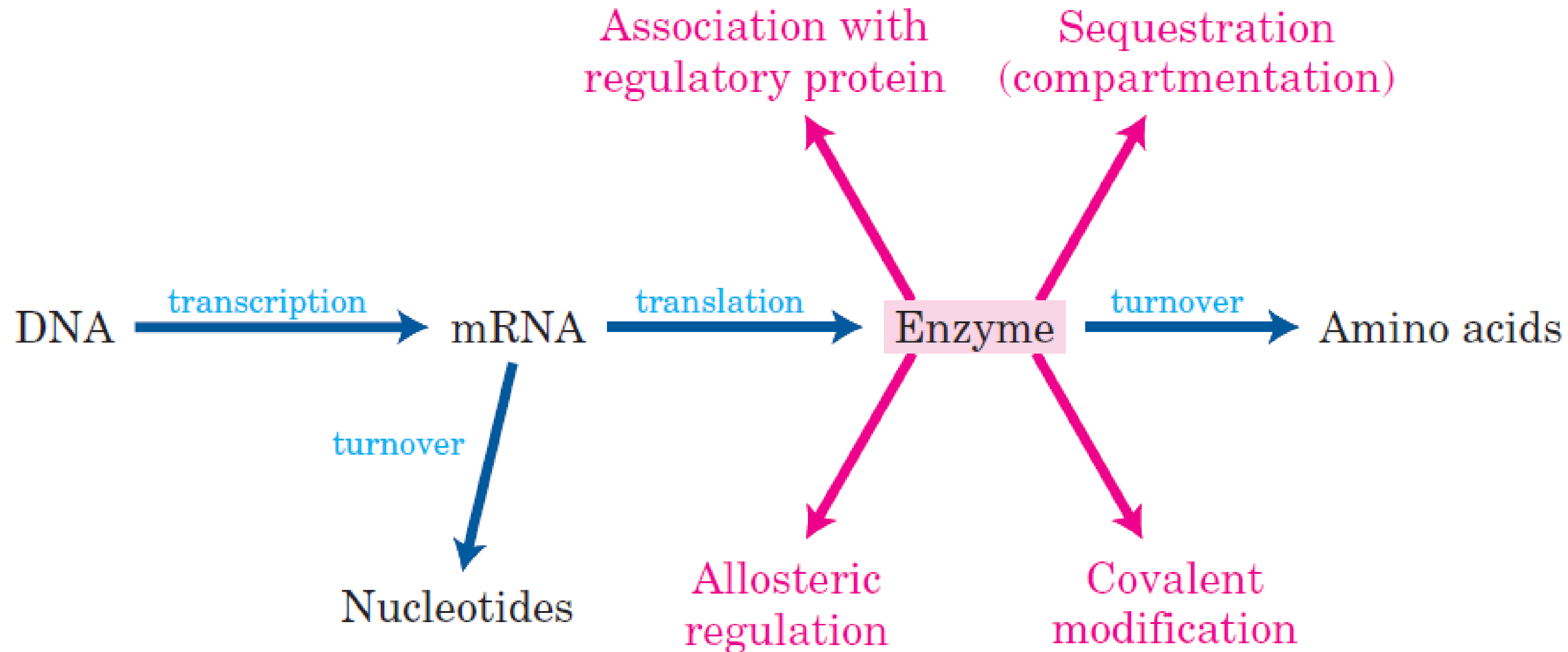
Extreme pHs can affect amino acid charge and denature proteins.





FACTORS THAT DIRECTLY AFFECT ENZYMATIC MOLECULES

In blue: control of the number of molecules.



In pink: control of the activity of the pre-existing enzyme.

COMPARTMENTALIZATION ALLOWS THE PHYSICAL LIMITATION OF THE SUBSTRATE

Enzymatic activity can be regulated by storing enzymes in a particular organelle of the cell in such a way that the enzymes are physically separated from their substrate.

A **substrate transporter is required** in the organelle membrane for the enzyme to have access to the substrate.

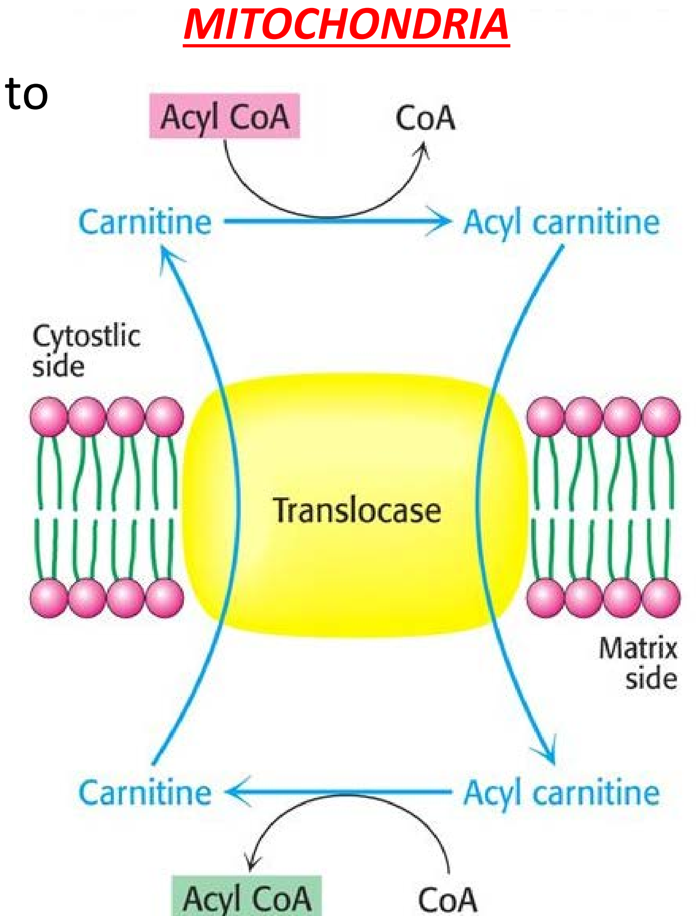
There is a physical limitation in substrate availability.

EXAMPLE: metabolic pathways/pathways

THE OXIDATION OF FATTY ACIDS IN MITOCHONDRIA **requires a shuttle** (e.g. carnitine) in mitochondrial membranes.

FIRST ENZYME OF THE PATHWAY: Acyl-CoA dehydrogenase

MITOCHONDRIA



TYPES OF INHIBITION AND EXAMPLES

An ***INHIBITOR*** is any molecule that slows down an enzyme-catalyzed reaction; they can be natural or artificial molecules, or proteins.

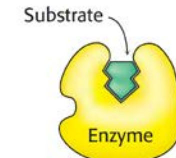
Irreversible:

Permanent inactivation by covalent bonds.

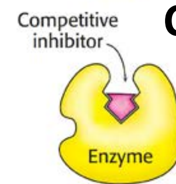
These inhibitors are common in pharmacology for treating highly diverse pathologies.

Reversible:

Binding to the enzyme by weak or non-covalent bonds. The EI (enzyme-inhibitor) complex is in equilibrium with the free forms and the inhibition is broken by dilution or the addition of large amounts of substrate.



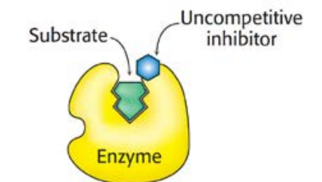
COMPETITIVE



The physiological inhibitors in the REGULATION OF METABOLISM are:

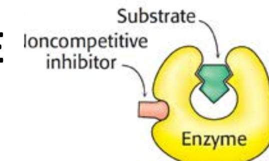
A) **Competitive**
B) **Non-competitive**
C) **Uncompetitive**

UNCOMPETITIVE



Allosteric effectors: positive and negative

NON-COMPETITIVE

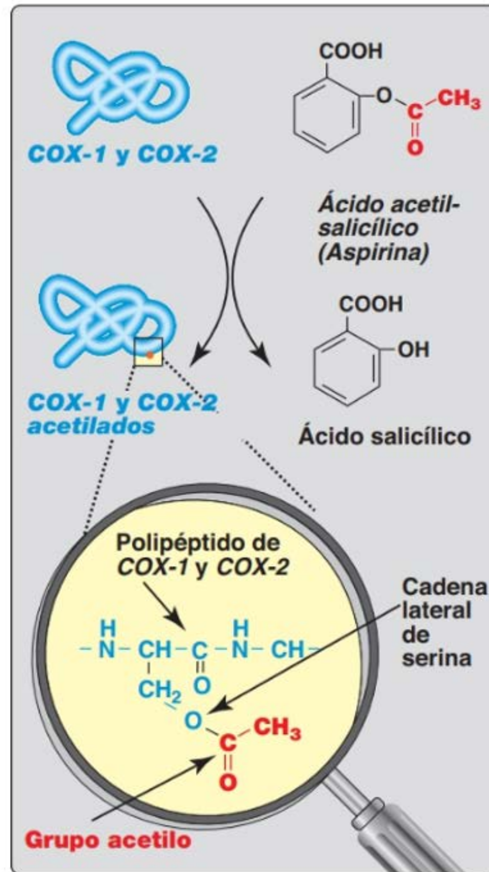


EXAMPLES OF TYPES OF INHIBITION

IRREVERSIBLE INHIBITORS: SUICIDE INHIBITORS

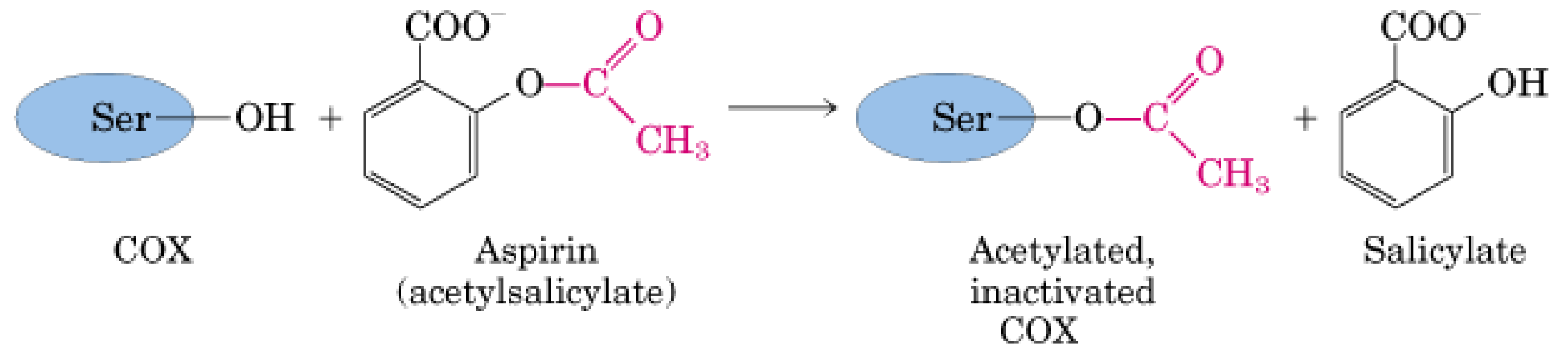
SUBSTRATE ANALOGUES

When catalyzed, these **bind COVALENTLY** to the enzyme irreversibly. They are also called "suicide" inhibitors.



Aspirin (acetylsalicylate)

Irreversibly inhibits COX. The **synthesis of prostaglandins (PG) and thromboxanes (TXA2) is blocked** from the arachidonic acid of cell membranes (released by PLA2, which is activated during inflammatory processes).

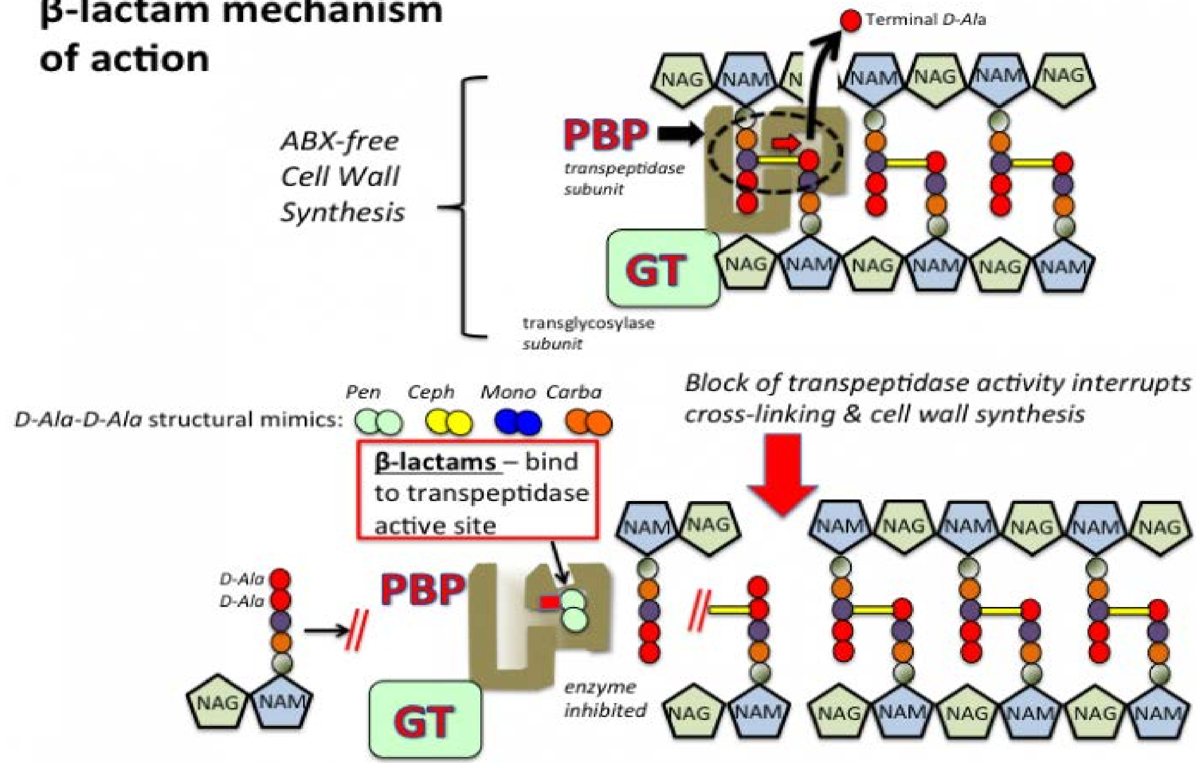


COX is acetylated in Serine

EXAMPLES OF IRREVERSIBLE INHIBITORS: β-LACTAM INHIBITORS

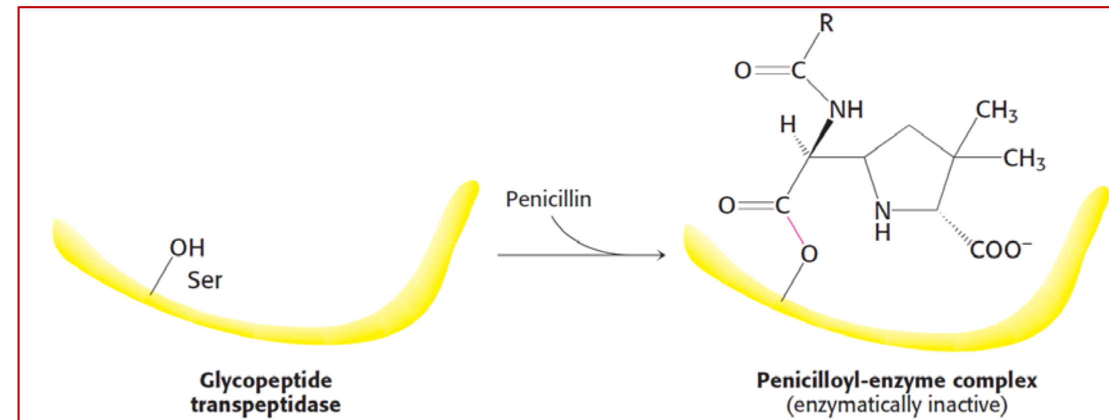
PENICILLIN is a β-lactam drug peptidemimetic that **irreversibly inhibits transpeptidase**, which intersects the peptides that maintain the peptidoglycan on the cell wall of bacteria.

Loss of the wall and death from cell lysis



Heteropolysaccharides

Covalent binding of penicillin to the serine of the active center



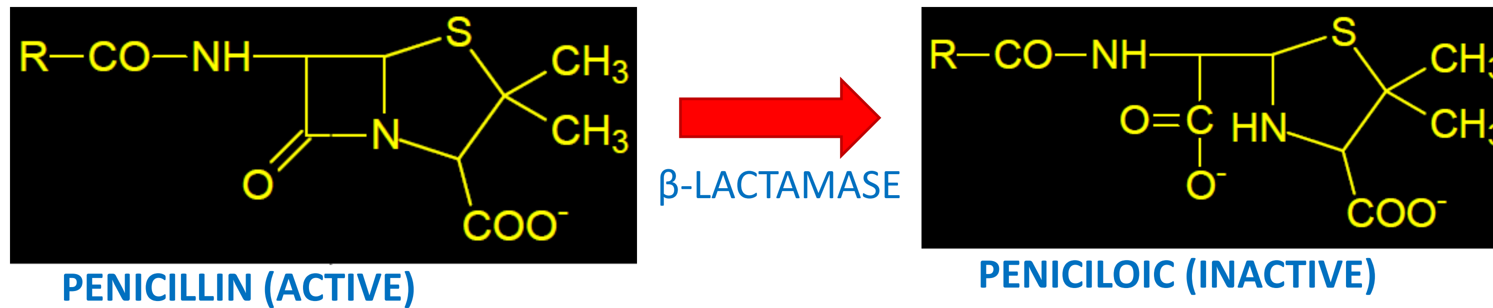
IRREVERSIBLE INHIBITORS: SUICIDE INHIBITORS IN THE β-LACTAMASE SYSTEM

The massive use of β-lactam antibiotics (penicillins and derivatives): RESISTANCE.

Microorganisms resistant to these antibiotics are resistant to producing an enzyme, **β-lactamase**, which inactivates β-lactam antibiotics.

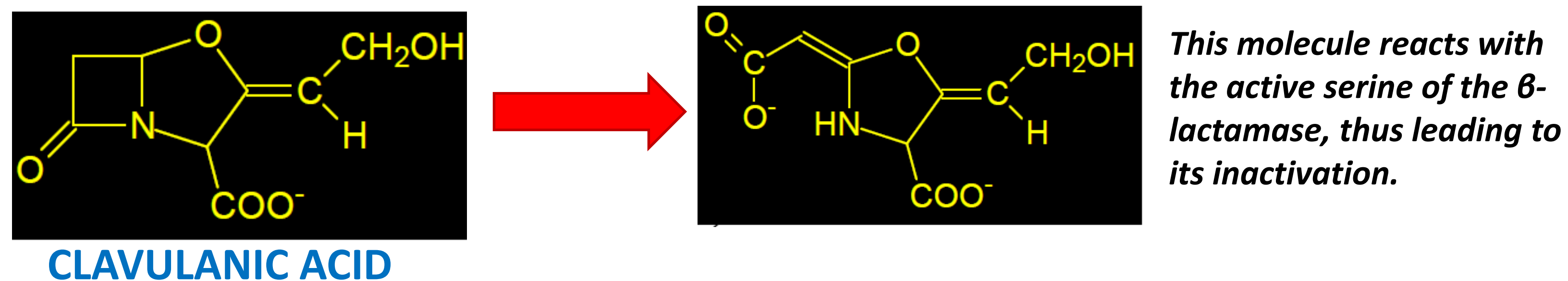
Adding clavulanic acid to penicillin and derivatives prevents resistance.

CLAVULANIC ACID (suicide inhibitor of β-lactamase).



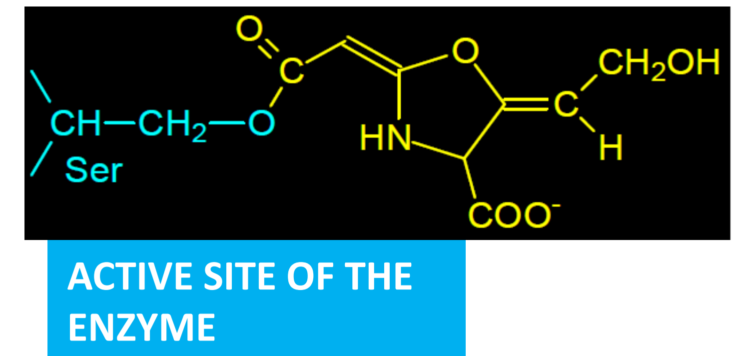
PENICILLIN (ACTIVE)

PENICILOIC (INACTIVE)



CLAVULANIC ACID

This molecule reacts with the active serine of the β-lactamase, thus leading to its inactivation.



ENZYME INHIBITORS AS DRUGS

Angiotensin-converting enzyme (ACE) inhibitors circulate in plasma.
They inhibit angiotensin I to form angiotensin II (vasoconstrictor).
Pro-inflammatory effects and cell death also occur.

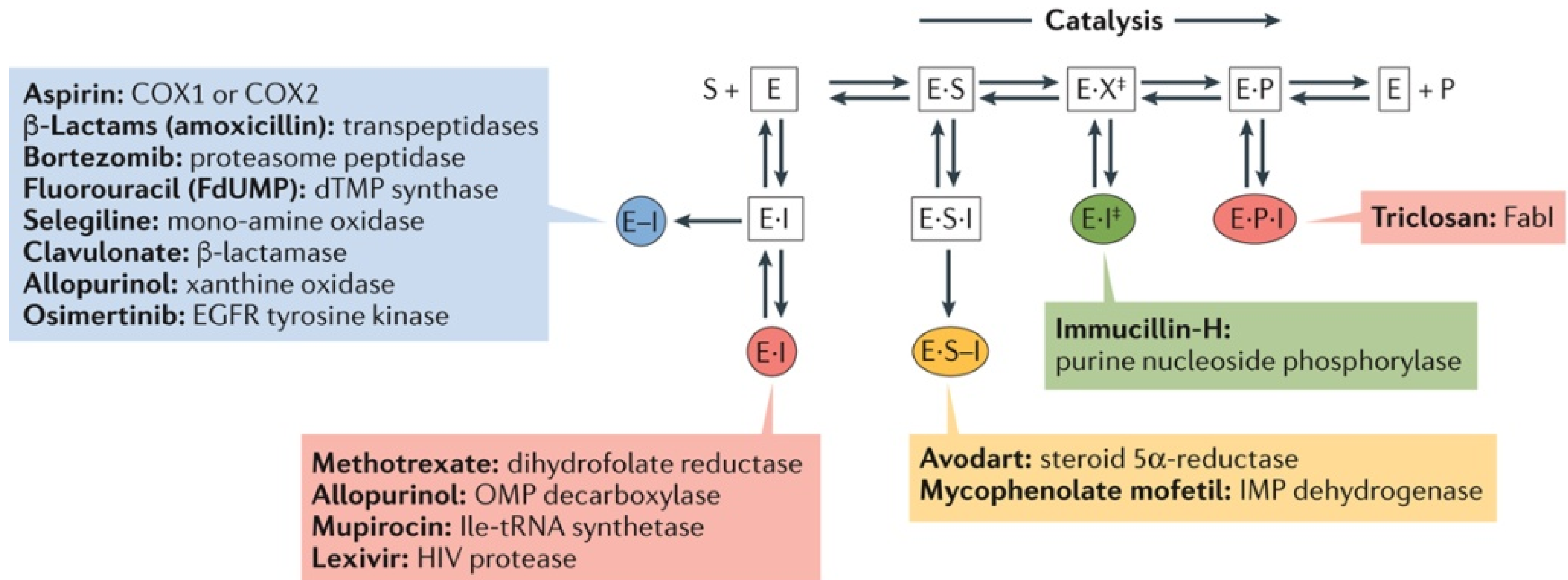
DRUGS: captopril, enalapril and lisinopril cause vasodilation and therefore reduce blood pressure.

ENZYME INHIBITORS WITH UTILITY AS DRUGS

Inhibitor	Pathology	Target enzyme
Aciclovir	Herpes	Viral DNA polymerase
Allopurinol	Gout	Xantine oxidase
Fluorouracil	Cancer	Thymidylate synthase
DuP450	AIDS	HIV Protease
Methotrexate	Cancer	Dihydrofolate reductase
Zidovudine	AIDS	HIV Reverse transcriptase
Omeprazol	Gastric ulcer	H+,K+ -ATPase
Phenelzine	Depression	Monoamino oxidase
Nitecapone	Parkinson's	Catechol-O-methyltransferase
Sorbinil	Diabetic retinopathy	Aldose reductase

MECHANISM OF ENZYME INHIBITORS USED AS DRUGS

Drugs can be designed if the enzymatic mechanisms are known.



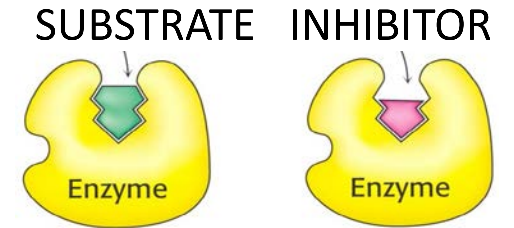
Inhibitors bind irreversibly or reversibly to any of the enzymatic forms during catalysis, thus blocking enzymatic progression.

Holdgate, G., Meek, T. & Grimley, R. Mechanistic enzymology in drug discovery: a fresh perspective.
***Nat Rev Drug Discov* 17,** 115–132 (2018). https://doi.org/10.1038/nrd.2017.219

REVERSIBLE INHIBITION: EXAMPLES AND KINETIC BEHAVIOR

A. COMPETITIVE INHIBITION

The inhibitor binds reversibly to the same site and competes with the substrate.

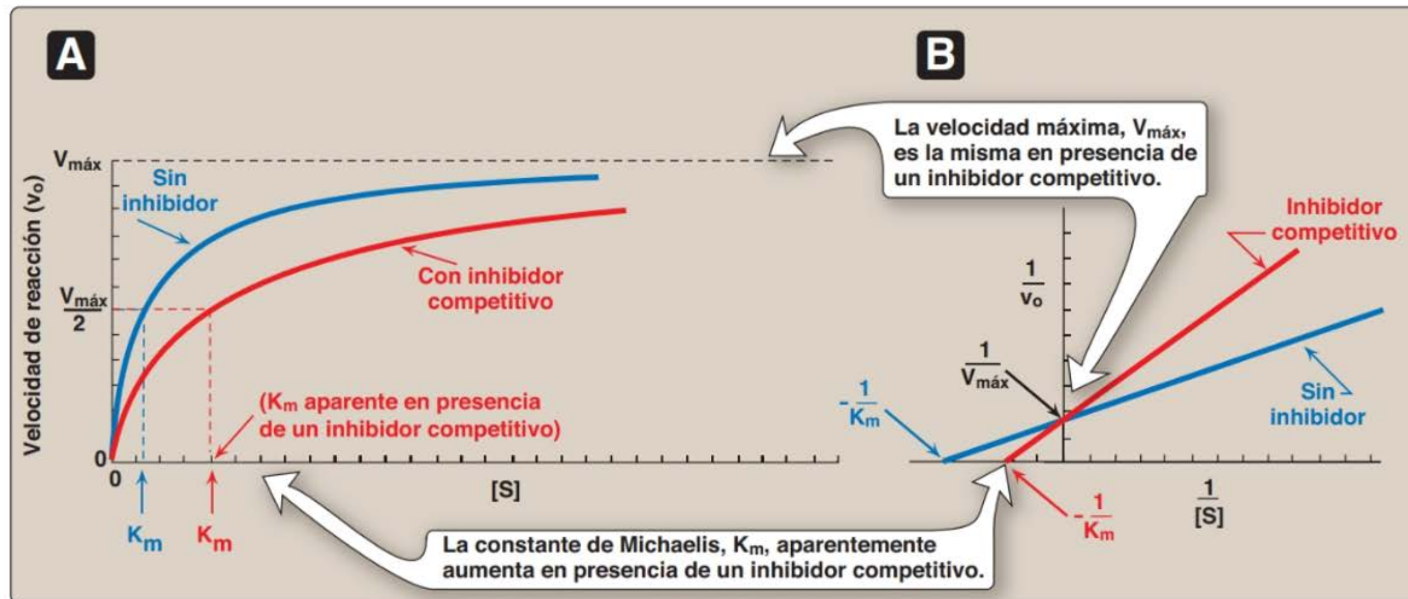


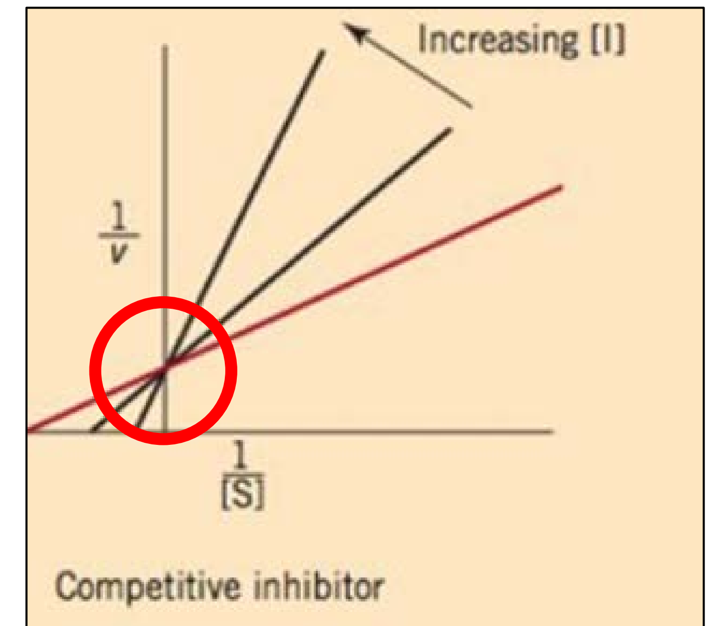
1. The effect of competitive inhibition on Vmax: VMAX DOES NOT CHANGE

This is reversed by increasing the concentration of the substrate. At a sufficiently high [S], the reaction rate reaches the Vmax observed in the absence of the inhibitor, i.e. the Vmax does not change.

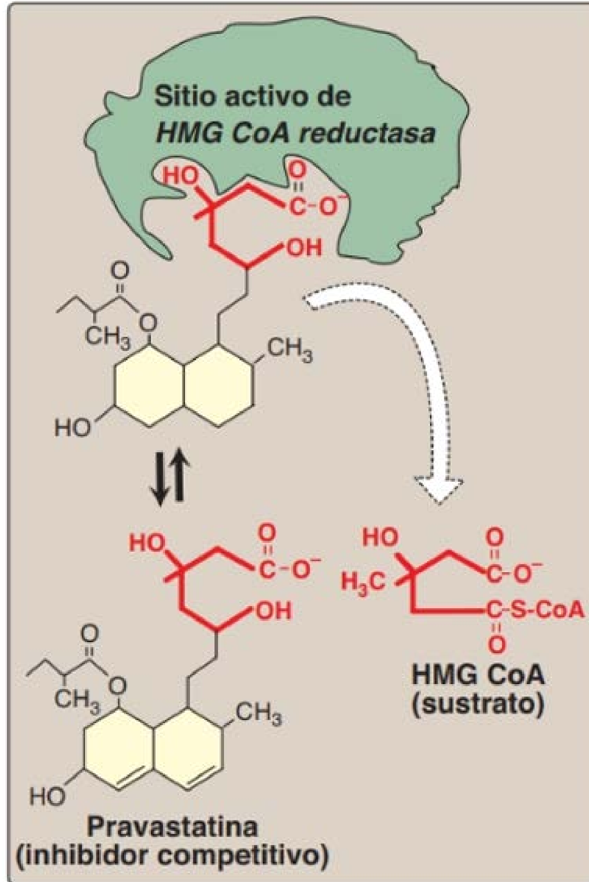
2. Effect on Km: A competitive inhibitor **increases the apparent Km** for a given substrate. This means that, in the presence of a competitive inhibitor, more substrate is required to reach half of the Vmax.

3. Effect on the Lineweaver-Burk graph: the lines with and without the inhibitor **meet at 1/Vmax.**





Active site of the HMG CoA reductase



STATINS AS AN EXAMPLE OF A COMPETITIVE INHIBITOR

Statins are anti-hyperlipidemic or lipid-lowering agents.
They competitively inhibit the rate-limiting step in cholesterol biosynthesis: hydroxymethylglutaryl coenzyme A reductase (HMG CoA reductase) enzyme.

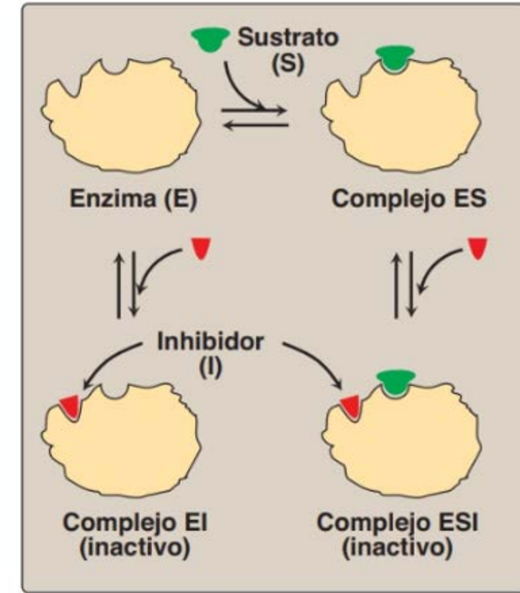
Statins, such as atorvastatin (Lipitor) and pravastatin (Pravachol):
Structural analogues of the natural substrate of this enzyme compete for the binding in the liver.

They inhibit *de novo* synthesis of cholesterol and:

1. reduce plasma and liver cholesterol levels.
2. induce the expression of the lipoprotein receptor in the liver.
3. cause the levels in the plasma to be further reduced.

B. NON-COMPETITIVE INHIBITION

This type of inhibition is recognized by **its characteristic effect on Vmax.**
The inhibitor and the substrate **bind at different sites** in the enzyme.

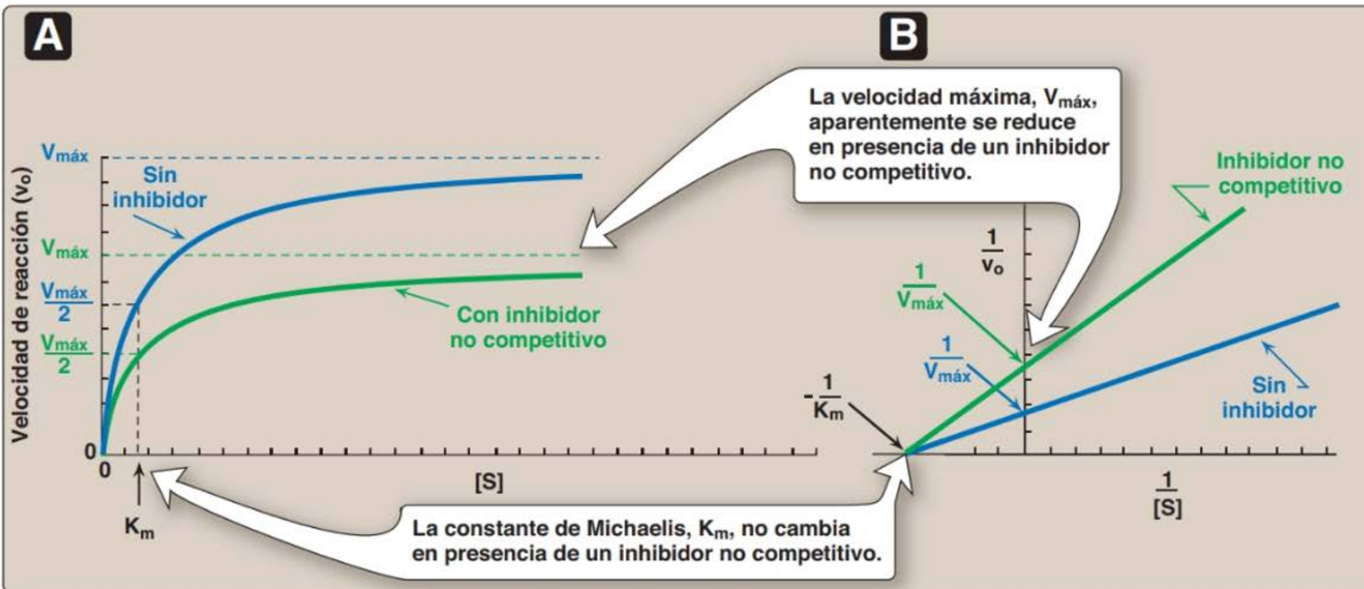


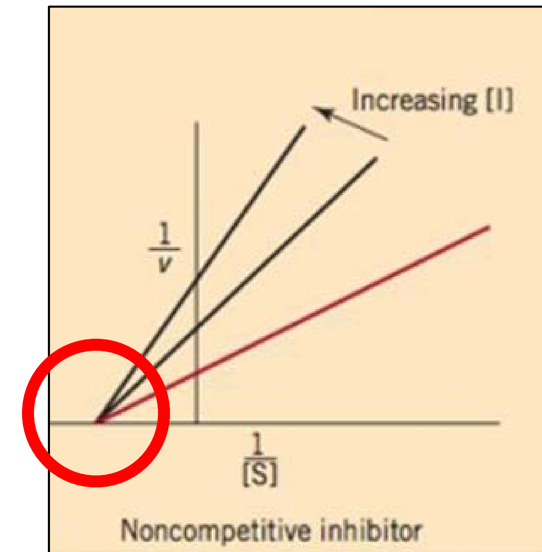
It may bind to the free enzyme or to the enzyme/substrate complex and makes the enzymatic reaction difficult.

1. Effect on Vmax: Vmax is reduced (apparent reaction). Inhibition cannot be overcome by increasing the concentration of the substrate.

2. Effect on Km: Km Enzyme (E) is not affected. The ES complex can bind the Inhibitor (I) forming ESI complex.

3. Effect on the Lineweaver-Burk graph: the lines with I and without I **meet at 1/Km.**



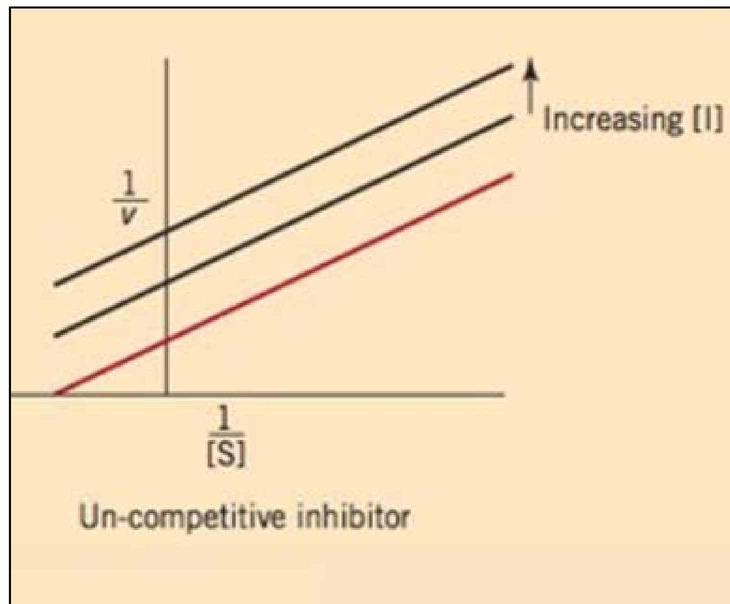


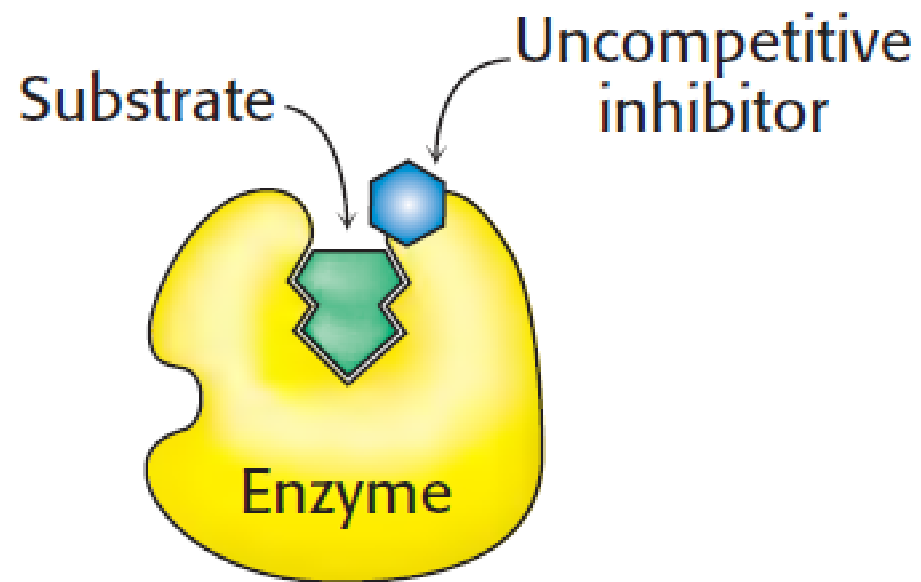
C) UNCOMPETITIVE INHIBITORS :

- These inhibitors join the **ES complex to form a ternary complex.**
- Increasing [S] does not decrease inhibitor binding.
- The inhibitors have affinity for the enzyme bound to S.

- The Km of the substrate is decreased.

- Vmax is also decreased.





REGULATION OF ENZYMATIC ACTIVITY IN ALLOSTERIC ENZYMES

Some enzymes (allosteric enzymes) respond to a more specialized regulation carried out by **EFECTORS.**
Their kinetic behavior does **not** follow the Michaelis-Menten equation.

EFFECTORS: ACTIVATORS (POSITIVE EFFECTORS) OR INHIBITORS (NEGATIVE EFFECTORS).

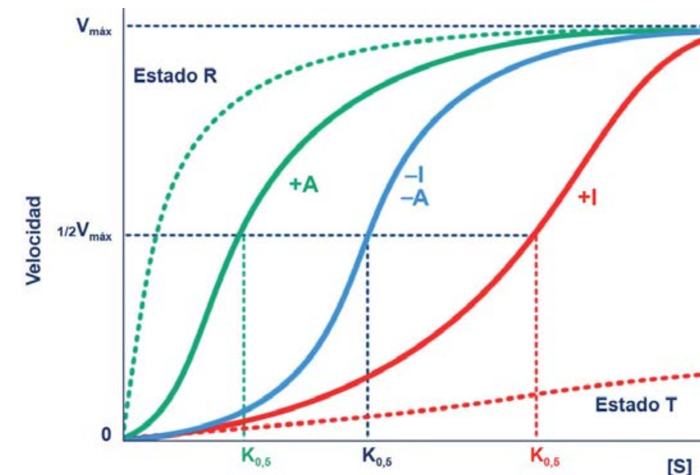
CHARACTERISTICS OF ALLOSTERIC ENZYMES:

1. Several subunits: OLIGOMERIC OR MULTIMERIC.

2. Allosteric binding/regulator/sites for different effectors with high specificity.
The **binding of the allosteric regulator** to the allosteric site of the enzyme produces a conformational change, i.e. it ALTERS THE ACTIVITY.
3. They catalyze certain limiting and important steps in metabolic pathways.

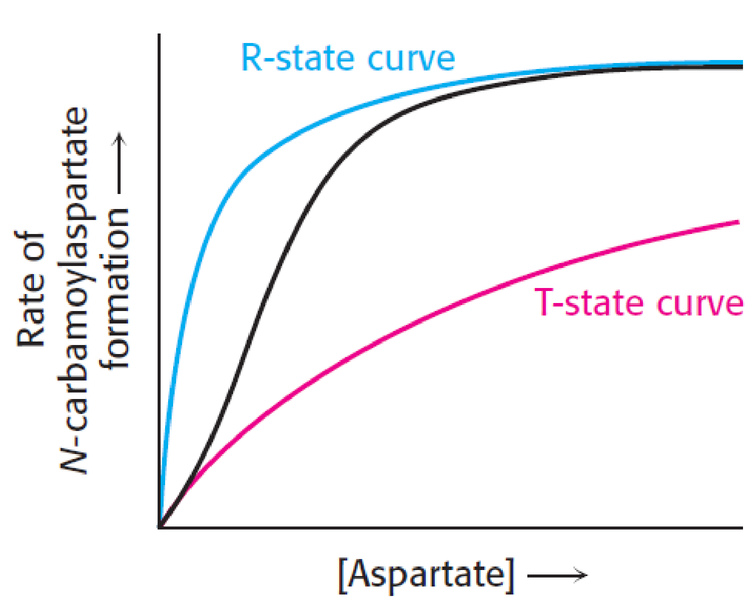
4. EFFECTORS can alter the **Vmax** of the Km reaction (substrate-enzyme affinity).

5. Non-Michaelian kinetic behavior: cooperativity.
Allosteric enzymes have sigmoid-type kinetics (V_0front to [S]).



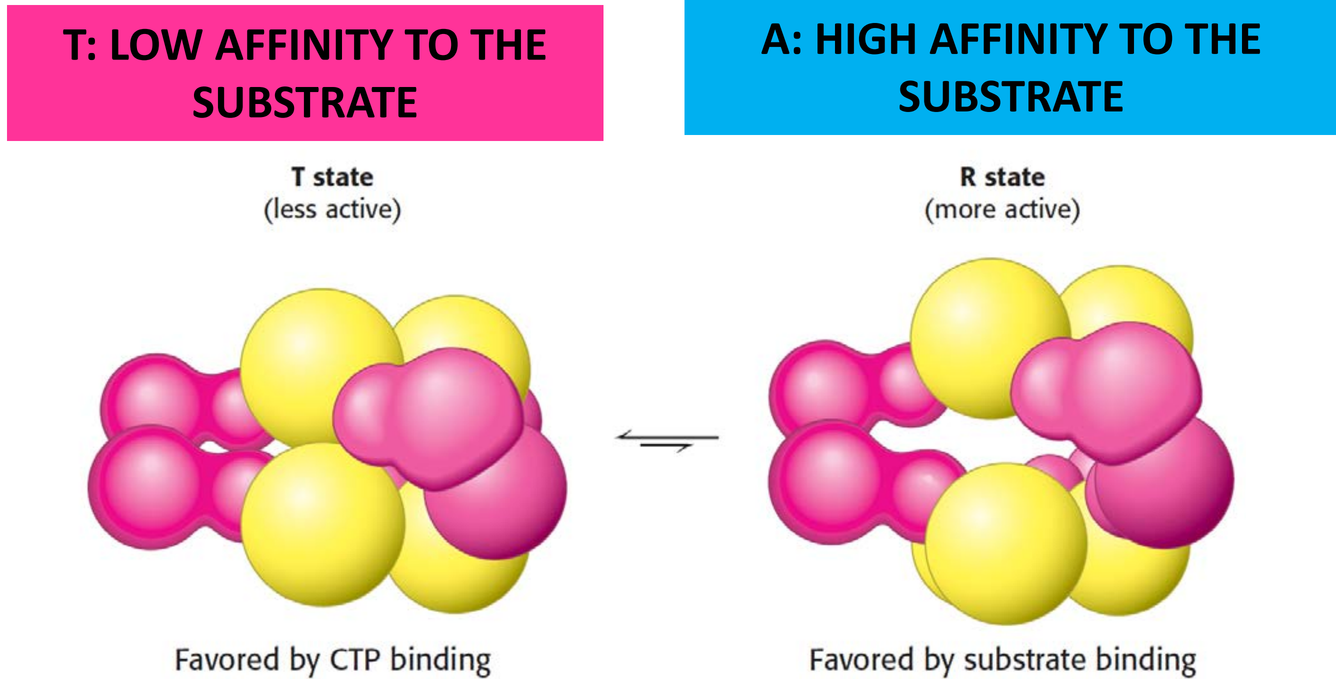
KINETIC BEHAVIOR AND COOPERATIVITY OF ALLOSTERIC ENZYMES: SIGMOID-TYPE KINETICS.

Cooperativity and sigmoid behavior in enzymatic kinetics appear in allosteric enzymes. The SUBSTRATES **have equivalent binding sites** for substrate binding **but are not independent.**



T: LOW AFFINITY TO THE SUBSTRATE

A: HIGH AFFINITY TO THE SUBSTRATE



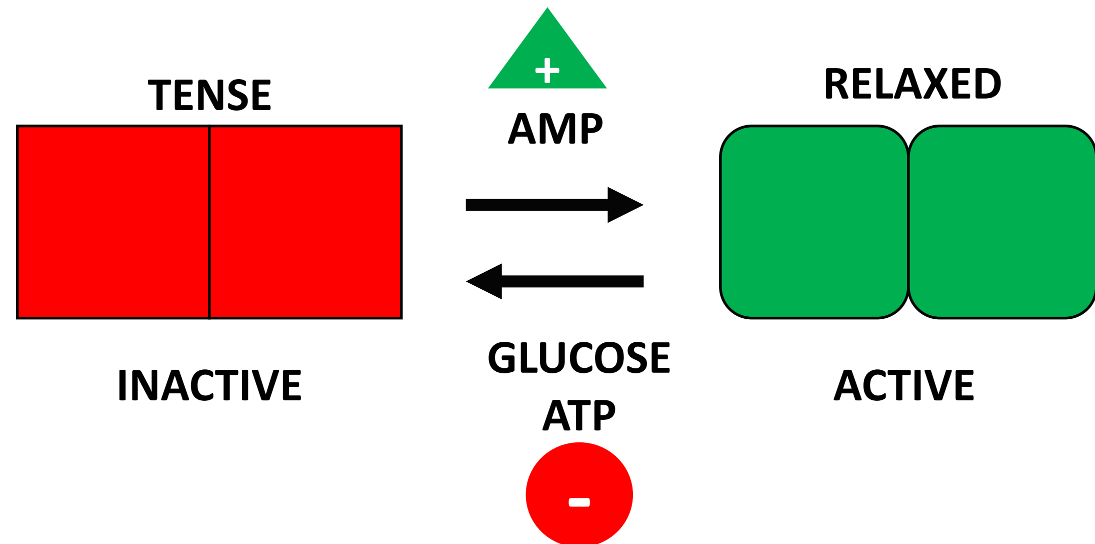
EXAMPLES OF REGULATION BY ALLOSTERISM: GLYCOGEN PHOSPHORYLASE

Glycogen phosphorylase has an allosteric regulation system that responds to a low energy load.

Glucose and ATP: Favorable energy conditions shift the balance of the enzyme towards the tense-form negative allosteric effectors/inhibitors.

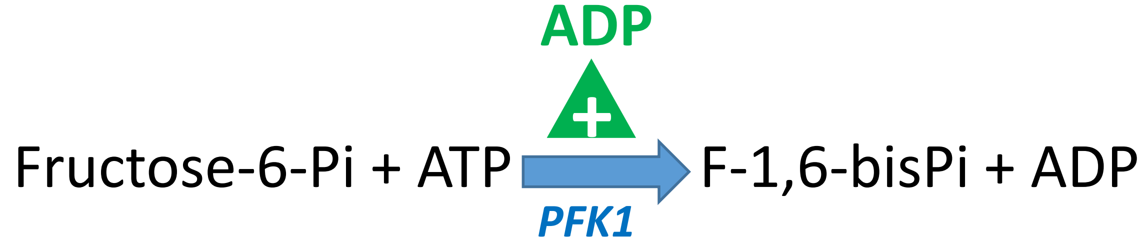
AMP: Poor energy conditions shift the balance towards the relaxed and active form. AMP is an allosteric effector, an activator.

GLYCOGEN PHOSPHORYLASE: A KEY ENZYME IN GLYCOGENLYSIS

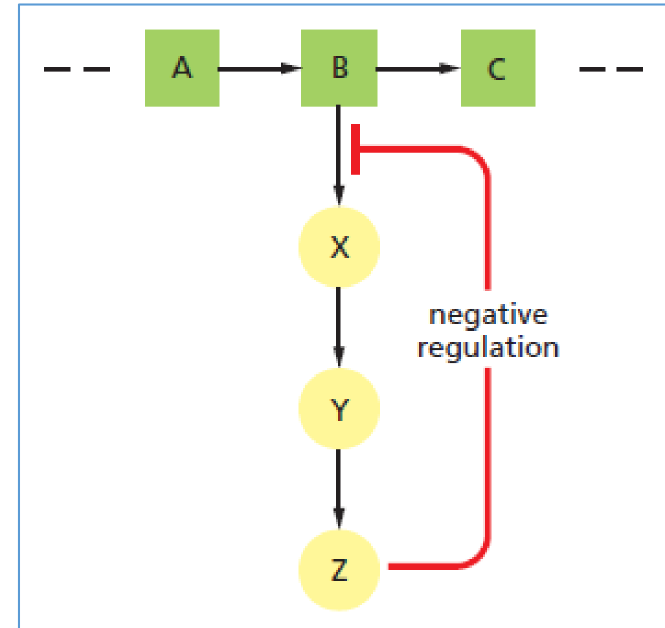


EXAMPLES OF REGULATION BY ALLOSTERISM: phosphofructokinase 1 (PFK1)

PHOSPHOFRUCTOKINASE 1 IN GLYCOLYSIS (PFK1): Glycolytic enzymes are inhibited in allosteric form by citrate, which is not a substrate for the enzyme.



FEEDBACK CITRATE INHIBITION:

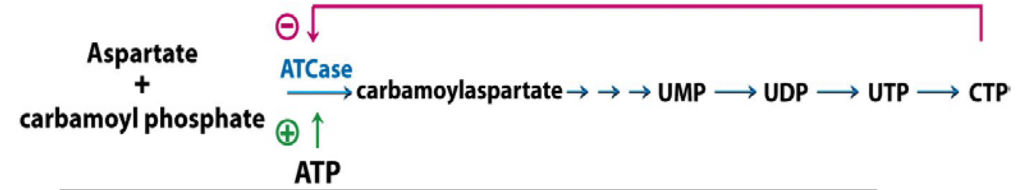


Inhibition of PHOSPHOFRUCTOKINASE (PFK1): KEY ENZYME OF GLYCOLYSIS

In a feedback negative inhibition a metabolic intermediate of a metabolic pathway connected to glycolysis, the Krebs Cycle.

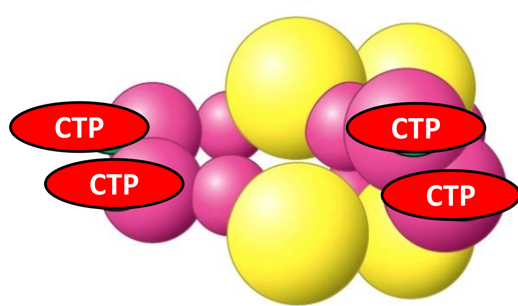
AN ALLOSTERIC ENZYME: ASPARTATE TRANSCARBAMOYLASE IS AN IMPORTANT ENZYME OF NUCLEOTIDE SYNTHESIS.

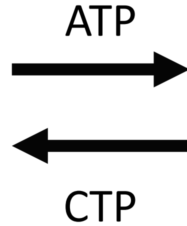
ATCase catalyzes the limiting stage of the pathway, is inhibited by the final product **CTP,** and is activated by **ATP.**

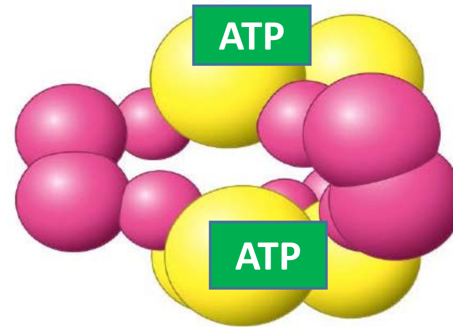


CTP inhibition mechanism stabilizes TENSE forms.

ATP Activation Mechanism: Stabilizes RELAXED forms.

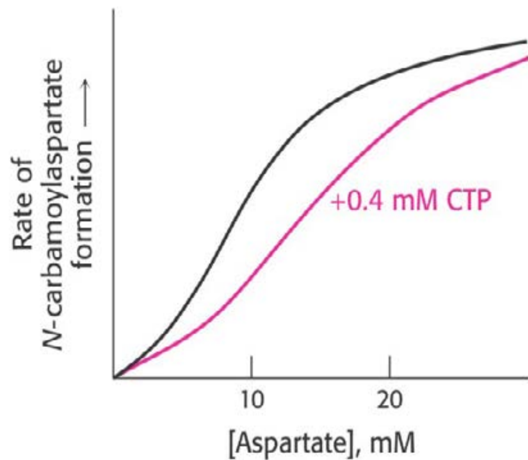




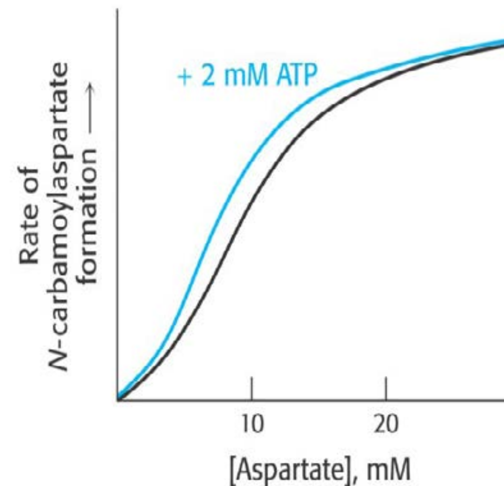


ALLOSTERIC SUBUNIT: YELLOW

CATALYTIC SUBUNIT: PINK



Lower activity and a curve that is less sensitive to substrate concentration.



Increased activity and a curve that is more sensitive to substrate concentration.

OTHER MECHANISMS OF ENZYMATIC REGULATION

Other mechanisms enable the activity to adapt to physiological needs, developmental states or environmental conditions.

1. TEMPORAL REGULATION:

Regulation of the genes that encode these enzymes by circadian or developmental hormones.

a) Slow (long-term) regulation mechanisms: in hours or days.

Control availability by developmental variable hormones, the circadian cycle or others.

Stability and **half-life of the enzyme,** degradation by **proteolytic systems.**

b) Rapid (short-term) regulation : in seconds or in minutes

- Allosteric control (Citrate, AMP: PFK1; AMP, ATP, glucose: Glycogen phosphorylase). - Covalent modification: irreversible, reversible.

2. SPATIAL REGULATION:

Compartmentalization: cytosolic enzymes, organelles, mitochondria, ER.

Isoenzymes in various tissues (lactate dehydrogenase).

Isoenzymes with different affinities or pH conditions act differently on the same substrate (hepatic **hexokinase and glucokinase**; a**cidic or alkaline phosphatase**).

INTEGRATION OF REGULATORY MECHANISMS INTO METABOLIC PATHWAYS

1. OBJETIVES

1. The regulatory mechanisms **adapt the metabolic pathway** to cellular needs and integrate the metabolism into the entire organism.

2. They regulatory mechanisms **coordinate the synthesis and degradation** pathways of a given substance in order to optimize metabolic functioning and substance storage.

2. **MECHANISMS**

Allosteric regulation (seconds or less).
Hormonal regulation (minutes).
Gene expression (specific genes that change metabolic status; hours).

3. OVERVIEW OF THE REGULATION OF METABOLIC PATHWAYS

1. On the routes **a low number of REGULATORY ENZYMES** catalyze irreversible and pathway-limiting reactions.

2. SPECIFIC ISOENZYMES of each tissue **OR WITH DIFFERENT CHARACTERISTICS** allow tissue compartmentalization. The lack of enzymes in certain tissues or the existence exclusively determines certain routes of degradation and synthesis.

3. HORMONAL REGULATION allows the metabolic integration/coordination of different tissues, organs and physiological systems. It enables the ORGANISM TO BE PLACED IN A METABOLIC STATE OF ENERGY OEXPENDITURE PRESERVATION.

4. **SPATIAL REGULATION**: **synthesis and degradation** in different organelles within the cell.

MECHANISMS OF METABOLIC REGULATION

1. TEMPORAL REGULATION:

HALF-LIFE AND AVAILABILITY OF METABOLIC ENZYMES

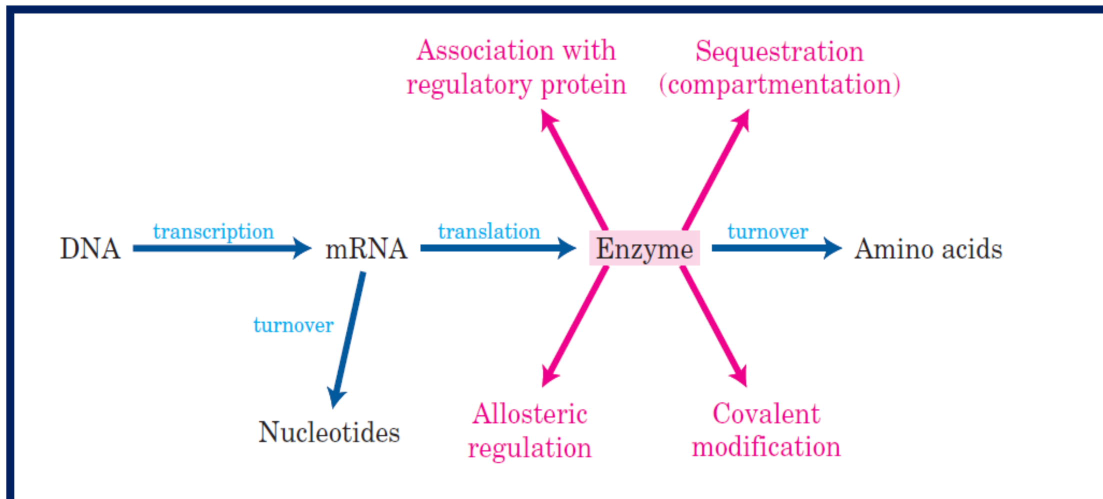
In cells, constant protein turnover affects the half-life of proteins and enzymes. The concentration of enzymes is regulated by their synthesis and degradation.

Slow response:

_1 Through the regulation of its synthesis: regulation of transcription and translation. Hormones and gene regulators affect gene expression.
2. Through the regulation of its degradation: mediated by ubiquitin and executed by the proteasome.

Quick response:

_- Reversible or irreversible covalent modifications: phosphorylation, synthesis as zymogens (inactive forms). Hormones also produce a rapid response.



PINK: MINUTES, SECONDS
BLUE: HOURS

MECHANISMS OF METABOLIC REGULATION

Rapid response in the regulation of metabolism:

- Reversible or irreversible covalent modifications: phosphorylation, synthesis as zymogens (inactive forms)

Irreversible

Activation by proteolytic breakdown of inactive precursors (zymogens)

Digestion enzymes

Coagulation cascade

Activation of caspases in apoptosis

Reversible

Covalent binding of a chemical group that alters the catalytic properties of the enzyme

1. Phosphorylation-dephosphorylation

2. Oxidation-reduction

3. Acetylation-deacetylation

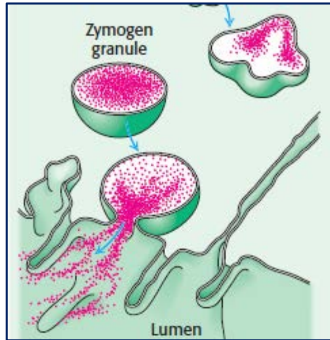
Rapid response in the regulation of metabolism: IRREVERSIBLE

ZYMOGENS: enzyme precursors or inactive enzymes

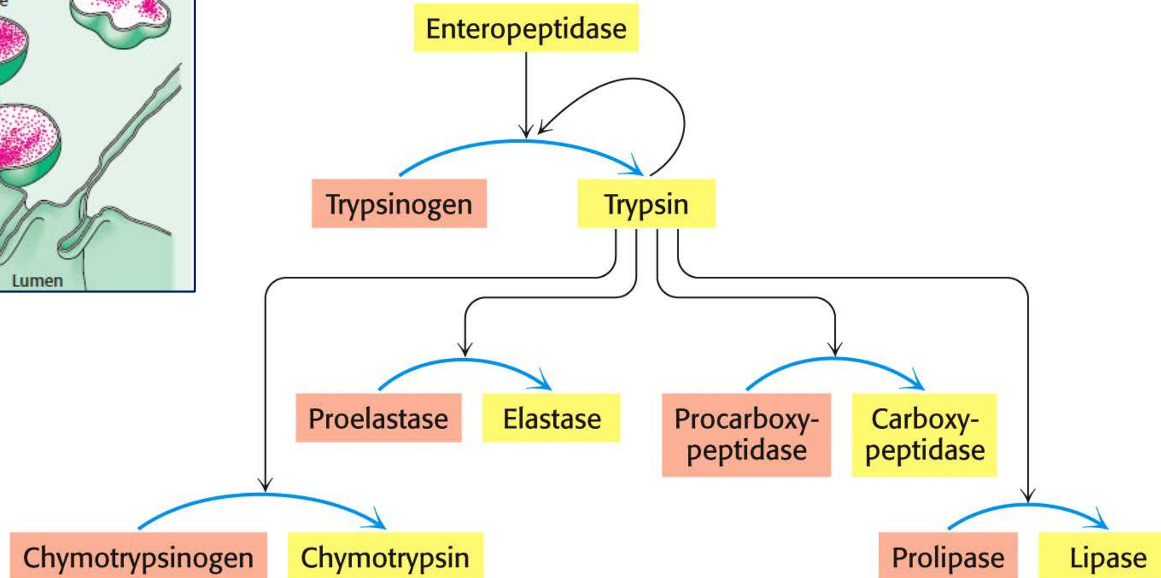
- These are inactive protein molecules that need to be activated to become active enzymes.

-Purpose:

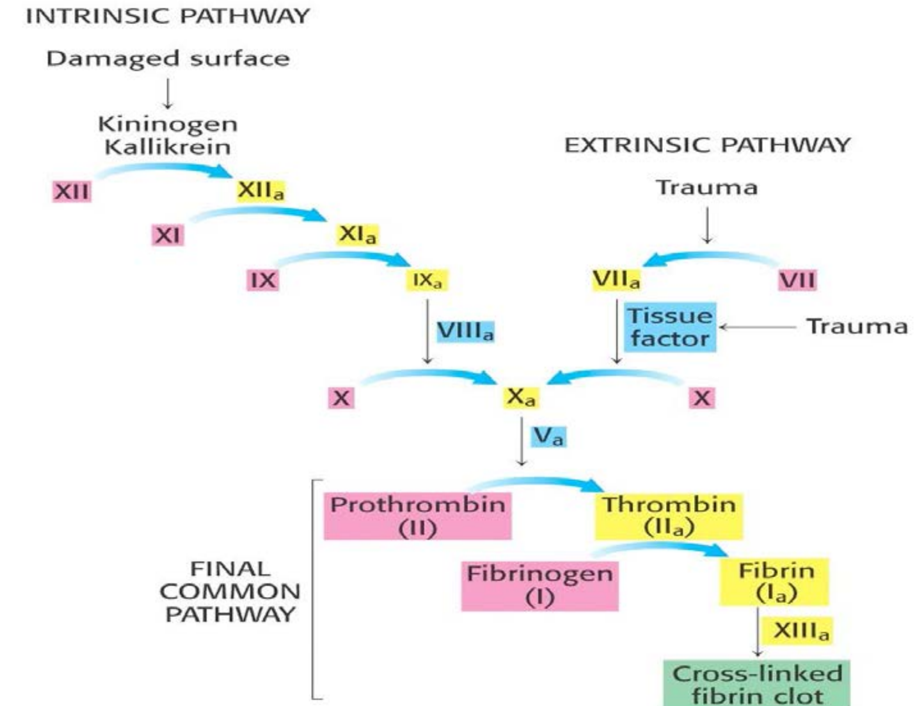
1. Safety mechanism to avoid catalysis in a suitable compartment or time.
2. Immediacy.



DIGESTIVE ENZYMES



COAGULATION FACTORS



Rapid response in the regulation of metabolism: REVERSIBLE

REVERSIBLE COVALENT MODIFICATION: 1) phosphorylation 2) adenylation 3) ADP-ribosylation 4) methylation

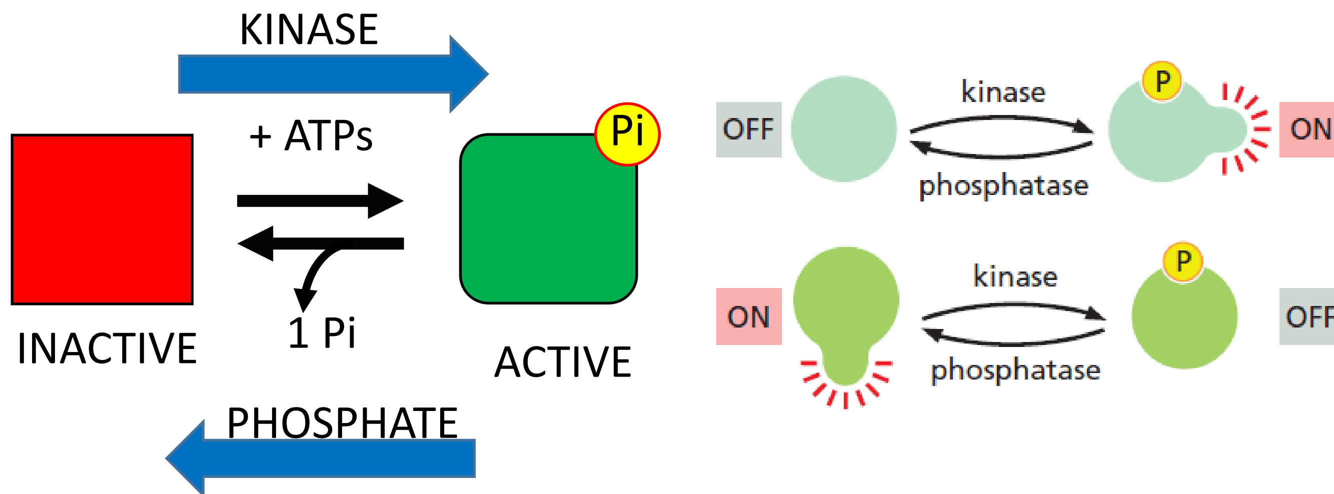
Table 10.1 Common covalent modifications of protein activity

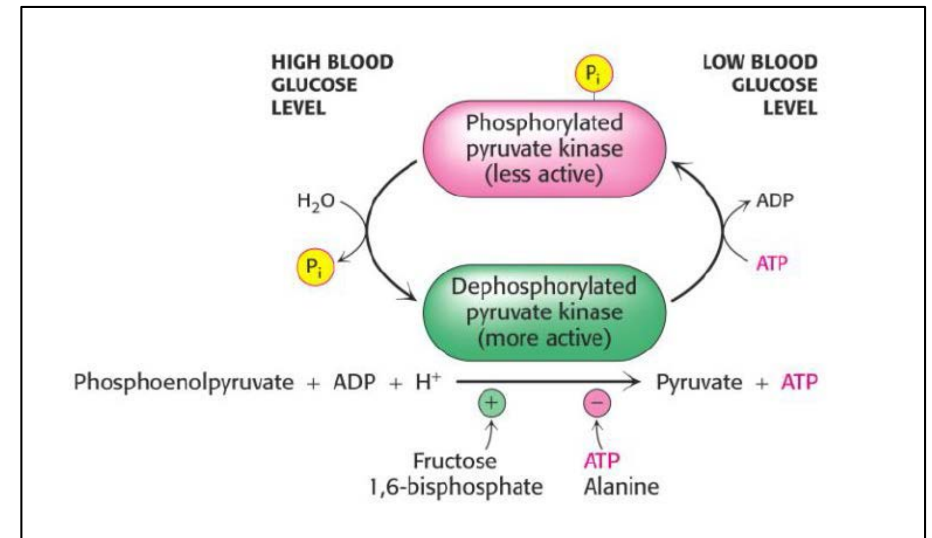
Modification	Donor molecule	Example of modified protein	Protein function
Phosphorylation	ATP	Glycogen phosphorylase	Glucose homeostasis; energy transduction
Acetylation	Acetyl CoA	Histones	DNA packing; transcription
Myristoylation	Myristoyl CoA	Src	Signal transduction
ADP ribosylation	NAD^+	RNA polymerase	Transcription
Farnesylation	Farnesyl pyrophosphate	Ras	Signal transduction
γ-Carboxylation	HCO_3^-	Thrombin	Blood clotting
Sulfation	3′-Phosphoadenosine-5′-phosphosulfate	Fibrinogen	Blood-clot formation
Ubiquitination	Ubiquitin	Cyclin	Control of cell cycle

Rapid response in the regulation of metabolism: REVERSIBLE

PHOSPHORYLATION/DEPHOSPHORYLATION:

1. Involves enzymatic activation/inactivation.
2. Catalyzed by PROTEIN KINASES that transfer phosphoryl groups from ATP to OH groups of the residues of Ser, Thr or Tyr.
3. These modifications often trigger amplified and cascading effects: a kinase activates many other enzymes, each of which activates many others.
4. Enzymatic activity is reversed dephosphorylation mediated by a PROTEIN PHOSPHATASE that removes the phosphoryl/PHOSPHATE group.





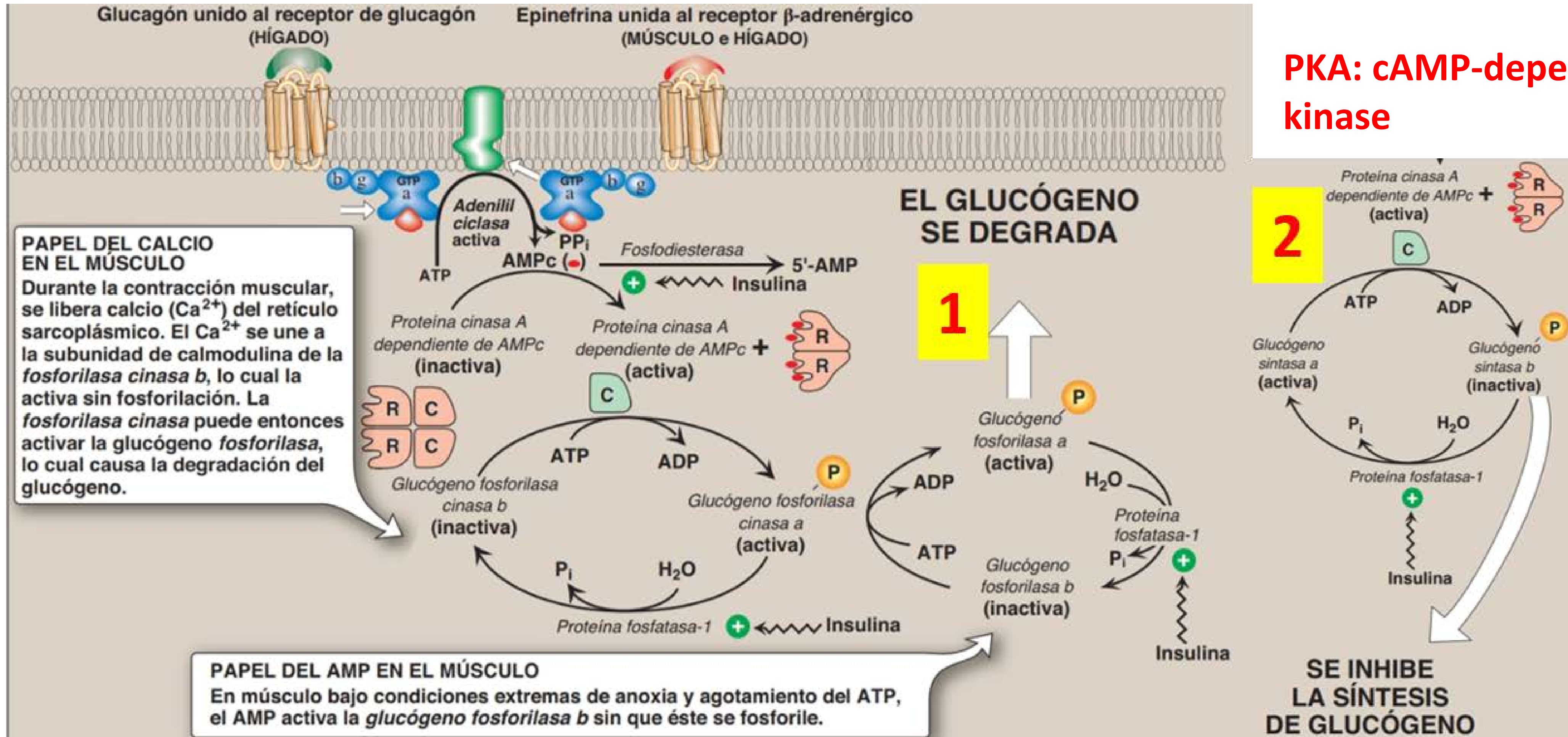
MECHANISMS FOR REGULATING ENZYMATIC ACTIVITY: RAPID HORMONAL RESPONSE

HORMONAL INTEGRATION. EXAMPLE: GLUCAGON/ADRENALINE-SIGNALLED BLOOD GLUCOSE NEED

GLUCAGON:

ADRENALINE: STRESS SITUATION

PKA: cAMP-dependent protein kinase



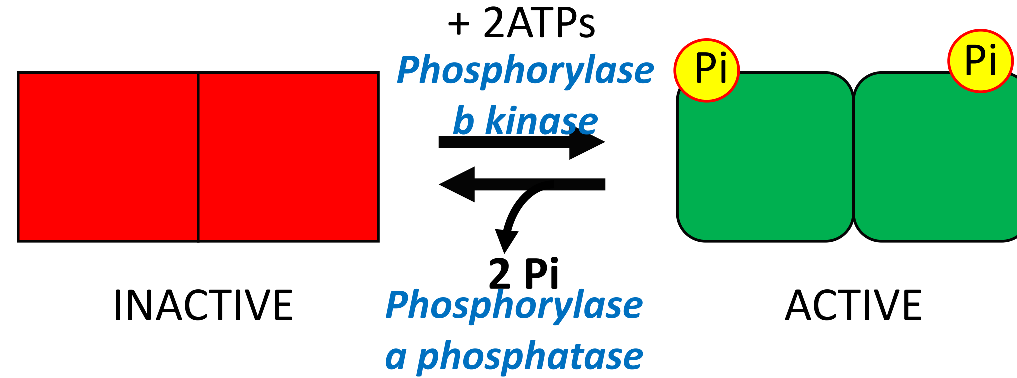
1 ↑ **GLYCOGENOLYSIS: glycogen phosphorylase activation.**

2 ↓ **GLYCOGENESIS: glycogen synthase inhibition.**

INTEGRATED REGULATORY MECHANISMS: GLUCOGEN PHOSPHORYLASE

Glycogen phosphorylase is an enzyme that catalyzes the first step in liver glycogenolysis to provide glucose.

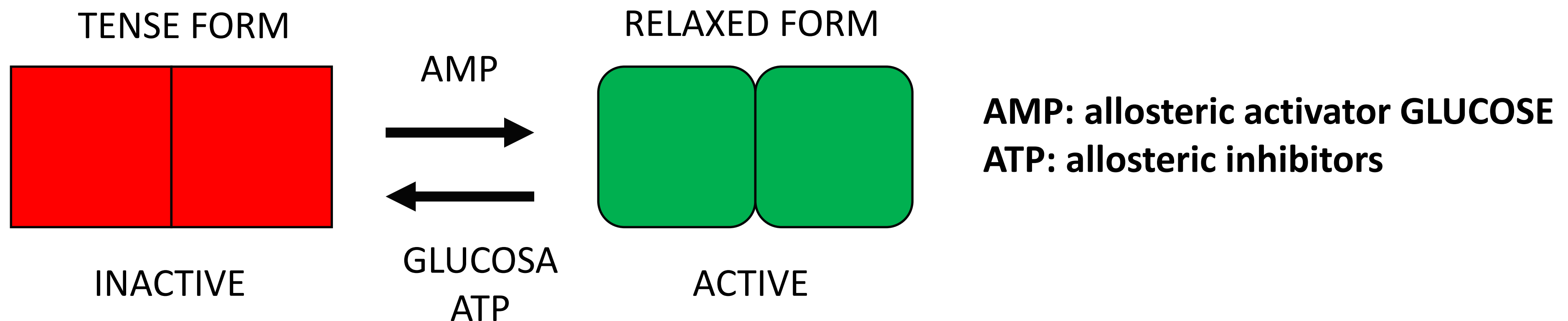
A) COVALENT MODIFICATION REGULATION



B) REGULATION:

Adrenaline/glucagon →→ phophorylation cascades until ***phosphorylase b kinase*** is activated and generates the active form.

C) ALLOSTERISM

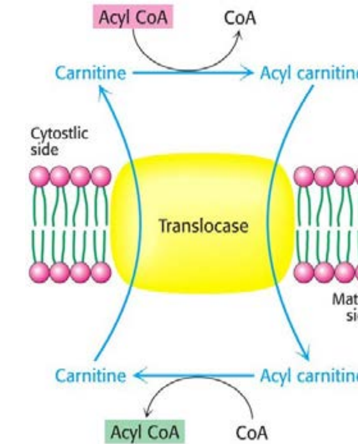


MECHANISMS FOR REGULATING ENZYMATIC ACTIVITY

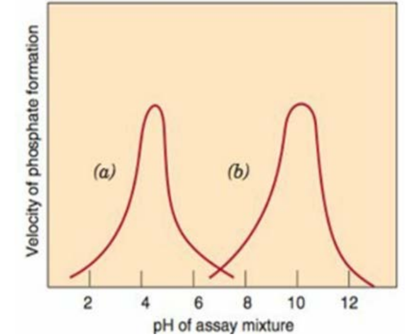
2.Spatial regulation:

COMPARTMENTALIZATION ALLOWS PHYSICAL LIMITATION OF THE SUBSTRATE

Organelle-specific enzymes. Physical limitation. Example of **β-oxidation**.



ACID/ALKALINE PHOSOHATASE



ISOENZYMES

1. These are homologous enzymes of the same organism.

2. They are tissue-specific or from different stages of development: they meet specific metabolic needs.

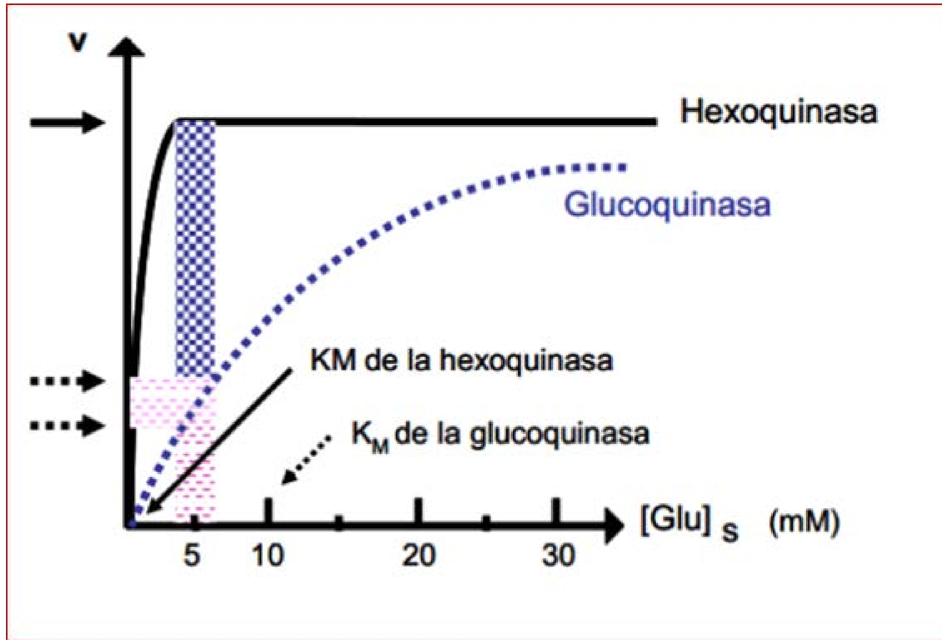
3. Catalysis of the same reaction with different characteristics: acid or basic catalysis.

4. Slight differences: affinity to the substrate, type of substrate, size, load, thermostability, Km, specificity substrates, immunoreactivity.

5. They are usually closely related structural variants of enzymes.

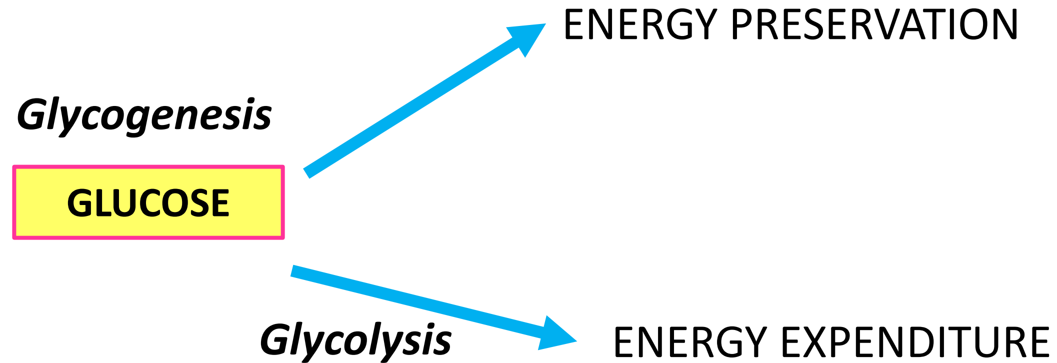
6. They represent enzymes from different genes whose products catalyze the same reaction.

LIVER



The Km of hexokinase is very low 0.1 mM; this enzyme works for glycolytic purposes when the glucose concentration is low.
The Km of GLUCOKINASE is 5 mM and will operate at high concentrations and for GLUCOGENIC PURPOSES (storage).

THE EXISTENCE OF ISOENZYMES WITH DIFFERENT Km ENABLES THE CONTROL AND COORDINATION OF THE METABOLIC PATHWAYS.



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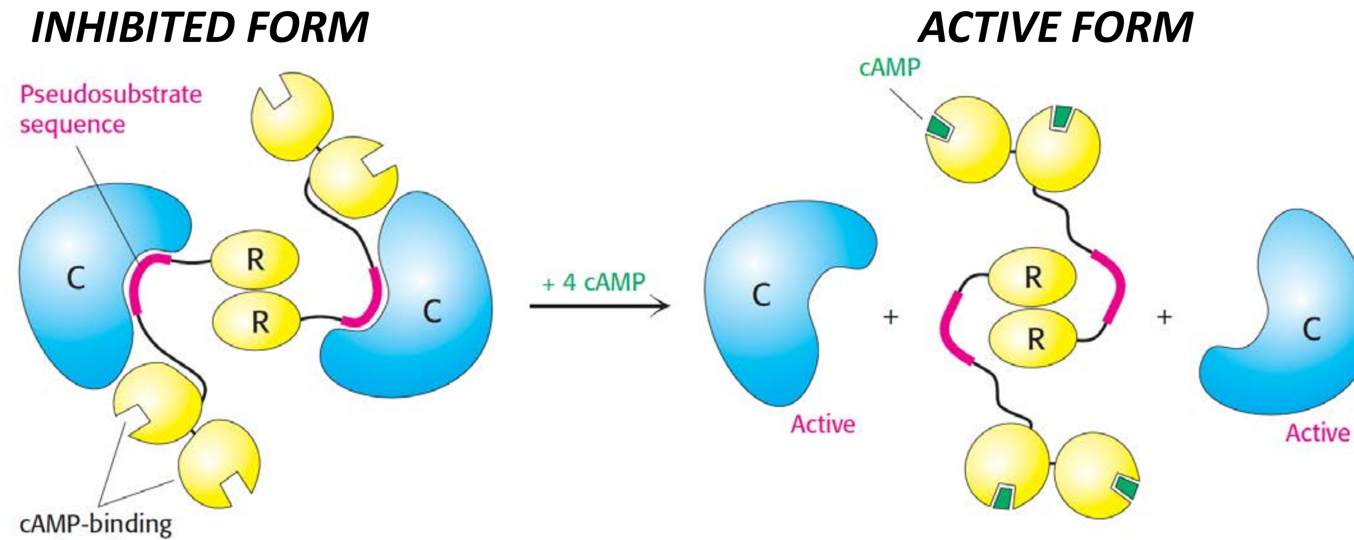
ANNEX

PKA AS AN EXAMPLE OF ALLOSTERIC REGULATION INTEGRATION AND PHOSPHORYLATION

R: REGULATORY SUBUNIT
C: CATALYTIC SUBUNIT WITH KINASE ACTIVITY

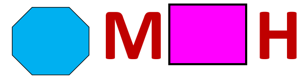
PKA ACTIVATION BY AMPc



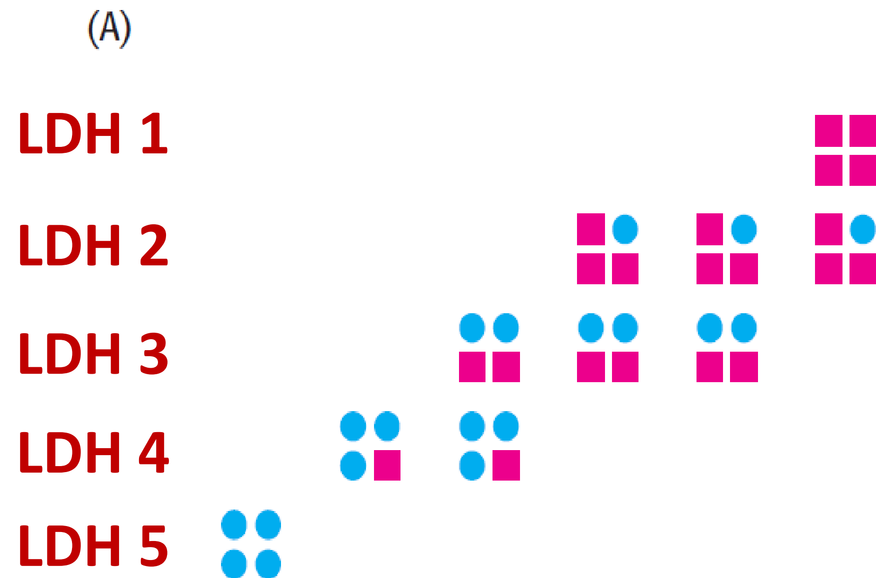
A "pseudosubstrate" sequence in the R subunit hides the active center where phosphorylable substrates enter in C. cAMP releases the R subunit and the region with kinase activity in C is exposed and can accept substrates to phosphorylate them.

LACTATE DEHYDROGENASE ISOENZYMES (LDH)

The LDH is made up of four subunits of two types.



Isoenzymes combine subunits with different tissue distributions.



(B)

Heart Kidney Red blood cell Brain Leukocyte Muscle Liver

H_4

H_3M

H_2M_2

HM_3

M_4

THE DIAGNOSTIC VALUE OF PLASMA ENZYMES IN CLINICAL BIOCHEMISTRY

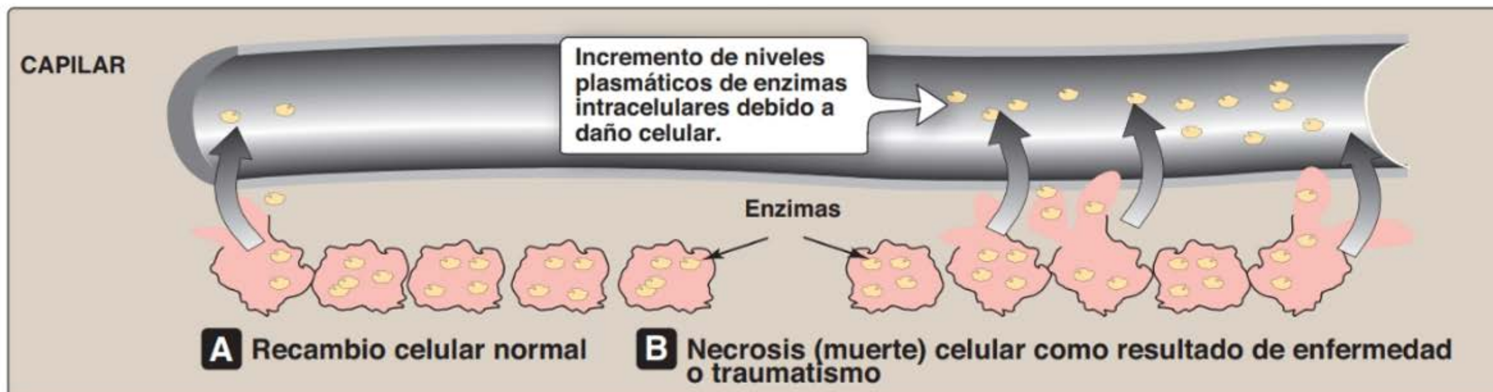
PLASMA:

Plasma is the liquid part of the blood that contains metabolites in solution : amino acids, glucose, mineral salts, vitamins, proteins and hormones.

AMONG these proteins are certain enzymes that are released by the tissues.

An increase in the plasma levels of this enzyme may indicate tissue damage.

The enzyme **alanine aminotransferase** is abundant in the liver. Elevated levels of ALT in plasma signal possible damage to liver tissue.



LESSON 23. Intermediate lipid metabolism (I)

E-mail: herminia.gonzalez@uv.es *Herminia González Navarro*

INDEX

1. Characteristics and properties of lipids
2. Lipid functions
3. Structure and function of complex saponifiable lipids:
    - 3.1. Properties, characteristics and structure of fatty acids
    - 3.2. Insoluble saponifiable lipids: acylglycerols and waxes
    - 3.3. Amphipathic saponifiable lipids: glycerolipids and sphingolipids
4. Simple non-saponifiable lipids:
    - 4.1. Isoprenoids: terpenoids and steroids
    - 4.2. Eicosanoids: prostaglandins, thromboxanes and leukotrienes
    - 4.3. Polypeptides

CHARACTERISTICS AND PROPERTIES OF LIPIDS

1. This heterogeneous group comprises organic molecules that are insoluble or poorly soluble in water. They can be **hydrophobic** (e.g. triglycerides) or **amphipathic** (e.g. phospholipids). **They are soluble in organic solvents** (e.g. benzene and chloroform).

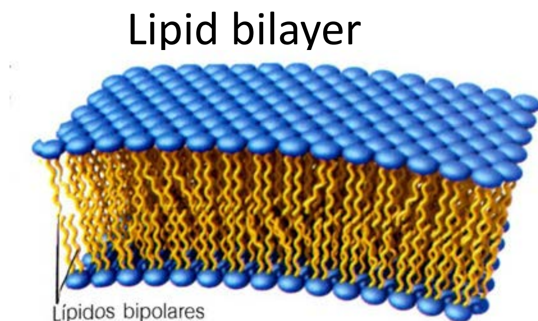
2. They are made up of C, H, O, S, P and N. They have a very high reducing potential, are anhydrous and have a very high energy content.

3. In water they form lipid bilayers in membranes, lipid droplets in cells and micelles. They are transported associated with proteins such as albumin, or form lipoproteins with apolipoproteins.

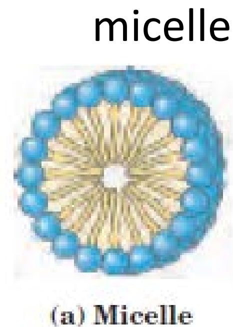
4. At room temperature their liquid state is **OILS** and their solid state is **FATS.**

5. Nutritional properties: **satiating, palatable**. They are highly oxidative and have a strong **energetic potential.**

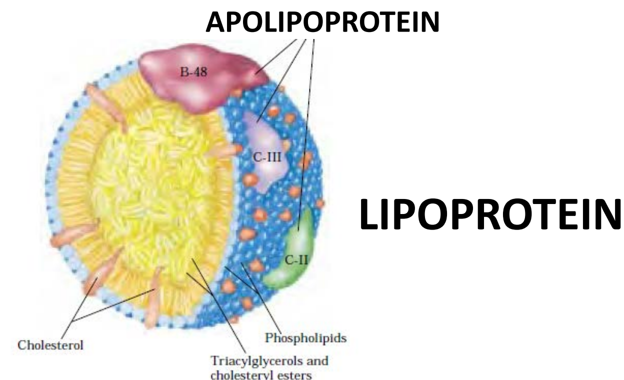
Lipid bilayer



micelle

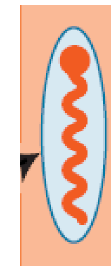

(a) Micelle

APOLIPOPROTEIN



LIPOPROTEIN

Fatty acid+ albumin



LIPID FUNCTIONS

ENERGY STORAGE: Important source of energy reserve: fatty acids, triglycerides.

STRUCTURAL:

- Hydrophobic barrier for compartmentalization PHOSPHOLIPIDS: the separation of aqueous contents of cells; cell and subcellular membranes.

- Surfactants: emulsifying bile acids.

- Important structures in the nervous system.

- Organ damping and insulation against heat loss.

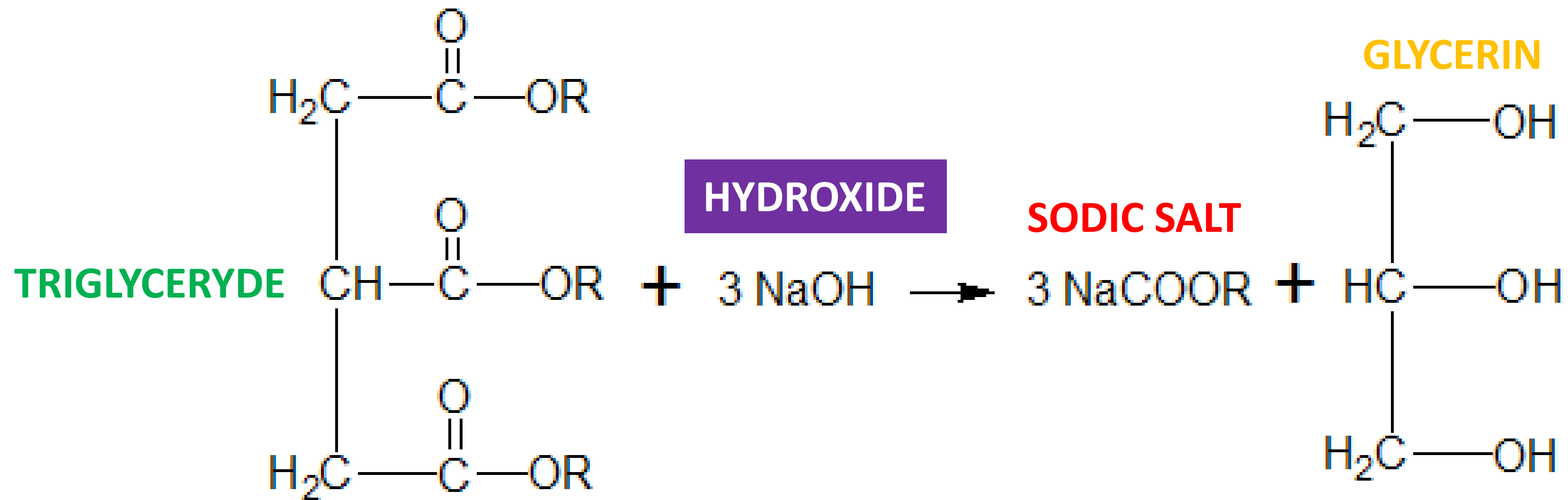
REGULATORS: fat-soluble vitamins, steroid hormones derived from cholesterol.

SIGNALING: Intracellular and extracellular mediators: PI3P (phosphatidylinositol triphosphate) and prostaglandins, thromboxanes and leukotrienes (eicosanoids).

TOXICS: antitumor polyketides.

COMPLEX LIPIDS ARE SAPONIFIABLE AND CONTAIN FATTY ACIDS IN THEIR STRUCTURE

THEY CAN PERFORM THE SAPONIFICATION REACTION

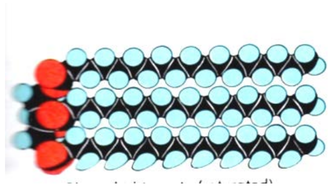


SIMPLE LIPIDS HAVE NO FATTY ACIDS IN THEIR STRUCTURE

COMPEX LIPIDS THAT CAN UNDERGO SAPONIFICATION REACTION:

FATTY ACIDS IN THEIR CHEMICAL STRUCTURE

INSOLUBLE
WAX AND ACYLGLYCERIDES



SOLUBLE: AMPHIPATHIC, POLAR HEAD
GLYCEROLIPIDS, SPHINGOLIPIDS

THESE MAY HAVE CARBOHYDRATE OR PHOSPHATE

GLYCEROPHOSPHOLIPIDS
GLYCEROGLYCOLIPIDS
SPHINGOPHOSPHOLIPIDS
SPHINGOGLYCOLIPIDS

FUNCTIONS OF FATTY ACIDS

FATTY ACIDS CAN BE FOUND : **1)** Esterifying saponifiable lipids or **2)** free

TRIGLYCERIDES: GLYCEROL + FATTY ACIDS

FUNCTIONS:

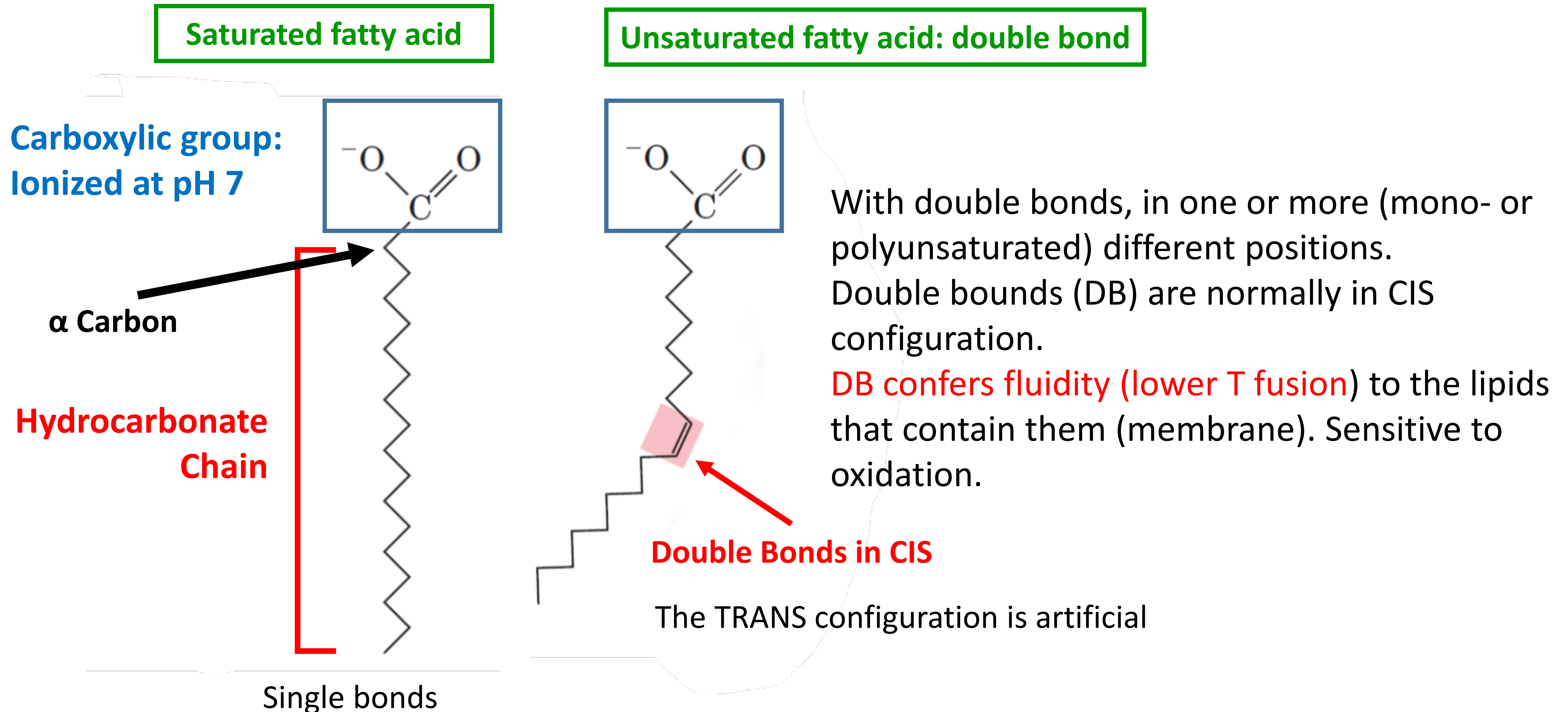
1. In TRIACYLGLYCERIDES, they are used as fuel with a high energy content (they save glucose).
2. They are precursors of eicosanoids (ARACHIDONIC ACID) and membrane lipids (phospholipids).
3. They are a fundamental component of acylglycerols and membrane lipids.

They give different characteristics to the lipids they esterify

FATTY ACIDS: STRUCTURE AND CHARACTERISTICS

They are carboxylic acids with hydrocarbon chains from **4 carbons to 36 (C4 C36).**

Structure: polar part, carboxylic group, and an apolar part, which is a hydrocarbonate chain variable in length.



FATTY ACIDS: NOMENCLATURE

NOMENCLATURE

1. The most frequent fatty acids have a common nomenclature: oleic, palmitic, linoleic, etc.

2. The number of carbons is indicated followed by a colon and the number of double bonds followed by parentheses with numbers indicating the positions of the double bonds from the **Carbon α.**

The positions of the double bonds are indicated in parentheses with Δ followed by the positions in superscript.

Examples:

Palmitic acid: is 16:0, i.e. 16 C with no double bonds.

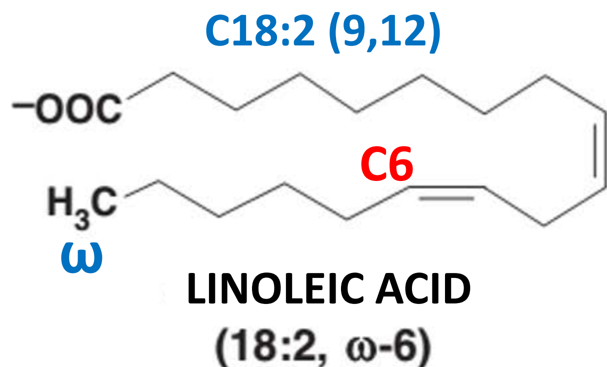
Oleic acid: is 18:1, i.e. with 18 C and 1 double bond.

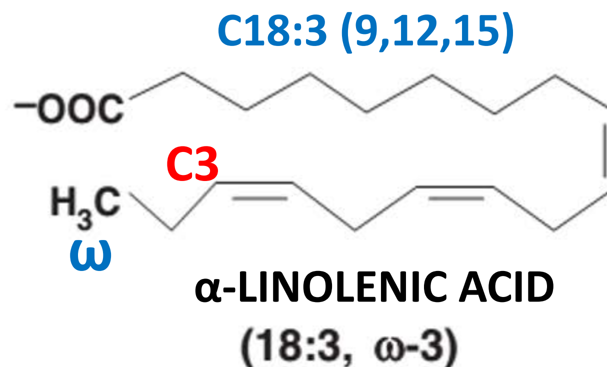
20:2 ($\Delta^{9,12}$) fatty acid, i.e. with 20 C and two double bonds in the C9 and C12 positions.

Arachidonic acid

20:4(5,8,11,14), i.e. 20 C and 4 double bonds (Carbons 5–6, 8–9, 11–12, 14–15).

ALTERNATIVE NOMENCLATURE: in long-chain fatty acids, the name refers the last carbon, called ω.





THE MOST COMMON FATTY ACIDS

MILK contains C14-C10 fatty acids

Structural lipids and TAG have mainly C16 fatty acids

NOMBRE COMÚN	ESTRUCTURA
Ácido fórmico	1
Ácido acético	2:0
Ácido propiónico	3:0
Ácido butírico	4:0
Ácido cáprico	10:0
Ácido palmítico	16:0
Ácido palmitoleico	16:1(9)
Ácido esteárico	18:0
Ácido oleico	18:1(9)
Ácido linoleico	18:2 (9,12)
Ácido α-linolénico	18:3 (9,12,15)
Ácido araquidónico	20:4 (5, 8,11,14)
Ácido lignocérico	24:0
Ácido nervónico	24:1(15)

Prostaglandin precursors

Essential fatty acids

The most common fatty acids with common proper names have an even number of carbons (16, 18 or 20).
The longest fatty acids (> 22 carbons) are found in the brain.
If unsaturated, they are mostly monounsaturated.

Essential fatty acids in the diet

Humans lack enzymes to synthesize these essential fatty acids.

Linoleic acid is the precursor of ω-6 arachidonic acid and is the substrate for the synthesis of prostaglandins.
α-linolenic acid is the precursor of ω-3 fatty acids and is for growth and development. It has a particularly high nutritional value thanks to its ability to lower cholesterol levels.

Plants, e.g. avocados, nuts and olives, provide us with these essential fatty acids.
ω-3 and ω-6 fatty acids are of NUTRITIONAL IMPORTANCE.

Ferrier, Denise R. *LIR. Bioquímica, 7th Edition*. Wolters Kluwer Health, 20170911.

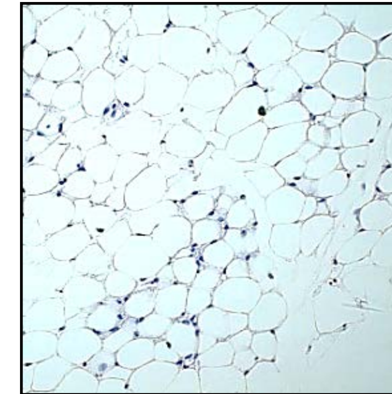
COMPLEX LIPIDS ARE SAPONIFIABLE AND CONTAIN FATTY ACIDS IN THEIR STRUCTURE

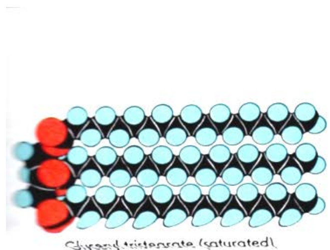
ACYLGLYCEROL= ACYLGLYCERIDES = GLYCERIDOLIPIDS

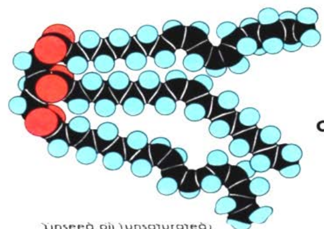
1) Energy storage: fatty acids
2) Fatty acid transport form
3) Protective function, organ insulator, body heat (brown adipose tissue)

TRIACYLGLYCERIDES (TAG) are only slightly soluble in water.
In white adipocytes they fuse and form oily droplets that are almost anhydrous.
They are the body's main energy reserve.
TAG stored in brown adipocytes serve as a heat source through thermogenesis

ADIPOSE TISSUE



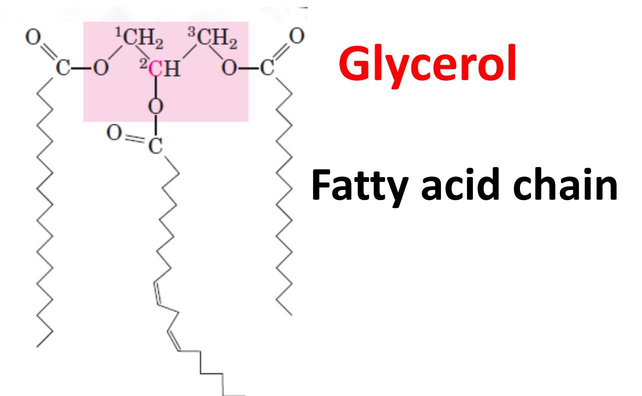




Monoglycerides: 1 fatty acid chains

Diglycerides: 2 fatty acid chains

Triglycerides: 3 fatty acid chains



Glycerol

Fatty acid chain

Glycerol esters with fatty acids: 1-3 molecules of fatty acid, the most common of which are those with carbon numbers from C16-C18.

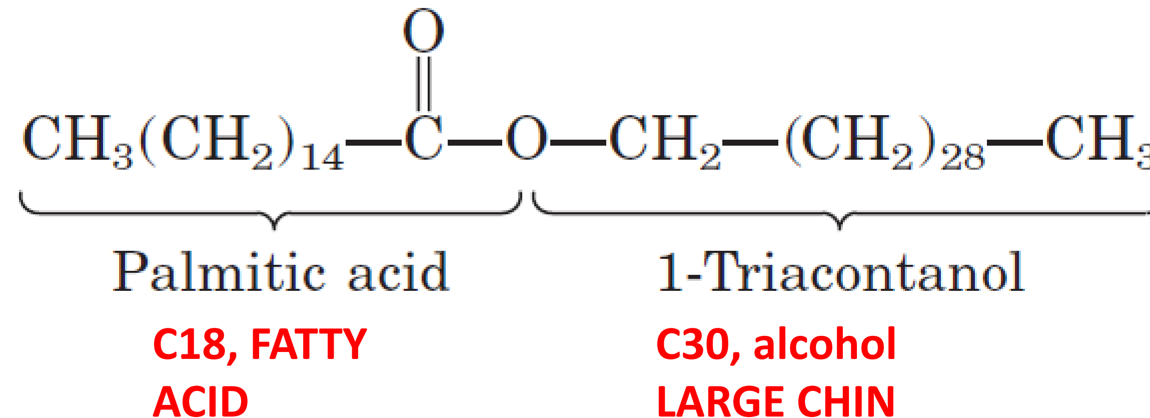
WAXES

THEY ARE VERY LONG FATTY ACIDS ESTERIFIED WITH ALCOHOLS WITH VERY LONG CHAIN:

1 molecule of saturated/unsaturated fatty acid with a very long chain with carbon numbers from C14-C36.

+

1 very long acyl (between C10-C36) alcohol molecule



C18, FATTY ACID

C30, alcohol LARGE CHIN

Honeycomb

Characteristics:

They have a very **high melting temperature**: 60–100º C.

They are an energy reservoir

They are waterproof (prevents evaporation)

They have firmness and consistency

MEMBRANE LIPIDS: SAPONIFIABLE AND AMPHIPATHIC LIPIDS

Glycerolipids:

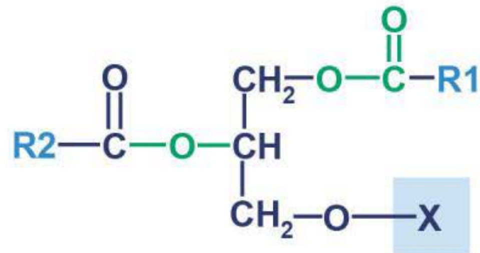
Esterified alcohol: Glycerol
GLYCEROPHOSPHOLIPIDS
GLYCEROGLYCOLIPIDS

Sphingolipids:

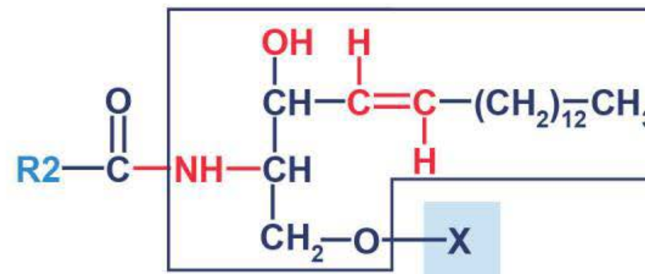
Esterified Alcohol: Sphingosine

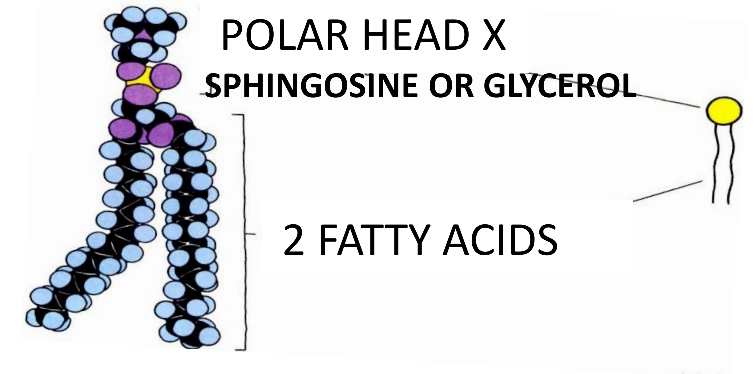
SPHINGOPHOSPHOLIPIDS
SPHINGOGOGLUCOLIPIDS

GLYCEROL



SPHINGOSINE



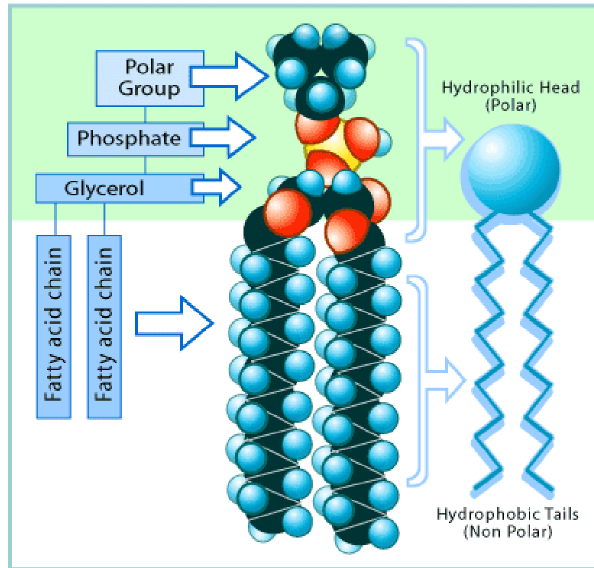


X: PHOSPHATE GROUP OR A GLUCID

Carbohydrate bound by a glycosidic ester-type bond.

Phosphate bound by a phosphoester bond.

MEMBRANE LIPIDS: GLYCEROLIPIDS AND (GLYCERO)PHOSPHOLIPIDS



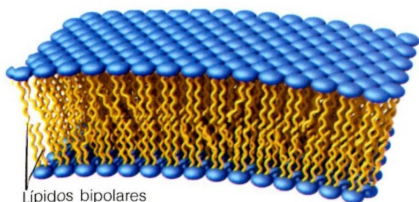
Polar: phosphate group

Non-polar: hydrophobic tail

1) Components of cell membranes: fluidity to membranes.
2) Source of polyunsaturated fatty acids.
3) Source of secondary messengers of cell signaling PI3P, I3P.

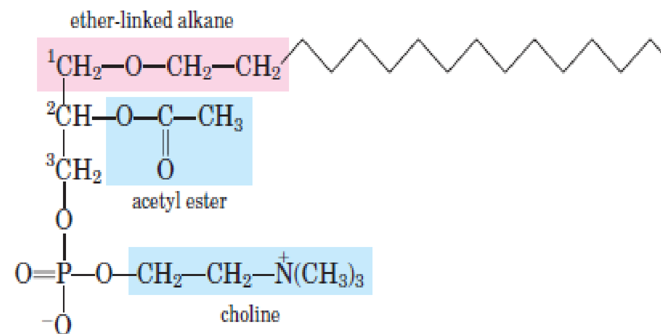
Most common: **phosphatidylcholine** or lecithin, **phosphatidylethanolamine** or cephalin, **phosphatidylserine** **and phosphatidylinositol**

LIPID BILAYER



OTHERS:

Some phospholipids have 1 ether group: platelet-activating factor (PAI)

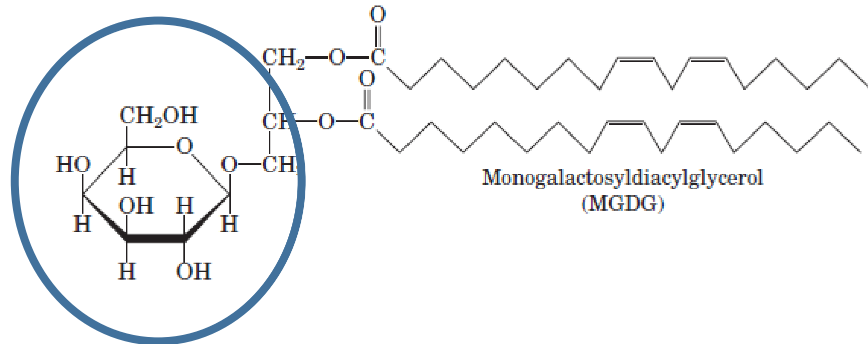


Platelet-activating factor

MEMBRANE LIPIDS: GLYCEROGLYCOLIPIDS

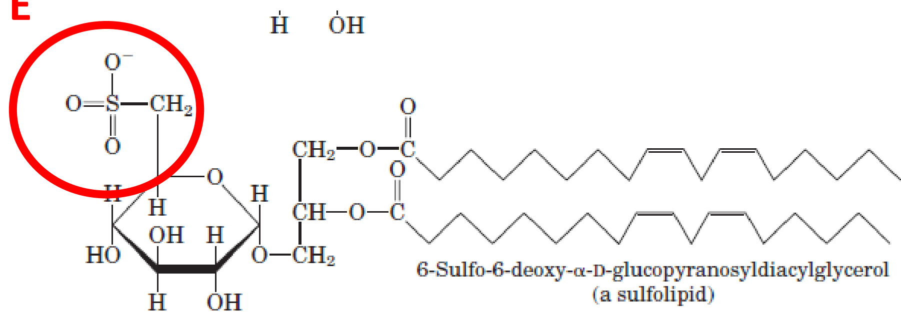
GALACTOLIPIDS AND SULFOLIPIDS: membranes of the chloroplast. These are important in photosynthetic function

SUGAR



GALACTOLIPIDS: 70-80% of thylakoid membranes

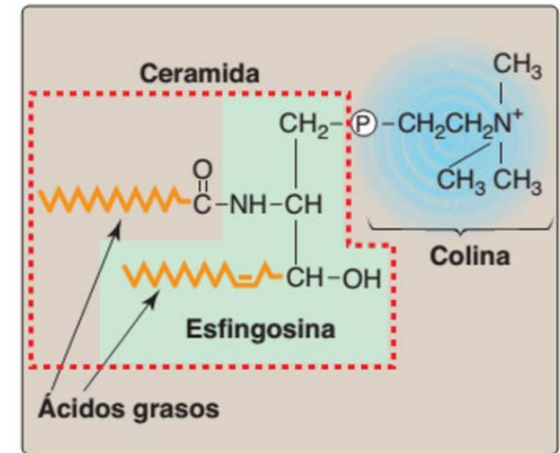
SULFATE



SULPHOLIPIDS (photosynthetic electron transport chain)

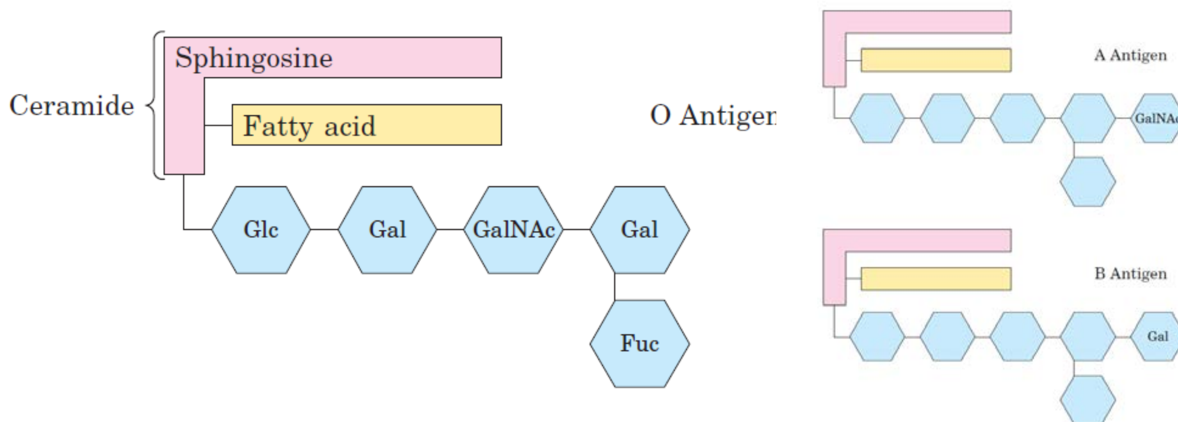
MEMBRANE LIPIDS: SPHINGOLIPIDS

Sphingomyelin



SPHINGOPHOSPHOLIPIDS: The most important of these in humans is sphingomyelin. **FUNCTION:** they are found in cell membranes, mainly in the myelin sheath of nerve cells.

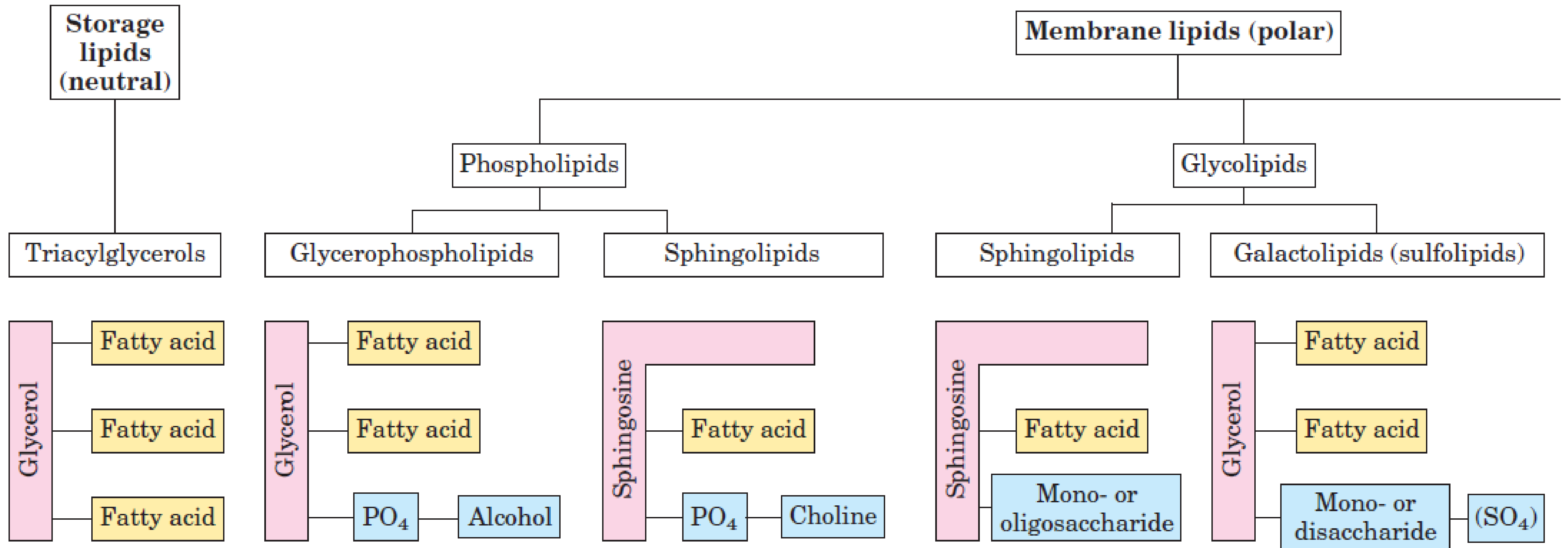
SPHINGOGLYCOLIPIDS: These are the most important glycolipids. They are located on the outer face of the cell membrane. They are important in cellular recognition. **CEREBROSIDES (1 mol. sugar), GLOBOSIDES (neutral) AND GANGLIOSIDES (negative charge).**



Examples:

IN CELL MEMBRANES THEY PARTLY DETERMINE BLOOD TYPE.

THE MOST IMPORTANT SAPONIFIABLE LIPIDS



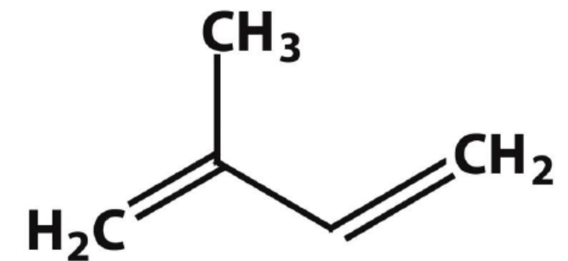
SIMPLE NON-SAPONIFIABLE LIPIDS HAVE NO FATTY ACIDS IN THEIR STRUCTURE

ISOPRENOIDS, STEROIDS, EICOSANOIDS, POLYKETIDES

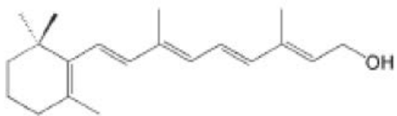
ISOPRENOIDS OR TERPENOIDS

These are **linear or cyclic molecules** that combine two or more isoprene molecules. These molecules that can be excited by light and emit energy.
Xanthophyll carotenes: light excitation.
Geraniol: natural aromas.
Squalene: synthesis of cholesterol.
Vitamins A, E, K: four molecules of isoprene

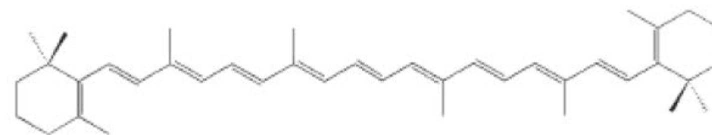
ISOPRENE



VITAMIN A

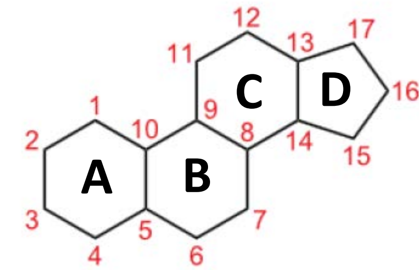


CAROTENE



STEROLS: 4-RING STEROID CORE

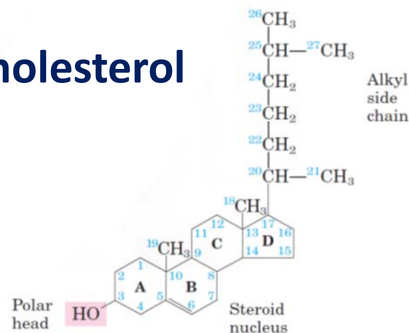
Cholesterol and derivatives: such as bile acids and their conjugates (salts).
Steroid hormones: estrogens, androgens, progestogens, glucocorticoids and mineralocorticoids.
Secosteroids: Cleavage of the B ring of the steroid nucleus. Vitamin D.



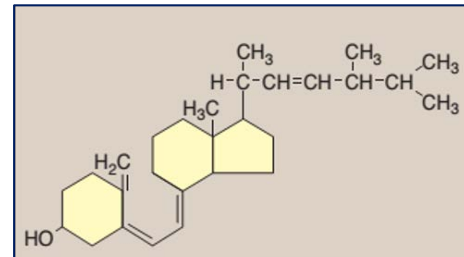
Cyclopentaneperhydrophenanthrene

FUNCTIONS:
- To provide membrane fluidity
- Hormonal functions.
- Formation of cofactors: vitamin D

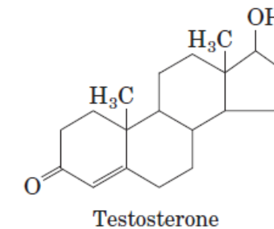
Cholesterol



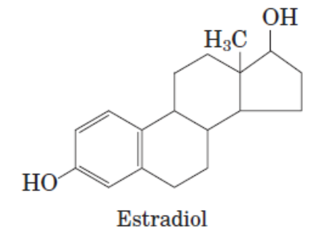
Vitamin D



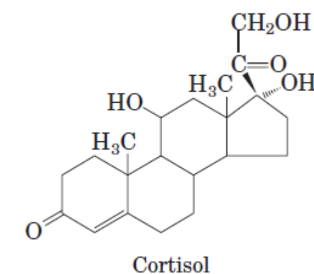
Steroid hormones



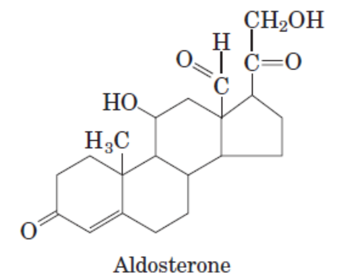
Testosterone



Estradiol



Cortisol



Aldosterone

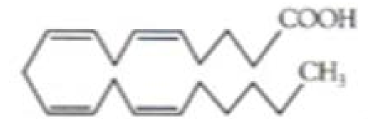
EICOSANOIDS: CYCLIC DERIVATIVES OF ARACHIDONIC FATTY ACID

These are derivatives of arachidonic fatty acid, **20 atoms of C and polyunsaturated: 20:4 (5,8,11,14).** They are **membrane phospholipids** that are cleaved and released from cellular membrane when phospholipase A2 is activated. They are synthesized from the essential fatty acid linoleic acid.

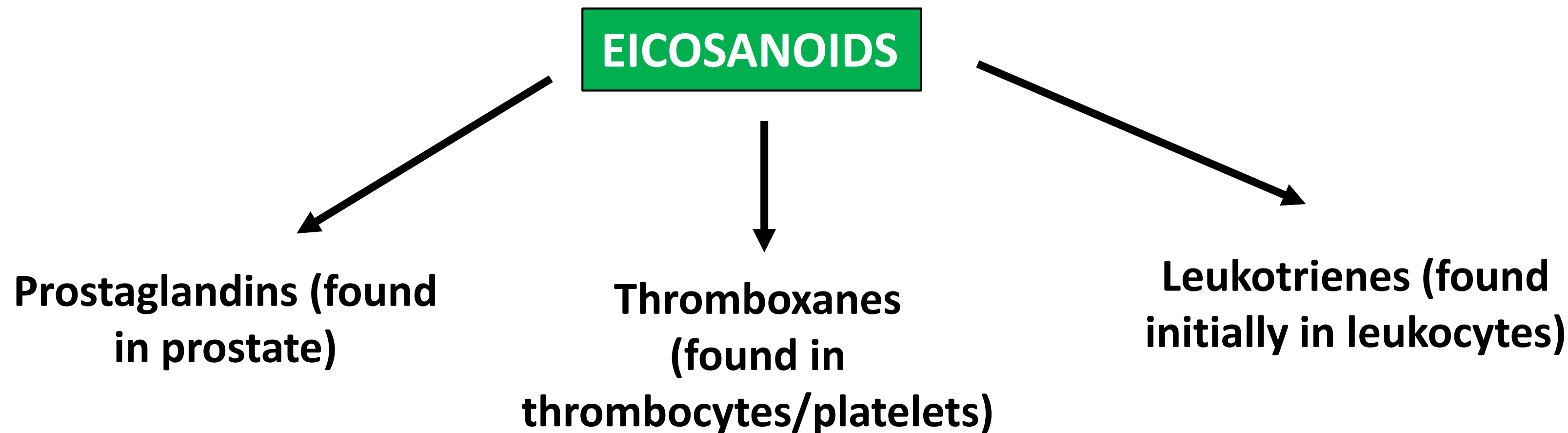
FUNCTIONS:

1) Inflammation, fever and pain associated with damage or illness.
2) Clot formation, blood pressure regulation.
3) Gastric secretion.
4) Reproductive function.

Arachidonic fatty acid



20:4 (5,8,11,14)



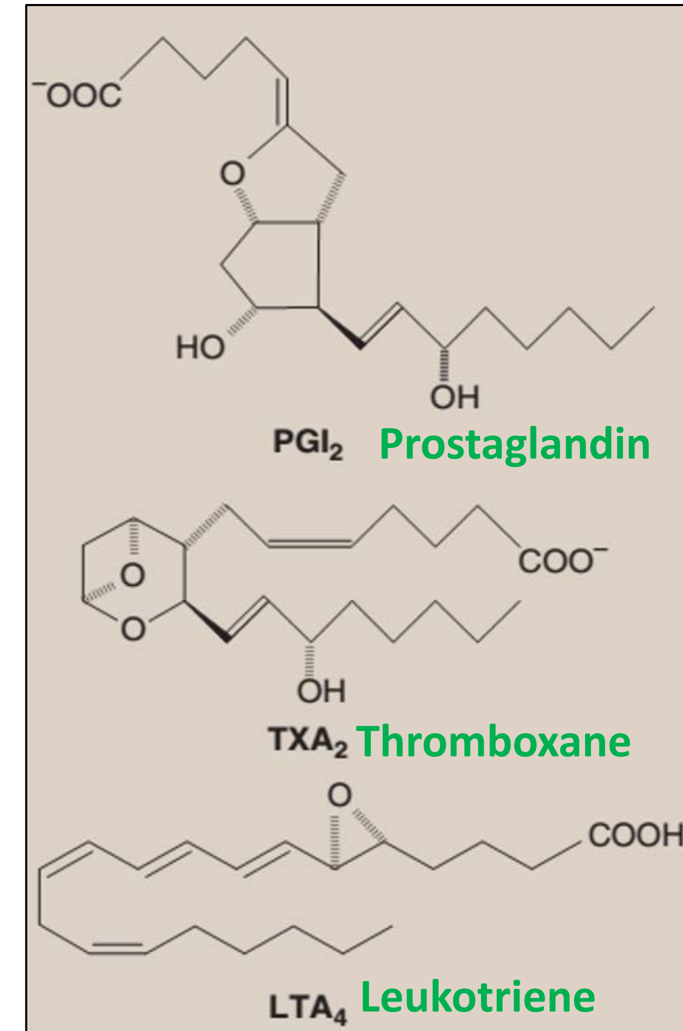
EICOSANOIDS

Prostaglandins: elevated temperature (fever), inflammation, pain, etc.

Thromboxanes are produced by platelets and act in the formation of thrombi. Nonsteroidal anti-inflammatory drugs (NSAIDS), aspirin and ibuprofen inhibit the synthesis of prostaglandins and thromboxane (cyclooxygenase inhibition).

Leukotrienes are powerful signaling molecules. They contract the smooth muscle that lines the lung. Excess produces asthma attacks. Prednisone inhibits the synthesis of leukotrienes.

CYCLIC FORMS OF ARACHIDONIC FATTY ACID



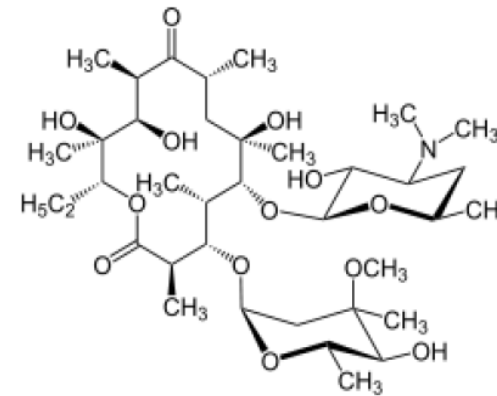
POLYPEPTIDES: SIMPLE LIPIDS WITH MEDICAL POTENTIAL

These are secondary metabolites of bacteria, fungi, plants and invertebrates.
Macrocyclic lactones (14-40 atoms) with ring system: acetyl CoA and propionyl CoA.

FUNCIONES:

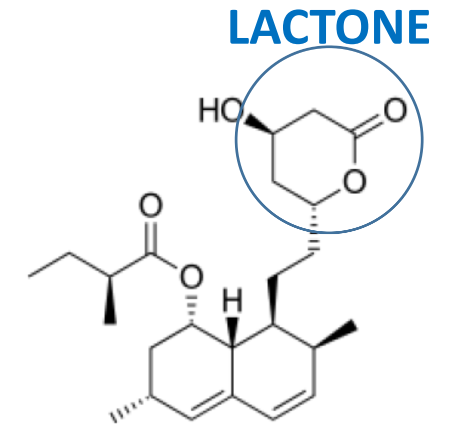
1) Antimocrobial, antiparasitic and antitumor.
2) Antibiotics: tetracyclines,
3) Anticholesterolemics: statins
4) Antitumors: avermectins

Some polypeptides are potent toxins: aflatoxins

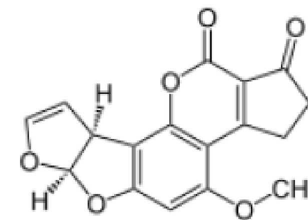


ERYTHROMYCIN A

LACTONE

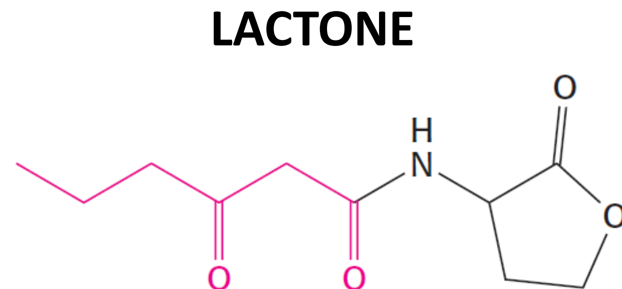


LOVASTATIN



AFLOTAXIN

LACTONE



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LESSON 24. LIPID METABOLISM (II): lipid origin, β fatty acid oxidation and ketone bodies metabolism

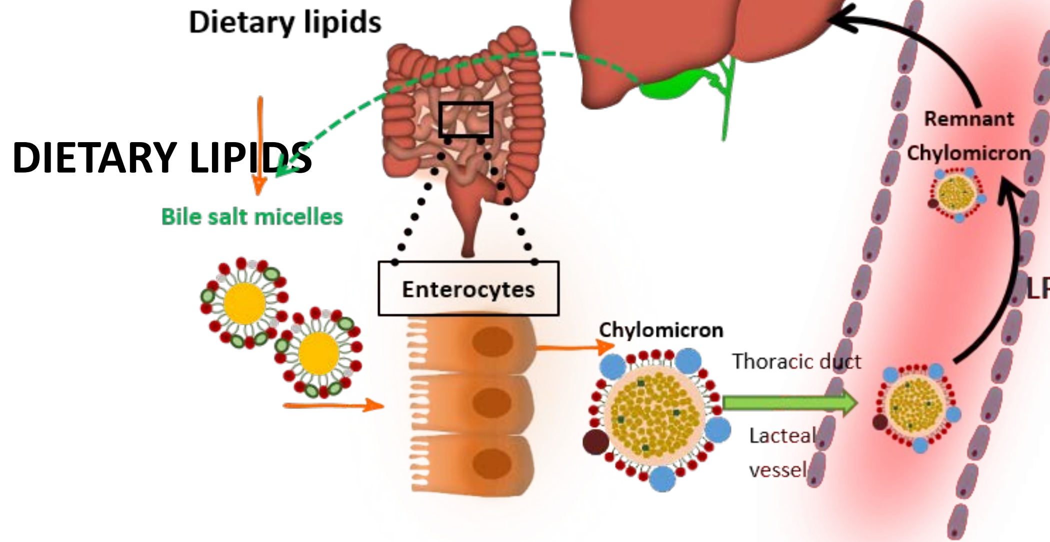
Herminia González Navarro

INDEX

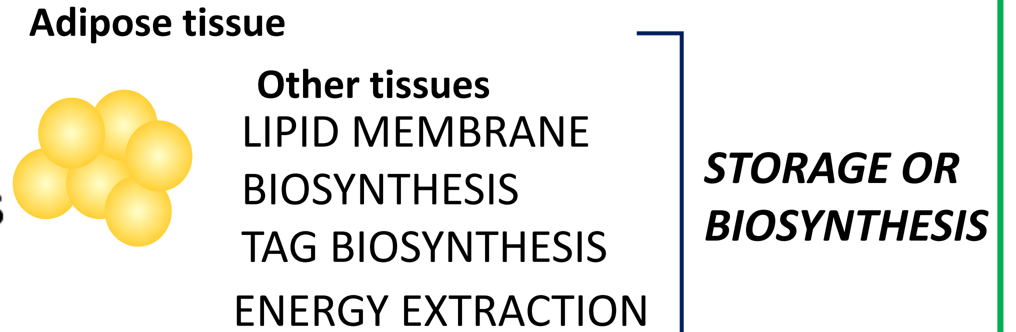
1. Dietary lipid metabolism
    - 1.1. Degradation and catabolism in the digestive system.
    - 1.2. Transport and destinations of lipids from the diet.
2. Endogenous lipid metabolism: fat mobilization
    - 2.1. Lipid mobilization of adipose tissue.
    - 2.2. The oxidation of fatty acids.
3. Catabolism of complex lipids and metabolism of eicosanoids.
4. Metabolism of ketone bodies: synthesis and uses.

METABOLISM OF DIETARY LIPIDS: GENERAL SCHEME

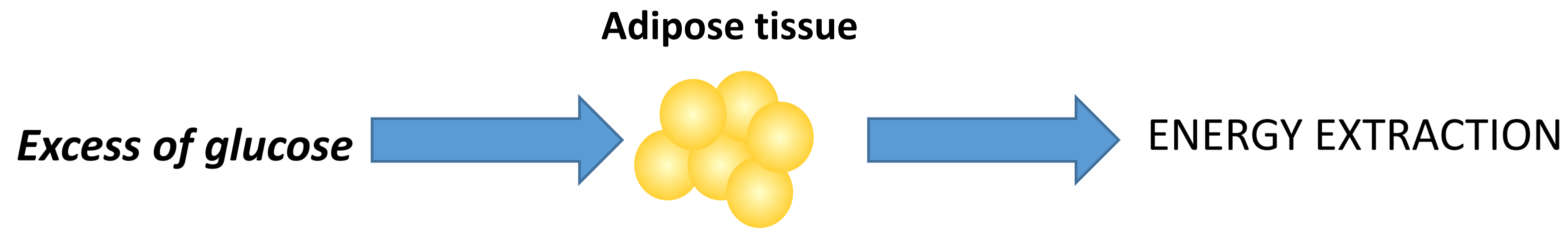
ORIGIN OF LIPIDS



DESTINATION OF LIPIDS



STORAGE OR BIOSYNTHESIS



DIETARY LIPID METABOLISM: DIGESTION

DIETARY LIPIDS:
- Over 90% of dietary lipids are triglycerides or triacylglycerols (TAG): three fatty acids bound to a glycerol molecule.
- Cholesterol, cholesterol esters, phospholipids and free fatty acids (non-esterified).

DIGESTION

STOMACH: digestion is limited by lingual and gastric lipases. Hydrolysis of TG with short-chain AG (<12 carbons).

SMALL INTESTINE (DUODENUM):
Emulsification: increase in lipid surface area by stabilization of lipid droplets for efficient catalysis.

Degradation with pancreatic enzymes: release of enzymes controlled by the peptide hormone cholecystokinin.

TAG: pancreatic lipase and colipase up to monoacylglycerol + 2 fatty acids.

Cholesterol: 10-15% of cholesterol is esterified and therefore it is hydrolyzed by cholesteryl ester hydrolase/cholesterol esterase. Requires bile salts to be active.

Phospholipids: Phospholipase A2 and requires bile salts. It generates a bisphospholipid that can degrade further.

AFTER DIGESTION: FATTY ACIDS, CHOLESTEROL AND BISPHOSPHOLIPIDS

DIETARY LIPID METABOLISM: ABSORPTION INTO ENTEROCYTES

SMALL INTESTINE (JEJUNUM): absorption of lipids and fat-soluble vitamins.
Formation of mixed micelles for absorption by the apical membrane of enterocytes.

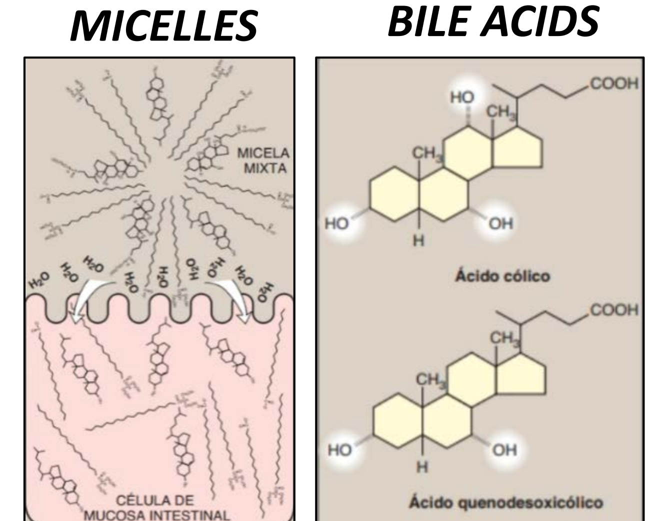
MICELLES IN THE JEJUNUM LUMEN

Free fatty acids, cholesterol and monoacylglycerols	+	Bile acids	+	Liposoluble vitamins (A, D, E, K)

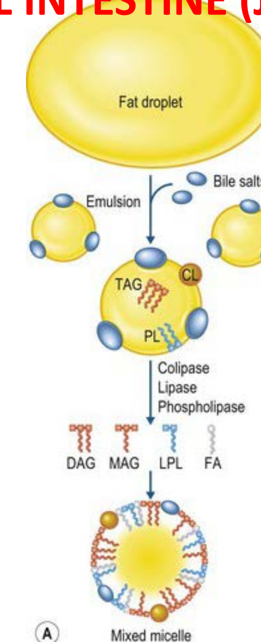
MICELLES cross the APICAL membrane of enterocytes.

Short and medium chain FA are not re-esterified. They are released into the portal circulation, where they are transported by serum albumin to the liver.

MICELLES ***BILE ACIDS***



SMALL INTESTINE (JEJUNUM):



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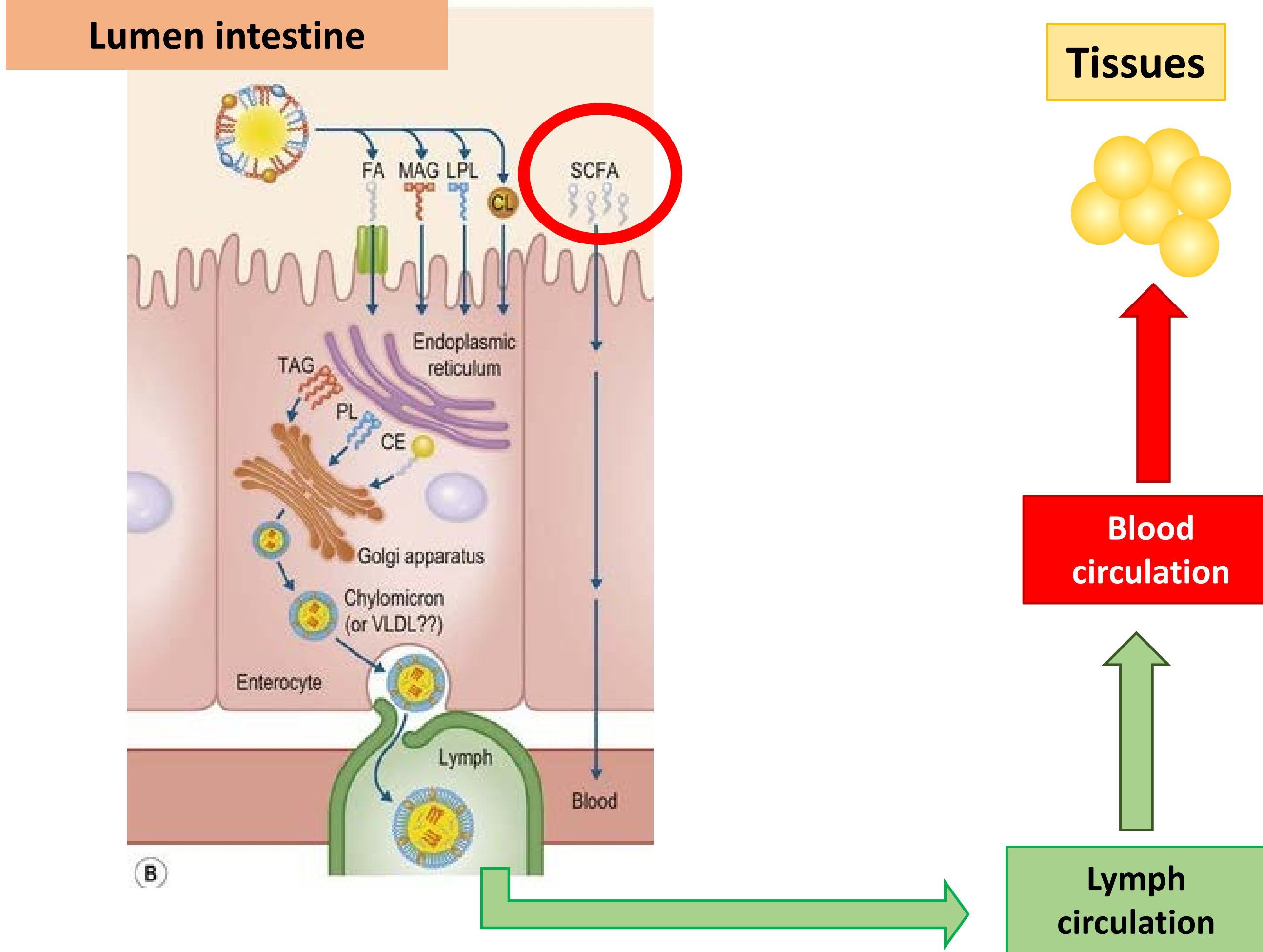
THE FORMATION OF CHYLOMICRONS IN ENTEROCYTES

ENTEROCYTES: Long-chain FAs are esterified to re-form TAGs, phospholipids, and cholesterol esters. **They are packaged in the ER and form CHYLOMICRONS.**



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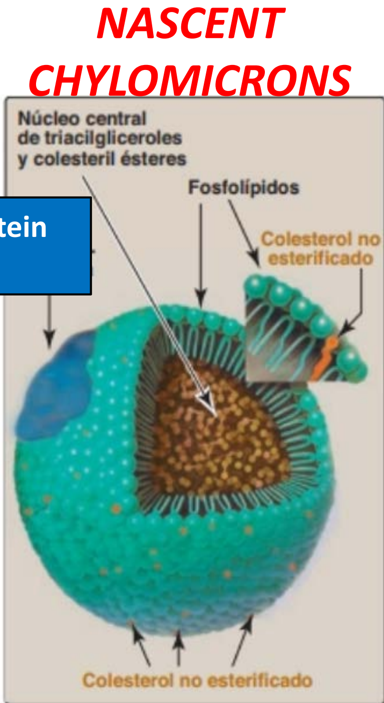
Tissues

Blood circulation

Lymph circulation

NASCENT CHYLOMICRONS

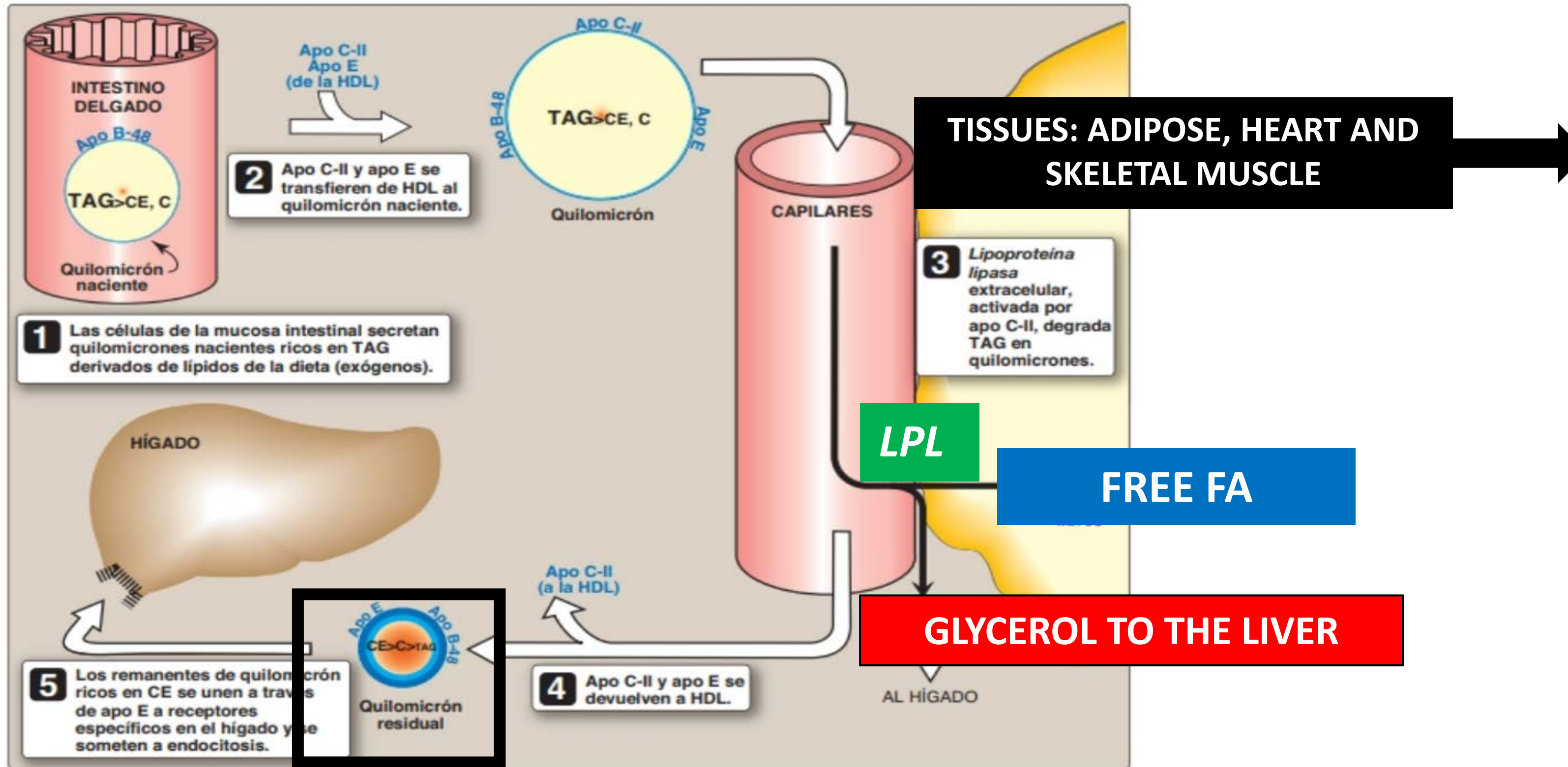
Apolipoprotein B48



NASCENT CHYLOMICRON: lipoprotein, which carries TAG (ALWAYS FROM A DIET), cholesterol and its esters, phospholipids and fat-soluble vitamins. Among other components, they contain apoB48 apolipoprotein.

TRANSPORT OF LIPIDS BY CHYLOMICRONS THROUGH THE CIRCULATION

Mature chylomicron: apoE and apoCII



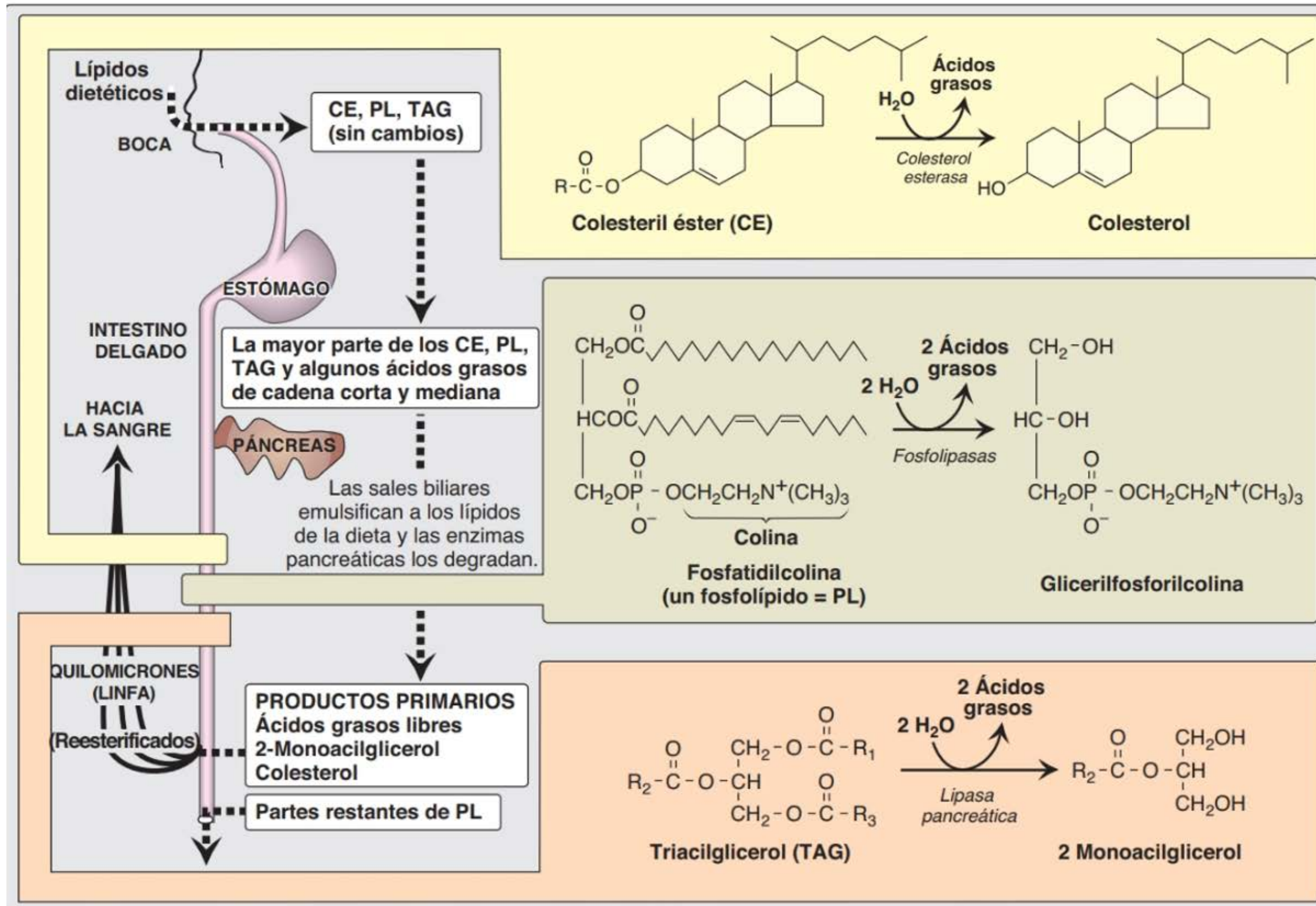
LIPOPROTEIN LIPASE: adipose tissue and cardiac and skeletal muscle. Hydrolyzes TAG to produce AG and GLYCEROL.

FATTY ACID DESTINATION:
BIOSYNTHESIS, OF
TAG and MEMBRANE LIPIDS

GLYCEROL: the carbonated skeleton is used in the liver for glycolysis or gluconeogenesis.

CHYLOMICRON REMNANTS ARE CAPTURED BY THE LIVER AND METABOLIZED

SUMMARY OF DIETARY LIPIDS



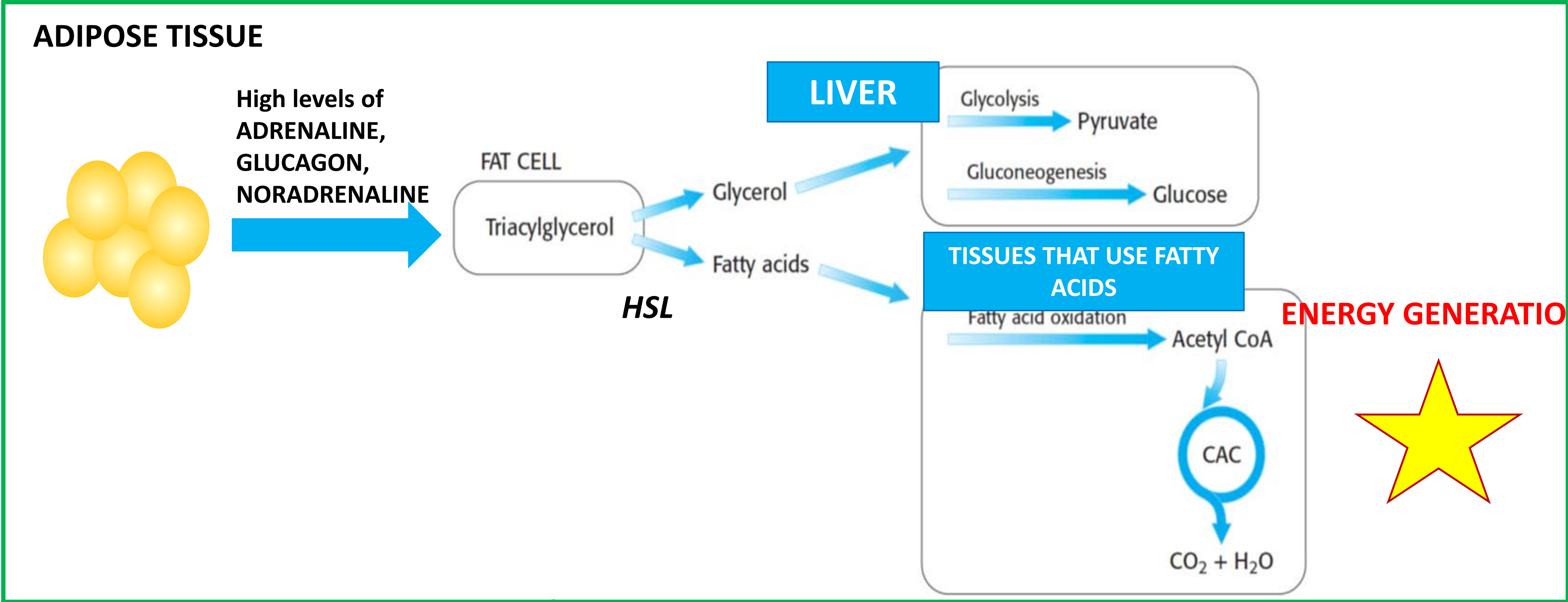
DIGESTION: small intestine

DUODENUM

ABSORPTION: small intestine JEJUNUM

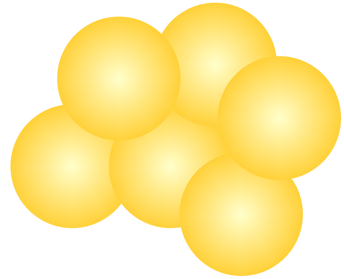
ENDOGENOUS LIPID METABOLISM: LIPID MOBILIZATION

LIPOLYSIS

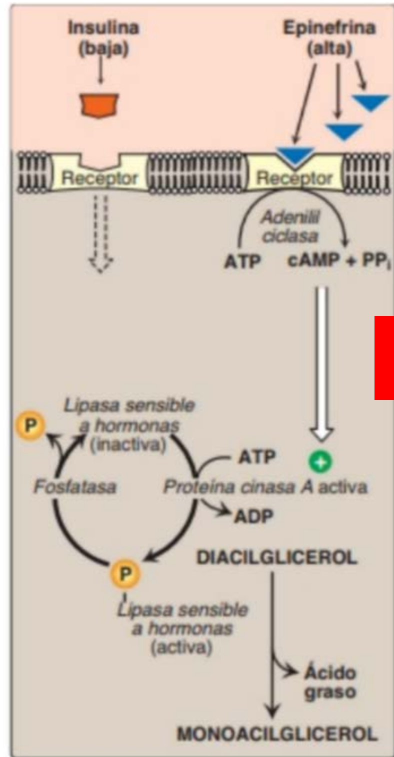


THE HORMONAL REGULATION OF LIPOLYSIS

ADIPOSE TISSUE

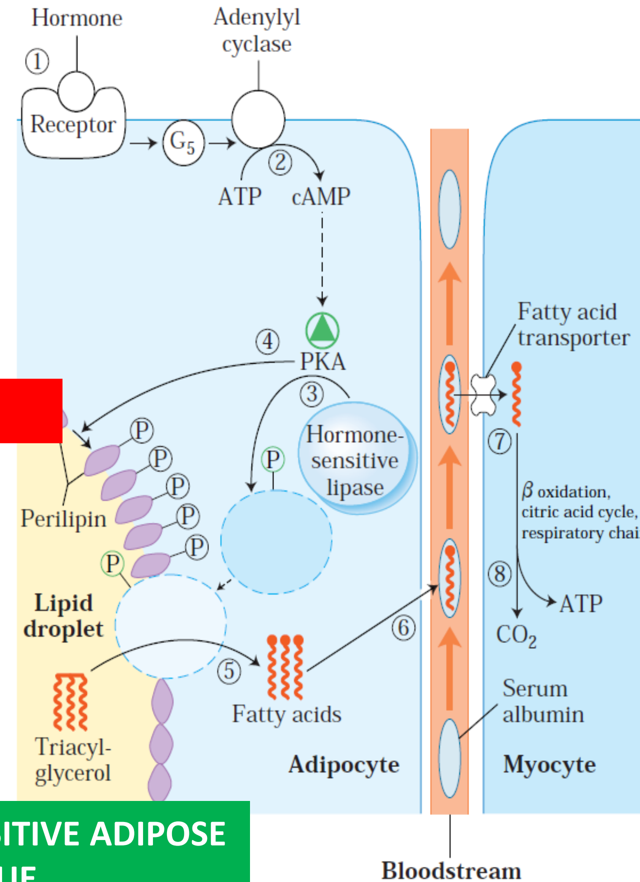


GPCR → G PROTEIN → AC → cAMP → PKA → HSL / PERLIPIN



HSL → FREE FATTY ACIDS / GLYCEROL

PERLIPIN



HORMONE-SENSITIVE ADIPOSE TISSUE

PERIPHERAL TISSUE CAPABLE OF PERFORMING β-OXIDATION

PERLIPIN is an adipocyte lipid droplet surface protein that protects from lipolysis by HSL. After the action of PKA by signaling glucagon or β-adrenergic receptors (ADRENALINE), it is phosphorylated, changes conformation, and allows the action of HSL.

DESTINATION OF MOLECULES FROM LIPOLYSIS

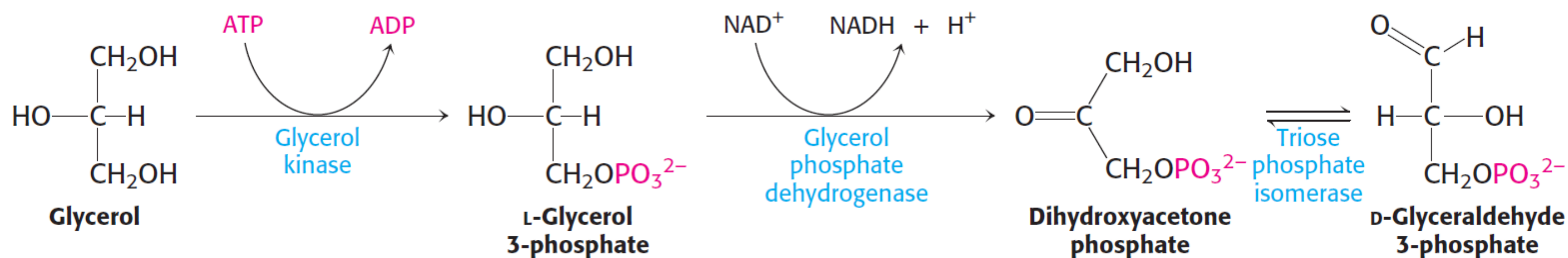
ADIPOSE TISSUE

FREE FATTY ACIDS

GLYCEROL

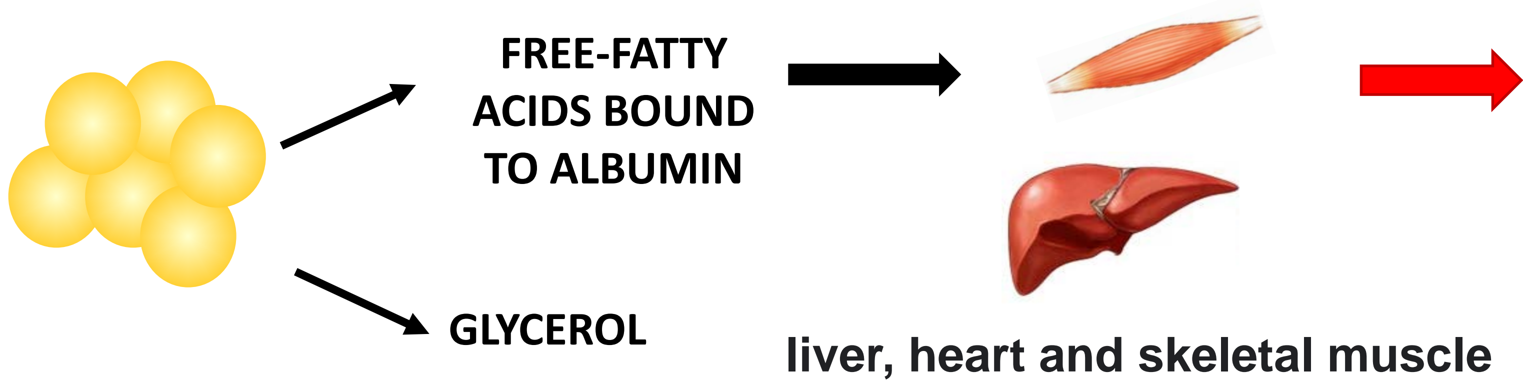
GLYCEROL → → GLUCONEOGENESIS OR GLYCOLYSIS

FATE OF GLYCEROL: Glycerol is incorporated into glycolysis or gluconeogenesis after conversion to **dihydroxyacetone Pi (DHAPi). TRIOSE STEP OF THE GLYCOLYSIS.**

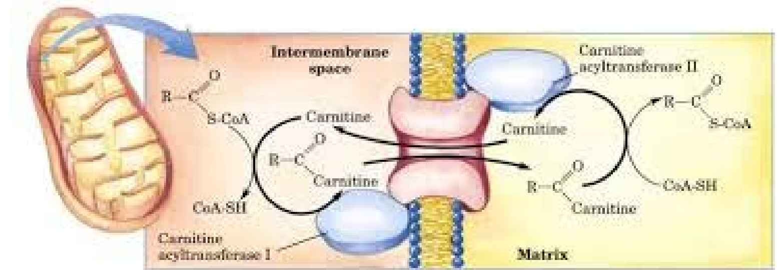


GLUCONEOGENESIS OR GLYCOLYSIS

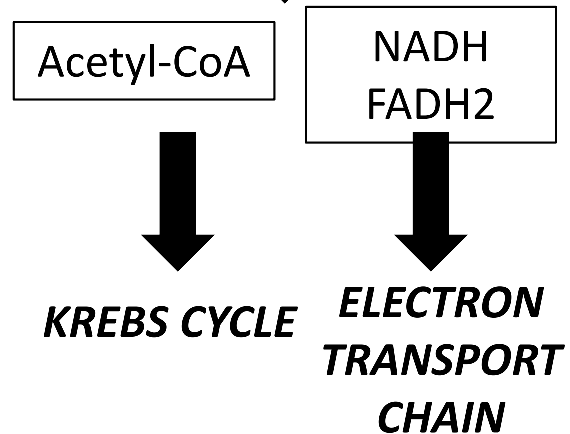
FATE OF LIPOLYSIS MOLECULES: β FATTY ACID OXIDATION (FAO)

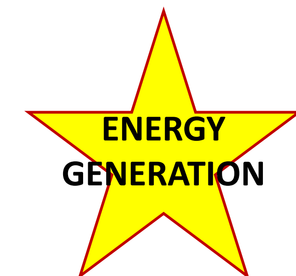


Entry into cells and mitochondria for β oxidation



β-OXIDATION





DESTINATION OF FREE FATTY ACIDS (FFA):

Free fatty acids move across the cell membrane of the adipocyte, bind to serum albumin, and are transported to tissues that can metabolize them.

β fatty acid oxidation: liver, kidney, heart and muscle

β oxidation does not occur in erythrocytes or in the brain.

THE β OXIDATION OF FATTY ACIDS TAKES PLACE IN THE MITOCHONDRIA

The β oxidation of fatty acids is a **spiral metabolic pathway** that **catabolizes/oxidizes fatty acids** in different tissues for energy.
It takes place in the **mitochondria.**

Processes that regulate the β-oxidation:

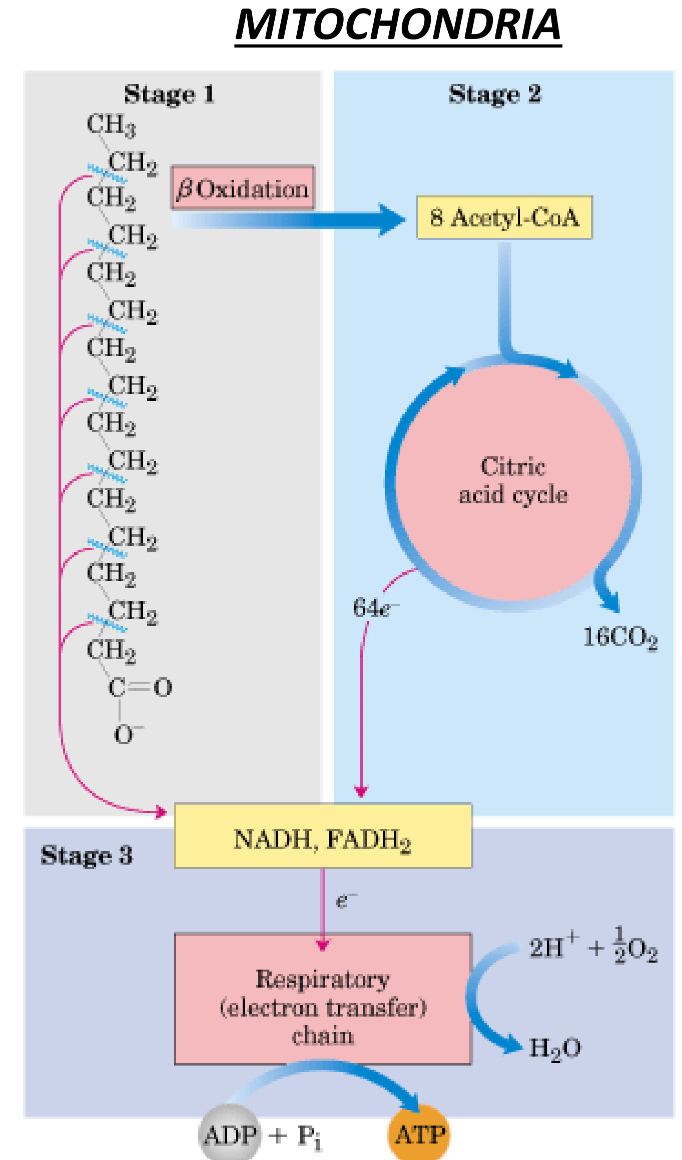
- The β-oxidation depends on the availability of fatty acids.
- Efficient transport of fatty acids into mitochondria is carried out by the CARNITINE shuttle.

- Energy state: Acetyl-CoA/CoA and NADH/NAD+ ratio.

- In general, the synthesis of fatty acids inhibits oxidation, and vice versa.

Carnitine acyltransferase deficiency: *symptoms of muscle weakness during prolonged exercise.*

MITOCHONDRIA



β FATTY ACID OXIDATION: ENTRY INTO CELLS AND MITOCHONDRIA IN THREE STEPS

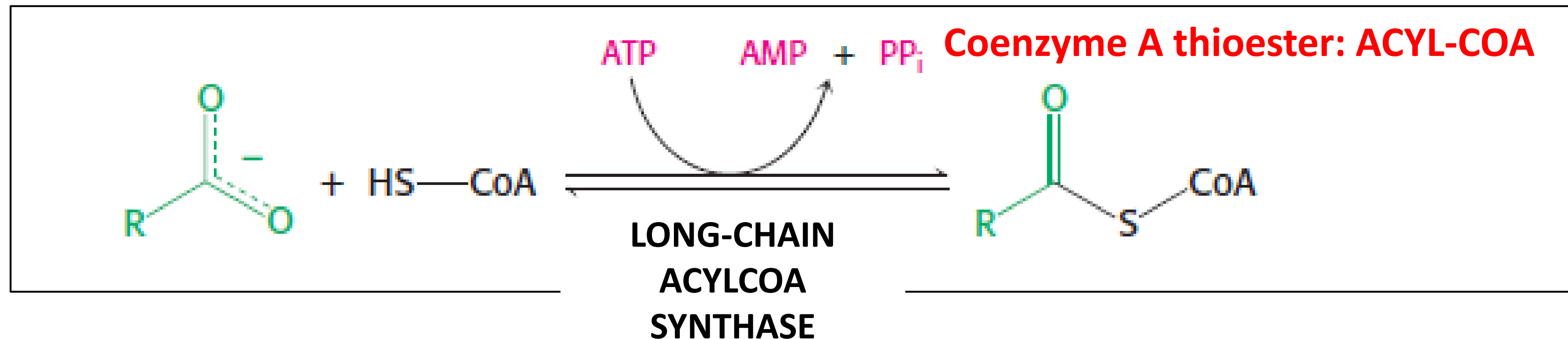
The steps are:

1. Internalization of fatty acids by transporters: **FATP, translocase CD36/FAT, FABP** of the cell membrane.

2. Activation of long-chain fatty acids >12 Carbons: **coenzyme A** binding before entering the mitochondria.

3. Transport into the mitochondria matrix by the **CARNITINE SHUTTLE.**

COST: 2 ATP



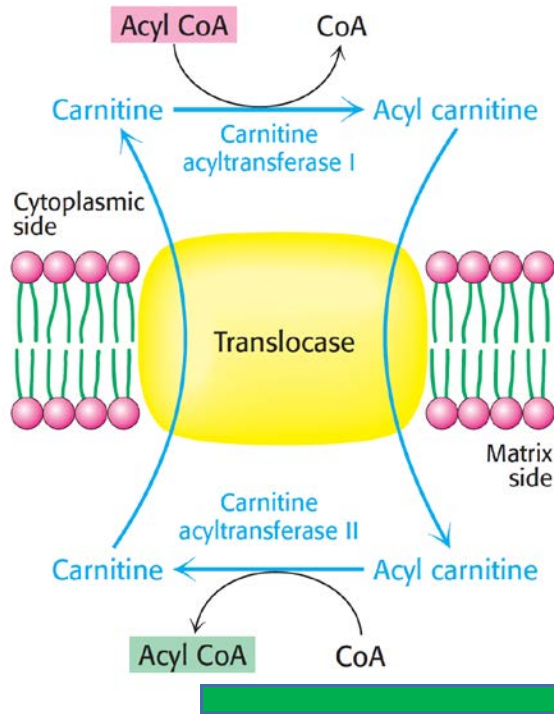
β FATTY-ACID OXIDATION: TRANSPORT TO THE MITOCHONDRIA

3. Transport into the mitochondria matrix by the **CARNITINE SHUTTLE.**

OUTSIDE THE MATRIX: ACYLCoA is added to the carnitine by **CARNITINE ACYL TRANSFERASE I** **(CoA is lost and Carnitine is bound).**

Caritine-Acyl fatty acid crosses the membrane.

INSIDE THE MATRIX: AcylCarnitine is transformed **back into Acyl-CoA** by **CARNITINE ACYL TRANSFERASE II.** The mitochondrial matrix enters **the β oxidation**.



Short-chain AAs (<12 C) cross the inner membrane of the mitochondria **without** needing to convert to acyl-CoA or requiring the help of **carnitine translocase**.

They are **activated with CoA in the mitochondrial matrix** to enter the β-oxidation**.**

β FATTY-ACID OXIDATION

THE β OXIDATION OF FATTY ACIDS IS THE MAIN WAY TO OBTAIN ENERGY WHEN GLUCOSE IS SCARCE

The β oxidation of fatty acids is the main route for the catabolism of fatty acids, It is also the main way to obtain energy in situations of high energy needs (intense sport) or in the absence of glucose as an energy source. Oxidation occurs in the mitochondria.

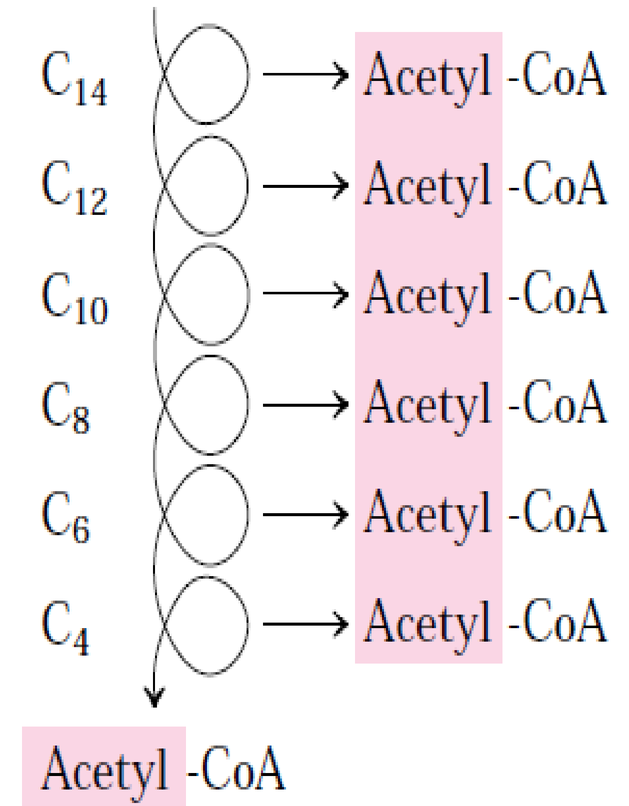
FOR EACH β OXIDATION CYCLE AND THE RELEASE OF ACETYL-CoA:

- There are **4 reactions:** oxidation by FAD, hydration, oxidation by NAD, and thiolysis by Coenzyme A.

- The removal of 2 carbons from the acyl-CoA fat from the carboxyl end produces:

a) Acetyl-CoA (Krebs cycle), NADH and FADH2 (electron transport chain).

b) Shortening by 2 CARBONS of the Acyl-CoA: a fat Acyl-CoA with carbon number of n-2 that re-enters the cycle of the β oxidation of the 4 reactions (where n is the number of carbons of the initial fat Acyl-CoA).



β FATTY-ACID OXIDATION: CHEMICAL REACTIONS

1. Acyl-CoA dehydrogenase (chain-length specific):

DEHYDROGENATION FAD →**FADH2**

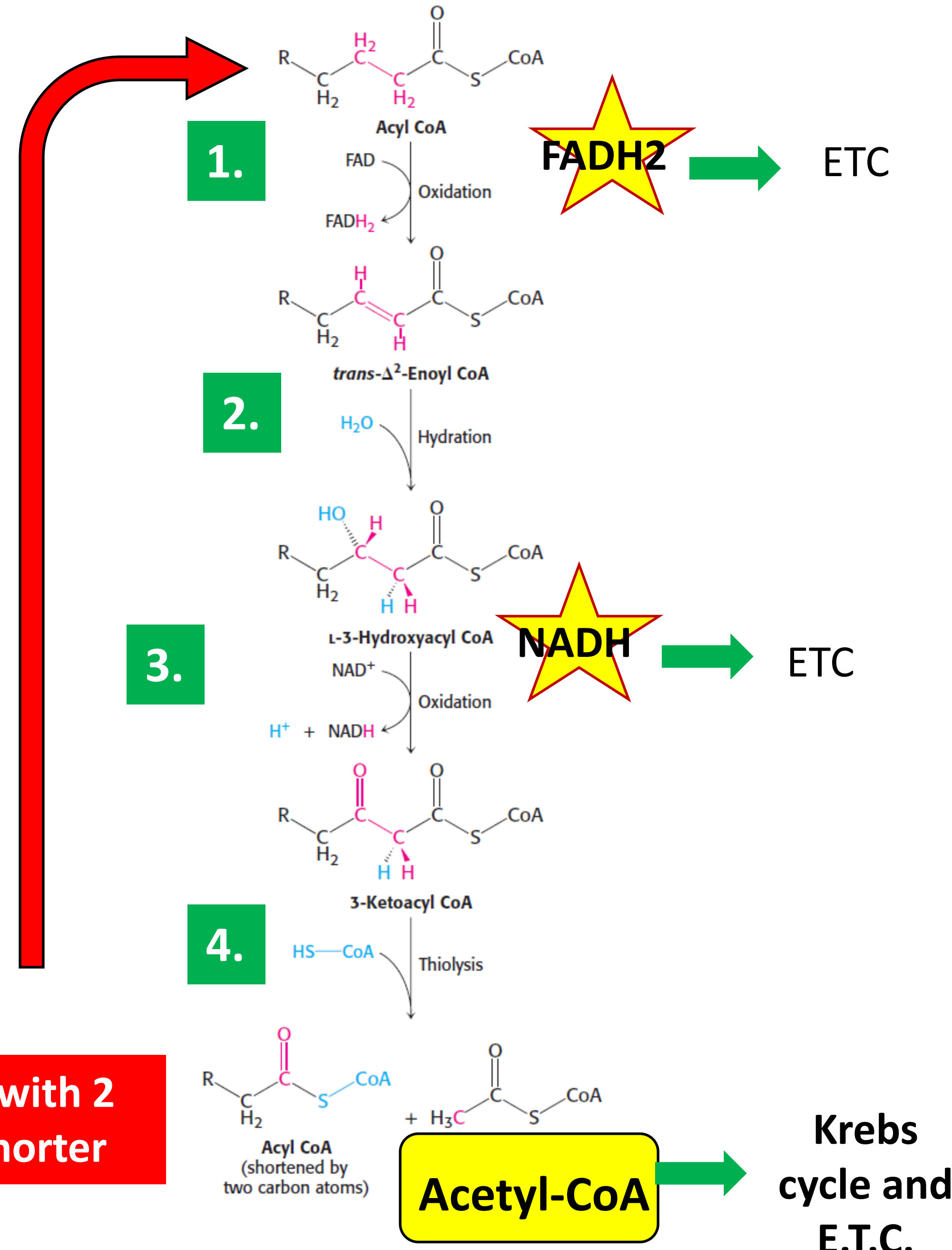
2. 2,3-Enoyl-CoA hydratase: **HYDRATION** (H2O).

3. 3-Hydroxyacyl CoA dehydrogenase: **DEHYDROGENATION.**

NAD+ →NADH

4. 3-Ketoacyl CoA Tyolase: **EXCISION** (HS-CoA): AcCoA

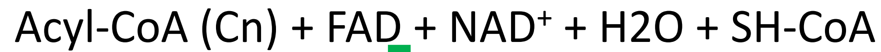
REPETITION OF THE CYCLE UNTIL THE ACYLCoA is 4 carbons. In **the last cycle, 2xAcetyl-CoA** are generated



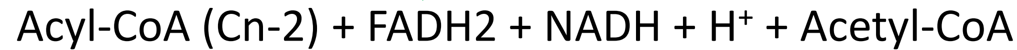
ACYL-CoA with 2 carbons shorter

β FATTY-ACID OXIDATION: STOCHIOMETRY AND PERFORMANCE

STOCHIOMETRY OF A β OXIDATION CYCLE OF AN ACYL-CoA:

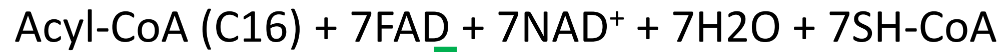


Acyl-CoA (Cn) + FAD + NAD^+ + H2O + SH-CoA

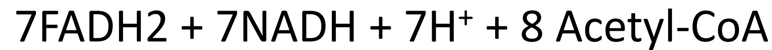


Acyl-CoA (Cn-2) + FADH2 + NADH + H^+ + Acetyl-CoA

TOTAL STOCHIOMETRY OF THE β OXIDATION OF LONG-CHAIN SATURATED FATTY ACID Palmitoyl-CoA (C16):



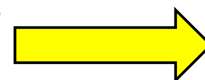
Acyl-CoA (C16) + 7FAD + $7NAD^+$ + 7H2O + 7SH-CoA



7FADH2 + 7NADH + $7H^+$ + 8 Acetyl-CoA

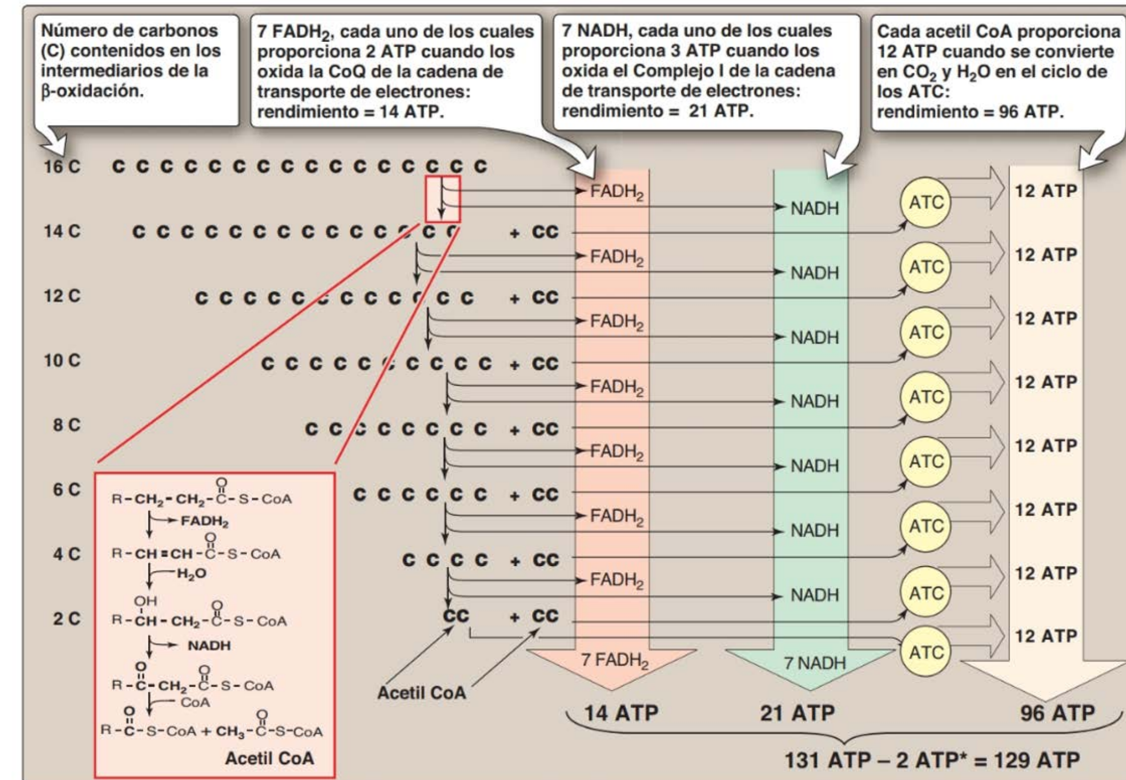
ENERGY PERFORMANCE IN ATP OF THE β OXIDATION OF Palmitil-CoA (C16):

1 FADH2= 2 ATP → 7X2=14 ATP
1 NADH= 3 ATP → 7X3=21 ATP
1 Acetyl-CoA= 12 ATP → 12x8= 96 ATP



131-2 ATP (PalmitoylCoA)= 129 ATPs

PALMITIC ACID C16



ENERGY PERFORMANCE IN ATP FOR THE FATTY ACID OF N CARBONS

ENERGY PERFORMANCE IN ATP OF THE β OXIDATION OF Palmitoyl-CoA (C16):

1 $FADH_2$= 2 ATP → 7X2=14 ATP
1 NADH= 3 ATP → 7X3=21 ATP
1 Acetyl-CoA= 12 ATP → 12x8= 96 ATP

131-2 ATP (Palmitoyl-CoA)= 129 ATPs

Number of NADH and $FADH_2$

(n/2)-1

Number of Acetyl-CoA

n/2

1 Acetyl-CoA= 12 ATP → 12XN/2
1 $FADH_2$= 2 ATP → 2X(N/2-1)
1 NADH= 3 ATP → 3X(N/2-1)

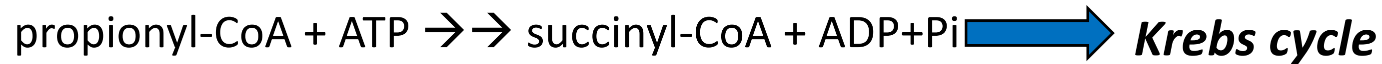
ENERGY PERFORMANCE: as it is saturated and misses one $FADH_2$ production step, the whole process has -2 ATP

THE β OXIDATION OF ODD-NUMBERED FATTY ACIDS: ADDITIONAL CHEMICAL REACTIONS

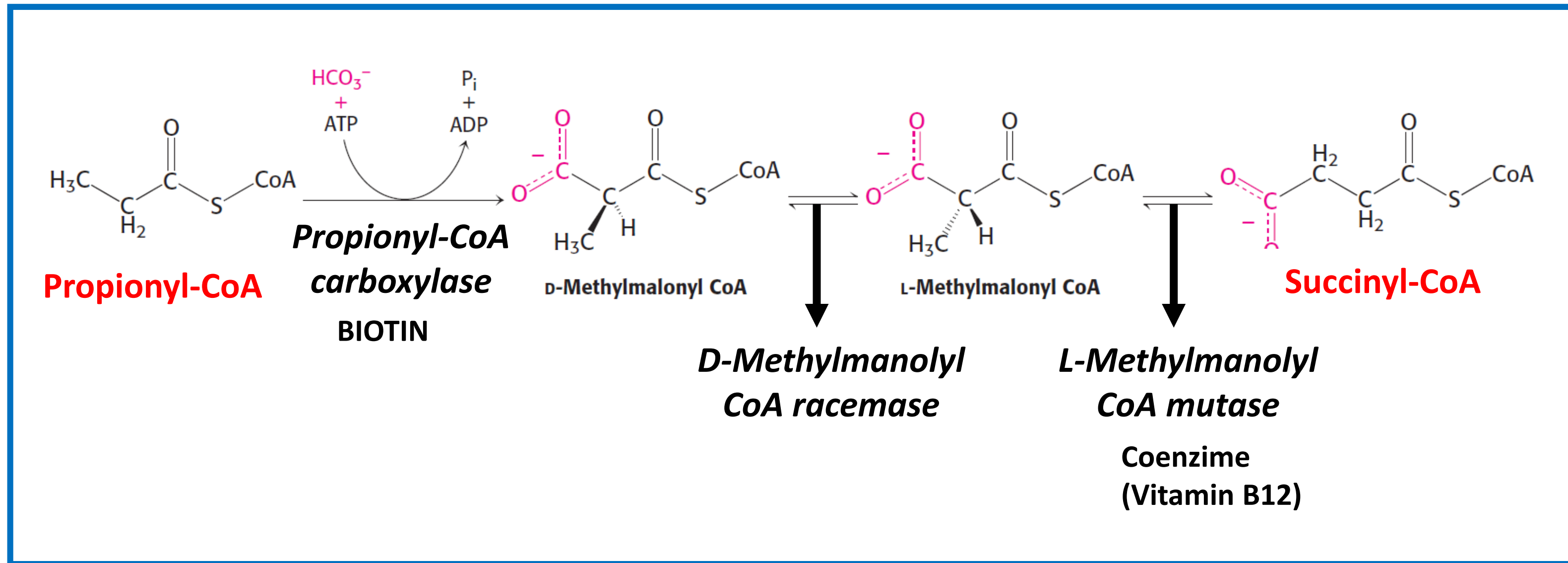
- 4 Reactions per β oxidation cycle.
- LAST CYCLE: generates of propionyl-CoA, **WHICH ENTERS THE KREBS CYCLE.**
- Metabolic reactions of the transformation of:

COST: 1 ATP

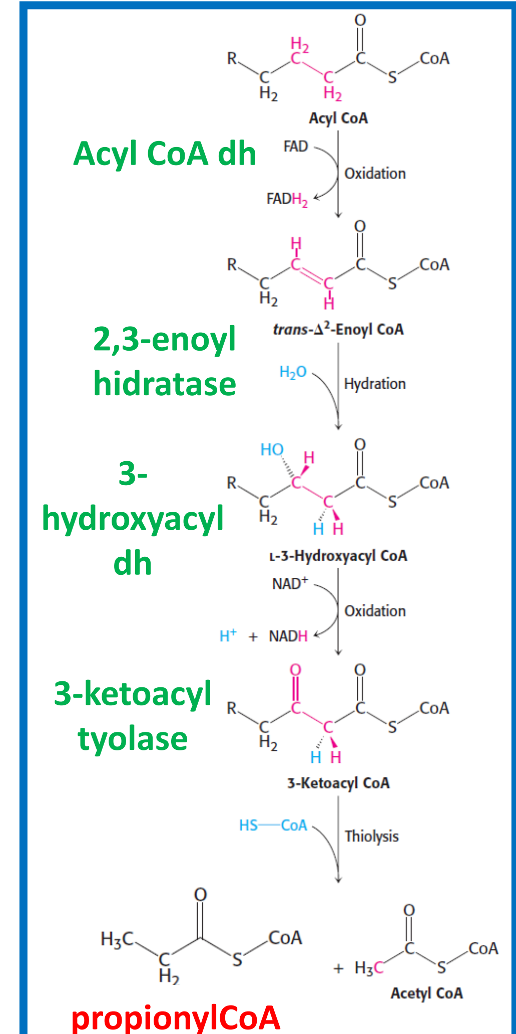
propionyl-CoA + ATP →→ succinyl-CoA + ADP+Pi



Krebs cycle



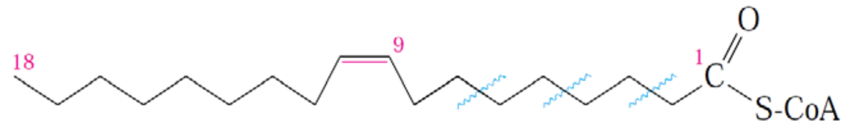
Last oxidation cycle



ENERGY PERFORMANCE is much lower when succinyl-CoA enters later into the Krebs cycle (- 2NADH and 1 ATP less)

THE β OXIDATION OF UNSATURATED FATTY ACIDS: ADDITIONAL CHEMICAL REACTIONS

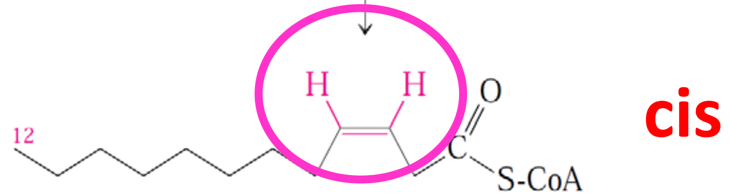
-**Metabolic reactions of double-bond transformation:** ISOMERIZATION OR REDUCTION OF THE DOUBLE BOND



Oleoyl-CoA

β oxidation (three cycles)

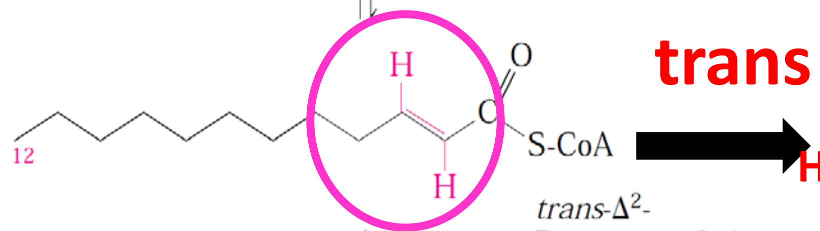
3 Acetyl-CoA



cis

cis-Δ^3-Dodecenoyl-CoA

3,2-enoyl isomerase



trans

trans-Δ^2-Dodecenoyl-CoA

Hydratase substrate of the β oxidation

β oxidation (five cycles)

6 Acetyl-CoA

18:1(9) OLEIC ACID, fatty acid with one unsaturated bond in the odd position.

- ***Enoyl-CoA isomerase*** changes position on the double bond.
- The resulting Acyl-CoA is hydrate substrate and can follow the β oxidation.
- **Performance is lower** as the first step does not generate FADH2.

The missing of one FADH2 = **2 ATPs** less per double bond.

ENERGY PERFORMANCE: as saturated but minus 2 ATP due to the reduction in 1 FADH2 per double bond.

THE β OXIDATION OF VERY LONG CHAIN FATTY ACID OXIDATION > 22 C IN PEROXISOMES

β-oxidation in peroxisomes:

- β-oxidation to shorten the fatty acids of chains of 22 or more carbons.
- When shorter, Acyl-CoA enters the mitochondria.

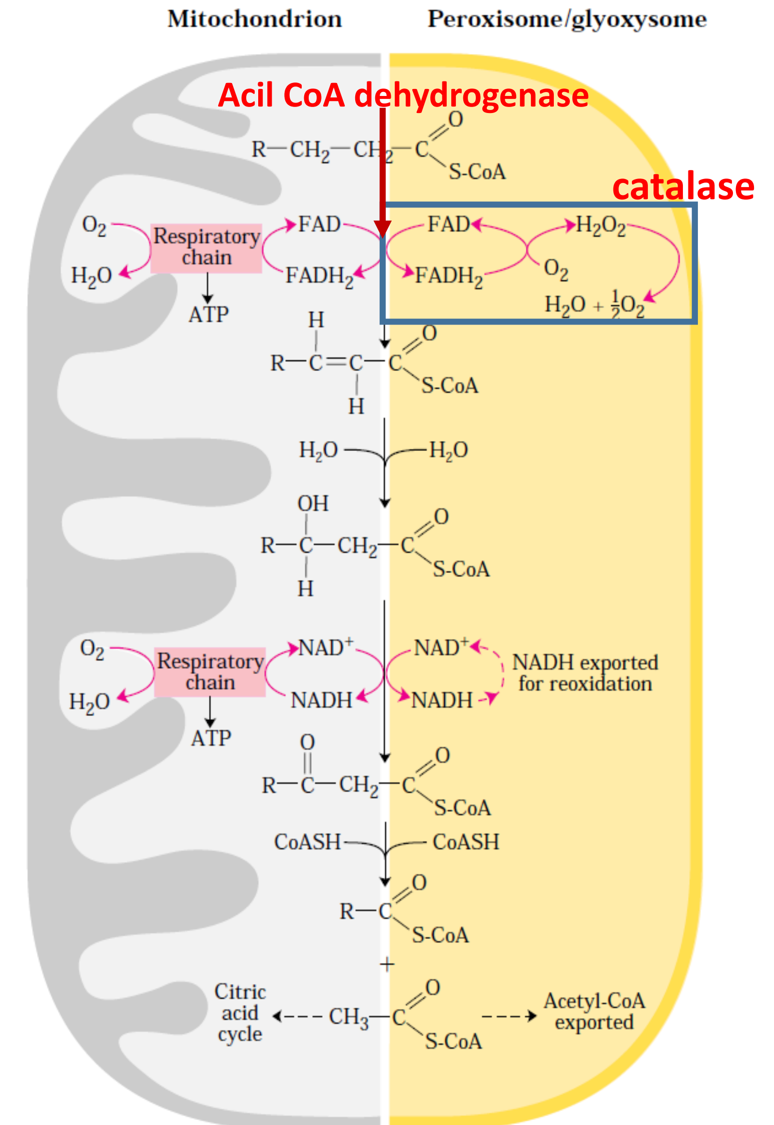
SPECIFIC **Acyl-CoA dehydrogenase** of 22 or more carbons is **specific to PEROXISOMES**.

DIFFERENCES WITH MITOCHONDRIA:

- The FADH2 of dehydrogenation is oxidized by O2, which generates H2O2 that is reduced to H2O by a catalase.
- The generated NADH, acetylCoA and shortened AcylCoA are transported to the mitochondria.

The yield is 2 ATPs less.

In PEROXISOMES also takes place the α-oxidation, and the ω-oxidation for branched fatty acids. The ω-oxidation begins with ω C



METABOLISM OF KETONE BODIES

ADIPOSE TISSUE

High levels of ADRENALINE, GLUCAGON, NORADRENALINE

HIGH ENERGY DEMAND AND LOW GLUCOSE LEVELS

FAT CELL

Triacylglycerol

HSL

Glycerol

Fatty acids

LIVER

Glycolysis → Pyruvate

Gluconeogenesis → Glucose

TISSUES THAT USE FATTY ACIDS

Fatty acid oxidation → Acetyl CoA

ENERGY GENERATION

OAA

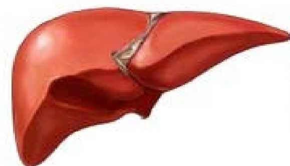
CAC

ENERGY GENERATION

$CO_2 + H_2O$

WHAT DO THE OTHER TISSUES CONSUME?

AcCoA accumulation



FORMATION OF KETONE BODIES:
Acetoacetate, 3-hydroxybutyrate and acetone (which has no metabolic purpose).

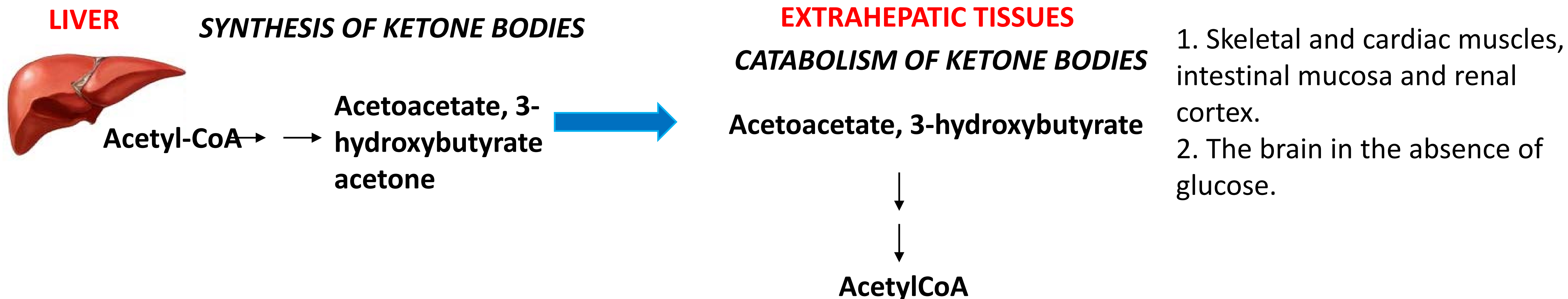
METABOLISM OF KETONE BODIES: SYNTHESIS AND USE

- Ketone bodies are activated when **glucose levels are low** (intense exercise, prolonged fasting or illness).

- Ketone bodies are produced in the ***MITOCHONDRIA OF THE LIVER*** from the accumulated Acetyl-CoA generated b the oxidation of fatty acids.

-Acetyl CoA produces two ketone bodies: **acetoacetate, 3-hydroxybutyrate and acetone**.

- Ketone bodies are water-soluble and are **transported to TISSUES that consume Ketone bodies.**



METABOLISM OF KETONE BODIES: KETOGENESIS CHEMICAL REACTIONS

1. Condensation 2 Acetyl-CoA **TYOLASE**: ACETOACETYL-CoA

2. Synthesis of 3-hydroxy-3-methylglutaryl-CoA:
The **3-hydroxy-3-methylglutaryl (HMG) CoA Synthase** combines a third molecule of acetyl-CoA with acetoacetyl-CoA to produce HMG CoA.

3. **HMG CoA lyase** breaks down HMG CoA to produce acetoacetate and acetyl-CoA.

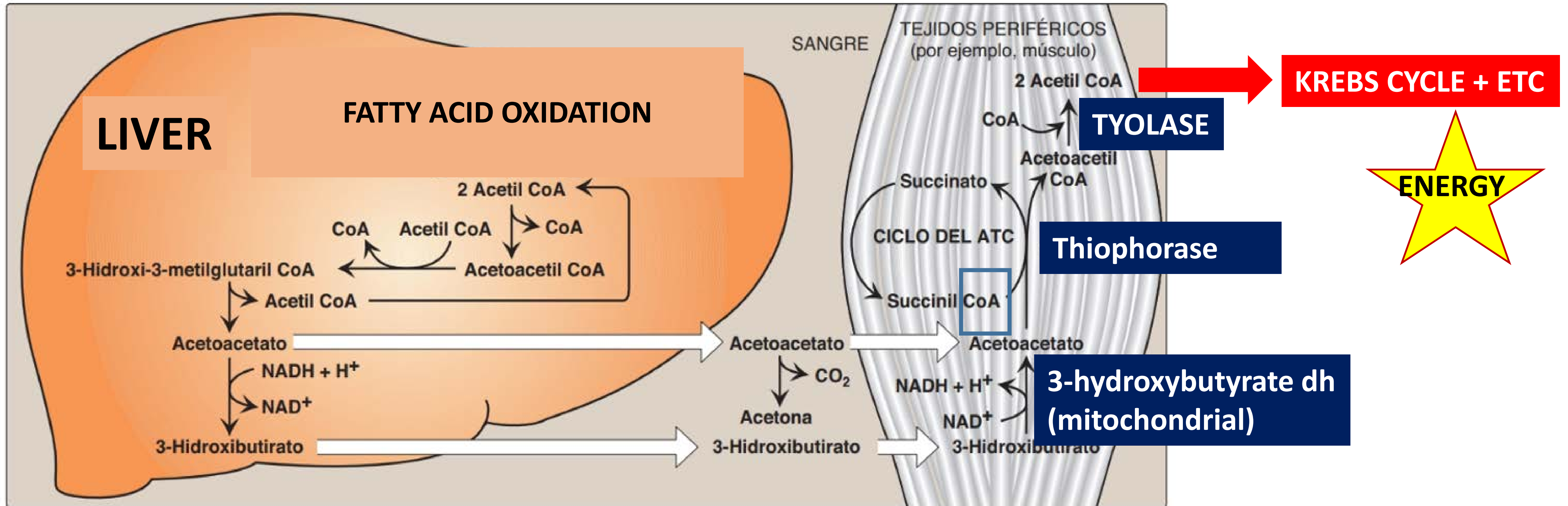
4. FROM ACETOACETATE:
Spontaneous decarboxylation : ACETONE + CO2

3-Hydroxybutyrate DH NADH-dependent dehydrogenation:
3-HYDROXYBUTYRATE + NAD+

The equilibrium between acetoacetate and 3-hydroxybutyrate is determined by the NAD+/NADH ratio.

2 Acetyl-CoA
TYOLASE
CoA-SH
Acetoacetyl-CoA
Limiting step
HMGCoA SYNTHASE
HMG-CoA synthase
Acetyl-CoA + H_2O
CoA-SH
β-Hydroxy-β-methylglutaryl-CoA (HMG-CoA)
HMGCoA LYASE
HMG-CoA lyase
Acetyl-CoA
Acetoacetate
SPONTANEOUS REACTION
acetoacetate decarboxylase
CO_2
Acetone
REVERSIBLE REACTION
NADH + H^+
NAD$^+$
D-β-hydroxybutyrate dehydrogenase
D-β-Hydroxybutyrate

METABOLISM OF KETONE BODIES: CHEMICAL REACTIONS; KETOLYSIS



<u>USE OF KETONE BODIES</u>: by tissues with mitochondria such as skeletal muscle, kidneys, heart and, in cases of prolonged fasting, also the brain.

<u>NON-USE OF KETONE BODIES</u>: liver due to lack of **β-ketoacetylCoA transferase** and erythrocytes due to lack of mitochondria.

β-ketoacetylCoA transferase: tiophorase

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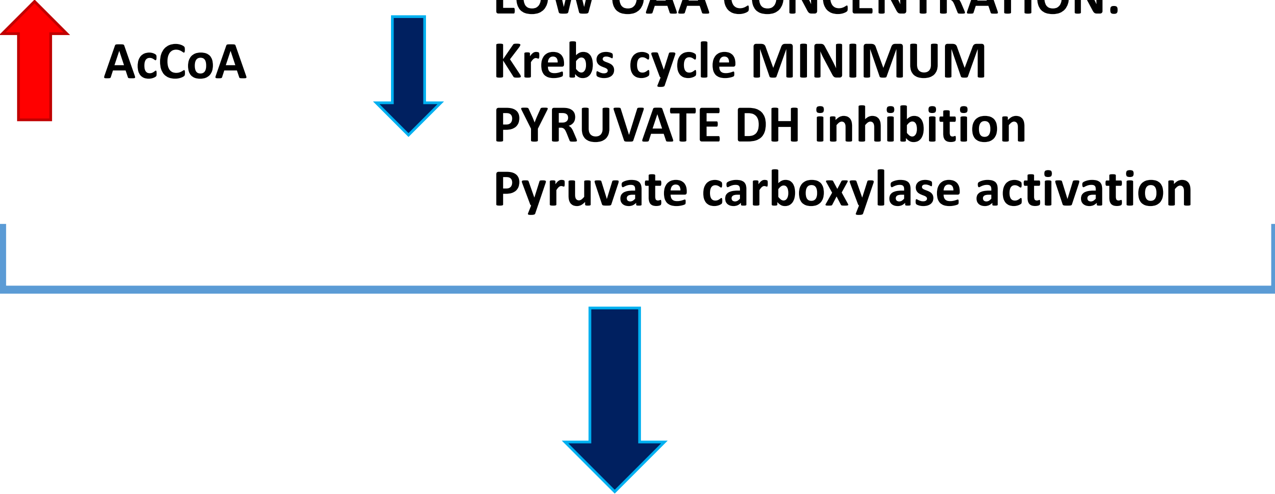
ANNEX

SITUATIONS OF HIGH ENERGY DEMAND AND LOW GLUCOSE LEVELS: FASTING AND DIABETES MELLITUS

ACTIVATED LIPOLYSIS → β OXIDATION → **LIVER: Acetil-CoA ACCUMULATION**

FASTING OR LACK OF GLUCOSE: MOBILIZATION OF TAG

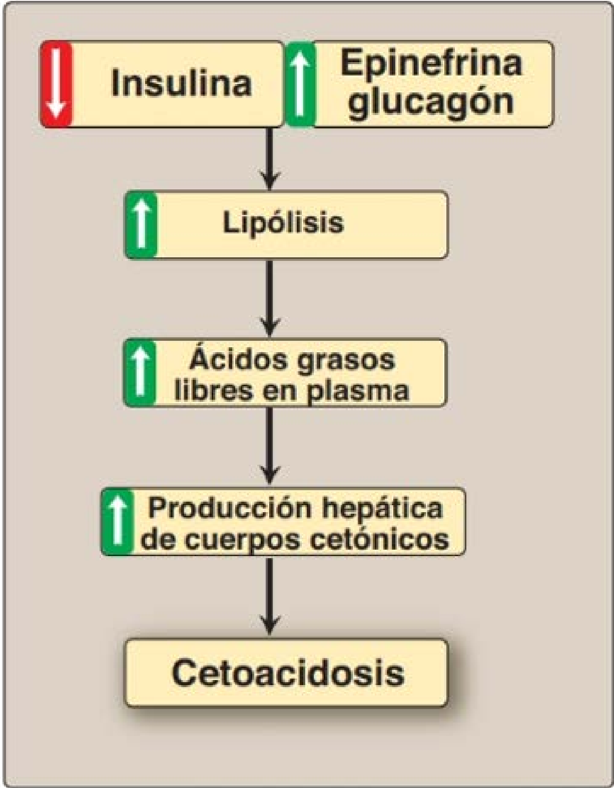
LIVER



AcCoA

LOW OAA CONCENTRATION:
Krebs cycle MINIMUM
PYRUVATE DH inhibition
Pyruvate carboxylase activation

AcCoA metabolism: FORMATION OF KETONE BODIES



Excessive production of ketone bodies in uncontrolled type-1 diabetes mellitus

T1DM: As there is no secretion of insulin, **glucose circulates** in the blood, where it cannot be used and where **its concentration increases**.

Fatty acids are mobilized as an alternative source of energy. **Excessive AcetylCoA production and accumulation in the liver leads to the overproduction of ketone bodies.**

Elevated levels of ketone bodies in the blood and later in urine: KETONEMIA and KETONURIA.

ELEVATION IN BLOOD: LOW pH

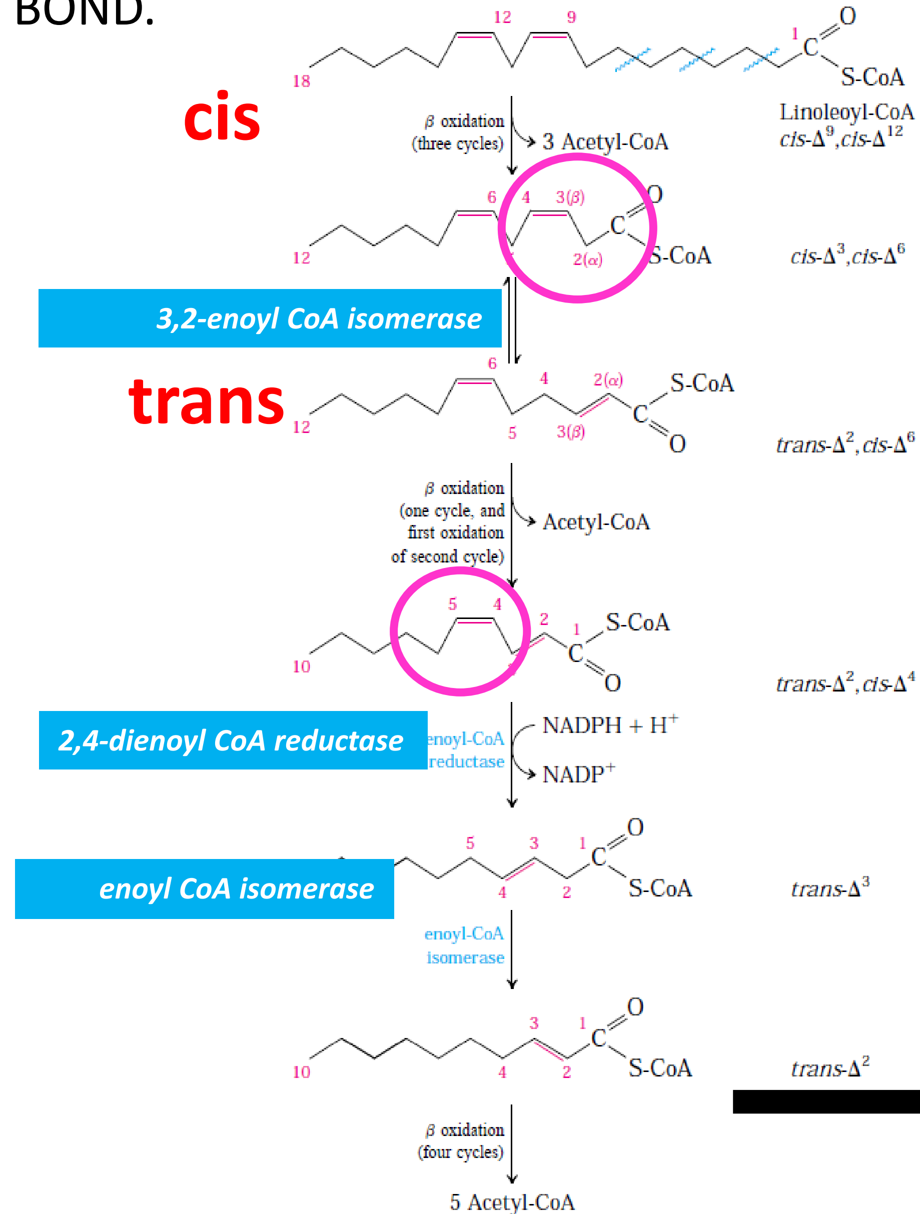
KETONE BODIES. The carboxyl group has a pKa de ~ 4. For each ketone body, there is a one-proton (H+) increase in the blood, which reduces blood pH. **ACIDEMIA (KETOACIDOSIS).**

URINE GLUCOSE AND KB INCREASE: DEHYDRATION.

Ketoacidosis may also be observed in cases of **prolonged fasting.**

THE β OXIDATION OF UNSATURATED FATTY ACIDS: MORE CHEMICAL REACTIONS

- **Metabolic reactions** of the transformation of double bonds: ISOMERIZATION OR REDUCTION OF THE DOUBLE BOND.



8:2(9,12) LINOLEIC ACID, A.G. polyinstaurated in an even position.

- The **double bond in an odd position is isomerized** with enoyl-CoA isomerase.

- The double bond in an even position is reduced at the expense of the **oxidation of a NADH**.

- **Performance is lower** when the first step generated by FADH2 is not performed.

2 ATPs less per each odd double bond.
3 ATPs less for the energy expenditure of a NADH.

Substrate of the hydratase of the β oxidation

LESSON 25. LIPID METABOLISM (III): BIOSYNTHESIS OF LIPIDS

Herminia González Navarro

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INDEX

1. Biosynthesis of fatty acids
2. Biosynthesis of triglycerides
3. Biosynthesis of complex lipids
4. Biosynthesis of eicosanoid lipids

BIOSYNTHESIS OF FATTY ACIDS: OVERVIEW

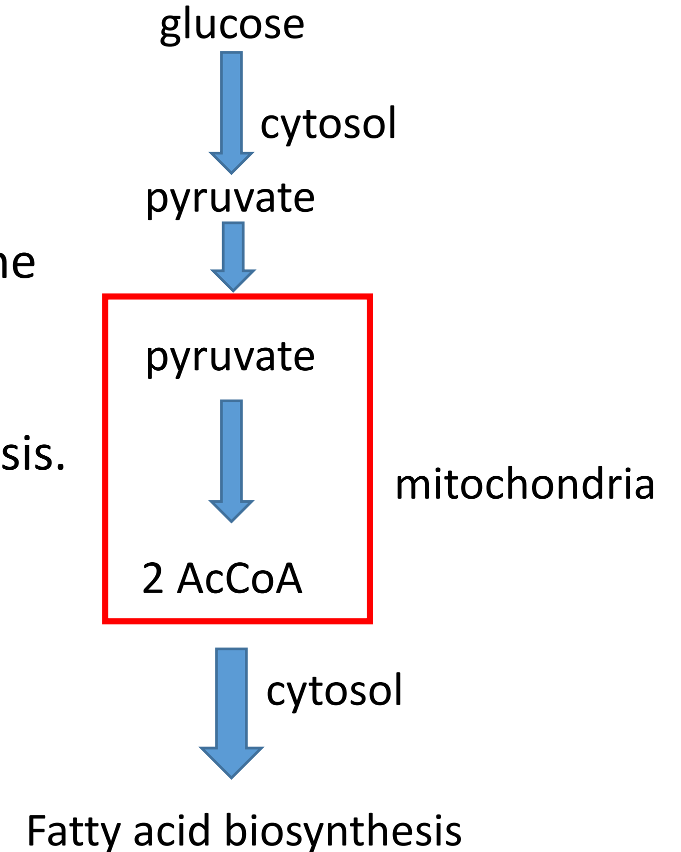
The metabolic pathway consists of the cyclic (SPIRAL) condensation of Acyl-CoA and Malonyl-CoA (3C acyl-CoA); 2C is added (1C from malonyl is not condensated) in each cycle.

4 biochemical reactions:
Condensation, reduction, dehydration and reduction.
Cycle reactions that **lengthen the fatty acid chain by 2 carbons** (acetate) from the Malonyl-CoA molecule.

Malonyl-CoA is formed from **AcCoA** from the oxidation of **pyruvate** from glycolysis.

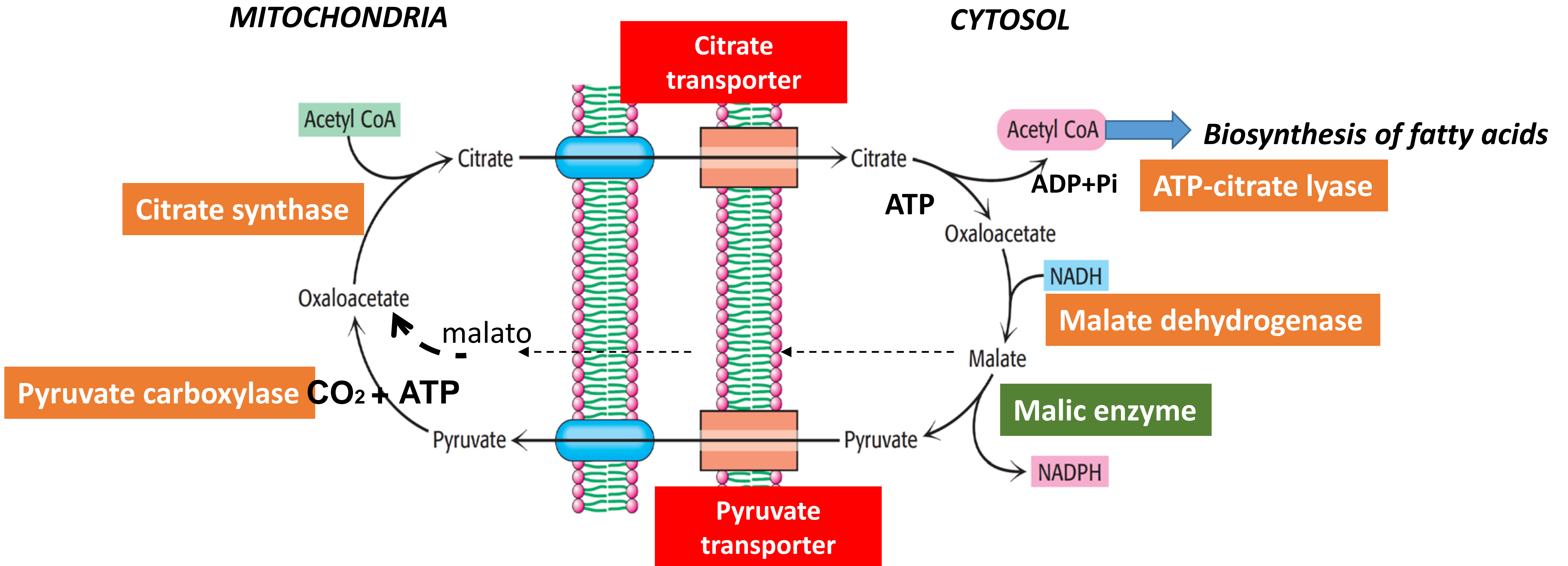
CELLULAR COMPARTMENT: CYTOSOL
ONE ENZYMATIC COMPLEX: FATTY ACID SYNTHASE

TISSUES: adipose tissue, liver, mammary gland, kidney and lung.



BIOSYNTHESIS OF FATTY ACIDS: STAGES

STAGE 1: AcCoA mitochondrial output: citrate/malate/pyruvate shuttle



The accumulation of citrate, ATP and NADH (generated by the excess of nutrients and glucose) favors the synthesis of fatty acids. Such molecules are substrates of the biosynthesis pathway.

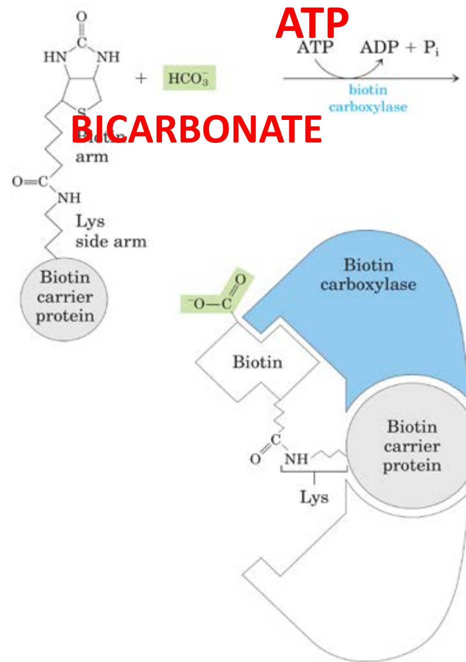
STAGE 2: The formation of Malonyl-CoA, which requires the enzyme Acetyl-CoA Carboxylase and consumes 1 ATP

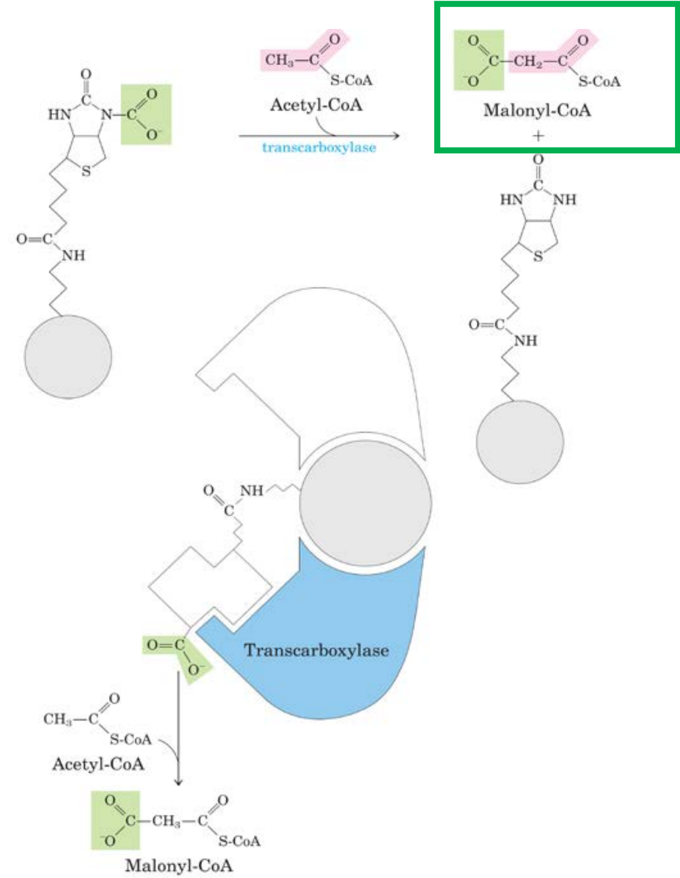
BIOTIN-CARRIER BIOTIN

ATP

BICARBONATE

ACETYL-CoA





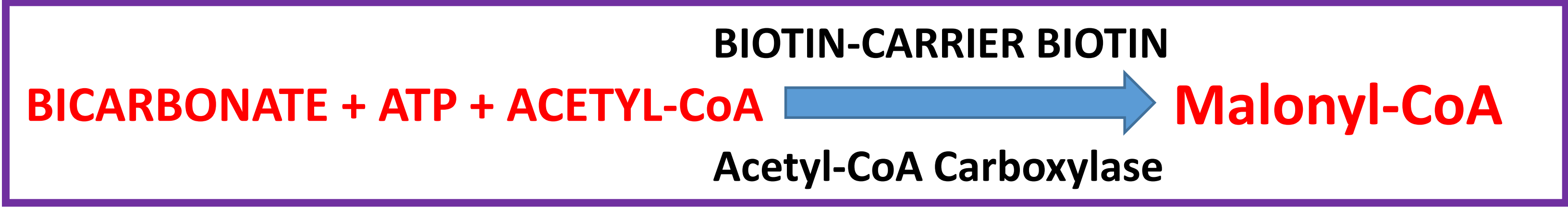
Malonyl-CoA
+

Malonyl-CoA

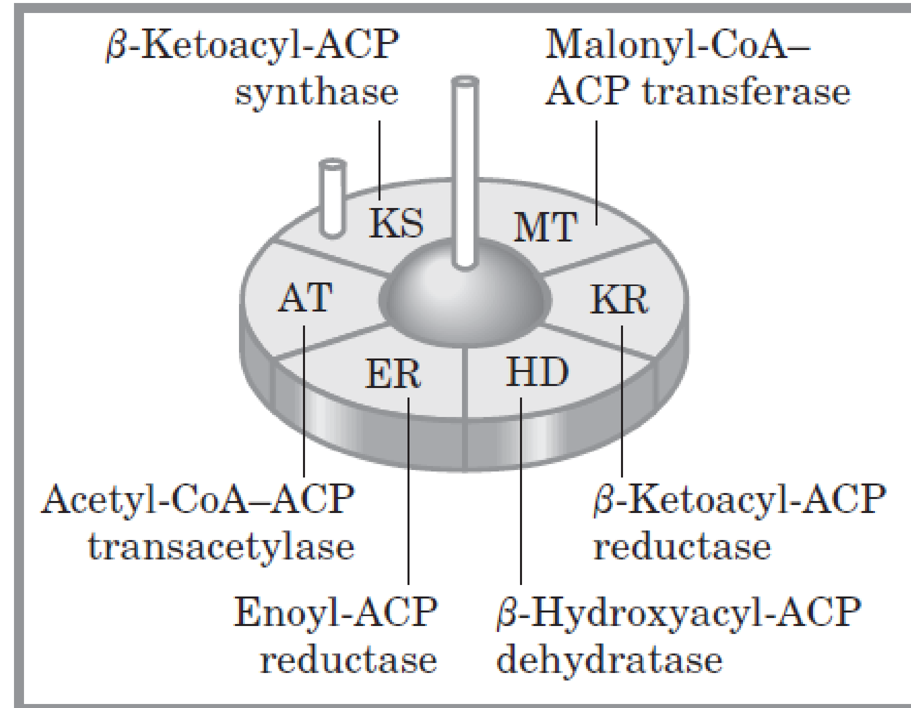
1. **BIOTIN CARBOXYLASE**: It carboxylates to the biotin-carrying protein.

2. The **BIOTIN CARRIER PROTEIN** transfers CO_2-BIOTIN to **TRANSCARBOXYLASE.**

3. **TRANSCARBOXYLASE: transfers** CO2 to acetyl-CoA, thus forming Malonyl-CoA and releasing the BIOTIN carrier protein.



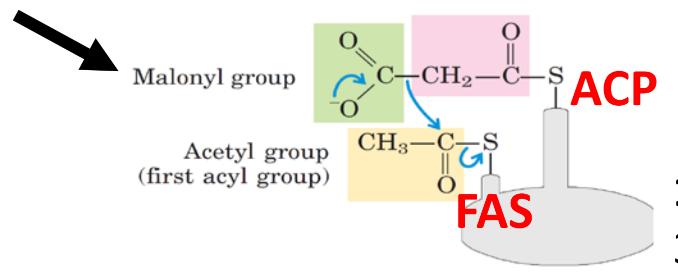
ENZYME COMPLEX FATTY ACID SYNTHASE



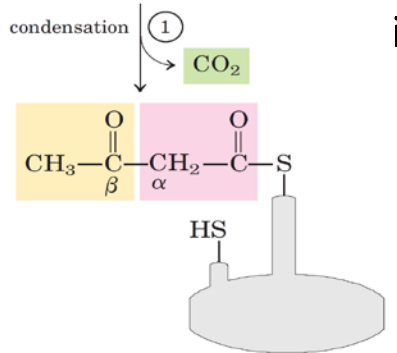
STAGE 3. Condensation of Acetyl-CoA and Malonyl-CoA (4 REACTIONS) and elongation of the chain up to 16 carbons.

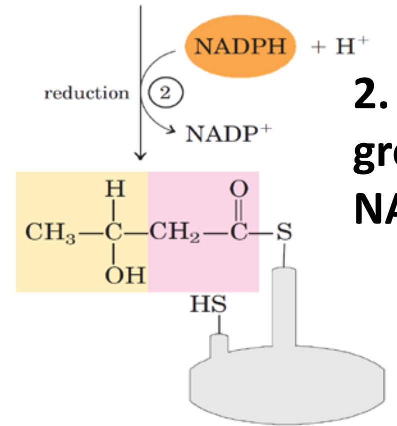
ENZYME COMPLEX: FATTY ACID SYNTHASE: enzyme complex with 4 activities. This has an acyl carrier protein (ACP) that contains the phosphopantotheine group.

INCORPORTADTED GROUP:

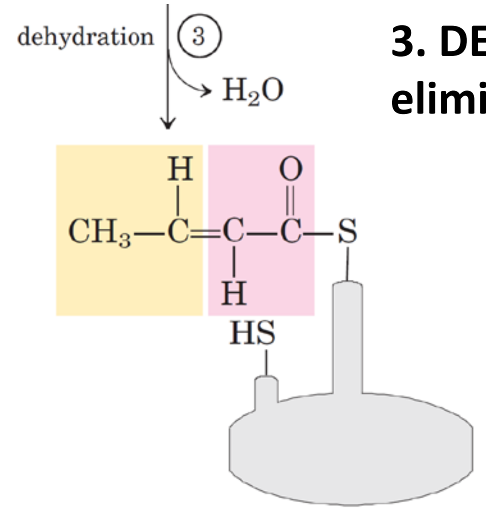


1. CONDENSATION: 2C of the 3C of the malonyl are incorporated.

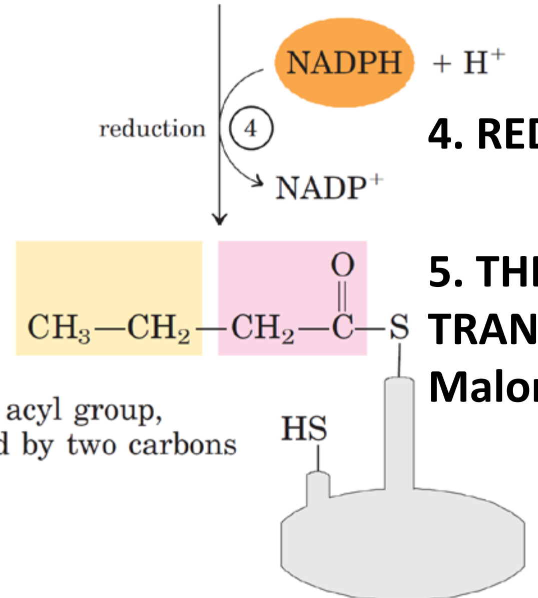




2. REDUCTION of the keto group in β position with a NADPH



3. DEHYDRATION OF C β to eliminate oxygen (reduction).



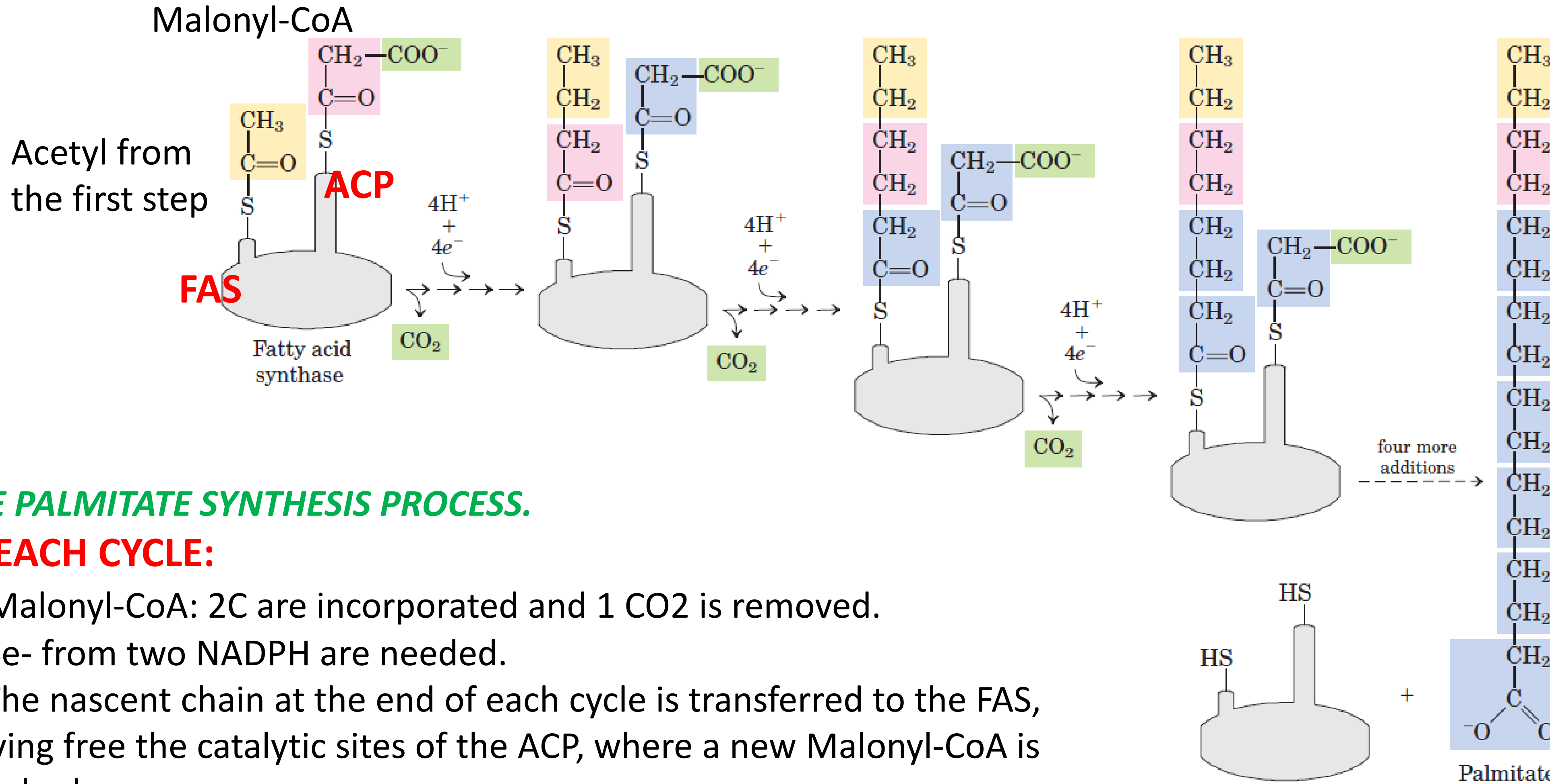
4. REDUCTION OF Cβ and Cα

5. THE NASCENT FATTY ACID IS TRANSFERRED TO THE FAS and Malonyl-CoA binds to the ACP.

ELONGATION TO THE FATTY ACID OF C16 PALMITATE

FAS: FATTY-ACID SYNTHASE
ACP: ACYL CARRIER PROTEIN

THIOESTERASE ACTIVITY: PALMITATE RELEASE



THE PALMITATE SYNTHESIS PROCESS.

IN EACH CYCLE:

1. Malonyl-CoA: 2C are incorporated and 1 CO2 is removed.

2. 4e- from two NADPH are needed.

3. The nascent chain at the end of each cycle is transferred to the FAS, leaving free the catalytic sites of the ACP, where a new Malonyl-CoA is attached.

4. In the first cycle, the binding of Acetyl-CoA in the ACP is required in order to begin the process.

BIOSYNTHESIS OF FATTY ACIDS: STOCHIOMETRY OF THE PALMITATE SYNTHESIS PROCESS

1 Acetyl-CoA + 7 Malonyl-CoA + (NADPH + H^+) + 7 ATP → Palmitate (C16) + 8 CoA-SH + 14 $NADP^+$ + 7 ADP + 7 Pi + 6 H_2O

FOR THE BIOSYNTHESIS OF PALMITATE :

- 1 Acetyl-CoA at the beginning and 7 malonyl-CoA, each of which incorporates two atoms of C.
- 7 ATPs for the formation of the 7 Malonyl-CoA.
- 14 NADPH, 2 for each cycle for the two reductions (4 electrons).
- 7 molecules of H_2O and 8 molecules of CoA-SH are produced.

OTHER FATTY ACIDS; SHORTER, ODD-CHAIN FATTY ACIDS

1) The synthesis of saturated fatty acids of <C16 (shorter fatty acids) follows the same process (thioesterase releases **fatty acids sooner**).

2) Biosynthesis of odd-chain fatty acids: biosynthesis begins with propionyl-ACP rather than acetyl-ACP and continues with the incorporation of Malonyl-CoA units.

BIOSYNTHESIS OF > 16C FATTY ACIDS AND UNSATURATED FATTY ACIDS

PALMITATE: precursor of SATURATED and UNSATURATED fatty acids of >16C chains.

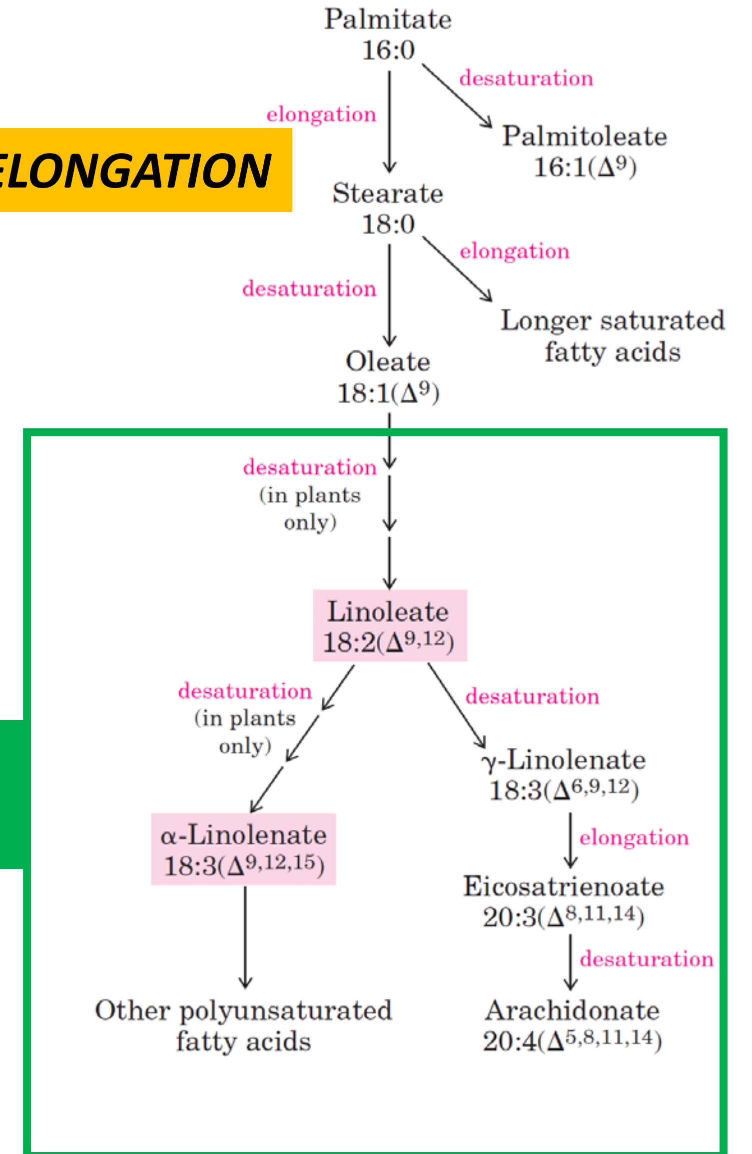
ELONGATION

MITOCHONDRIAL ELONGATION:
Adding and reducing acetyl units inversely to β-oxidation. Uses NADPH rather than FADH2.

ELONGATION IN THE ENDOPLASMIC RETICULUM:
Condensation of **MalonylCoA units.**
BRAIN: MORE THAN **C22** precursors of **brain lipids.**

ELONGATION

ONLY IN PLANTS: DESATURASE



INCORPORATION OF DOUBLE LINKS:

This occurs in endoplasmic R. by desaturases that incorporate bonds in CIS up to C9 **USUALLY IN C18** (ESTEARIC ACID).

IMPORTANT ASPECTS OF FATTY ACID BIOSYNTHESIS:

1) **Malonyl-CoA CONDENSATION** is a favorable reaction driven by the energy released from decarboxylation (CO_2 loss).

2) NADPH is used as a reducing agent for reduction reactions.

NADPH is consumed in biosynthetic reactions and NADH is generated in energy-producing reactions.

3) One of the ***2 MOLECULES OF NADPH*** for each cycle comes from the **transport of Acetyl-CoA** to the cytosol, the conversion step of malate to pyruvate, and the other comes from the **phosphate pentose** pathway.

4) **ATP** comes from glycolysis.

5) CITRATE IS REQUIRED as it is a cataplerotic reaction. Keeping the Krebs cycle functioning requires an anabolic reaction. **PYRUVATE CARBOXYLASE generates OAA, a key anabolic reaction.**

REGULATION OF FATTY ACID BIOSYNTHESIS: ACETYL-COA CARBOXYLASE PLAYS AN IMPORTANT ROLE

ALLOSTERIC ENZYMES:

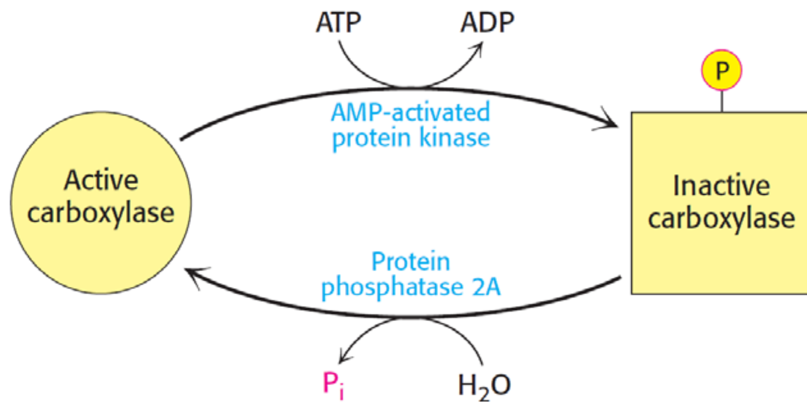
1) **CITRATE IS AN ACTIVATOR** (excess glucose) and a FA synthesis substrate.

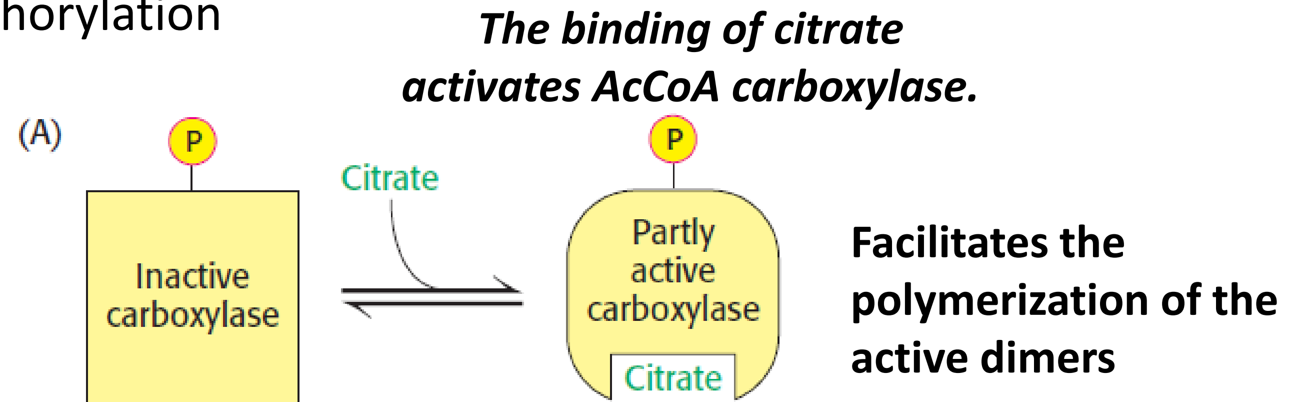
2) Acyl-CoAs and **palmitoyl-CoA inhibit synthesis.**

Covalent modifications of Acetyl-CoA Carboxylase (activation of Malonyl-CoA):

1) **Insulin activates** biosynthesis by dephosphorylation.
2) **Glucagon and adrenaline: inhibition** by phosphorylation

The binding of citrate activates AcCoA carboxylase.





Facilitates the polymerization of the active dimers

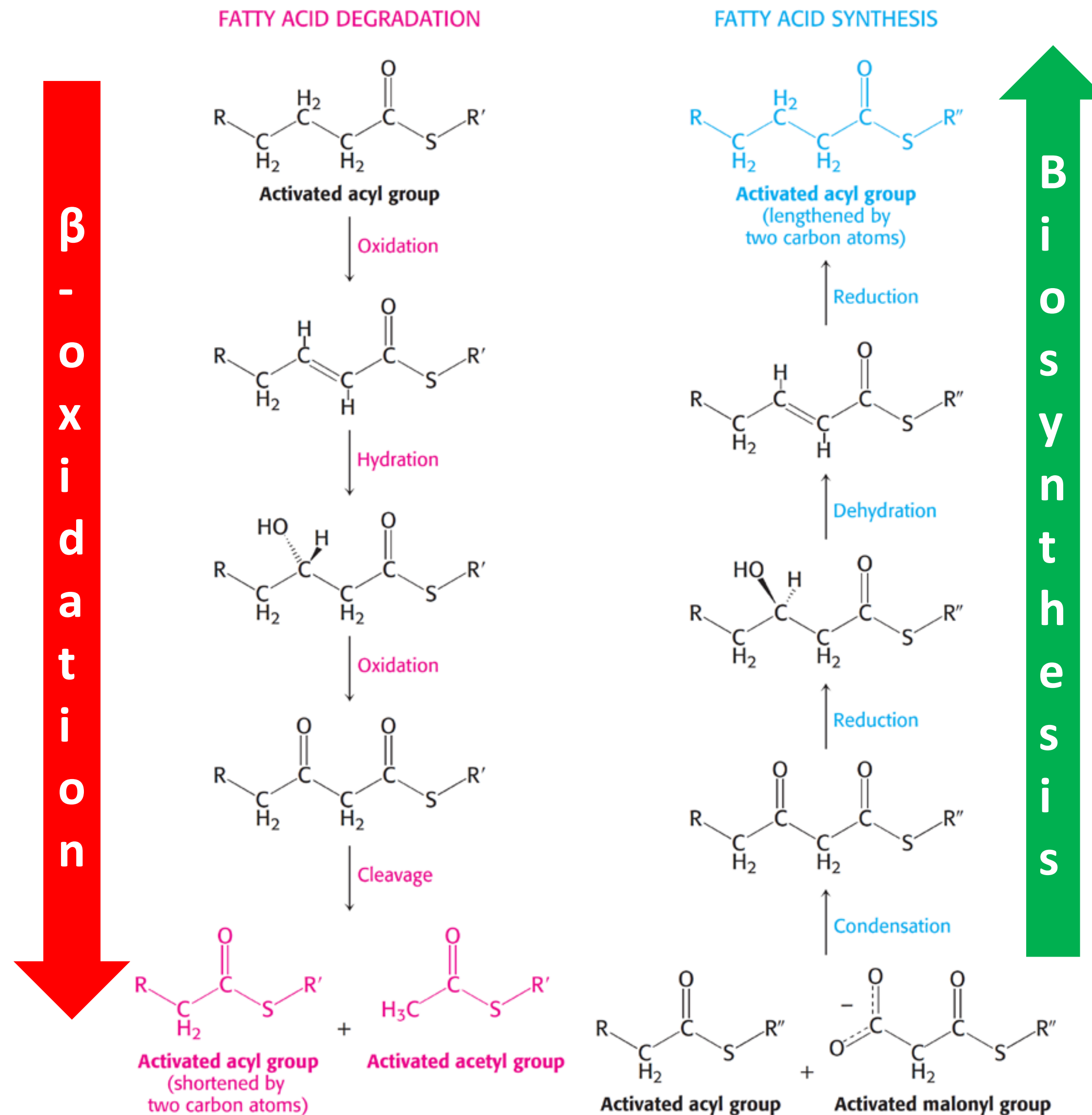
AMPK: cAMP-dependent protein kinase.

AMPK inhibits fatty acid synthesis by Pi of Acetyl-CoA Carboxylase.

OXIDATION AND SYNTHESIS COMPARISON

1. LOCATION: MITOCHONDRIA
2. OX/RED COFACTOR:NADH
3. ACETYL GIVER: ACETYL-CoA
4. ACYL CARRIER: SH-CoA

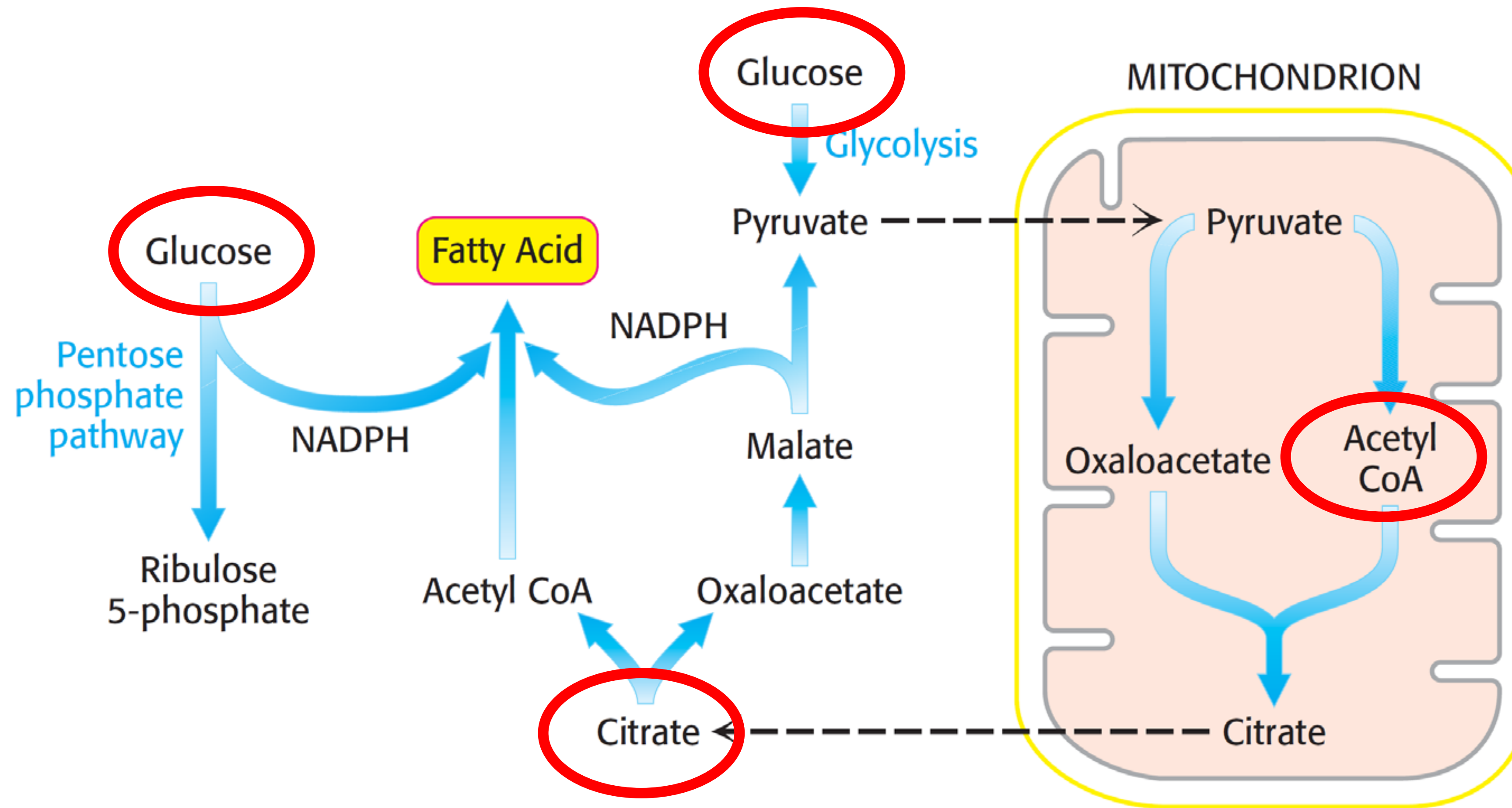
β-oxidation



Biosynthesis

1. LOCATION: CYTOSOL
2. OX/RED COFACTOR:NADPH
3. ACETYL GIVER: MALONYL-CoA
4. ACYL CARRIER: ACP

COORDINATION OF SEVERAL METABOLIC PATHWAYS FOR FATTY ACID SYNTHESIS



Accumulation of the substrates of the biosynthesis of fatty acids favors the process:
Excess Acetyl-CoA and its transport to the cytoplasm provides the **Carbons**.
The **phosphate pentose pathway** and the conversion **malate pyruvate provide** the **NADPH.**
The **glycolysis provides the ATP** for the activation of the MaolnylCoA pentose phosphate.

TRIGLYCERIDE BIOSYNTHESIS: FATTY ACID STORAGE IN LIVER AND ADIPOSE TISSUE

FATTY ACIDS ARE STORED AS TAG:

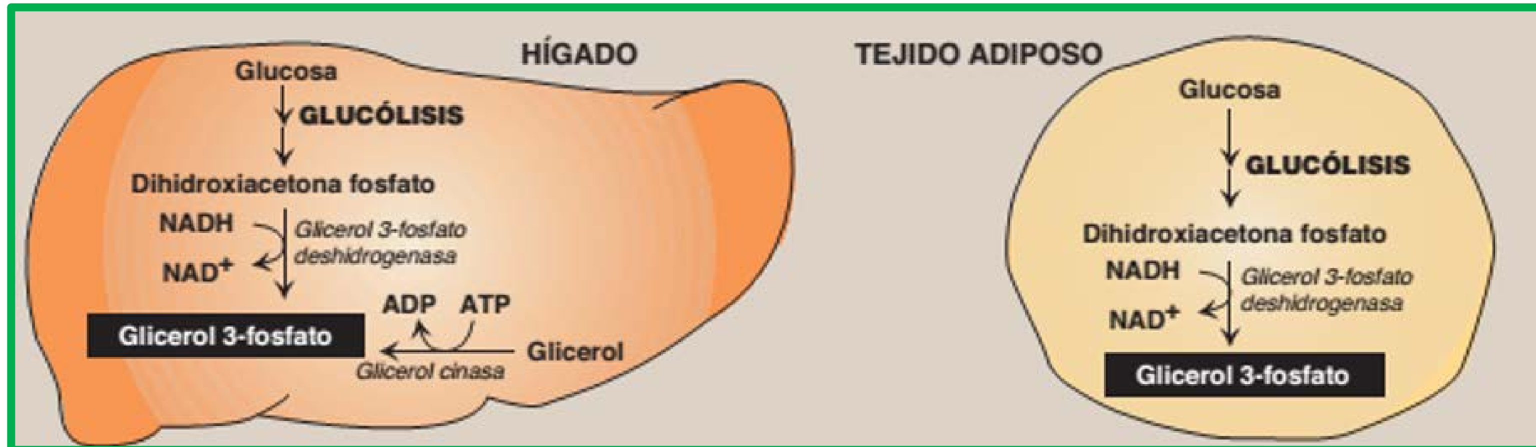
3 FATTY ACIDS STERIFY GLYCEROL

GLYCEROL-3-Pi + FATTY ACIDS (sterified in the carboxylic group) are required.

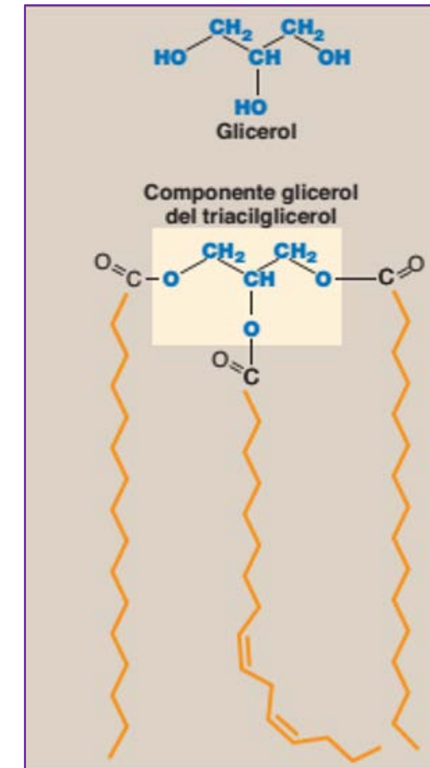
LIVER AND ADIPOSE TISSUE FROM GLYCOLYSIS

LIVER ALSO FROM GLYCEROL KINASE

ONLY WHEN THERE IS EXCESS GLUCOSE



TRIGLYCERIDE



IF LOW ENERGY STATUS:

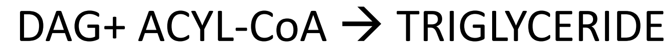
GLYCEROL-3-Pi used in glycolysis

FATTY ACIDS in β oxidation

STORAGE OF FATTY ACIDS IN TAG: SIMPLIFIED REACTIONS

ACYLTRANSFERASES PHOSPHATASE

ACYLTRANSFERASE



FATE OF TRIACYLGLYCEROL IN LIVER AND ADIPOSE TISSUE

In **white adipose tissue** TAG form fat droplets in the cellular cytosol and constitute the "fat deposit" until their mobilization.

The liver does not store fat under normal conditions.

The liver packages **TAGs** into nascent VLDL. VLDL is secreted into the blood stream where it matures and carry endogenously-derived lipids **to peripheral tissues.**

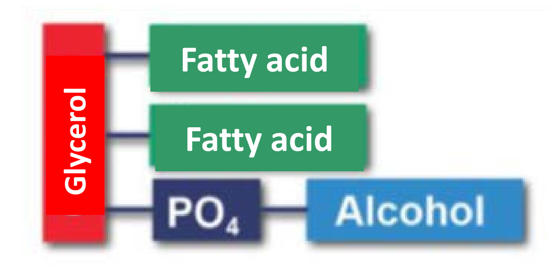
BIOSYNTHESIS OF MEMBRANE COMPLEX PHOSPHOLIPIDS

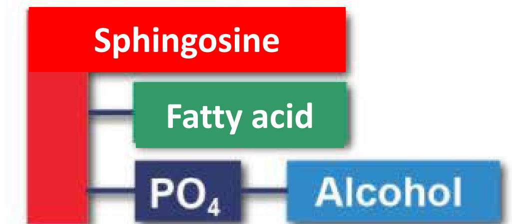
Glycerolphospholipids and sphingophospholipids

Most of these are synthesized **by combining the groups** of polar heads, fatty acids and skeletons of sphingosine or glycerol.

GENERAL SCHEME OF BIOSYNTHESIS:

1. Synthesis of the **glycerol or sphingosine molecule.**
2. **Binding of fatty acids** via ester or amide bond to the glycerol/sphingosine skeleton.
3. **Addition** of the hydrophilic group, **phosphate/carbohydrate** to the skeleton.
4. **Alteration of the polar group** that gives rise to the final phospholipid.





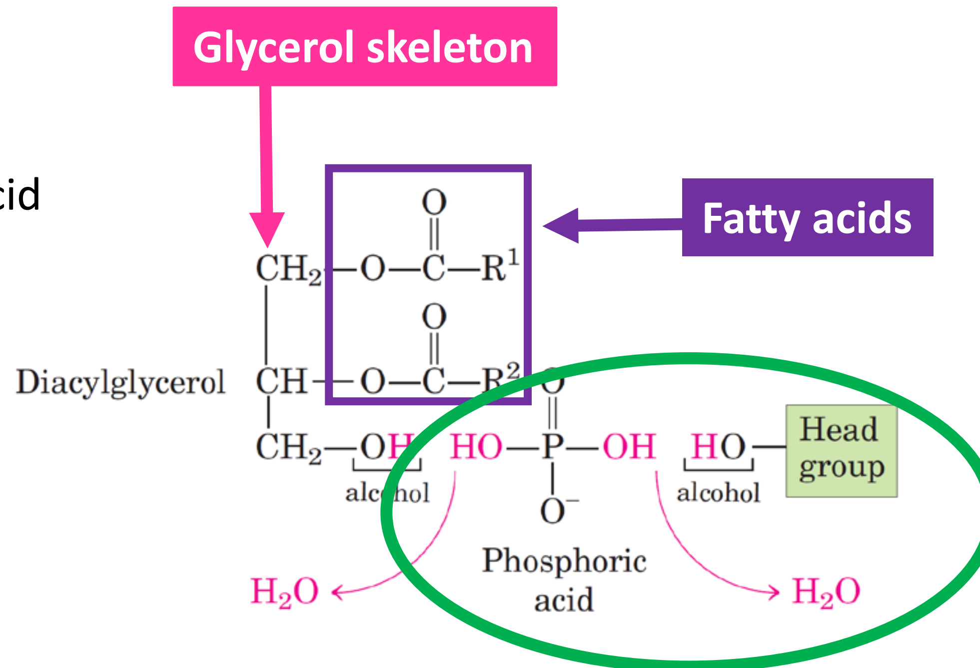
LOCATION OF THE BIOSYNTHESIS:

Biosynthesis is located in **most cell types** (unlike other lipids, e.g. TAG), in the smooth **ER, and in the mitochondrial** inner membrane.
Most phospholipids are transported to other cell sites.

BIOSYNTHESIS OF MEMBRANE COMPLEX PHOSPHOLIPIDS: GLYCEROPHOSPHOLIPIDS

The first steps involve the formation of **DIACYLGLYCEROL in C1 and C2 in Glycerol-3-Pi using the TAG synthesis pathway.**

C1: saturated fatty acid
C2: unsaturated fatty acid



Phospholipid polar head: cholinePi, inositolPi, enthanolaminePi

BIOSYNTHESIS OF MEMBRANE COMPLEX PHOSPHOLIPIDS: GLYCEROPHOSPHOLIPIDS

The synthesis **of glycerophospholipids requires activation with CTP** (cytidine triphosphate). The CDP (Cytidine diphosphate) provides the phosphate group of the phosphodiester bond.

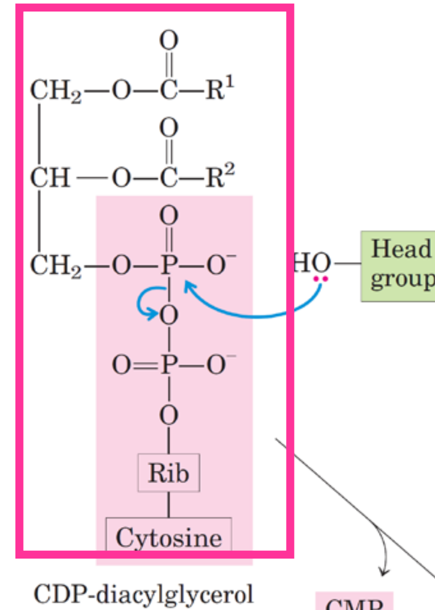
STRATEGY 1:

Formation of activated **CDP-DIACYLGLYCEROL.** The OH group of the polar head reacts with the Pi CDP.

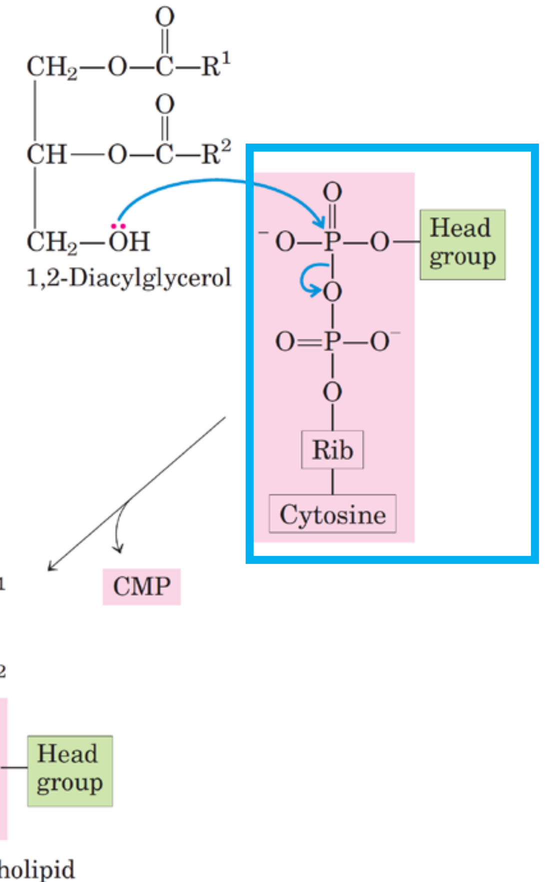
STRATEGY 2:

Formation of activated **CDP-ALCOHOL** (polar head). The OH of the DAG reacts with the Pi of the CDP.

STRATEGY 1:



STRATEGY 2:



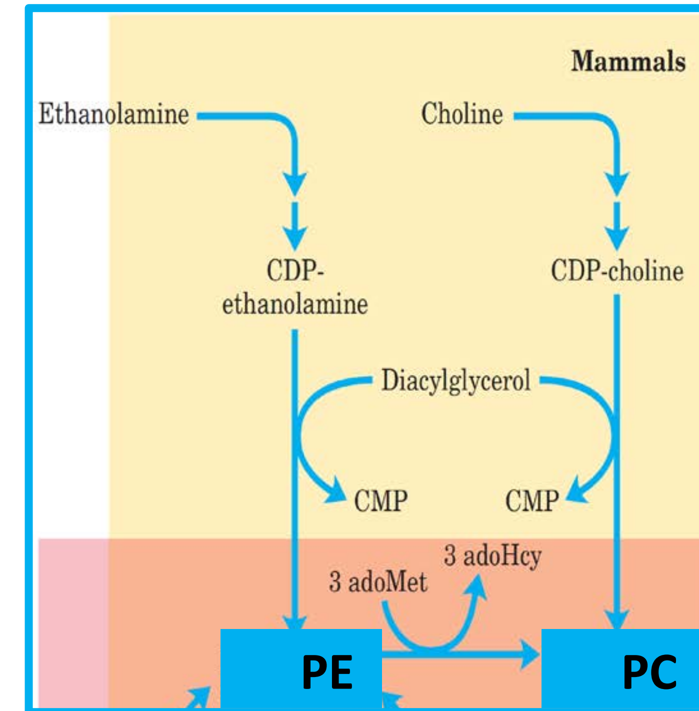
POLAR HEAD: CHOLINE, INOSITOL, ETHANOLAMINE

THE MOST IMPORTANT MEMBRANE COMPLEX PHOSPHOLIPIDS

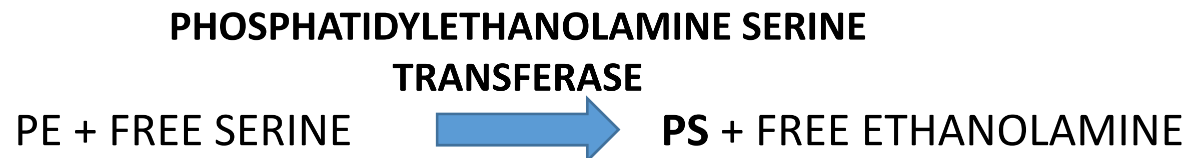
Phosphatidylcholine (PC) and Phosphatidylethanolamine (PE)
These **neutral phospholipids** are the most abundant ones in most eukaryotic cells.
Choline and ethanolamine are obtained from the **diet** or from the exchange of body phospholipids through
STRATEGY 2: the formation of alcohol-CDP.

Phosphatidylserine (PS) and Phosphatidylinositol (PI)
Negative charge
PI is synthesized from free inositol and CDP-DAG (**STRATEGY 1**).

PI is a rare phospholipid as it **contains stearic acid and arachidonic acid** in glycerol carbon 2.PI: **reservoir of arachidonic acid in membranes**; substrate for the synthesis of prostaglandins (PG).
PS is synthesized from PE by exchanging ethanolamine for free serine.



PHOSPHATIDYLETHANOLAMINE SERINE TRANSFERASE



BIOSYNTHESIS OF EICOSANOIDS: PROSTAGLANDINS, THROMBOXANES AND LEUKOTRIENES

These are synthesized **from the arachidonic acid of membrane phospholipids** (Pi-lipids).

They are **powerful signaling molecules** that act as second messengers in response to hormones or others.

Synthesis of prostaglandins:

1. From arachidonic acid **released by Phospholipase A2** from membrane phospholipids.
2. Synthesis in the **smooth endoplasmic reticulum (ER).**
3. **By the action of cyclooxygenase**, which catalyzes two reactions.

Arachidonic acid → Prostaglandins → TXA and leukotrienes

Arachidonic fatty acid: 20 Atoms of C and polyunsaturated: 20:4(5,8,11,14).

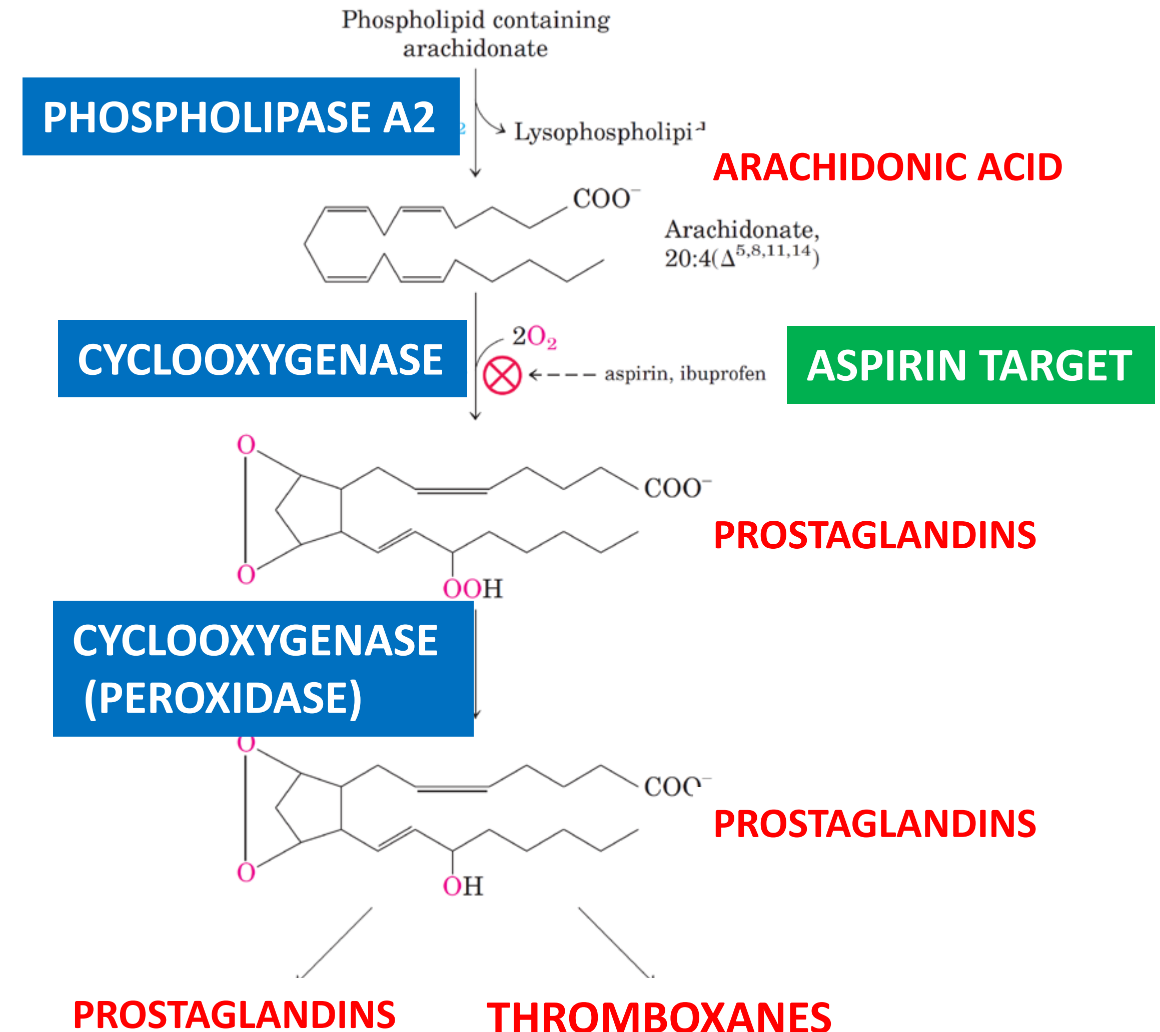
CYCLIC PATHWAY OF ARACHIDONIC ACID: PROSTAGLANDINS, THROMBOXANES

1. CYCLOOXYGENASE (COX) (bifunctional) catalyzes oxygen incorporation and peroxidation in two steps to produce PG from arachidonic acid.

2. The **THROMBOXANE SYNTHASE ENZYME** in platelets (thrombocytes) produces thromboxane A2 (TxA2) from PG.

Aspirin (acetylsalicylate) inactivates (is a suicidal inhibitor of) cyclooxygenase activity. It also prevents the production of thromboxanes and PG involved in inflammation and platelet aggregation.

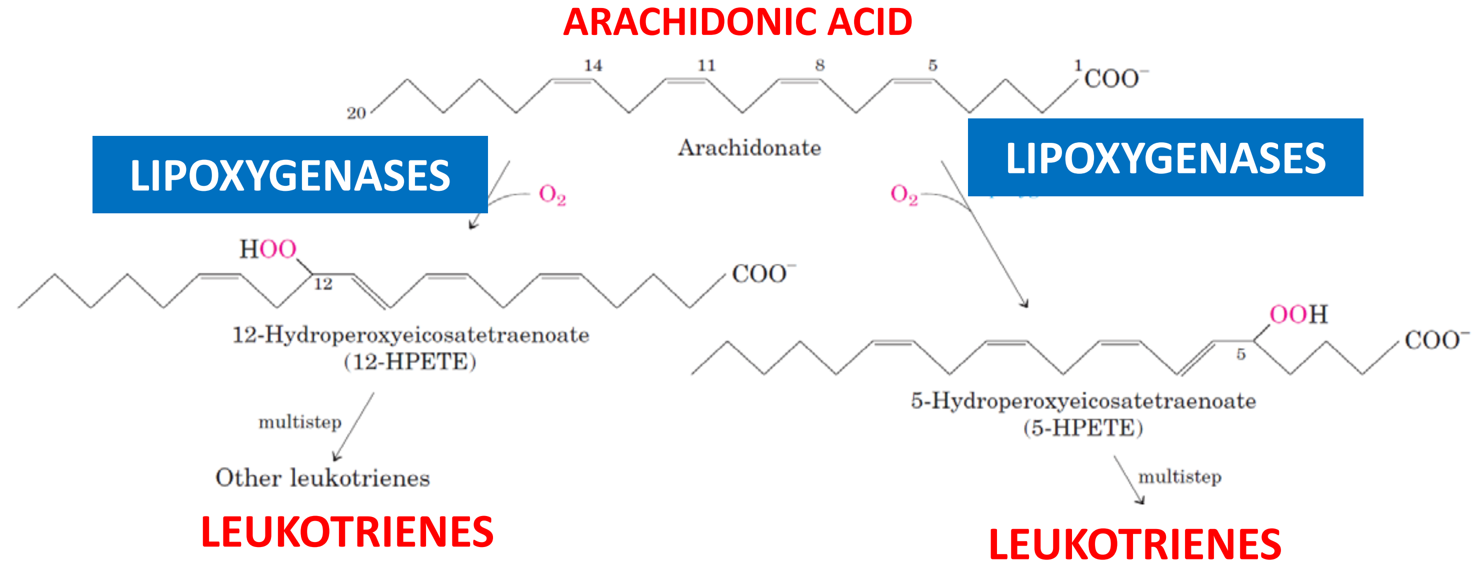
Low doses of aspirin reduce the likelihood of thromboembolic phenomena (reduce the likelihood of heart attacks and strokes) by reducing the production of thromboxanes.



LINEAR PATHWAY OF ARACHIDONIC ACID: SYNTHESIS OF LEUKOTRIENES (FOUND FIRST IN LEUKOCYTES)

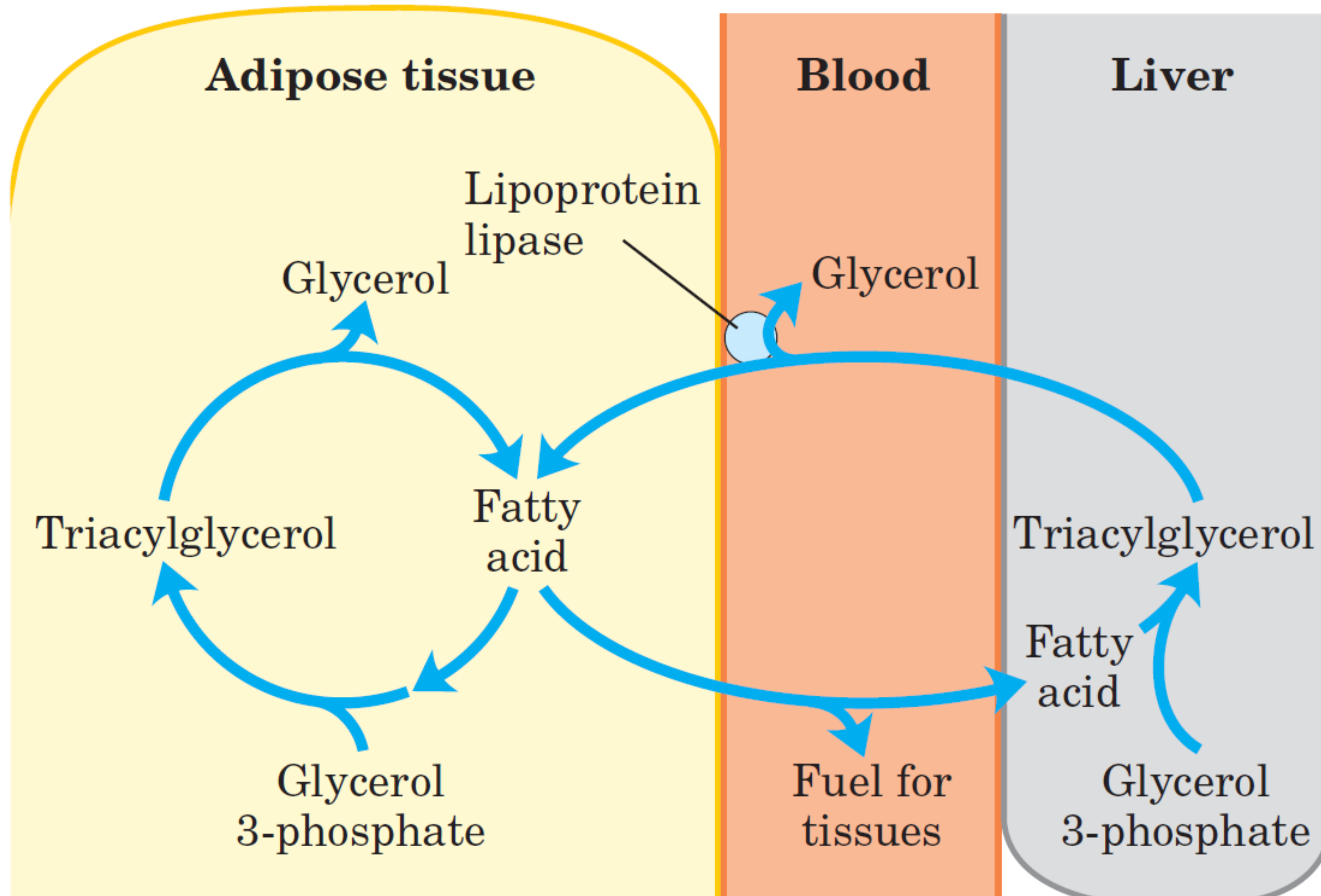
Leukotrienes are generated by the action of **LIPOXYGENASES.** They catalyze the incorporation of molecular oxygen in arachidonic acid.

CELL TYPES: leukocytes, heart, brain, lung and spleen.



They are not inhibited by acetylsalicylate.
Leukotrienes differ from each other by the position of the peroxide.

TRIACYLGLYCERIDE CYCLE



In mammals, the synthesis of TAG and its mobilization is hormonally regulated. ***When there are combustible nutrients,*** the release and circulation of fatty acids are low and, although TAG are hydrolyzed by lipases, they are re-esterified into adipose tissue and stored. The free fatty acids captured by the liver are incorporated into GADs and packaged in VLDLs for distribution.

In situations of lack of nutrients (CH, glucose) and production of glucagon adrenaline, lower glycolysis increases the gluconeogenesis and mobilization of GADs. Fatty acids are taken up by different tissues (muscle) to produce energy.

LESSON 26. METABOLISM OF LIPIDS (IV): CHOLESTEROL AND LIPOPROTEINS

Herminia González Navarro

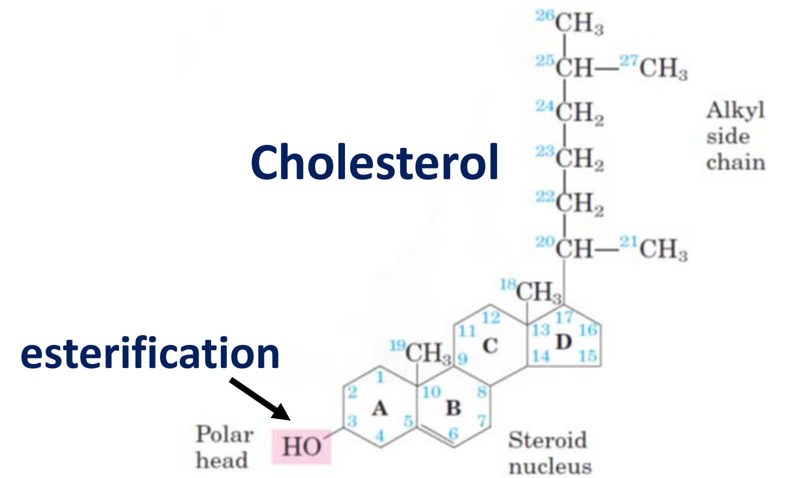
INDEX

1. Cholesterol metabolism:
    - 1.1. Functions of cholesterol
    - 1.2. Origin and biosynthesis of cholesterol
    - 1.3. Destinations of cholesterol
    - 1.4. Regulation of cholesterol biosynthesis
2. Lipoprotein metabolism
    - 2.1. Types of lipoproteins and composition
    - 2.2. Lipoprotein transport
3. Mechanisms of regulation of lipid and lipoprotein transport and pathological implications

CHOLESTEROL FUNCTIONS IN THE BODY

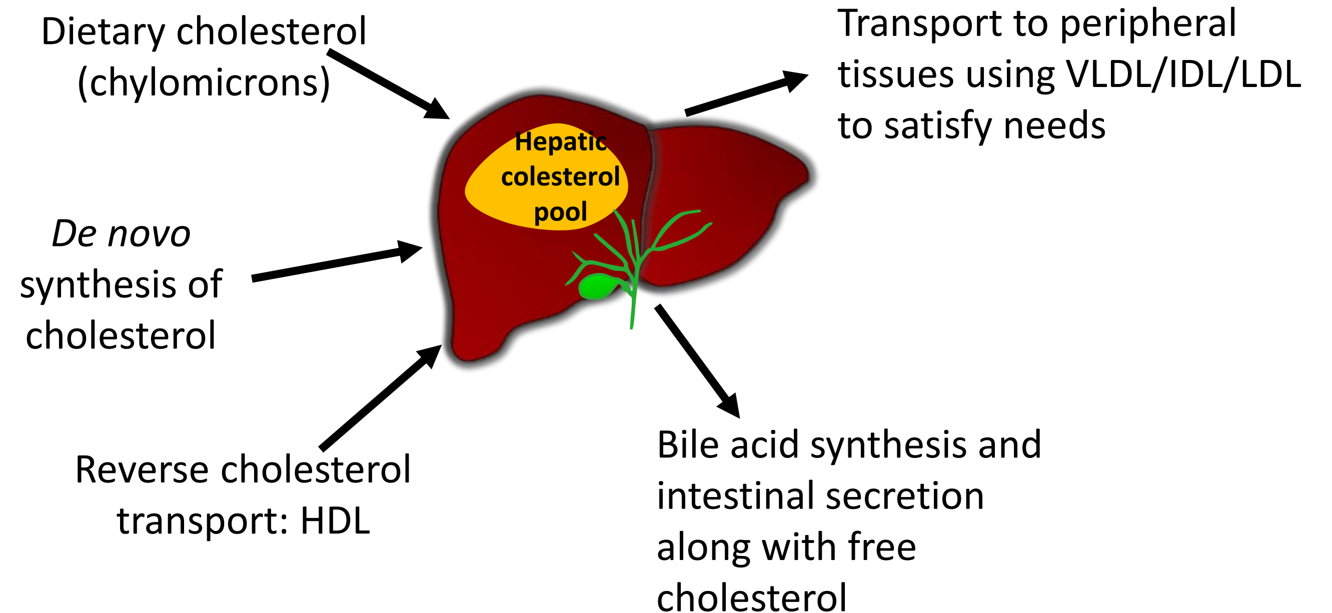
CHOLESTEROL

Cholesterol is an alcohol steroid with a ring structure that is characteristic of animal tissues.
It is the main sterol in animal tissues.
It is highly insoluble.
It forms cholesterol esters.
It is transported in Lps or emulsified with bile salts.



FUNCTIONS

Membrane Component
Bile Acid Precursor
Steroid Hormone Precursor and Vitamin D
Isoprenoid Synthesis

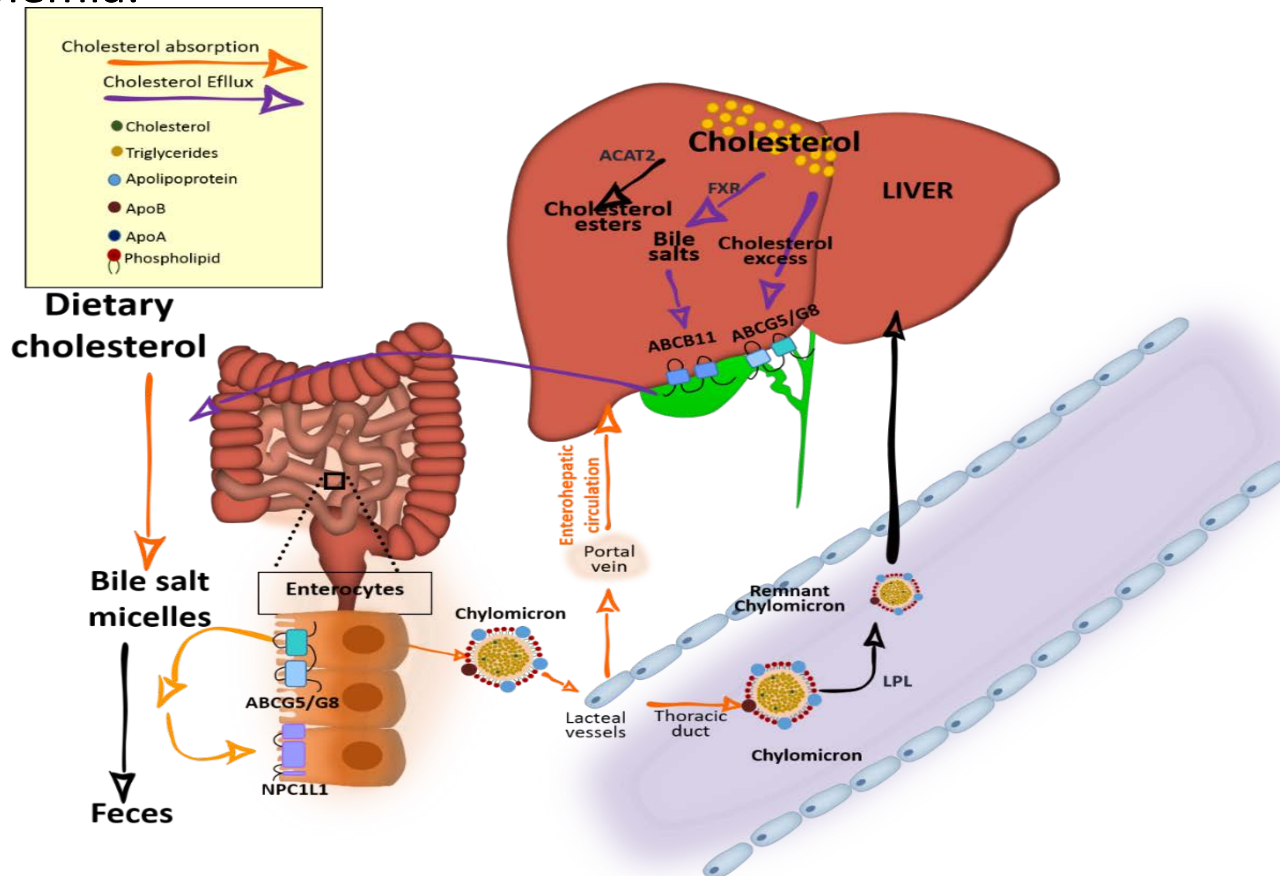


DIETARY CHOLESTEROL:

The absorption of dietary cholesterol is performed by Niemann-Pick C1-Like 1 (NPC1L1) protein which is expressed in the intestine.

The absorption of a 5% of plant sterols (phytosterols such as β-sitosterol) is also performed by .

Plant sterols are excreted back into the intestine by heterodimeric transporters, ABCG5/G8, whose defect produces sitosterolemia.



Adapted from: Aguilar-Ballester et al., *Nutrients* 2020

DE NOVO BIOSYNTHESIS OF CHOLESTEROL

LOCATION OF BIOSYNTHESIS

In all tissues but especially in the LIVER, INTESTINE, ADRENAL CORTEX, AND REPRODUCTIVE TISSUES (ovaries, testicles and placenta).
Synthesis requires enzymes in the cytosol, **smooth endoplasmic reticulum (REL) membrane, and peroxisome**.

BIOSYNTHESIS REQUIREMENTS

FROM **AcCoA**, while sharing the first two reactions with **ketone bodies biosynthesis.**
NADPH is used as a reducing potential.
The endergonic process is driven by the rupture of the Thioester bond (AcCoA) and ATP.

REGULATION

It is performed by a sensor mechanism of cholesterol pool that results in an adjustment between excretion and biosynthesis.

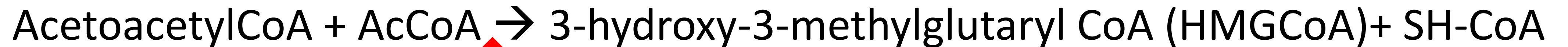
STAGES OF BIOSYNTHESIS:

1. Synthesis of 3-Hexyl-3-Methylglutaryl CoA, **HMGCoA: 3 molecules of AcCoA.**
2. Synthesis of **MEVALONATE.**
3. Condensation of **isoprenoids (5C)** and generation of **SQUALENE.**
4. Synthesis **of LANOSTEROL (first sterol)** and **production of CHOLESTEROL.**

STAGES OF DE NOVO BIOSYNTHESIS OF CHOLESTEROL

1. Synthesis of 3-hydroxy-3-methylglutaryl coenzyme A, HMGCoA:
The first two reactions are shared with the biosynthesis of ketone bodies.

Tyolase



HMGCoA synthase

2. Synthesis of MEVALONATE: performed by HMGCoA reductase which is the **limiting step** of cholesterol synthesis.

HMGCoA reductase

CHARACTERISTICS OF MEVALONATE SYNTHESIS

- Requires **2 NADPH** as a reducing agent.
- Is the **Irreversible and limiting step** in the synthesis of cholesterol.
-HMGCo A reductase **is a membrane protein of the REL but** the catalytic domain is oriented to the cytosol.

STAGES OF DE NOVO BIOSYNTHESIS OF CHOLESTEROL

3. Isoprenoid condensation (5C, isoprene reactions) and SQUALENE (30C) generation:

A) Mevalonate (6C) → (+2 ATPs) 5-pyrophosphomevalonate → (+ 1ATP, -CO_2) IPP (Δ^3isopentenyl pyrophosphate)

MEVALONATE

IPP: precursor of ISOPRENOIDS, 5 carbons
3 ATPs/IPP

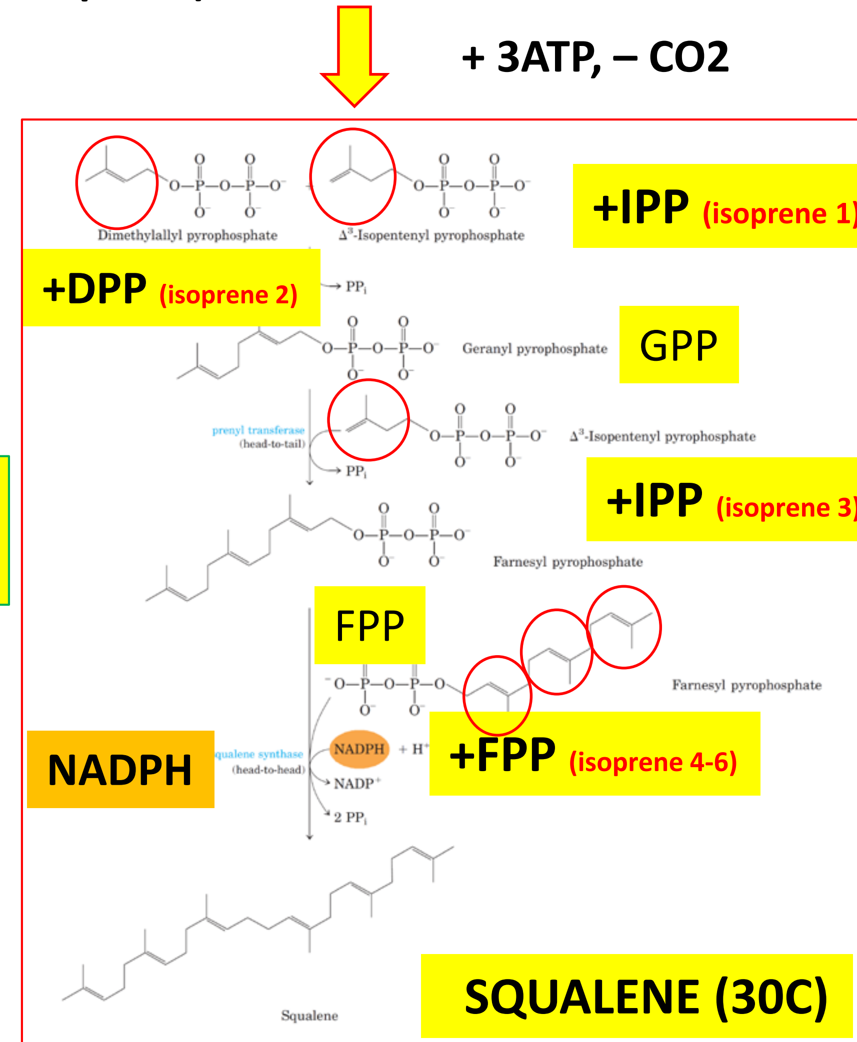
B) IPP+DPP (isomerized IPP)→ GeranylPP (10 C)

C) Geranyl PP + IPP → Farnesyl PP (15C)

Farnesyl PP: covalently binding to proteins, prenylation, to anchor them to membranes

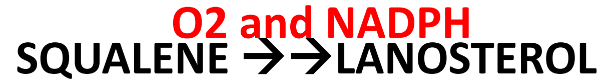
D) Farnesyl PP (15C) + Farnesyl PP (15C) + NADPH→ SQUALENE (30 C) + PPi

SQUALENE (30 C): a 6-unit polyisoprenoid that requires 18 ATPs (the combination of 6 IPP) and 1 NADPH for synthesis



STAGES OF DE NOVO BIOSYNTHESIS OF CHOLESTEROL

4. Synthesis of LANOSTEROL and CHOLESTEROL



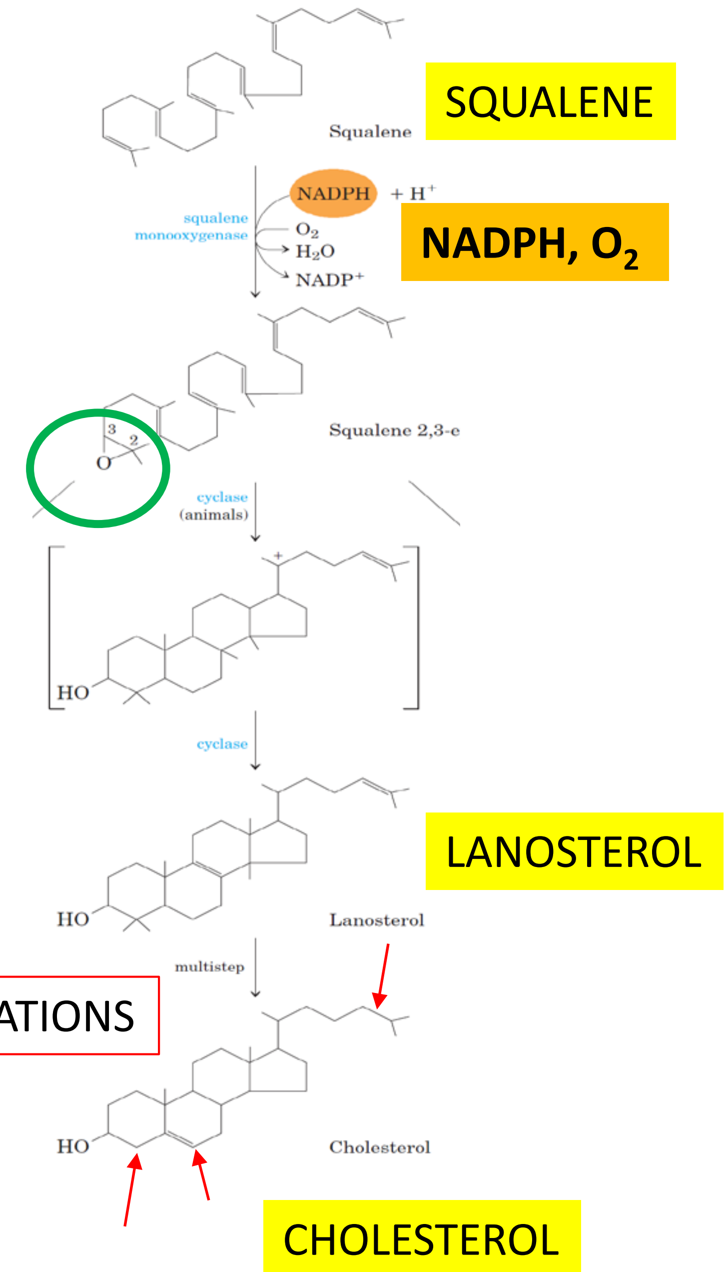
CYCLIZATION OF LANOSTEROL

SEVERAL PATHWAYS

Oxidative removal of methyl groups.
Reduction of 1 double link.
The migration of a double link.

The intermediate 7-dehydrocholesterol

VITAMIN D3 (SKIN)



SEVERAL MODIFICATIONS

SUMMARY OF DE NOVO BIOSYNTHESIS OF CHOLESTEROL

1. The CONDENSATION OF 3 AcCoA to give **mevalonate and its pyrophosphate** requires **2 NADPH.**

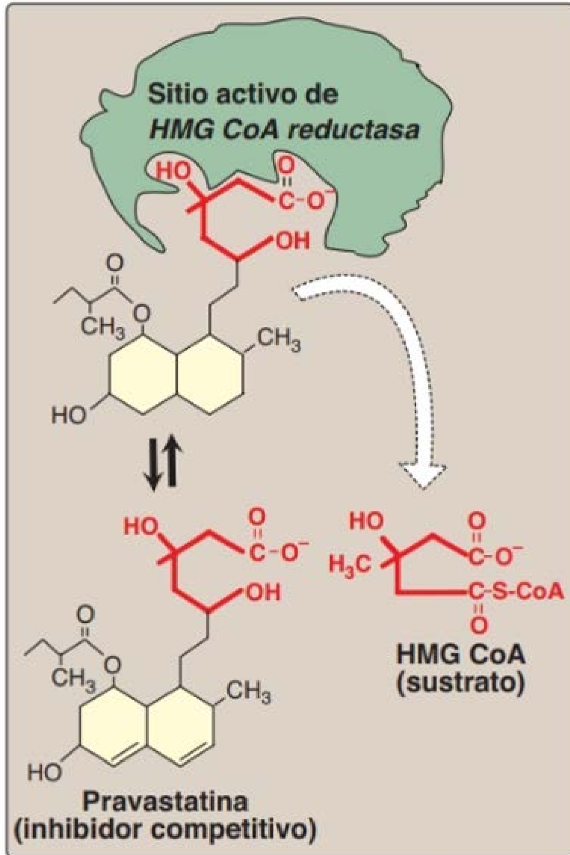
2. **PHOSPHOMEVALONATE PYROPHOSPHATE is decarboxylated**, generates **ISOPENTILPP (IPP),** and uses **3 ATPs**.

3. The **condensation of 6 UNITS OF ISOPRERENOID,** Δ^3ISOPENTENYLPYROPHOSPHATE, to generate **SQUALENE requires 18 ATPs and 1 NADPH.**

4. Transformation of **SQUALENE to LANOSTEROL cycled with NADPH and O2**.

LANOSTEROL generates CHOLESTEROL through three changes (the loss of methyls, the reduction of the double bond, and a change in the position of another double bond).

CHOLESTEROL SYNTHESIS IS INHIBITED BY STATINS: COMPETITIVE INHIBITORS



Antihyperlipidemic or lipid-lowering agents

These agents competitively inhibit the rate-limiting step in cholesterol biosynthesis: enzyme 3-hydroxy-3-methylglutaryl CoA reductase (HMG CoA reductase).

Statins, such as atorvastatin (Lipitor) and pravastatin (Pravachol)**: these are structural analogues of the natural substrate** of this enzyme and compete with HMGCoA for binding to the enzyme HGMCoA reductase in the liver.

Statins inhibit the *de novo* synthesis of cholesterol:

1. They reduce plasma and liver cholesterol levels.
2. They induce lipoprotein receptor expression in the liver.

Levels of liver sterols

LDLR: the receptor that clears cholesterol from the blood

Lowers plasma cholesterol

REGULATION OF CHOLESTEROL SYNTHESIS: HMGCoA REDUCTASE

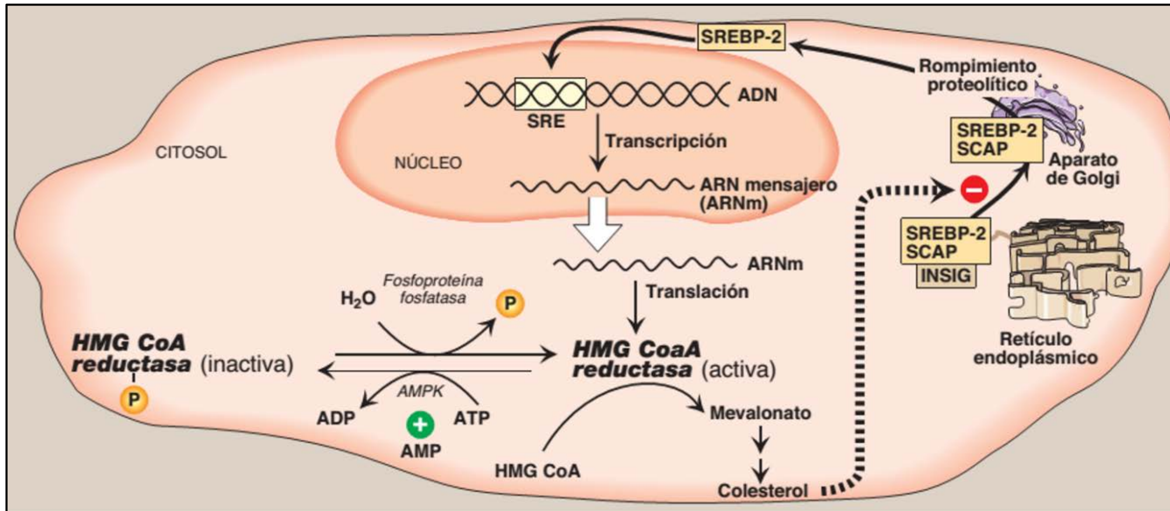
1. Levels of sterols in the smooth ER:

LOW LEVELS OF STEROLS smooth ER: SREBP (a sensor of cholesterol) is translocated to the nucleus and activates the HMGCoA.

ELEVATED LEVELS OF STEROLS smooth ER: Translocation of HMGCoA to the cytosol, ubiquitination and degradation in proteasomes.

2. Phosphorylation/dephosphorylation of HMGCoA independent of sterols:

REGULATION BY AMPK/phosphatase. Dependent on ATP concentrations



3. Hormonal regulation HMG CoA reductase:

Insulin: dephosphorylation of HMGCoA

Glucagon/adrenaline: phosphorylation HMGCoA

DESTINATION OF CHOLESTEROL

Most of the synthesis is hepatic: a small fraction goes to the membranes of hepatocytes.

The main DESTINATIONS ARE:

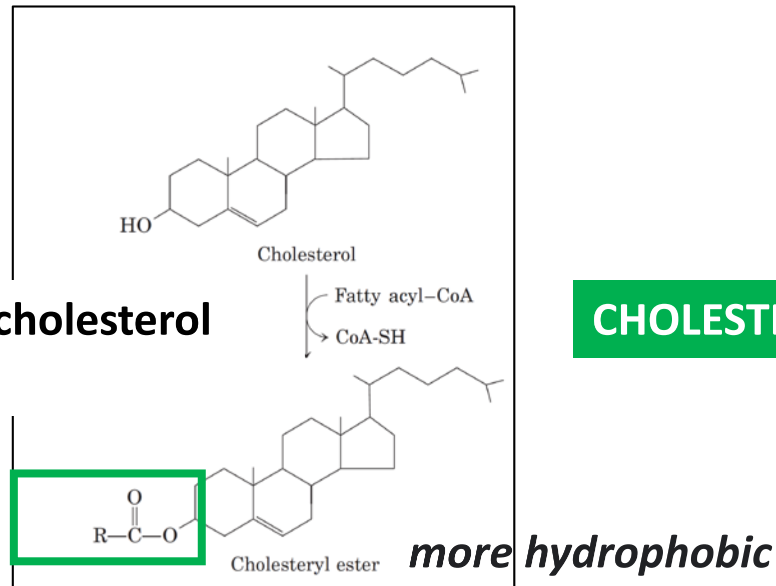
1. **BILIARY EXCRETION** by ABCG5/G8 heterodimer transporters.

2. **SYNTHESIS OF BILE ACIDS** (CYP7a) and **ISOPRENOIDS** (intermediates of their synthesis).

3. THE FORMATION OF **CHOLESTEROL ESTERS FOR** incorporation into **LIPOPROTEINS** and transport to cells with needs or LIVER STORAGE (cholesterol reservoir).

IN CELLS: SYNTHESIS OF VITAMIN D, STEROID HORMONES, INCORPORATION INTO MEMBRANES.

ACAT (1,2): AcylCoA cholesterol acyltransferase



more hydrophobic

CHOLESTEROL ESTERS

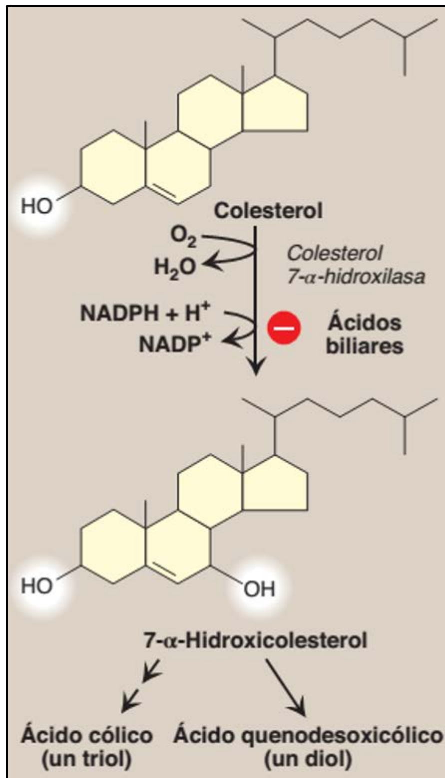
→ **HEPATIC STORAGE (pool of cholesterol)**

→ **PLASMA TRANSPORT: LIPOPROTEIN METABOLISM**

BIOSYNTHETIC DESTINATIONS OF CHOLESTEROL

BILE ACID SYNTHESIS: IN THE LIVER WITH THE MAIN ENZYME **CYP-7-α HYDROXYLASE**.

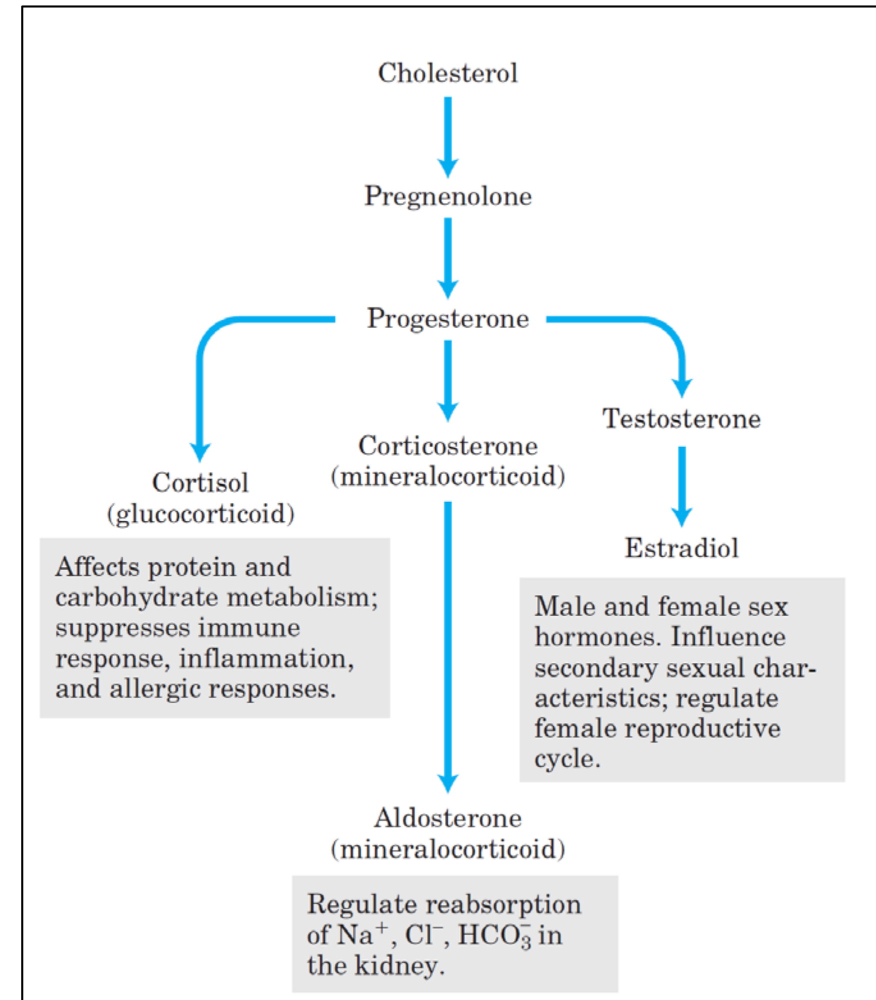
Emulsifiers/surfactants for fat-soluble vitamin and lipid absorption.



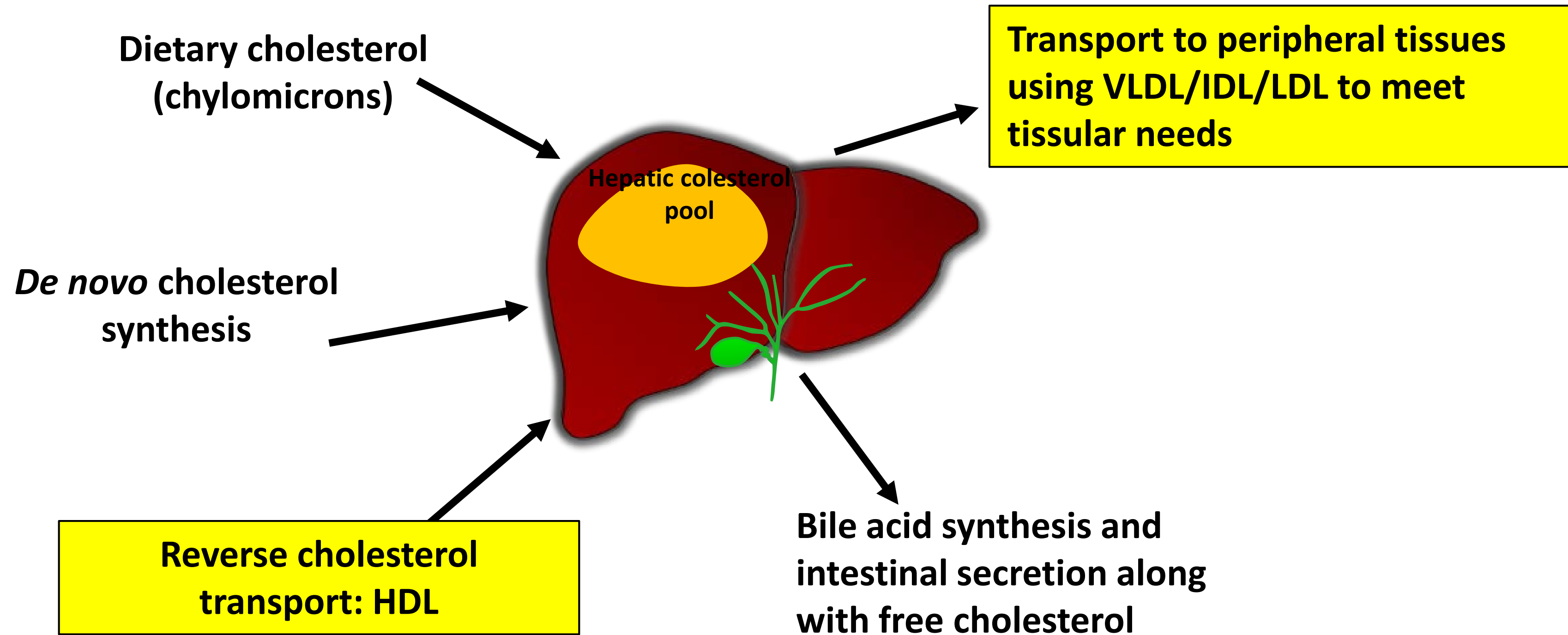
CHOLESTEROL

CHOLIC ACID
DEOXYCHOLIC ACID

SYNTHESIS OF STEROID HORMONES: adrenal glands, gonads and placenta.



BIOSYNTHETIC DESTINATIONS OF CHOLESTEROL

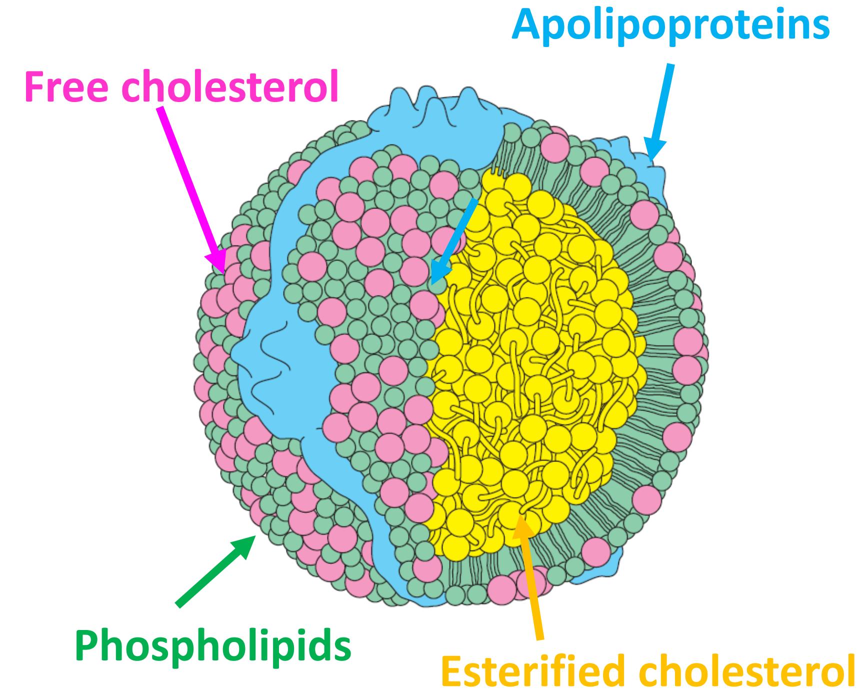


LIPOPROTEIN METABOLISM

LIPOPROTEINS

Lipoproteins are spherical macromolecular complexes made up of lipids, TAG, cholesterol and its esters and phospholipids, and apolipoprotein proteins. Their main function is the **transport of lipids to and from the tissues.**

GENERAL STRUCTURE OF LIPOPROTEINS



TYPES OF LIPOPROTEINS (LPS):

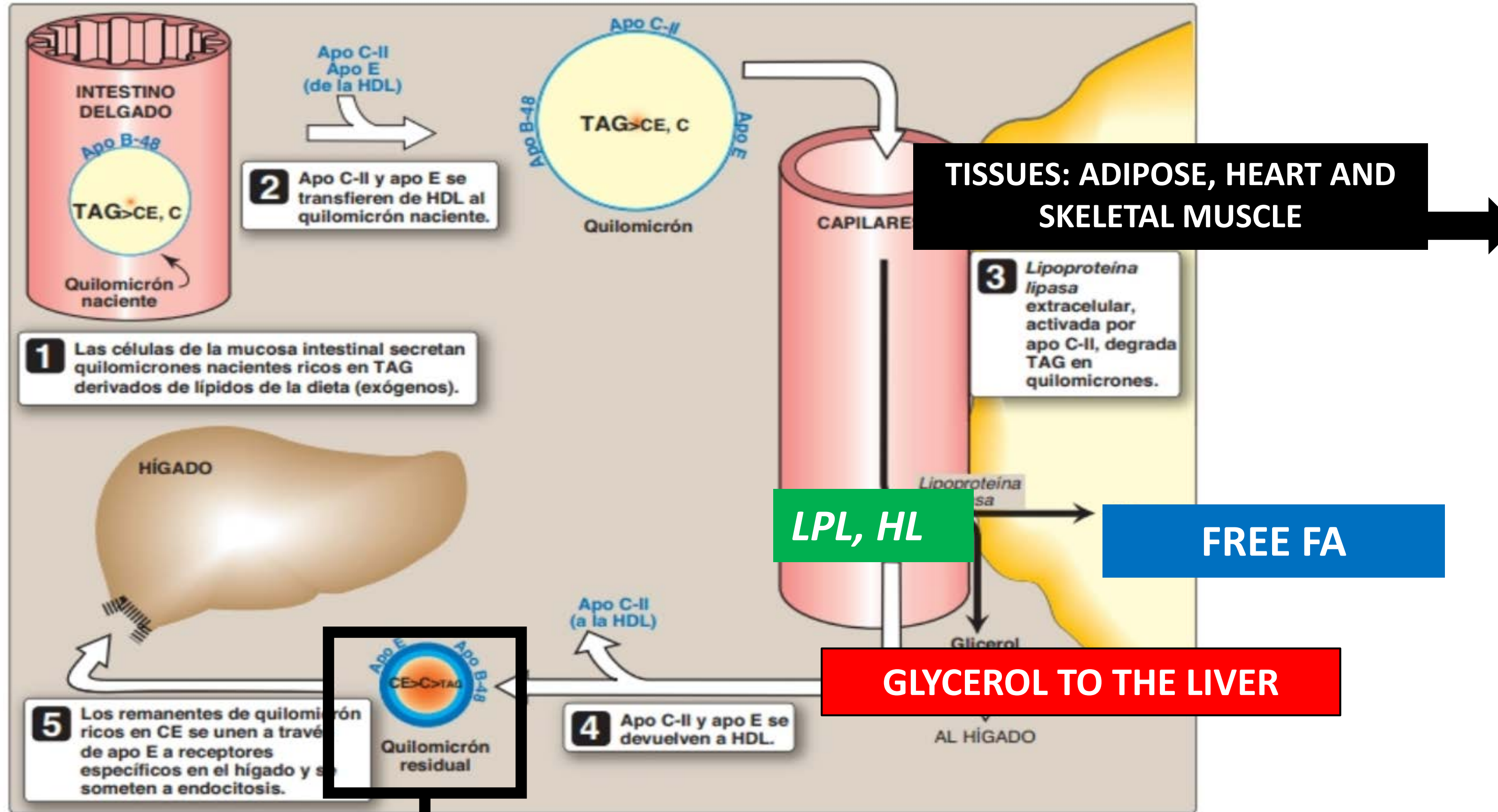
CHYLOMICRONS (postpandrial LPS, i.e. after food intake).
VLDL (very-low-density lipoprotein).
LDL (low-density lipoprotein).
HDL (high-density lipoprotein).

These lipoproteins differ in:

- lipid and protein composition and size
- transport, function and **the affinity for** receptors that mediate their uptake/endocytosis.

CHYLOMICRONS TRANSPORT THE DIETARY LIPIDS

Mature chylomicron: apoE and apoCII



CHYLOMICRON REMNANTS ARE CAPTURED BY THE LIVER AND METABOLIZED

LIPOPROTEIN LIPASE: adipose tissue and cardiac and skeletal muscle. Hydrolyzes TAG to give AG and GLYCEROL.

DESTINATION OF FATTY ACIDS: BIOSYNTHESIS OF TAG and MEMBRANE LIPIDS

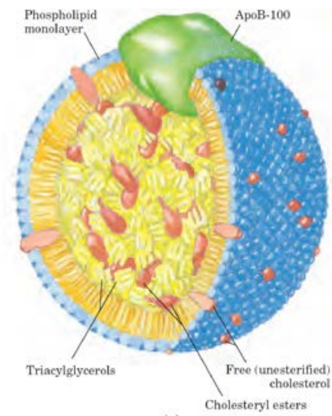
GLYCEROL: as a carbonated skeleton goes to the liver for glycolysis or gluconeogenesis.

CHYLOMICRONS: 86% of TAG, ApoB48, ApoCII and ApoE

TRANSPORT OF DE NOVO SYNTHESIZED LIVER LIPIDS: VLDL AND LDL

Excess dietary fat is transformed into TAGs, which are packaged together with cholesterol and cholesterol esters into VLDL (very low-density lipoprotein) in Golgi Apparatus.

Excess CH and glucose activates the biosynthesis of FA and TAGs and are exported as VLDLs.



VLDL

VLDL: **Rich in triglycerides (50%)**, low in cholesterol 14%.
Apolipoproteins: ApoB100, nascent and mature apoCI-III and apoE (from HDL).
Density and size: 1,006 g/mL and 30-80 nm.

FUNCTION: transport *de novo* synthesis of lipids to muscle and adipose tissue.
They become IDL (intermediate density lipoproteins); by the loss of apoCII and apoE VLDL are converted into LDL.
Excess remnants of VLDL are eliminated by the liver mediated by receptors (e.g. LDLR, VLDLR and LRP).

LDL

LDL: POOR in triglycerides, **RICH IN CHOLESTEROL** 46%.
Apolipoproteins: ApoB100.
Density and size: 1.019-1.063 g/mL and 18-25 nm.
LDL TRANSPORT: LIPIDS TO TISSUES possessing LDLR. Delivery of cholesterol. The excess is metabolized and eliminated in the liver by **LDLR.**

TRANSPORT OF DE NOVO SYNTHESIZED LIVER LIPIDS by VLDL AND LDL

HÍGADO
Apo B-100
TAG>CE,C
VLDL naciente
LDLR

Apo C-II
Apo E
(de la HDL)

Apo B-100
Apo E
TAG>CE,C
Apo C-II
VLDL

2 Apo C-II y apo E se transfieren de HDL al quilomicrón naciente.

1 El hígado secreta partículas nacientes, ricas en TAG, de VLDL que contienen principalmente lípidos de síntesis endógena.

CAPILARES

TEJIDOS
(por ejemplo, adiposo)

3 *Lipoproteína lipasa* extracelular, activada por apo C-II, degrada a TAG en VLDL.

HL: hepatic lipase and
LPL: lipoprotein lipase

Ácidos grasos libres

Glicerol

Al HÍGADO

5 LDL se une a receptores específicos en tejidos extrahepáticos y en el hígado y se somete a endocitosis.

TEJIDO EXTRA-HEPÁTICO
LDLR

Apo B-100
CE,C >TAG
LDL

Apo C-II
y apo E
(hacia HDL)

IDL

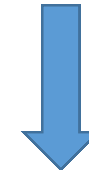
4 Apo C-II y apo E se devuelven a HDL.

VLDL AND IDL REMNANTS can be cleared from the blood by hepatic receptors through apolipoprotein E binding.

LDL is cleared by hepatic LDLR through binding of the apoB100.

VLDL

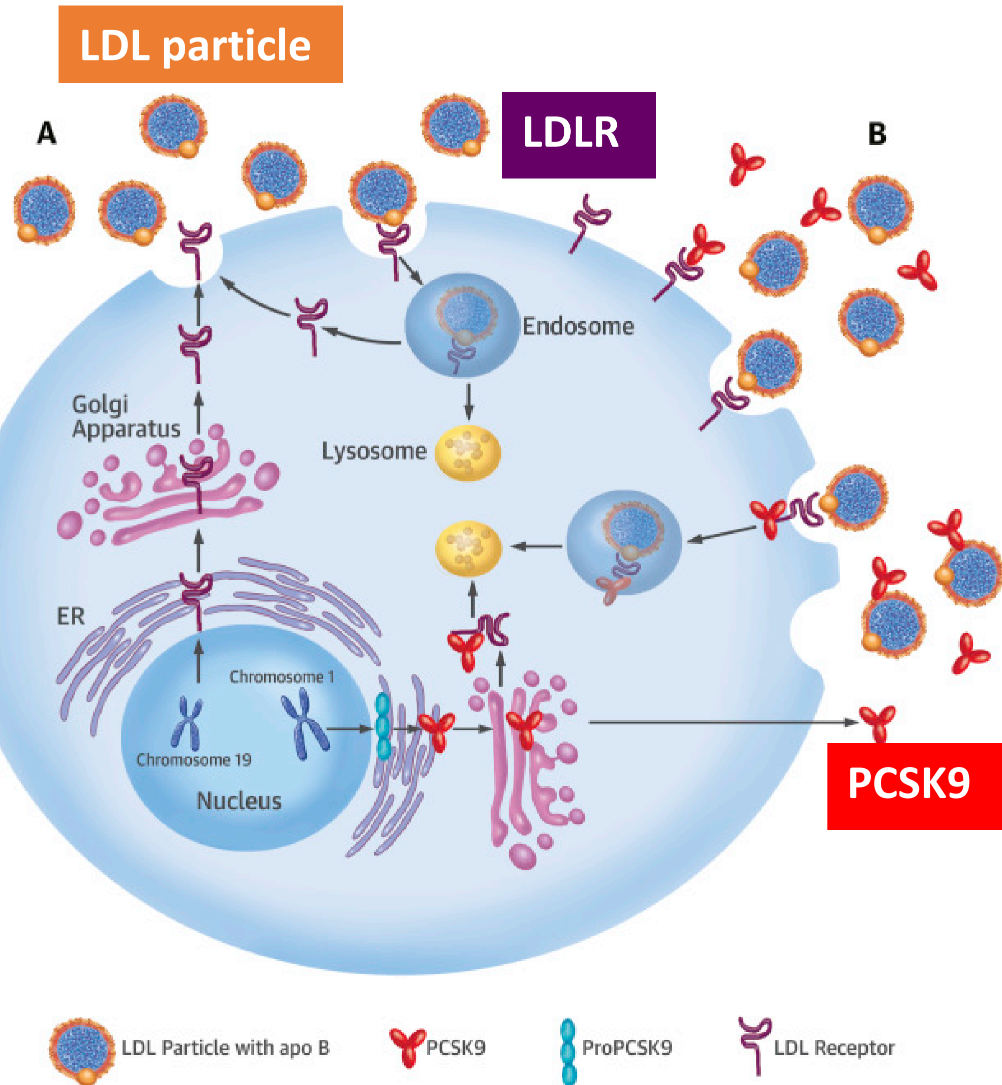
IDL



LDL

By the action of LPL and HL, (LOSS OF TAG AND PHOSPHOLIPIDS) the lipoproteins become denser and more cholesterol-rich.

THE REMOVAL OF LIPOPROTEINS FROM THE BLOOD IS MEDIATED BY RECEPTORS



FROM: RS Rosenson, RA Hegele, S.Fazio, CPCannon, **2018**, ***J Am Coll Cardiol***, 72, 314-329

LDLR receptor-mediated LDL UPTAKE:

1. LDL undergoes ApoB100-mediated enocytosis (though LDL can also bind to apoE).
2. Inside the cells, the vesicle fusions with lysosomes and recycling of components: release of fatty acids, cholesterol, and degradation of apoB.
3. Recycling of LDLR to the membrane.

LDR degradation is mediated by PCSK9:
The proprotein convertase subtilisin/kexin type 9 (PCSK9) promotes the LDLR internalization and lysosomal degradation of the receptor.

https://www.nejm.org/do/10.1056/NEJMdo005109/full/

"Inclisiran: *A New Approach to Targeting PCSK9*"

A Highly Durable RNAi Therapeutic Inhibitor of PCSK9

N Engl J Med **2017**; 376:41-51 DOI: 10.1056/NEJMoa1609243

https://www.nejm.org/do/10.1056/NEJMdo005109/full/

TRANSPORT OF LIPIDS MEDIATED BY HDL: CHOLESTEROL REVERSE TRANSPORT

HDL

HDL composition: **POOR** in triglycerides 10%, CHOLESTEROL UP TO 21%.
RICH IN Apolipoproteins: ApoAI-AII (50%).
Density and size: 1.063-1.21 g/mL and 8-20 nm.
HDL TRANSPORT: **REVERSE CHOLESTEROL TRANSPORT** MEDIATED BY APOAI AND AIDED BY ABCA1.

FUNCTIONS OF HDL: cholesterol reverse transport

1. Circulating reservoir of apo C-II and apo E (transfer to VLDL/IDL and chylomicrons)

2. Uptake of non-esterified cholesterol: nascent HDLs are rich in phospholipids and apoA, C and E.

THREE ENZYMES ACT IN THE MATURATION OF HDL:

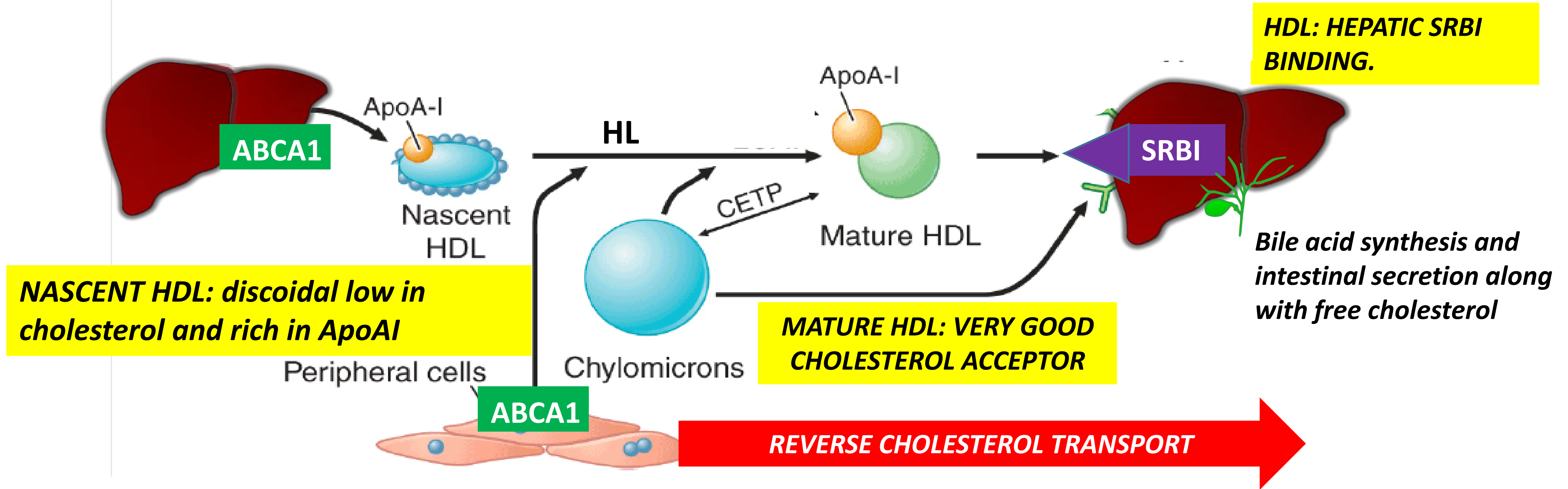
LCAT, which binds and acts on the nascent discoidal HDL and generates cholesteryl ester; HDL becomes HDL2 and then HDL3 as accumulates CE and then travels back to the liver.

HL, which degrades TAG and phospholipids, and participates in the conversion of HDL2 to HDL3.

CETP, which transfers some cholesteryl esters from HDL to VLDL in exchange for TAG.

HDL-MEDIATED LIPID TRANSPORT: REVERSE CHOLESTEROL TRANSPORT

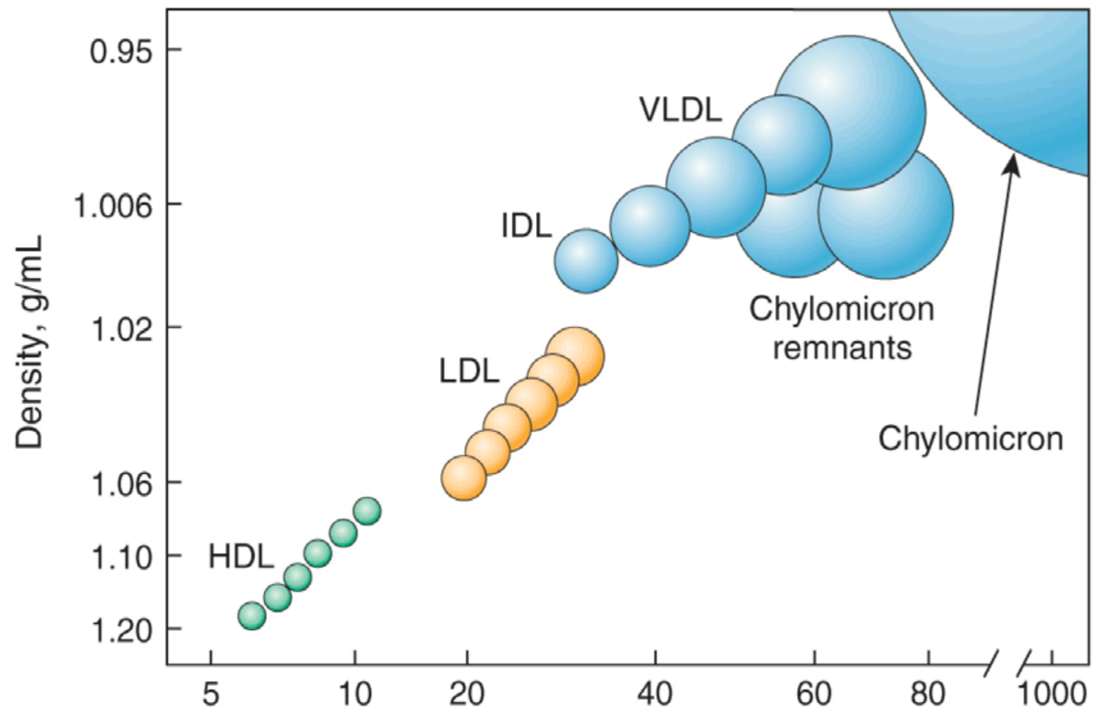
SRBI: scavenger receptor class B type I



Dan Rader and Helen Hobbs 2016
Disorders of Lipoprotein Metabolism | Thoracic Key

HDLS ARE BORN IN THE LIVER. IN THE BLOOD CIRCULATION VIA INTERACTION WITH ABCA1, THEY ACCEPT PERIPHERAL CHOLESTEROL FOR DELIVERY BACK TO THE LIVER VIA SR-BI. IN THE LIVER, HDL IS NOT INTERNALIZED AND SR-BI CAPTURES ONLY CHOLESTERYL ESTERS.

SUMMARY OF THE SIZE, COMPOSITION AND FUNCTION OF LIPOPORTEINS



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LPS	TAG	CHOLESTEROL	PHOSPHOLIPIDS	APOPROTEIN
CHYLOMICRONS	**86% RICH**	4%	8%	ApoB48, apoCII apoE
VLDL **IDL**	**50%** **38%** **RICH**	21% 38%	18% 23%	ApoB100 CI-CIII, apoE
LDL	10%	**46% RICH**	22%	apoB100
HDL	5-10%	14-21%	19-29%	**apoAI, AII RICH**

FUNCTION OF LIPOPROTEINS

CHYLOMICRONS: Dietary lipid distribution. Rich in TAG and low in cholesterol.
VLDL/IDL cholesterol: *De novo* synthetized lipid distribution. Rich in TAG and low in cholesterol.
LDL cholesterol: Transport of cholesterol *de novo* to peripheral tissues or back to the liver. Rich in cholesterol and poor in TAG.
HDL: Reverse transport of cholesterol. Rich in protein.

HYPERCHOLESTEROLEMIA AND PREMATURE CARDIOVASCULAR DISEASE

Elevated cholesterol levels decrease LDLR expression and affect the transport of remaining VLDL/IDL chylomicrons and LDL to the liver.

Mutations in LDLR (HF) and apoB affect the elimination of LDL cholesterol ABCA1 mutations (which decreases HDL cholesterol, *Tangier Disease*) and increase PCSK9 activity (which decreases hepatic LDLRs).

HYPERCHOLESTEROLEMIA: HIGH LEVELS OF CHOLESTEROL IN BLOOD PLASMA. CARDIOVASCULAR RISK FACTOR.

LDL CHOLESTEROL: “BAD CHOLESTEROL”

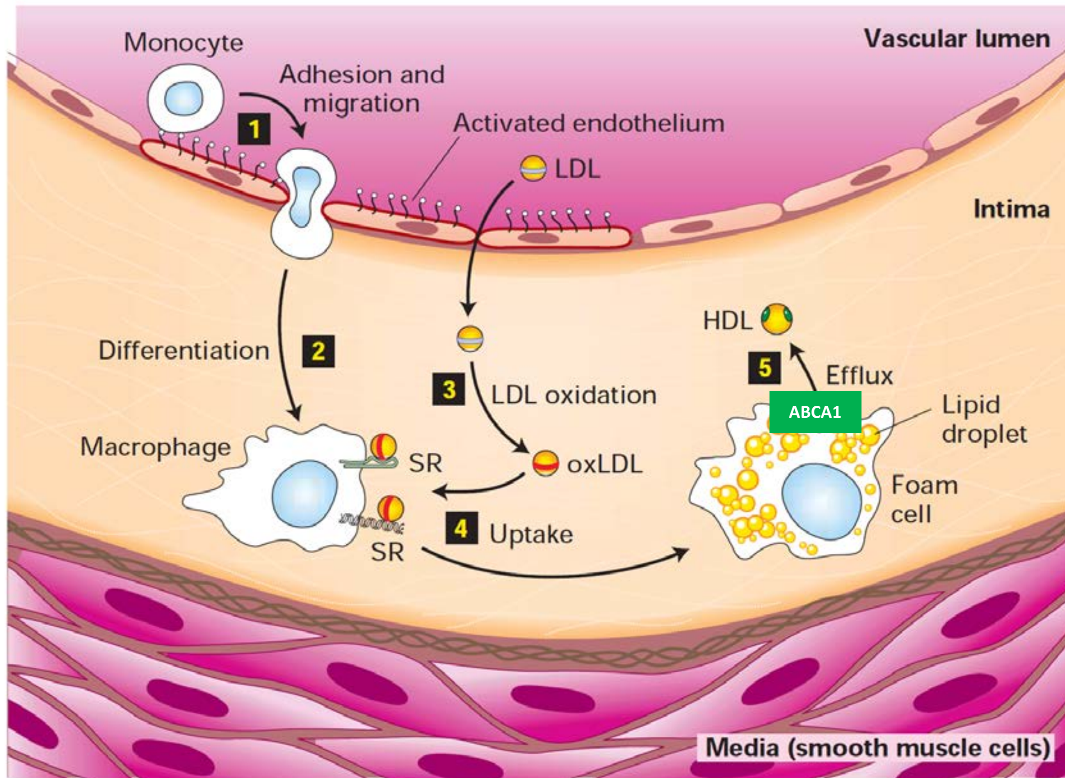
A decrease in LDLR in the tissues and liver produces an increase in LDL cholesterol in the blood that can accumulate and produce hypercholesterolemia. Similarly, VLDLs become LDLs if they are not eliminated by the liver.

HDL CHOLESTEROL: “GOOD CHOLESTEROL”

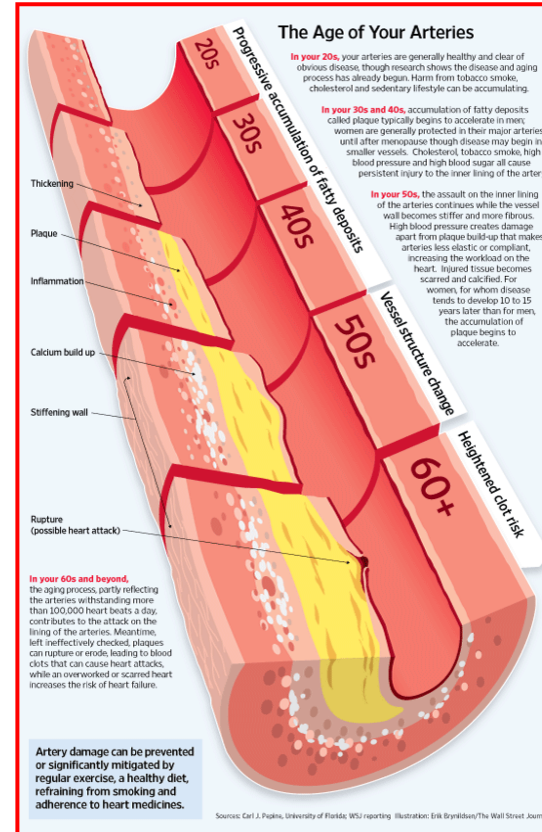
Functional HDLs can return excess cholesterol to the liver.

THE TRANSPORT SYSTEM FOR LIPOPROTEINS, ESPECIALLY CHOLESTEROL, IS NOT FULLY EFFICIENT AND HAS PATHOLOGICAL CONSEQUENCES FOR HUMANS.

ATHEROSCLEROSIS: A CHRONIC METABOLIC AND INFLAMMATORY DISEASE



Molecular Cell Biology: 5th Edition



WSJ reporting The Wall Street Journal

Atherosclerotic lesions are caused by the progressive accumulation of lipids and inflammatory cells in the vascular subendothelium of the arteries.

Macrophages in the subendothelial space

Oxidized LDL uptake (oxLDL) is mediated by scavenger receptor A (SR-A) and CD36. Lipids accumulate progressively in macrophages, which leads to the formation of foam cells. These accumulate and form theromatous lesions. The process is compensated by HDLs that capture cholesterol and return it to the liver. Over time, the lesions are aggravated through the participation of other types of immune cells (T lymphocytes), which can trigger thromboembolic phenomenon and acute events (e.g. infarction, stroke and embolism).

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ANNEX

REGULATION OF CHOLESTEROL SYNTHESIS: HMGCoA REDUCTASE

HMG CoA reductase (passage from HMGCoA to mevalonate): a limiting and the main step in the regulation of biosynthesis.

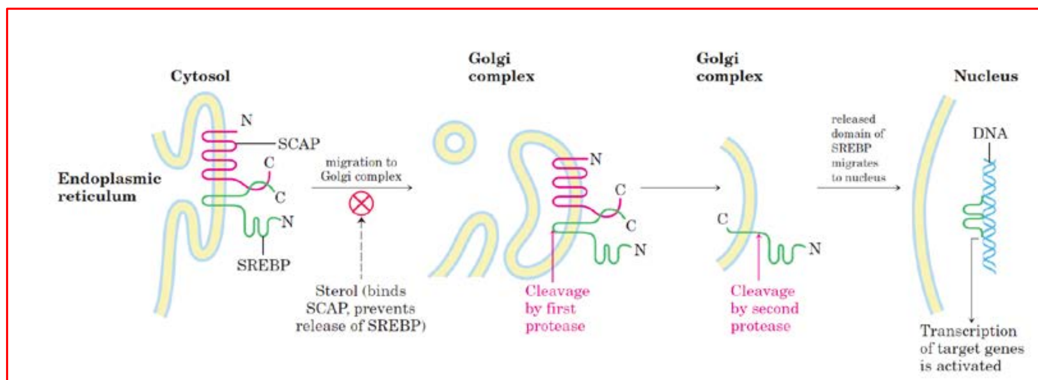
Regulation of the gene expression of the HMGCoA gene:

HMGCoA gene is activated by the transcriptional factor SREBP2; it has a sterol sensor site and a binding site to SRE sequences in DNA.

SREBP-2 is a smooth ER protein associated with another protein called SCAP (SREBP cleavage-activated protein).

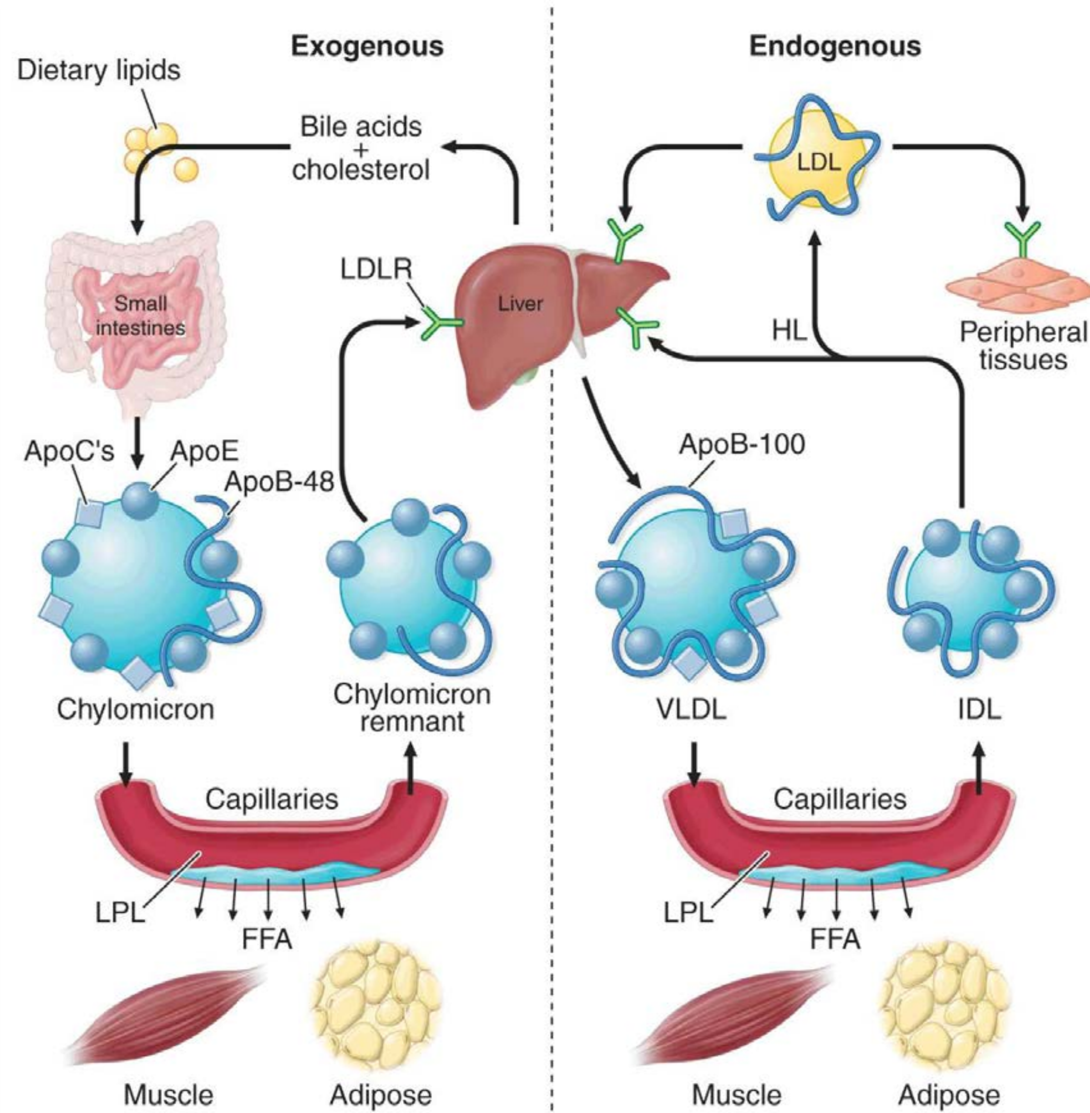
Low levels of sterols in the smooth ER: the SREBP-2-SCAP complex migrates to the Golgi apparatus. There, it is processed and released to SREBP, which is translocated to the nucleus and activates the HMGCoA gene, among others.
SREBP activates the expression of LDLR, which increases the uptake of LDL cholesterol in plasma.

High levels of sterols in the smooth ER: the sterols bind to SCAP and induce their binding to other proteins such as INSIG (insulin-induced gene) and the retention of the SCA-SREBP complex in the smooth ER.



REGULATION OF SREBP IN THE SMOOTH ENDOPLASMIC RETICULUM BY SCAP AND TRANSLOCATION TO THE NUCLEUS

LIPOPROTEIN TRANSPORT



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