Tema 7:

Mecanismos químicos de las reacciones enzimáticas

CLASE 1. OXIDORREDUCTASAS

CLASE 2 . TRANSFERASAS

CLASE 3. HIDROLASAS

CLASE 4 . LIASAS

CLASE 5. ISOMERASAS

CLASE 6 LIGASAS

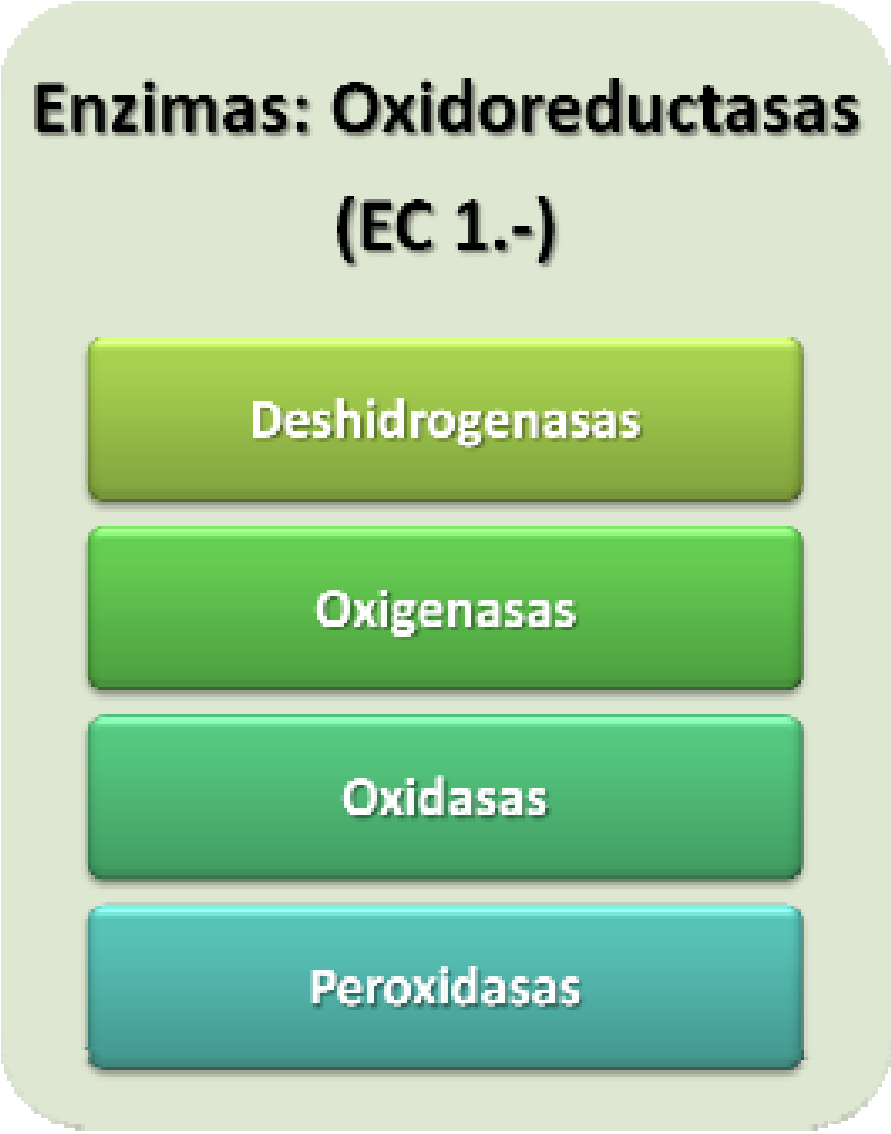
E.C. Enzyme Commission Numbers

KEGG

ENZYME: 1.3.1.31

Help

Entry	EC 1.3.1.31 Enzyme
Name	2-enoate reductase; enoate reductase
Class	Oxidoreductases; EC 1.3.1.31 Acting on the CH-CH group of donors; EC 1.3.1.31 With NAD+ or NADP+ as acceptor EC 1.3.1.31 BRITE hierarchy
Sysname	butanoate:NAD+ Delta2-oxidoreductase
Reaction(IUBMB)	butanoate + NAD+ = but-2-enoate + NADH + H+ [RN:R01689]
Reaction(KEGG)	R01689; (other) R02252 Reaction
Substrate	butanoate [CPD:C00246]; NAD+ [CPD:C00003]
Product	but-2-enoate; NADH [CPD:C00004]; H+ [CPD:C00080]
Comment	An iron-sulfur-flavoprotein (FAD). Acts (in the reverse direction) on a wide range of alkyl and aryl alphabeta-unsaturated carboxylate ions; but-2-enoate was the best substrate tested.
History	EC 1.3.1.31 created 1982
Pathway	ec00360 Phenylalanine metabolism ec01120 Microbial metabolism in diverse environments



E.C. Enzyme Commission Numbers

Cofactores

Iones metálicos

Cu^{2+}

Cu^{1+}

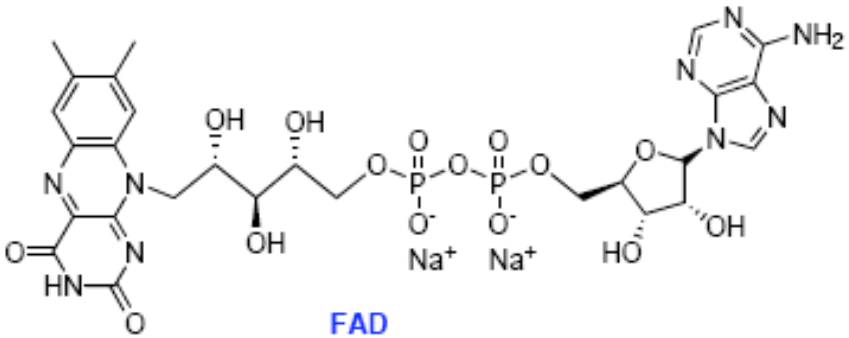
Fe^{2+}

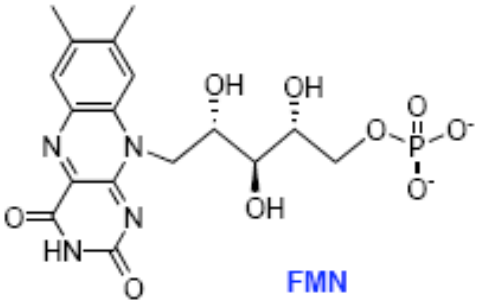
Co^{2+}

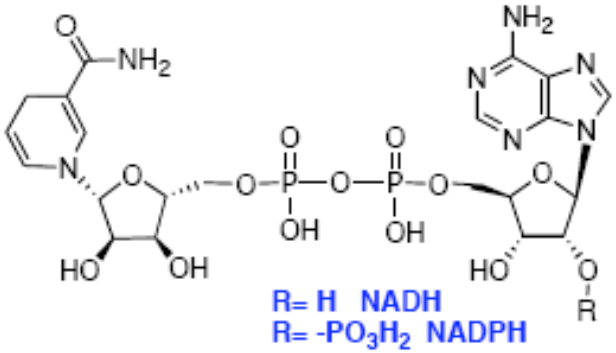
Mo^{2+}

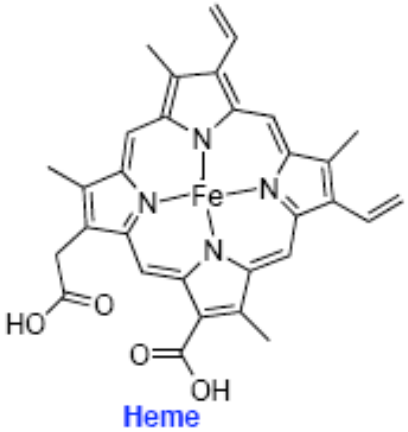
Zn^{2+}

Moléculas orgánicas: coenzimas → VITAMINAS







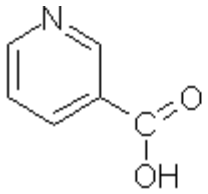


Cofactores redox

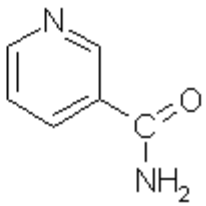
The Vitamins, Their Coenzymes, and Their Chemical Functions

Vitamin	Coenzyme	Reaction catalyzed	Human deficiency disease
Water-Soluble Vitamins			
Niacin (niacinate)	NAD^+, $NADP^+$	Oxidation	Pellagra
	NADH, NADPH	Reduction	
Riboflavin (vitamin B_2)	FAD, FMN	Oxidation	Skin inflammation
	$FADH_2$, $FMNH_2$	Reduction	
Thiamine (vitamin B_1)	Thiamine pyrophosphate (TPP)	Two-carbon transfer	Beriberi
Lipoic acid (lipoate)	Lipoate	Oxidation	—
	Dihydrolipoate	Reduction	
Pantothenic acid (pantothenate)	Coenzyme A (CoASH)	Acyl transfer	—
Biotin (vitamin H)	Biotin	Carboxylation	—
Pyridoxine (vitamin B_6)	Pyridoxal phosphate (PLP)	Decarboxylation	Anemia
		Transamination	
		Racemization	
		C_α—C_β bond cleavage	
		α,β-Elimination	
		β-Substitution	
Vitamin B_{12}	Coenzyme B_{12}	Isomerization	Pernicious anemia
Folic acid (folate)	Tetrahydrofolate (THF)	One-carbon transfer	Megaloblastic anemia
Ascorbic acid (vitamin C)	—	—	Scurvy
Water-Insoluble (lipid-soluble) Vitamins			
Vitamin A	—	—	—
Vitamin D	—	—	Rickets
Vitamin E	—	—	—
Vitamin K	Vitamin KH_2	Carboxylation	—

Niacina (Vitamina B3)



ácido nicotínico



nicotinamida

El ácido nicotínico (niacina) se convierte en el organismo en las coenzimas NAD+ y NADP+

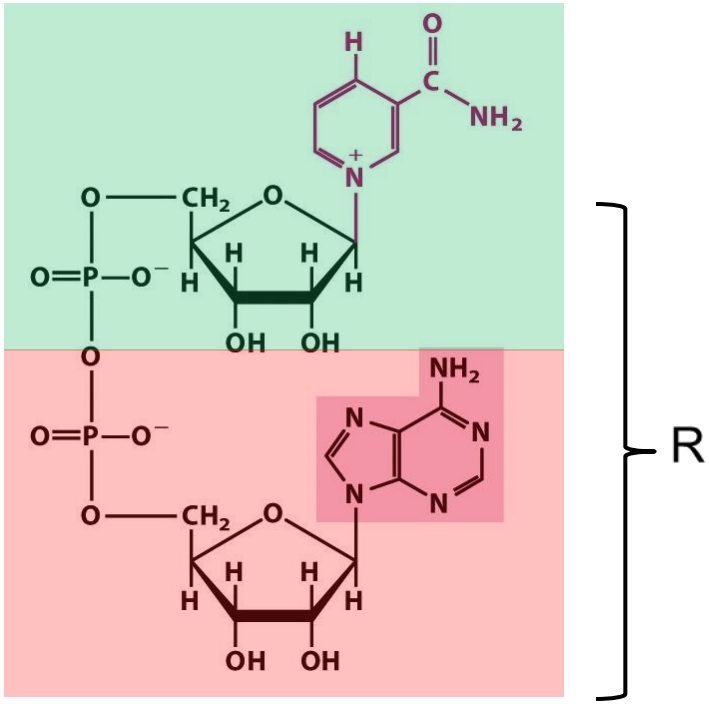
Requerimientos diarios: 18 mg (síntesis endógena a partir de Trp)

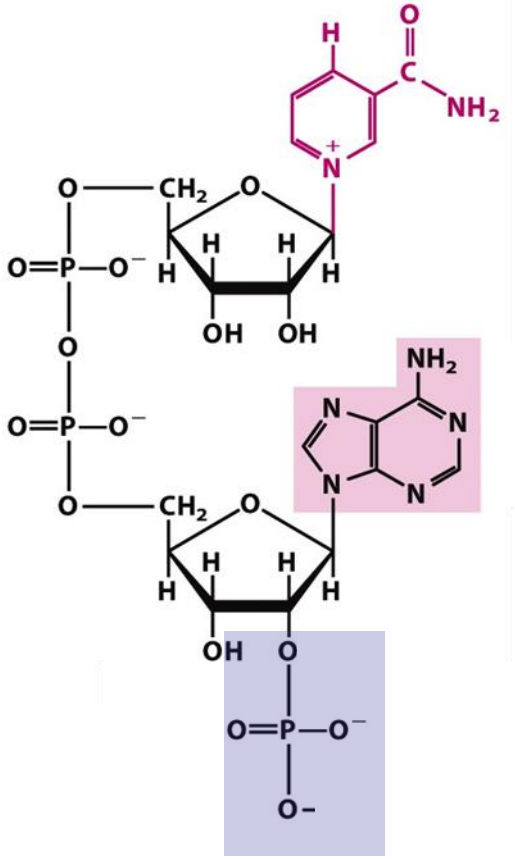
Fuentes naturales: levaduras y vegetales

Síntomas de carencia: Pelagra (enfermedad de las 3D: Dermatitis, diarreas, demencia)

su incidencia en los países occidentales es reducida gracias a que las harinas de trigo suelen estar enriquecidas con vitamina B

Bajo la **Ley 25630**, publicada en el Boletín Oficial N° 30213 del 14/08/02, las harinas para panificación en Argentina deberán estar adicionadas con 6,3 mg de tiamina, 1,3 mg de riboflavina, 13 mg de niacina, 2,2, mg de ácido fólico y 30 mg de hierro por kilo de harina





NAD$^+$

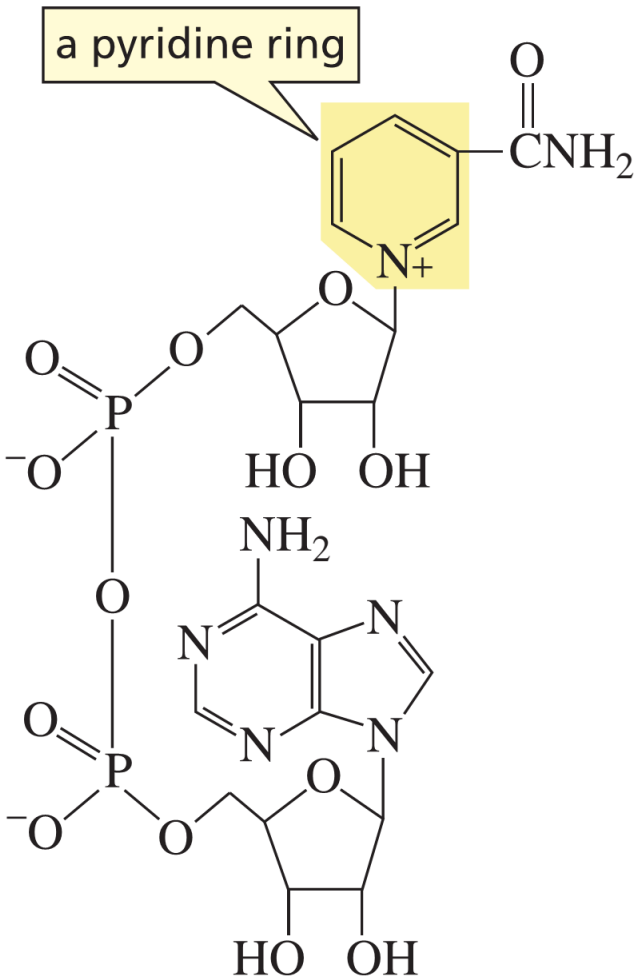
Nicotinamida Adenina Dinucleótido

NADP$^+$

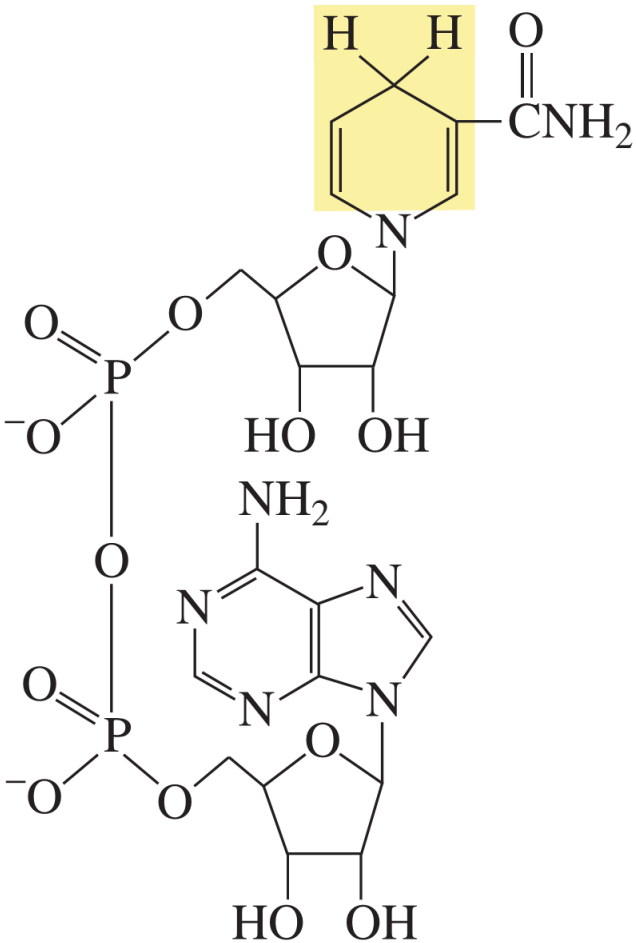
Nicotinamida Adenina Dinucleótido - Fosfato

$$\text{sustrato}_{\text{reducido}} + \text{NAD}^+ \underset{}{\overset{\text{enzima}}{\rightleftharpoons}} \text{sustrato}_{\text{oxidado}} + \text{NADH} + \text{H}^+$$

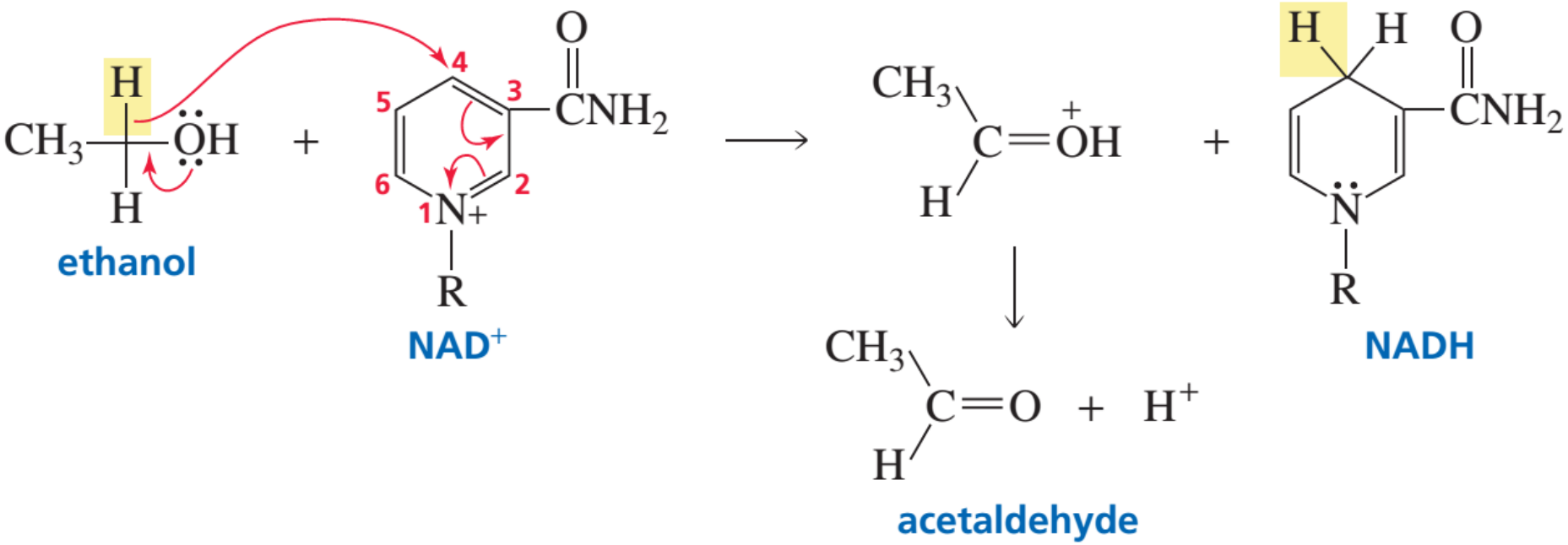
$$\text{sustrato}_{\text{reducido}} + \text{NADP}^+ \overset{\text{enzima}}{\rightleftharpoons} \text{sustrato}_{\text{oxidado}} + \text{NADPH} + \text{H}^+$$

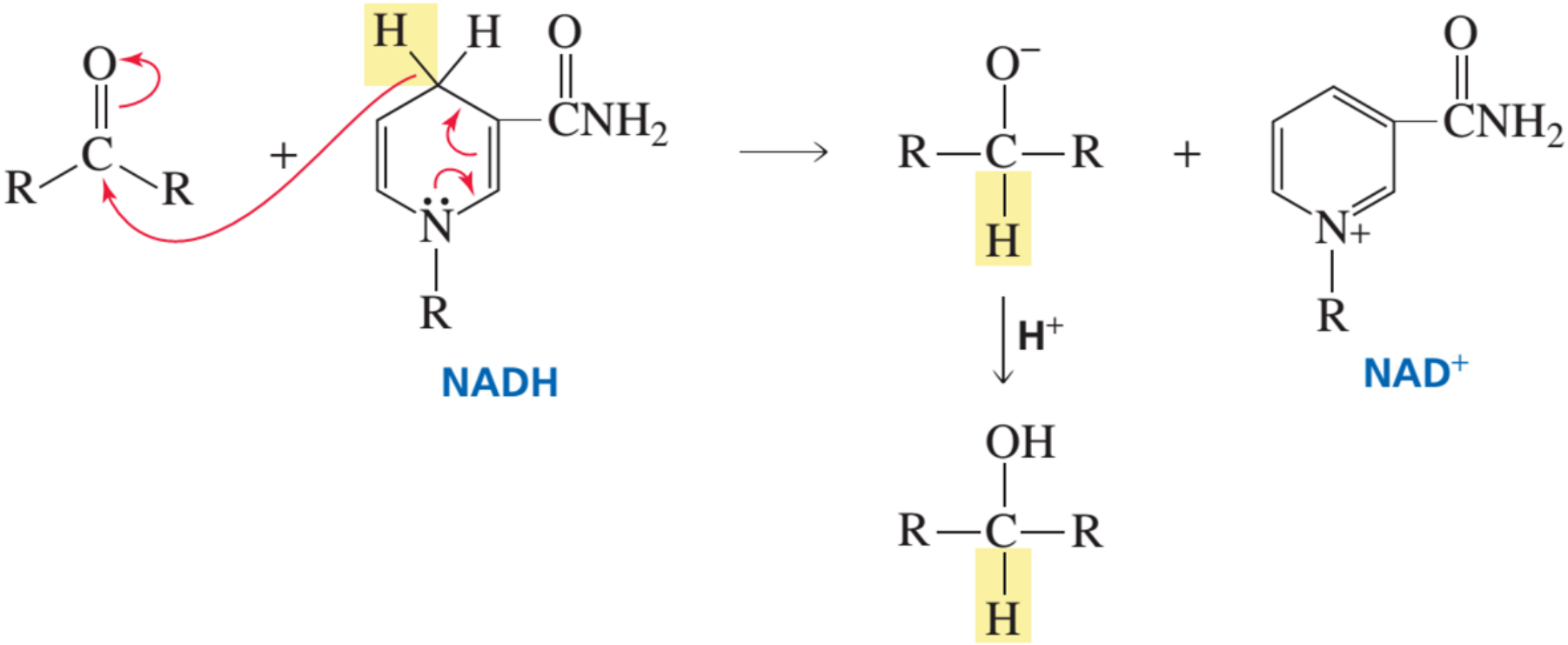


nicotinamide adenine dinucleotide
NAD^+

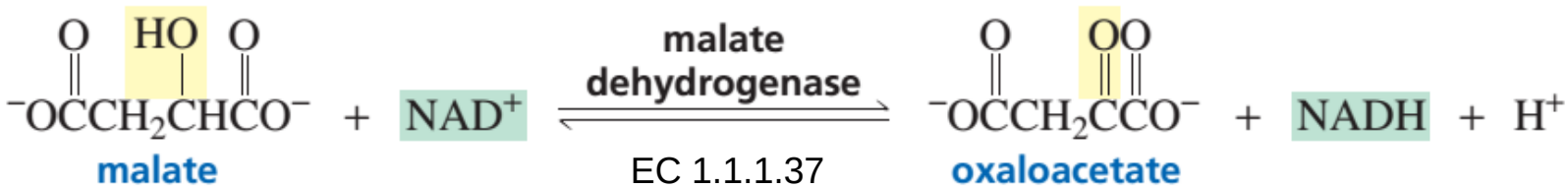


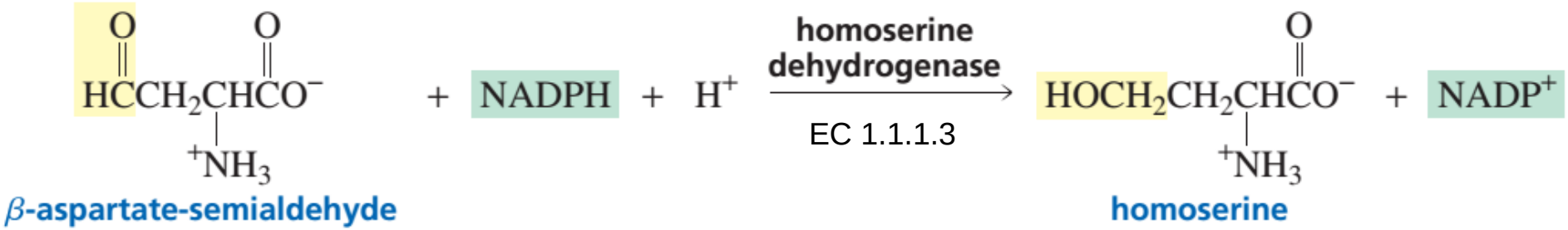
reduced nicotinamide adenine dinucleotide
NADH


H
CH3
OH
H
ethanol
+
O
CNH2
4
3
5
6
2
1
N+
R
NAD+
CH3
C=OH
H
+
H
H
O
CNH2
N
R
NADH
CH3
C=O
H
+ H+
acetaldehyde


O
C
R
R
+
H
H
O
CNH2
N
R
NADH
O⁻
R—C—R
H
H+
OH
R—C—R
H
+
O
CNH2
N+
R
NAD+

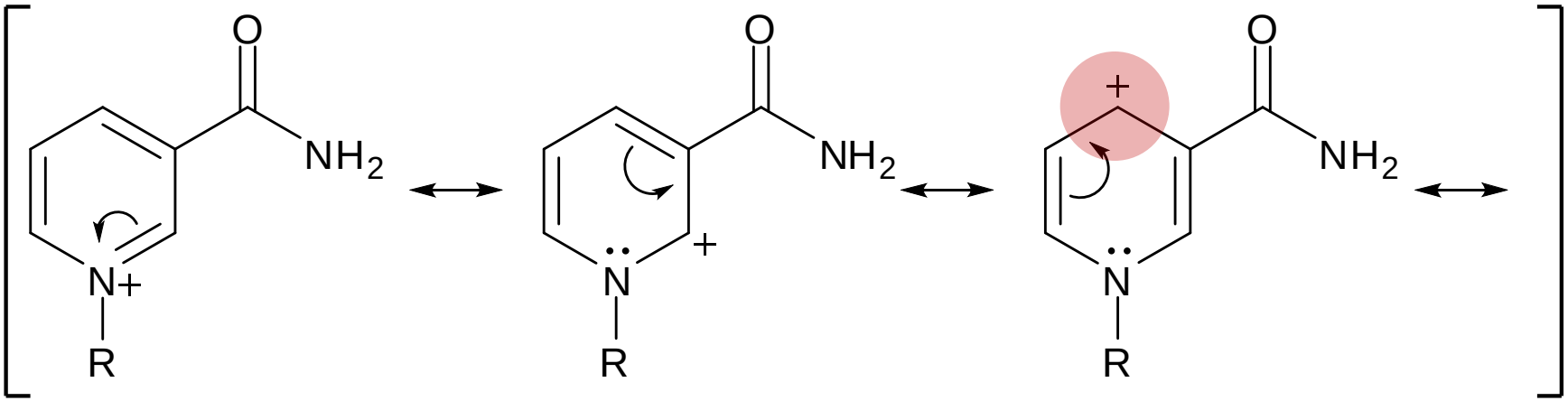
$[NAD^+]/[NADH] = 1000$

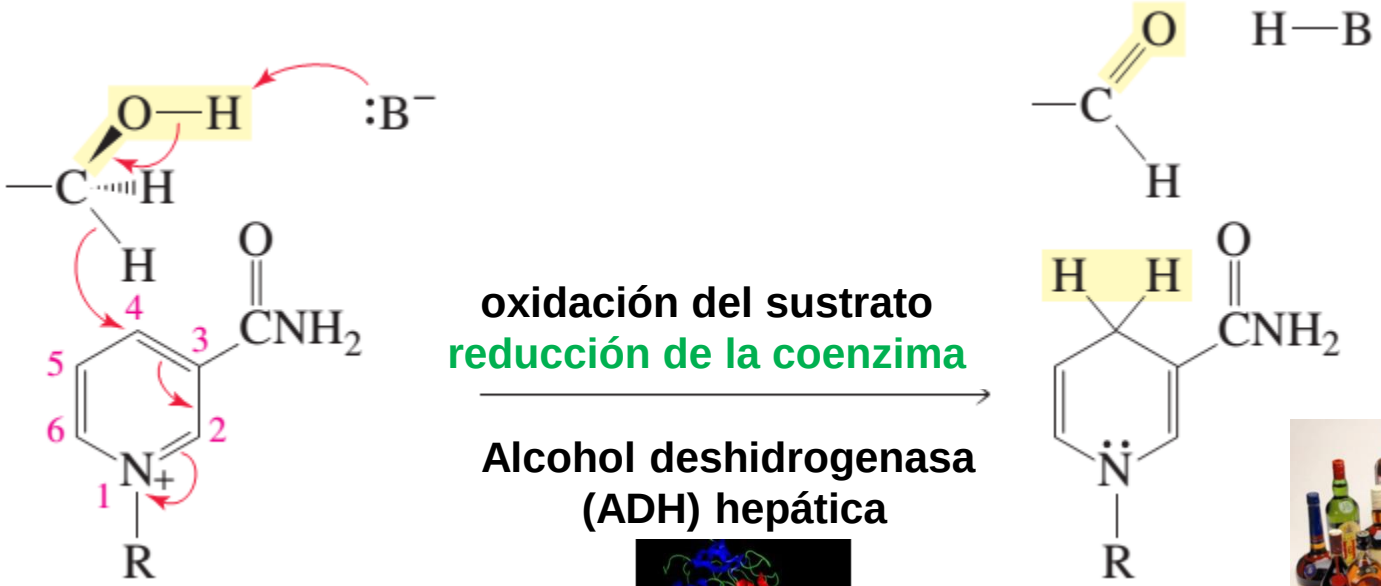




$[NADP^+]/[NADPH] = 0{,}01$

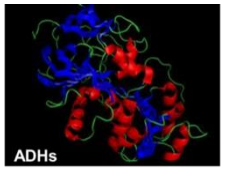
El **NAD(P)+** actúa como agente oxidante

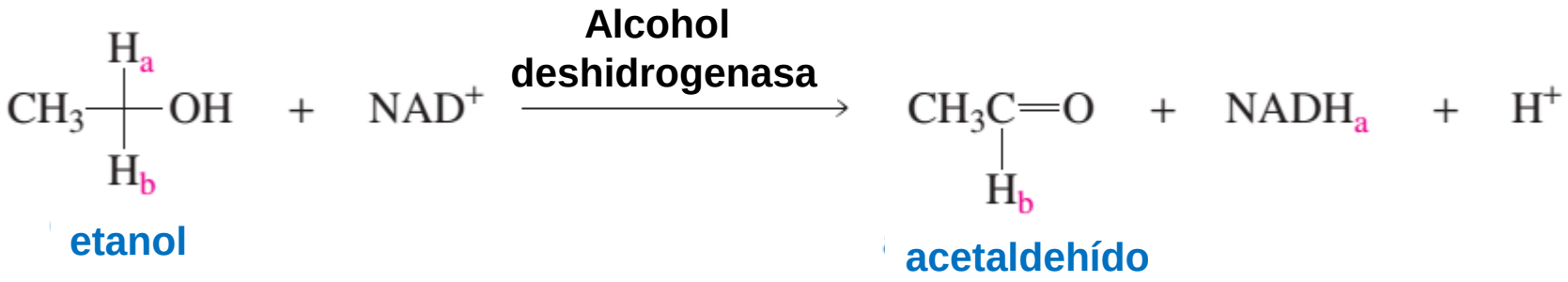


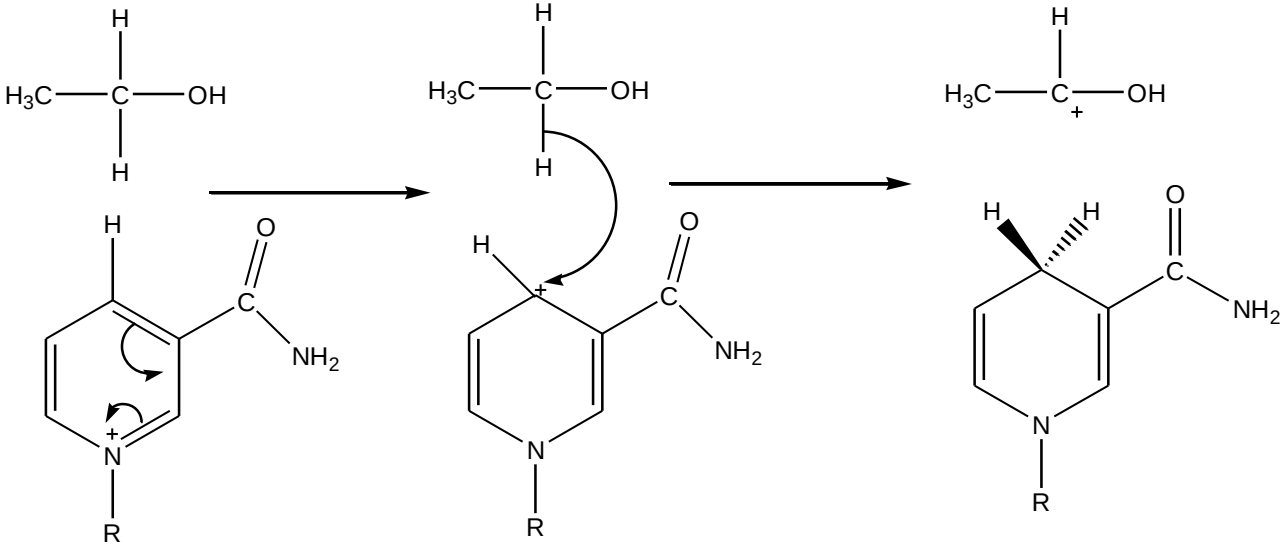


oxidación del sustrato
reducción de la coenzima

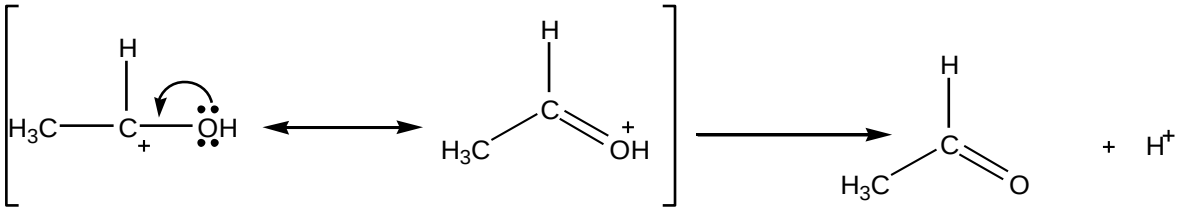
Alcohol deshidrogenasa (ADH) hepática



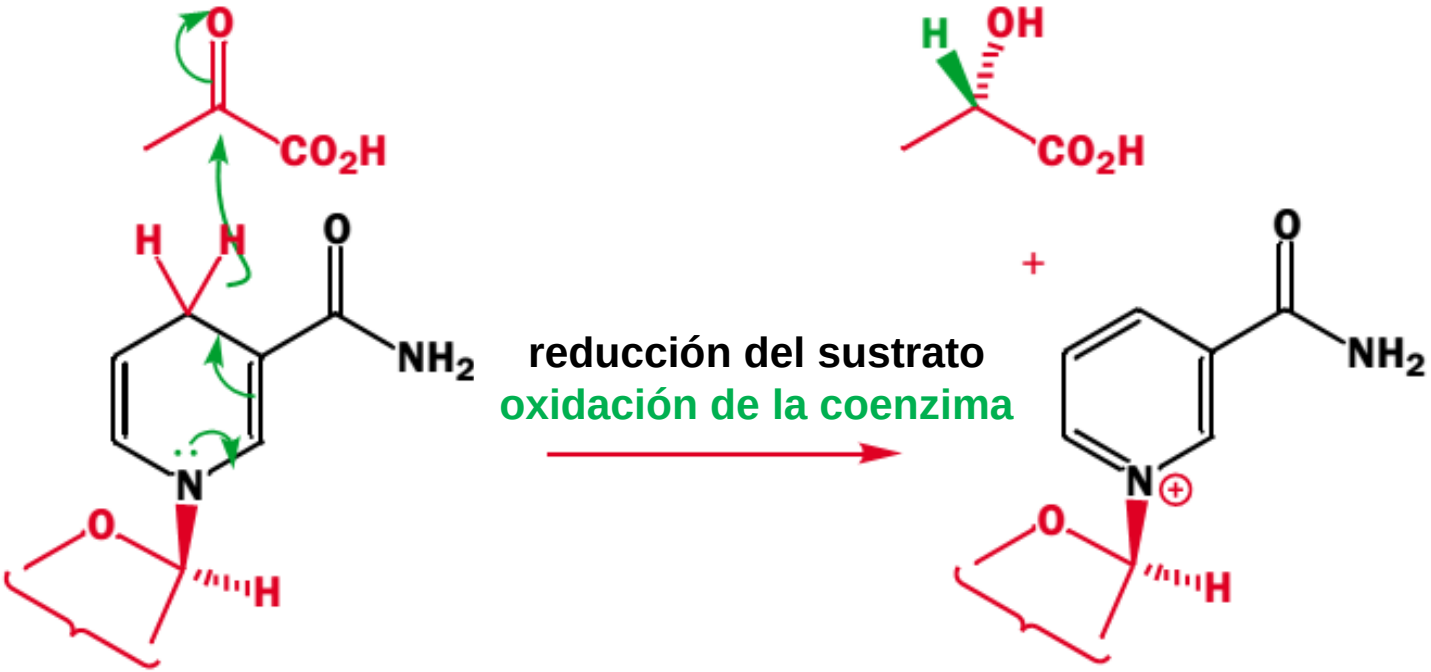




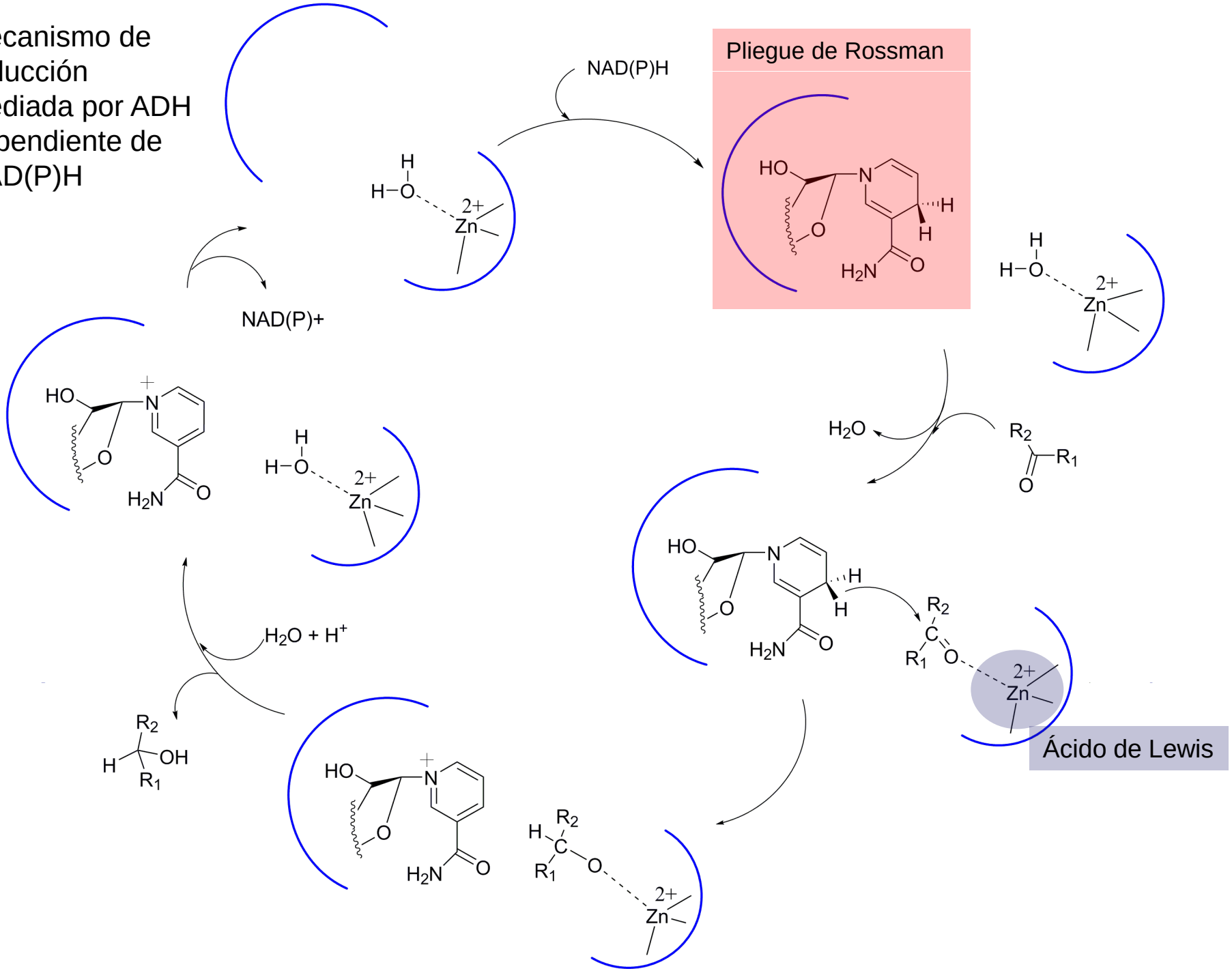
Por otra parte el carbocatión:



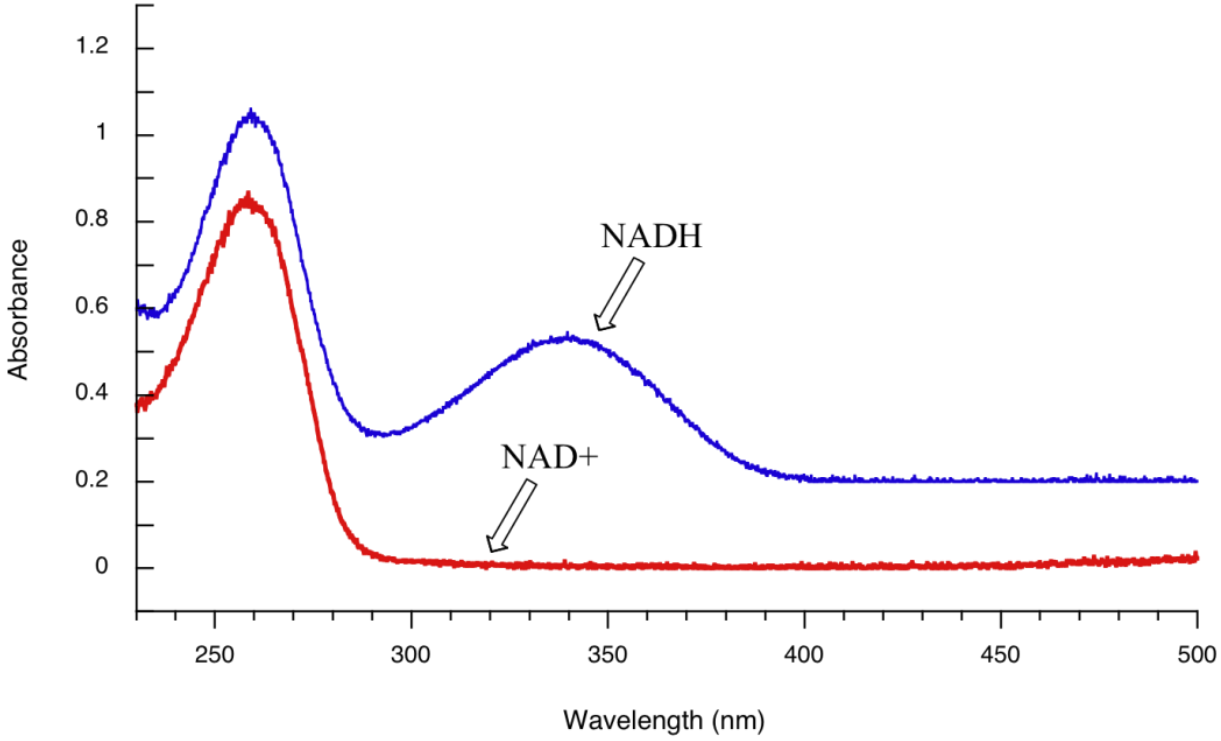
El **NAD(P)H** actúa como agente reductor



Mecanismo de reducción mediada por ADH dependiente de NAD(P)H

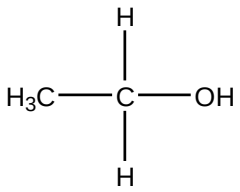


Se puede monitorear el progreso de una reacción enzimática redox midiendo la absorbancia de la mezcla de reacción a 340 nm determinando el NADH que se forma.

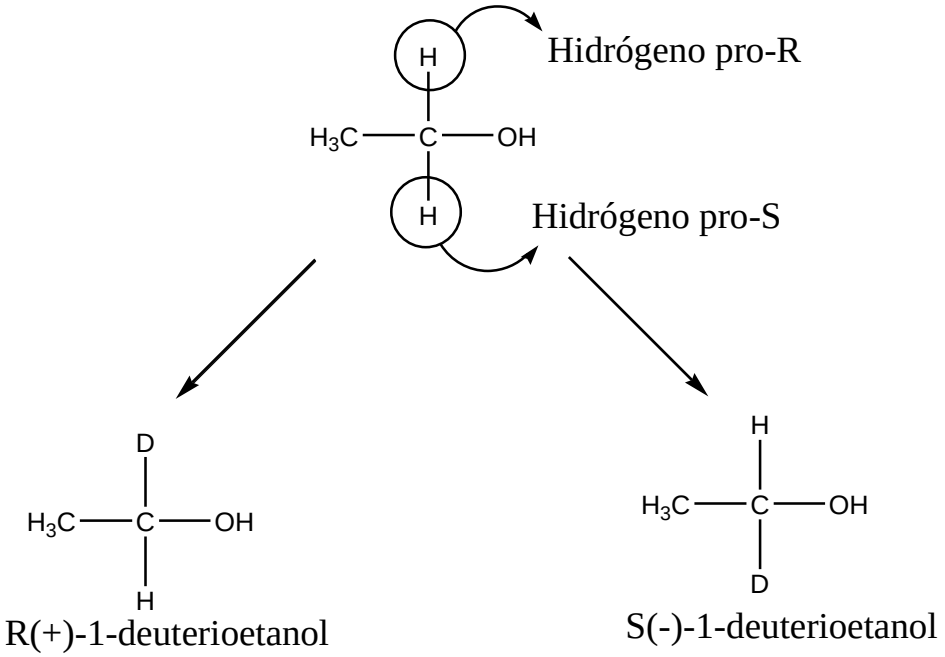


Estereoquímica de la reacción catalizada por la alcohol deshidrogenasa hepática

El sustrato de la alcohol deshidrogenasa es **NO QUIRAL:**

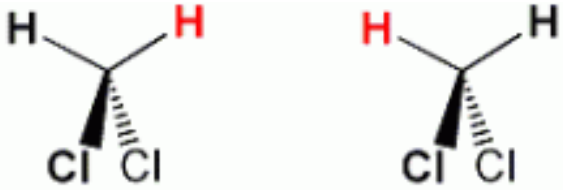


Sin embargo los α-hidrógenos son *enantiotópicos*, ya que reemplazando un H por D la molécula se torna quiral.

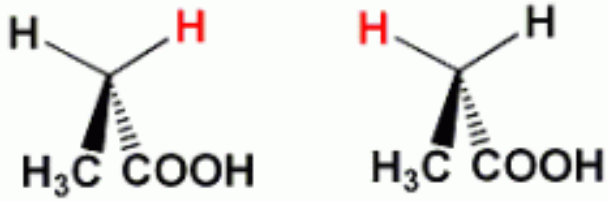


ENANTIÓMEROS

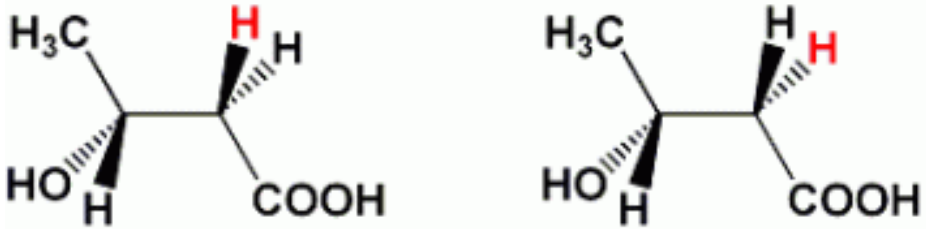
La alcohol deshidrogenasa es estereoselectiva y transfiere sólo el H pro *R* del etanol al NAD+



Homotópicos

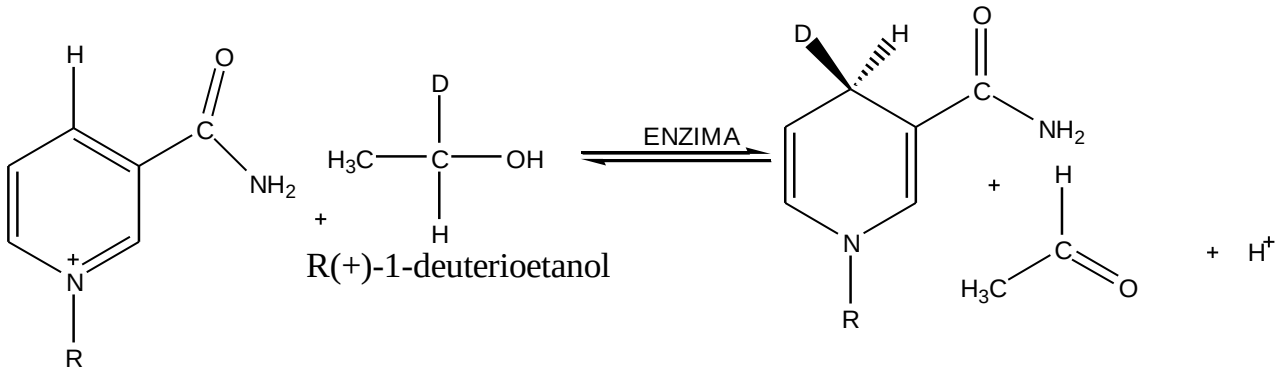


Enantiotópicos

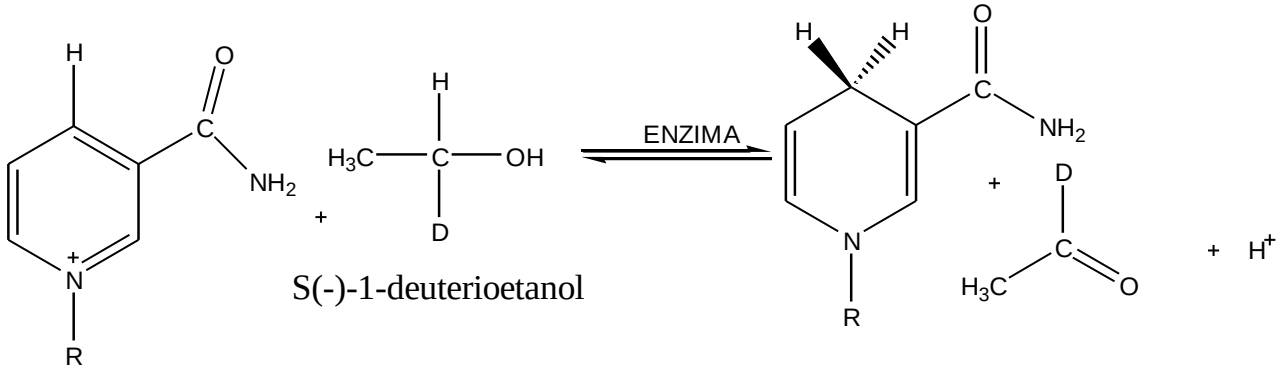


Diastereotópicos

La ADH hepática solo transfiere el D de R(+)-1-deuterioetanol al NAD^+

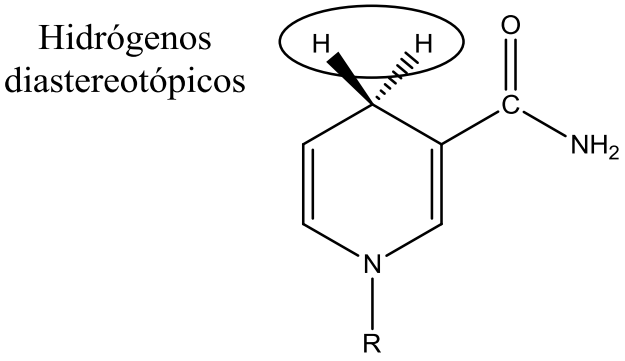


R(+)-1-deuterioetanol

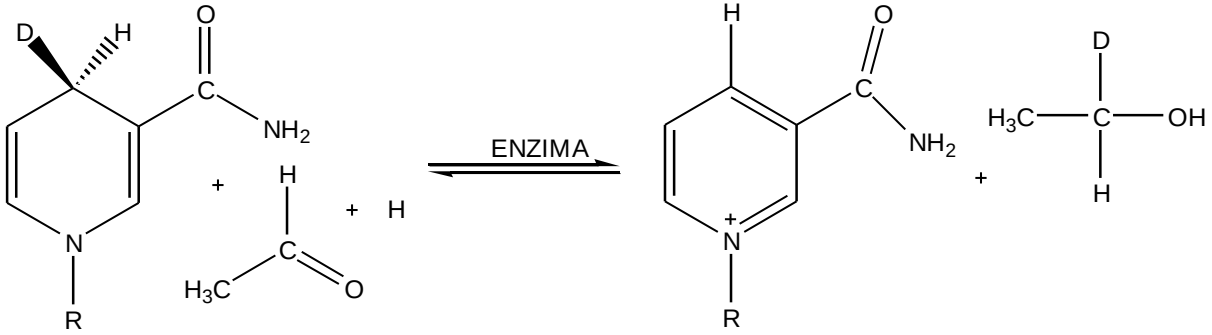


S(-)-1-deuterioetanol

Por otra parte, la enzima también distingue entre los hidrógenos unidos a C-4 del NADH en reacciones de reducción.



Así en la reacción:

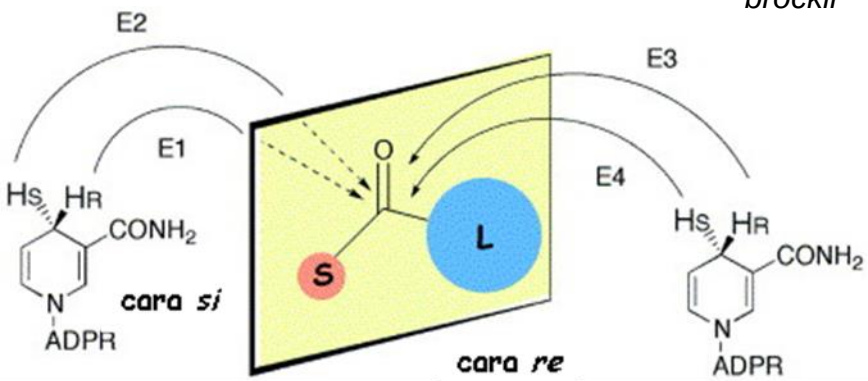


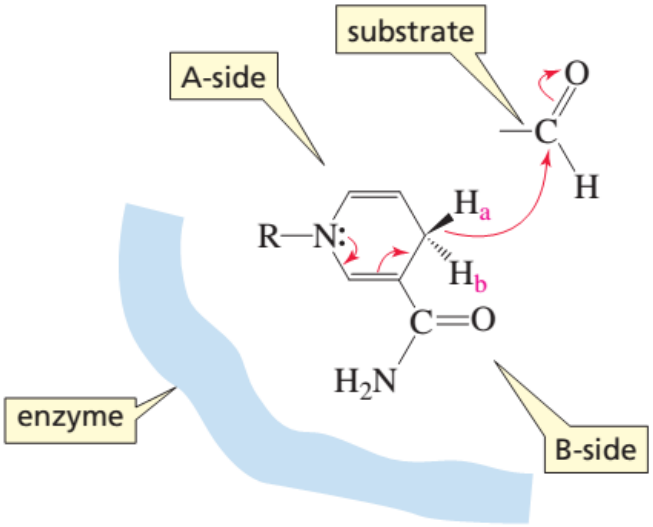
Se transfiere selectivamente el D desde el NADH para regenerar el R-(+)-1-deuterioetanol.

E1: ADH de *Pseudomonas* sp. y *Lactobacillus kefir*

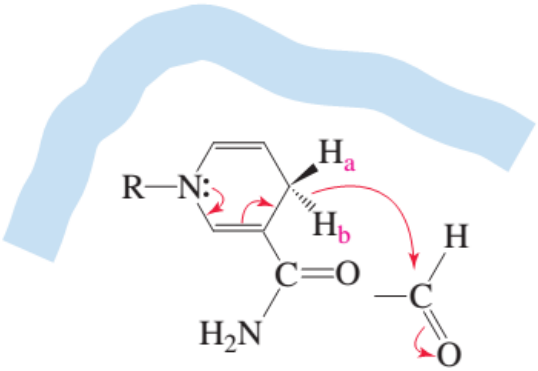
E2: Glicerol deshidrogenase de *Geotrichum candidum* y Dihidroxiactona deshidrogenasa de *Mucor javanicus*.

E3: ADH de levadura (*Saccharomyces cerevisiae*)
ADH de hígado de caballo
ADH de *Moraxella* sp. y *Thermoanaerobium brockii*



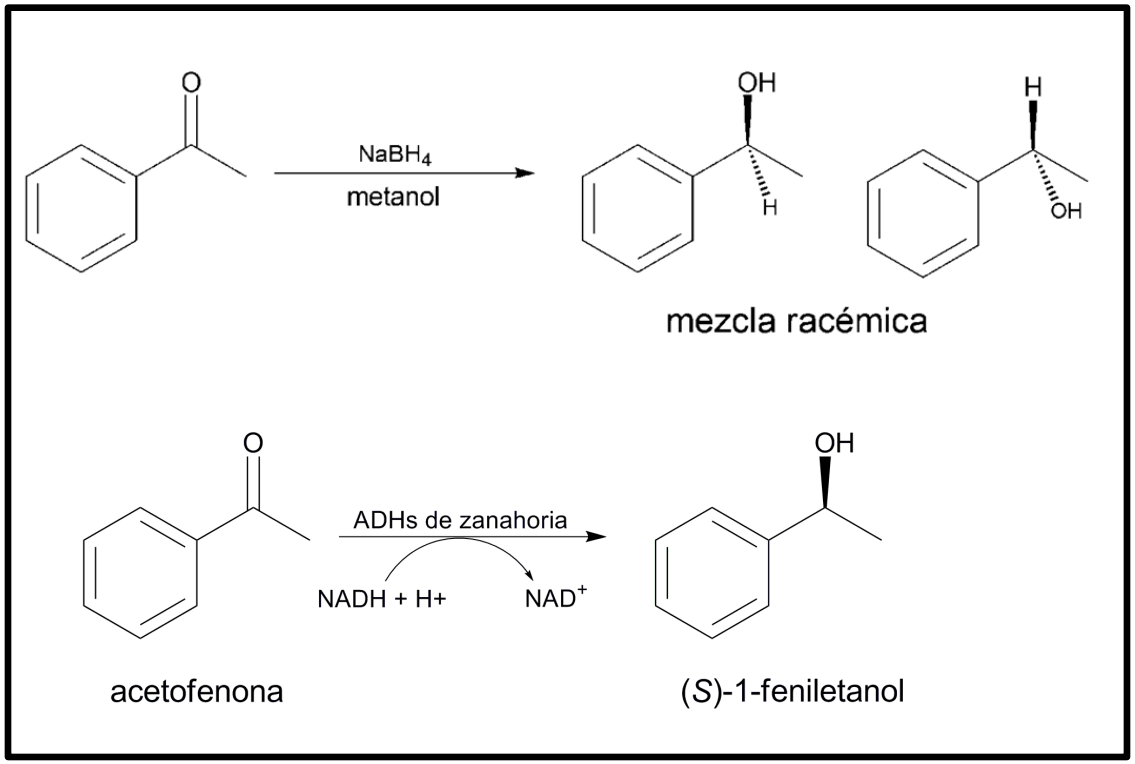


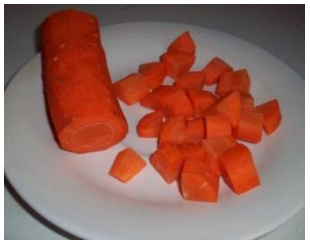
The enzyme blocks the B-side of the coenzyme, so the substrate binds to the A-side.

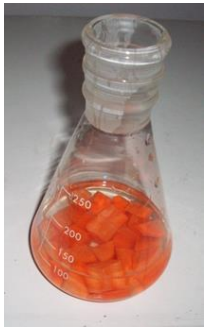


The enzyme blocks the A-side of the coenzyme, so the substrate binds to the B-side.

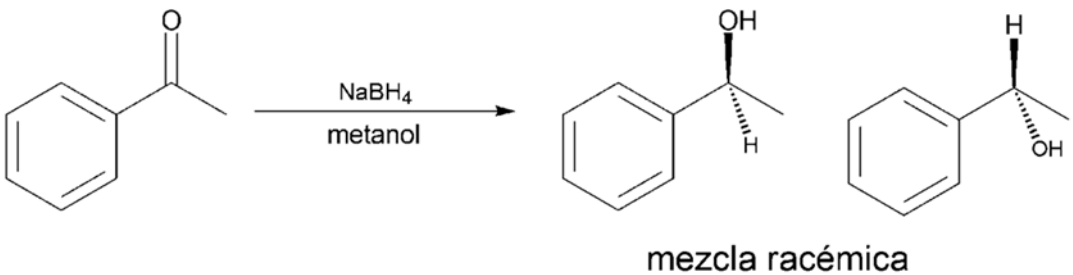
Trabajo Práctico de Laboratorio:
Reducción estereoselectiva de acetofenona mediada por alcohol-deshidrogenasas dependientes de NAD(P)H de zanahoria. Comparación con la reducción química.

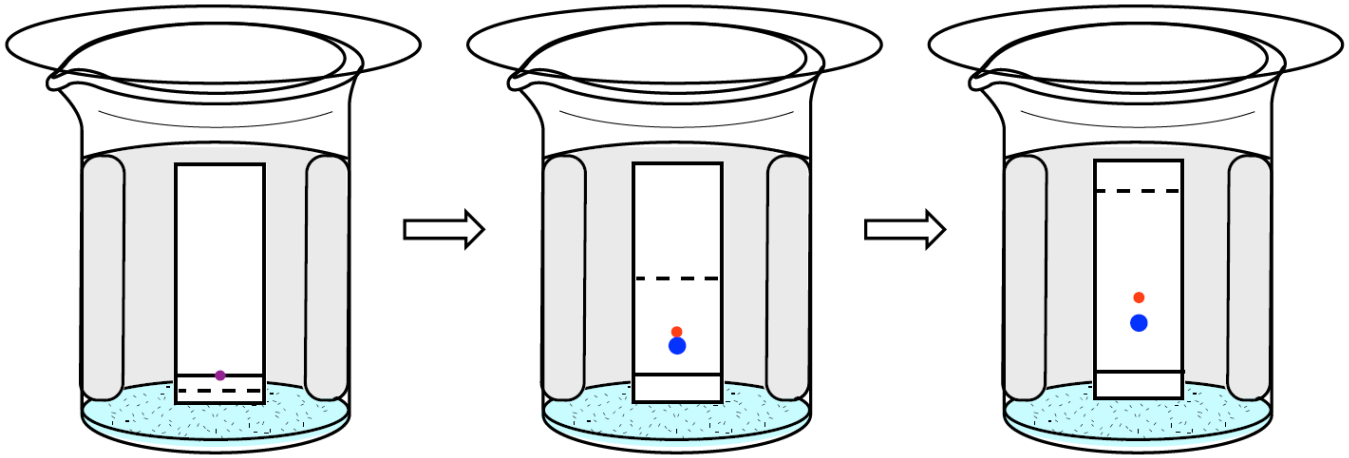






Análisis por TLC



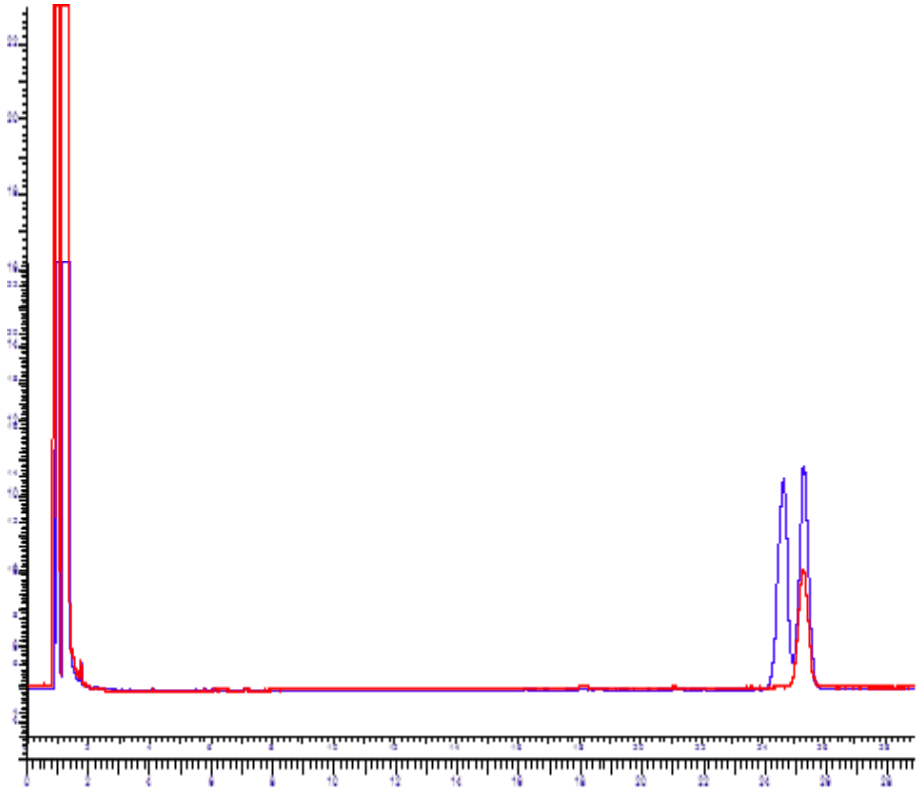


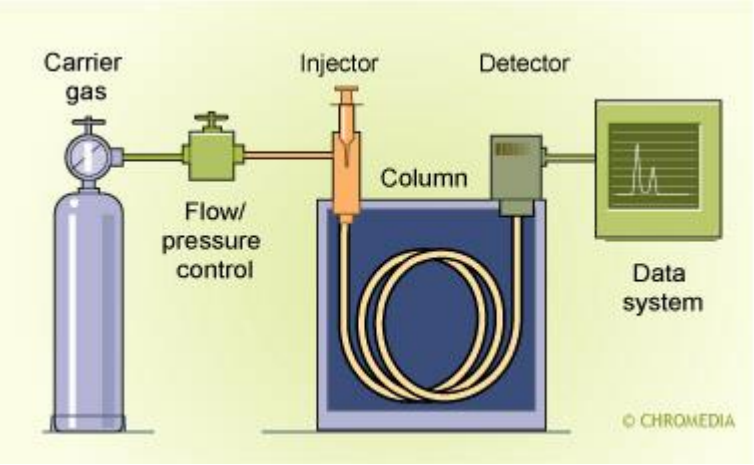
Análisis por cromatografía gaseosa y cálculo del exceso enantiomérico (*ee*)

El *ee* puede ser determinado si se conoce la cantidad de cada enantiómero producido:

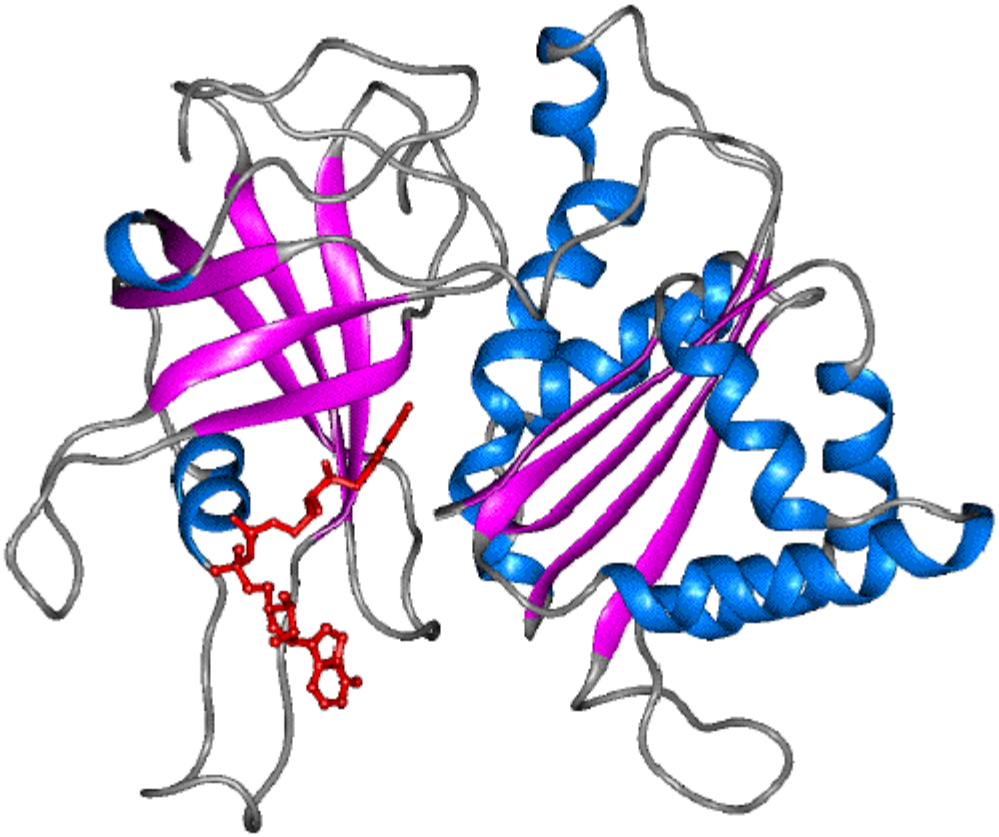
$$ee = ((R - S) / (R + S)) \times 100$$

R y *S* son las respectivas fracciones de los enantiómeros en una mezcla tal que $R + S = 1$





Flavoproteínas



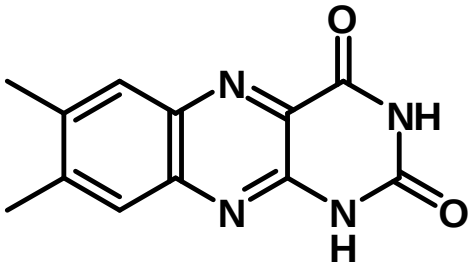
Vitamina B_2

Requerimientos diarios: 1,7 mg

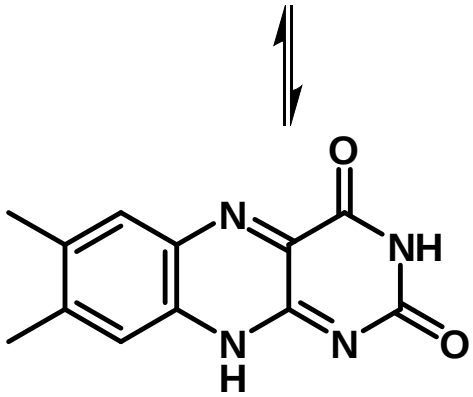
Fuentes naturales: en forma libre en microorganismos (levaduras) vegetales de hojas verdes

Síntomas de carencia: Dermatitis

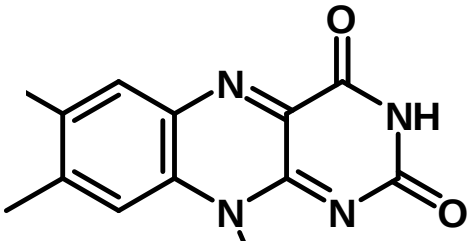
RIBOFLAVINA VITAMINA B_2

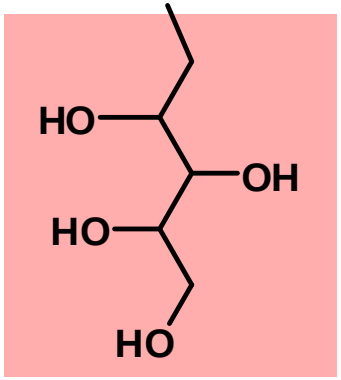


7,8-dimetil-aloxazina



7,8-dimetil-isoaloxazina
(flavina)
Del lat. flavus: amarillo



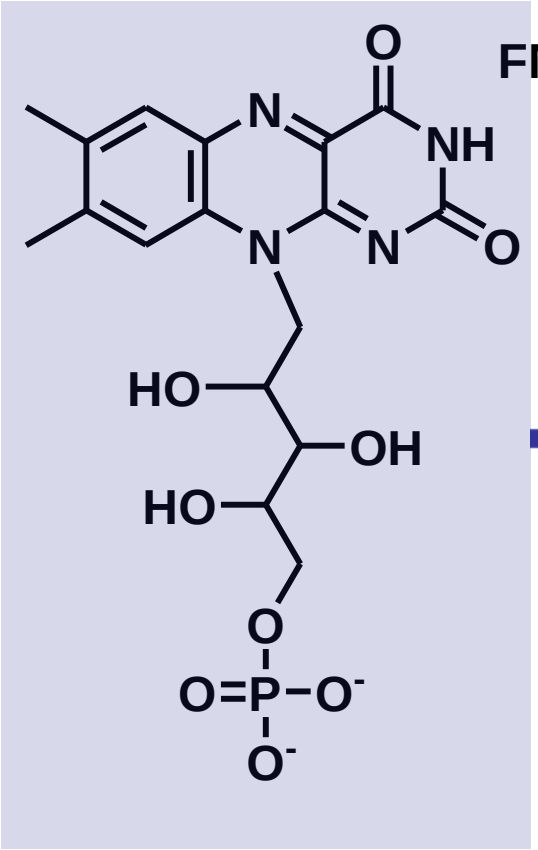


ribitol

RIBOFLAVINA VITAMINA B_2

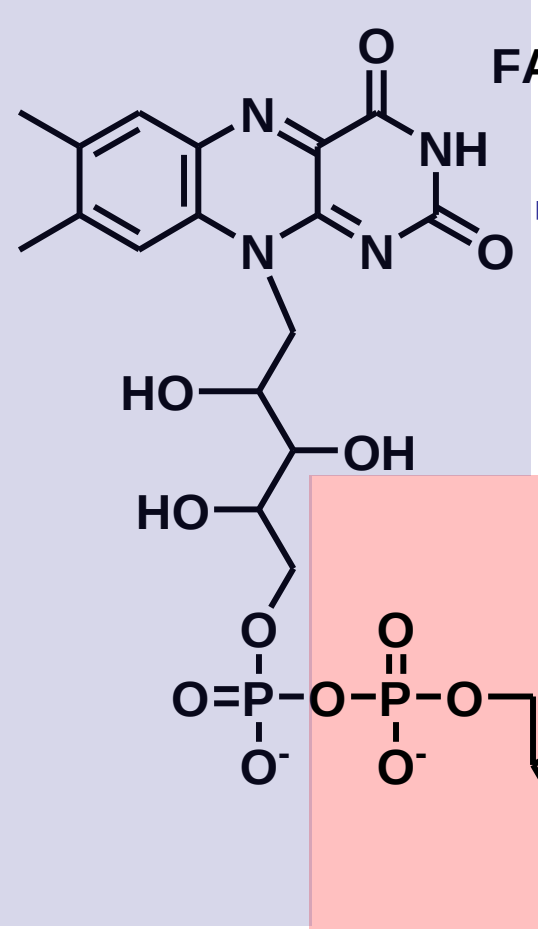
Coenzimas

Flavín adenín dinucleótido

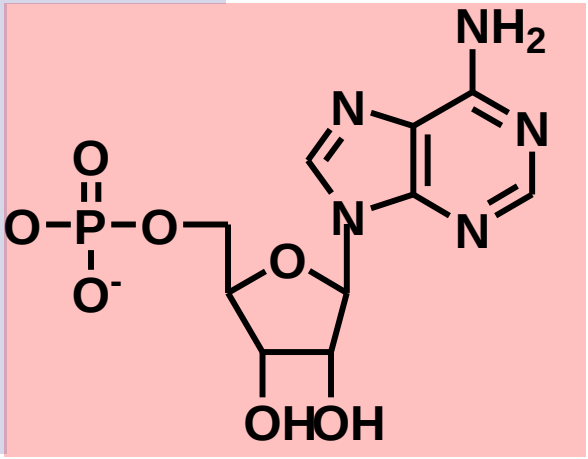


nucleótido

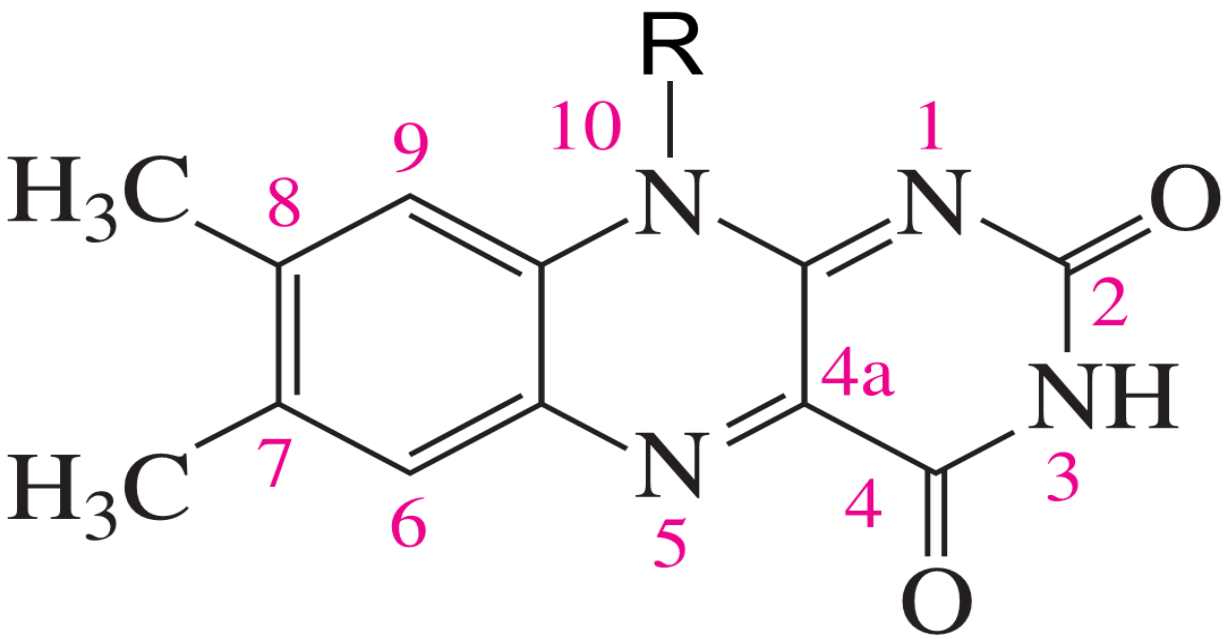
Flavín mononucleótido o riboflavina-5′-fosfato



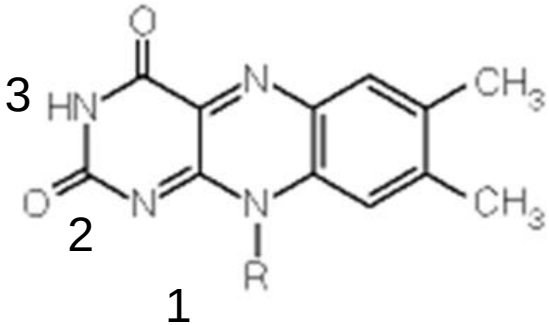
nucleótido

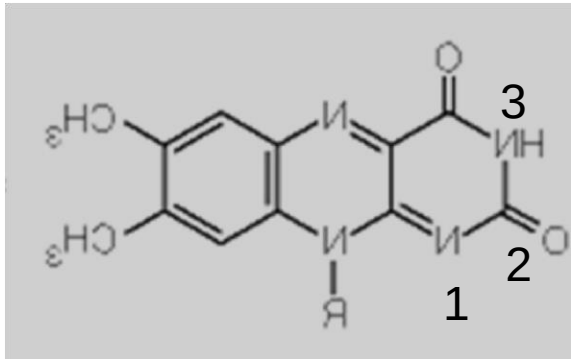


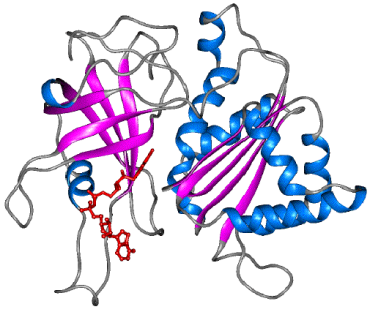
nucleótido

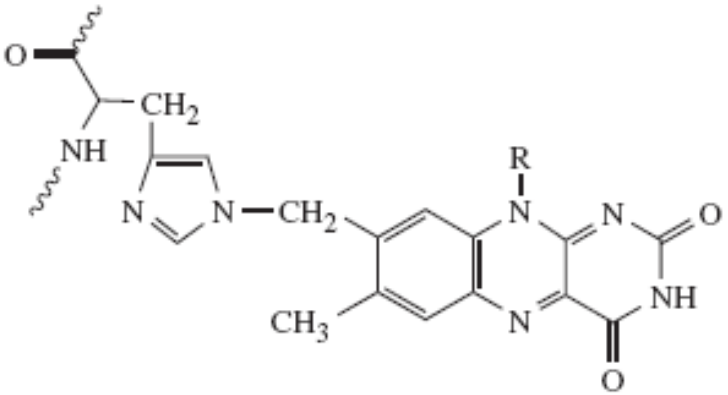


Atención! Formulaciones equivalentes en las cuales se ha rotado la molécula 180°

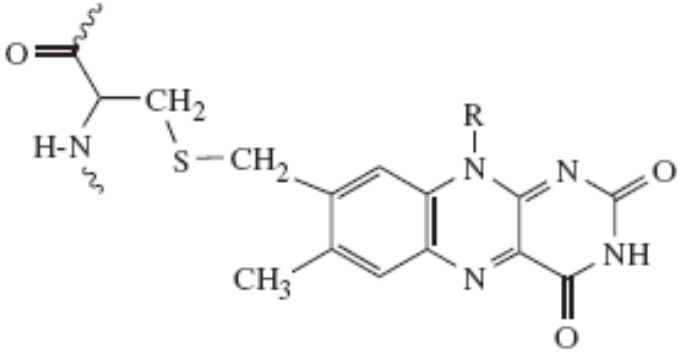








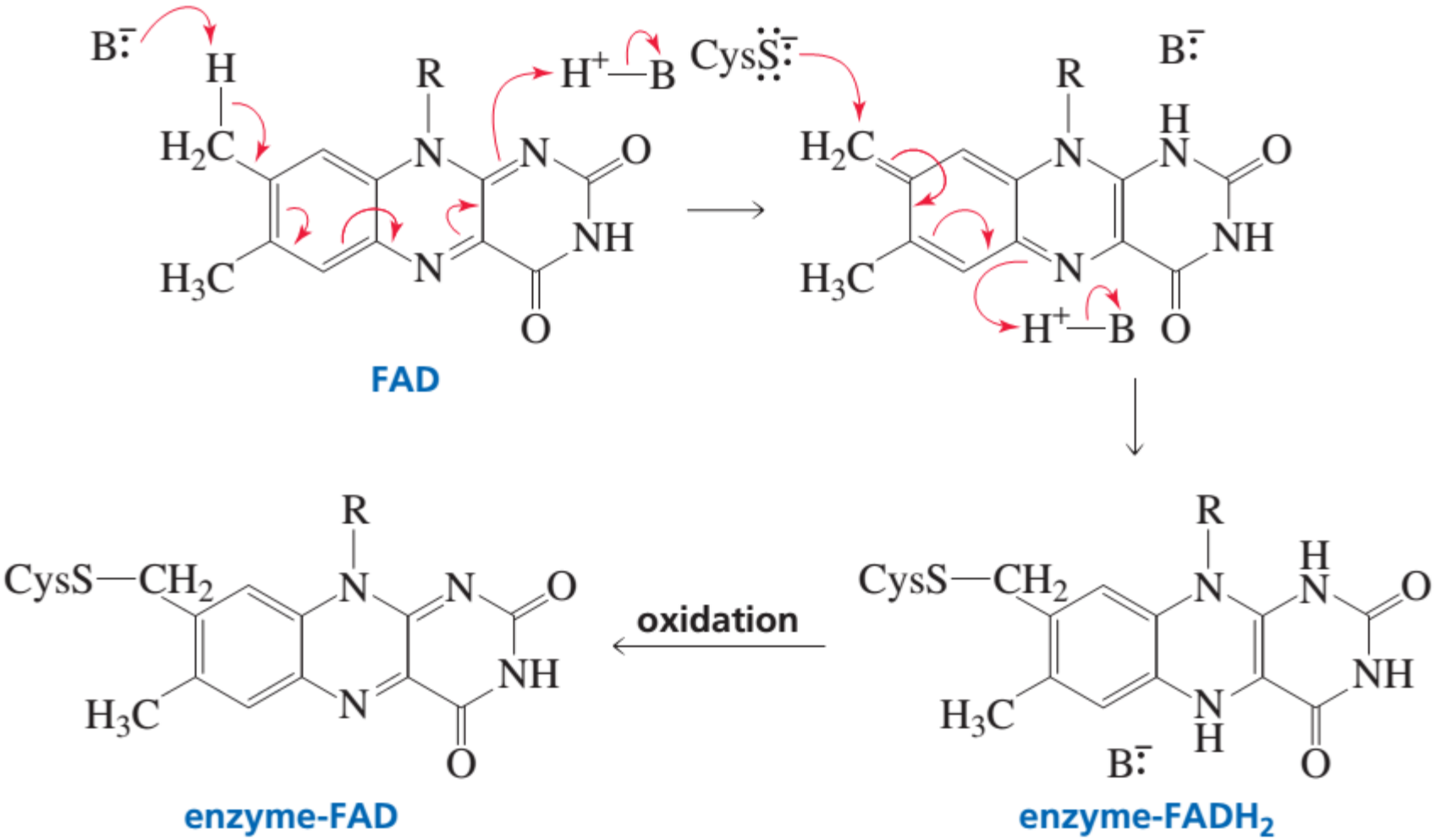
8α-(N3)-Histidylflavins
Succinate dehydrogenase



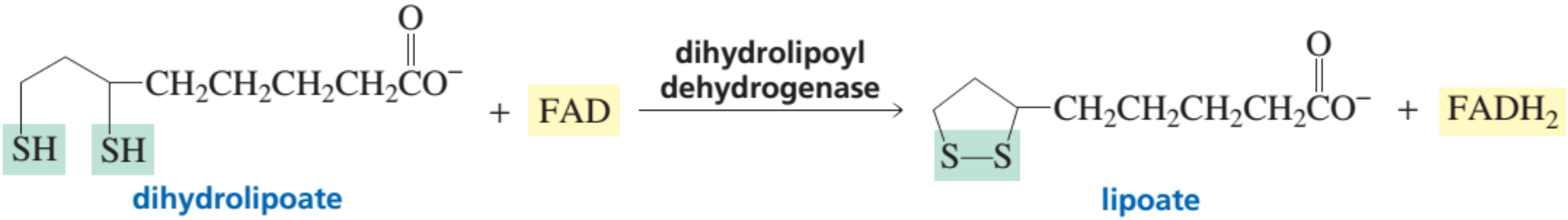
8α-Cysteinylflavin thioethers
Monoamine oxidase

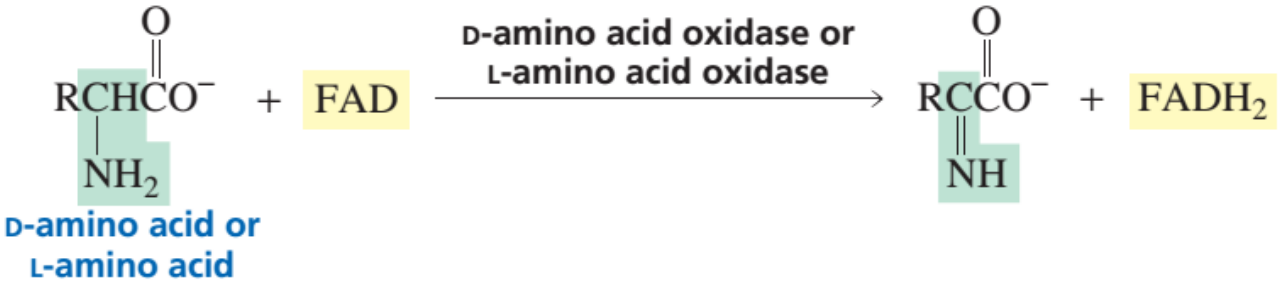
A diferencia de las coenzimas piridin dependientes, por lo general los nucleótidos de flavina se unen covalentemente a las proteínas- COFACTORES-

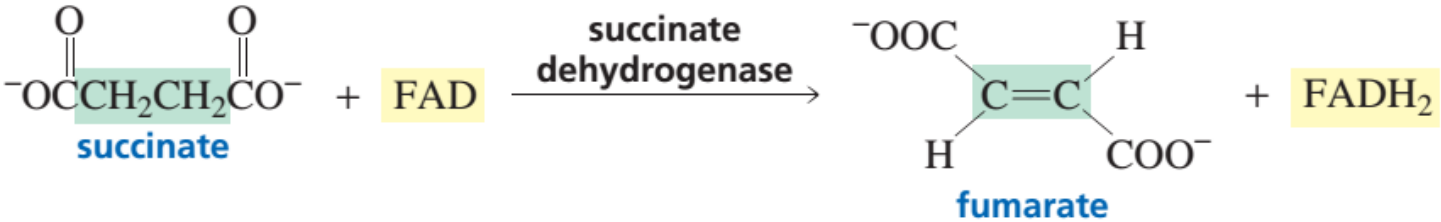
Mecanismo



Ejemplos de los tipos de reacciones en las que interviene el FAD

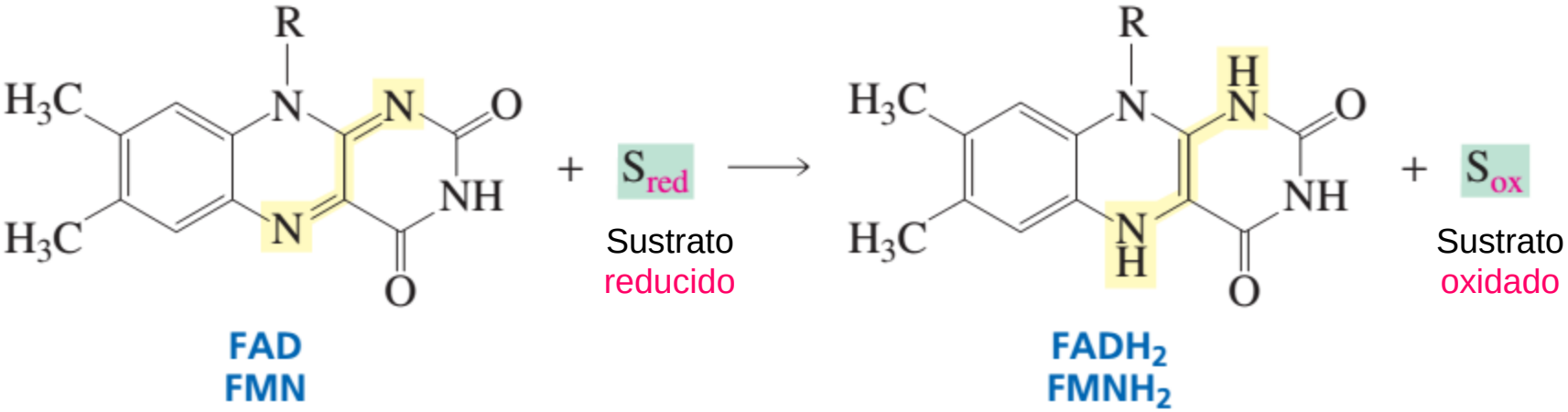




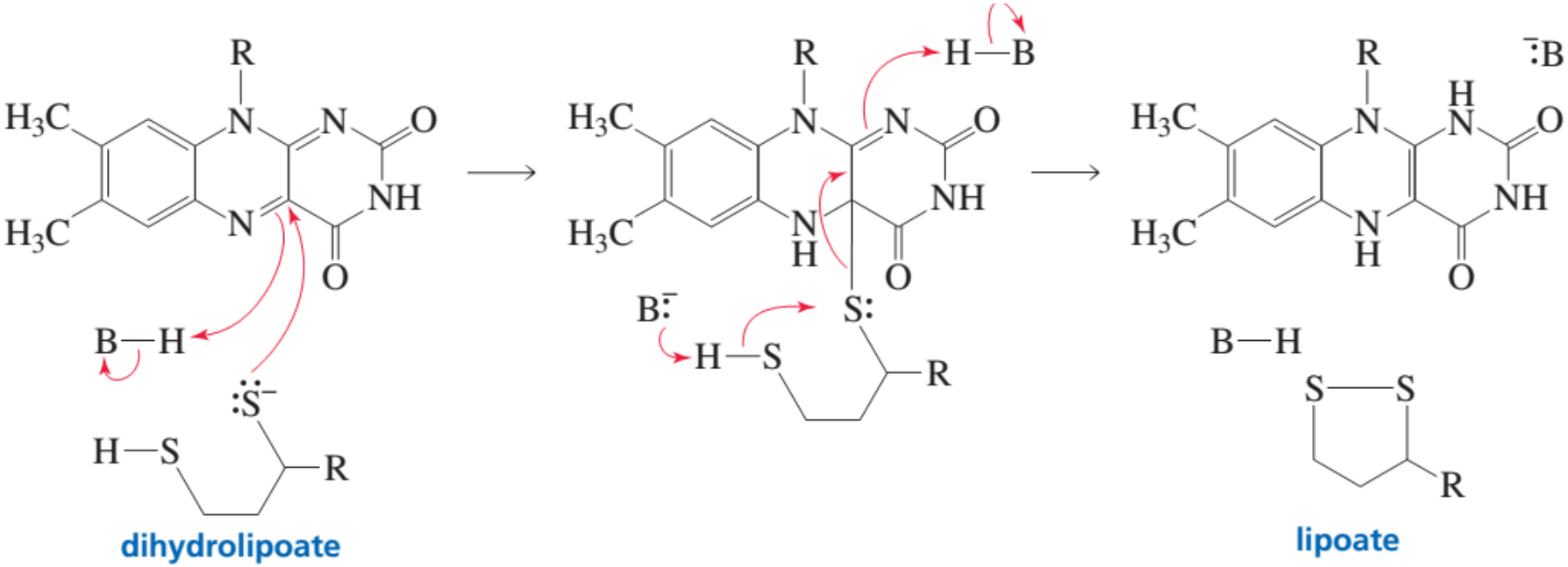


Como **regla general**, se puede decir que el **NAD(P)+** interviene en reacciones donde hay un grupo carbonilo involucrado. Ej. Oxidación de alcoholes a aldehídos, cetonas o ácidos carboxílicos.

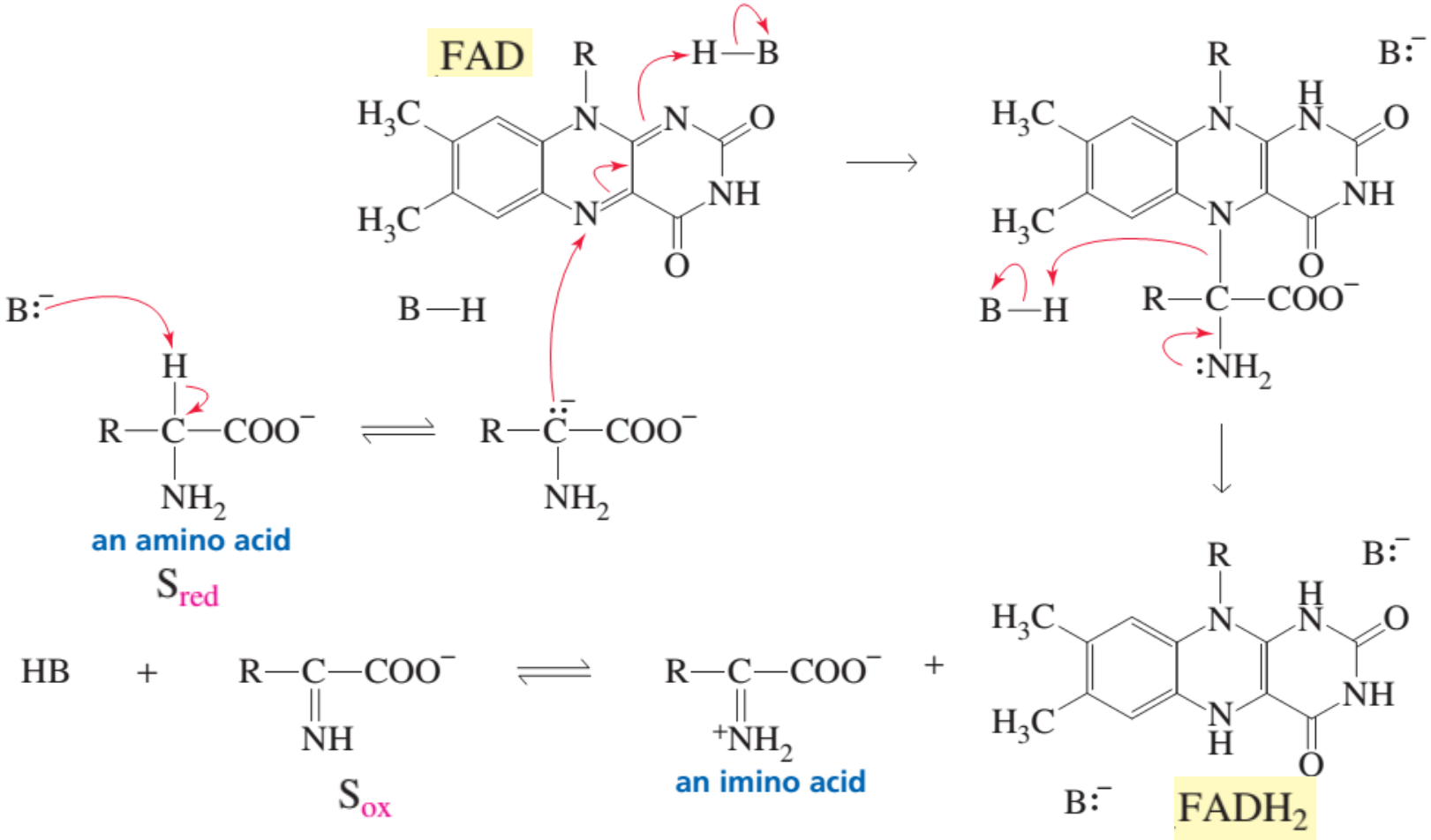
FMN o **FAD** interviene en otro tipo de oxidaciones

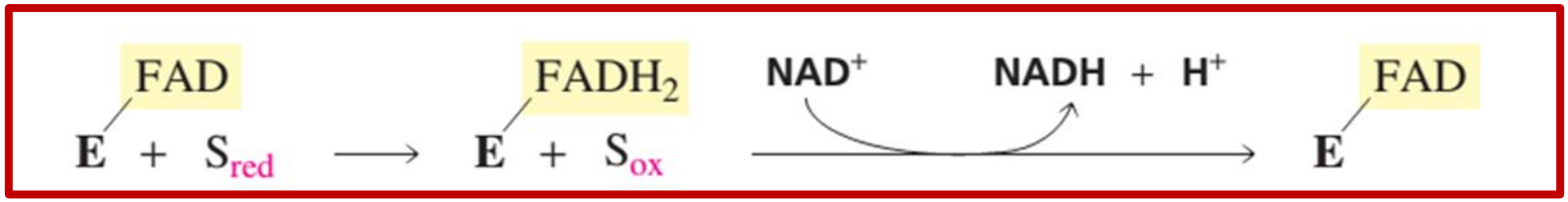

R
H3C
N
N
O
NH
H3C
N
O
+
Sred
Sustrato
reducido
FAD
FMN
R
H
H3C
N
N
O
NH
H3C
N
H
O
+
Sox
Sustrato
oxidado
FADH2
FMNH2

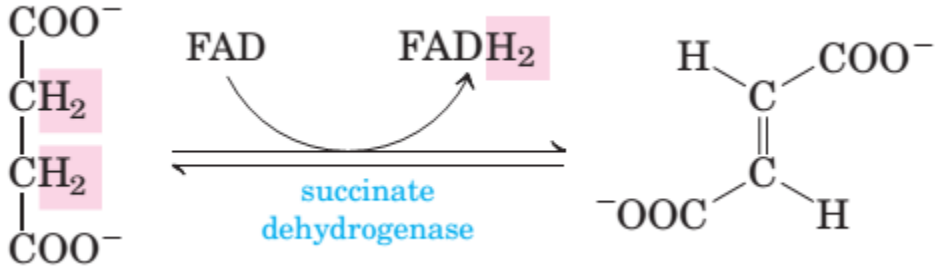
Mecanismo de la **dihidrolipoil deshidrogenasa**
(perteneciente al complejo piruvato deshidrogenasa)



Mecanismo de D- o L-aminoácido oxidasa

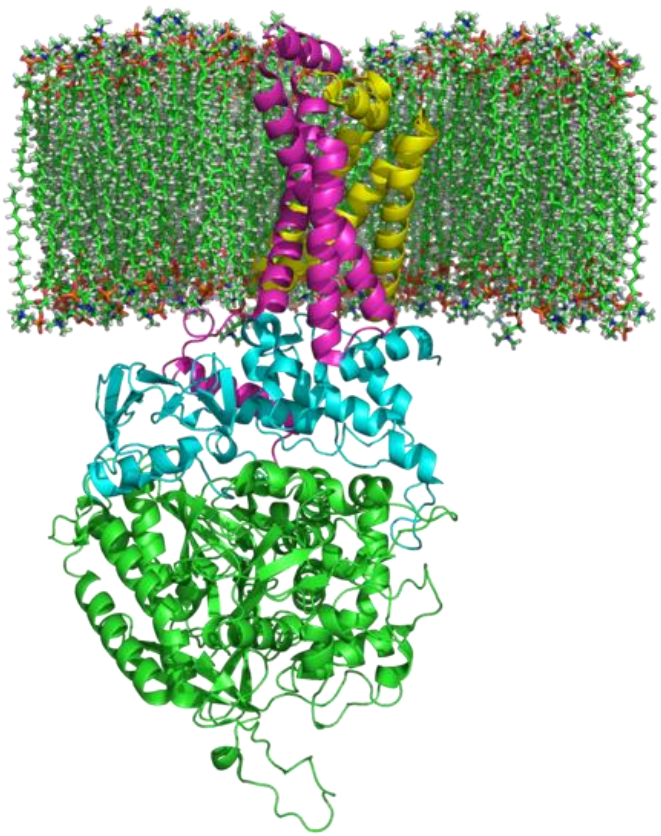


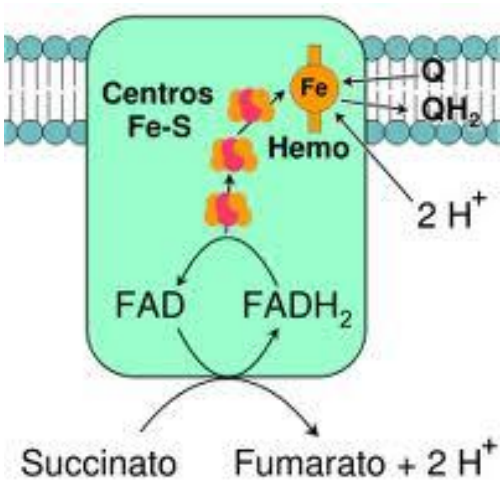


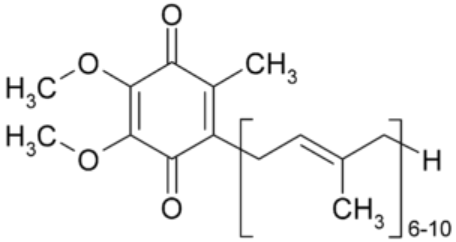


succinato

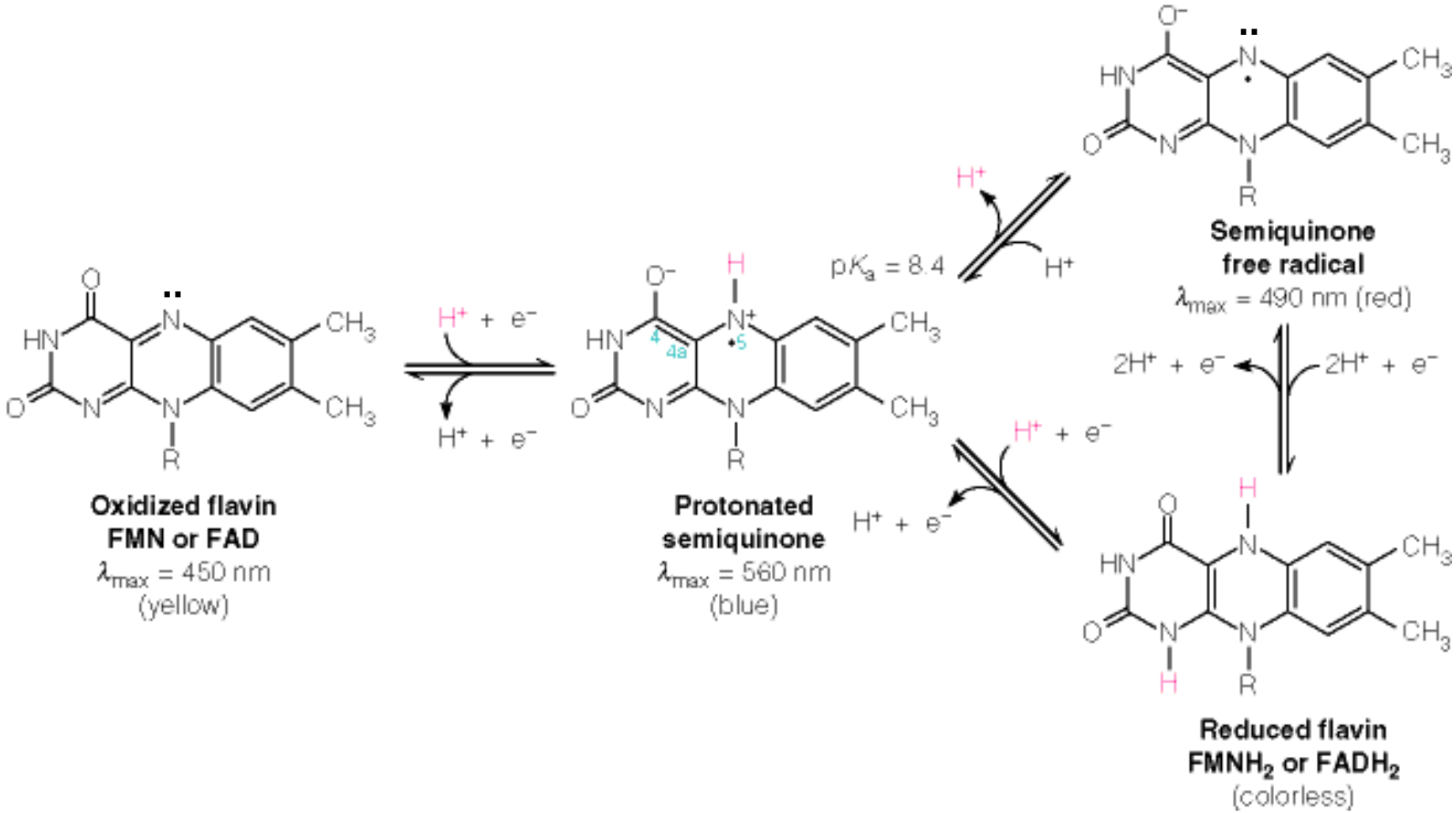
fumarato





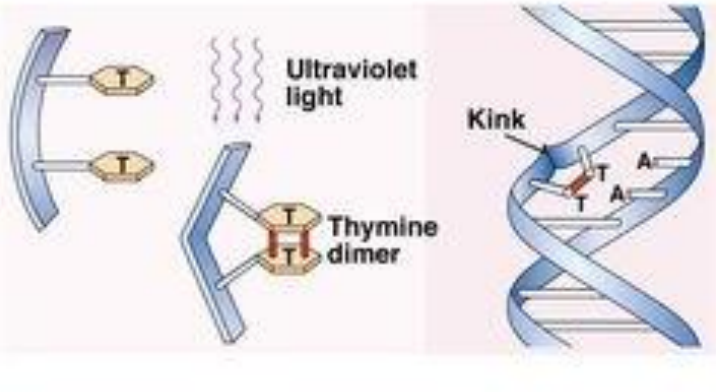


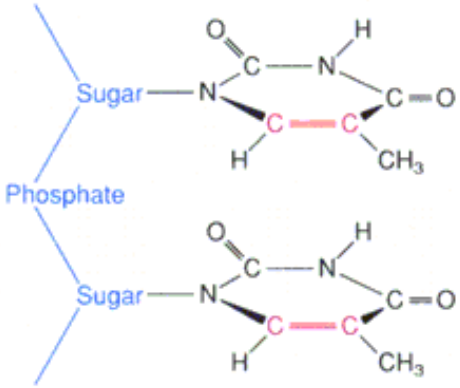
Mecanismo radicalario



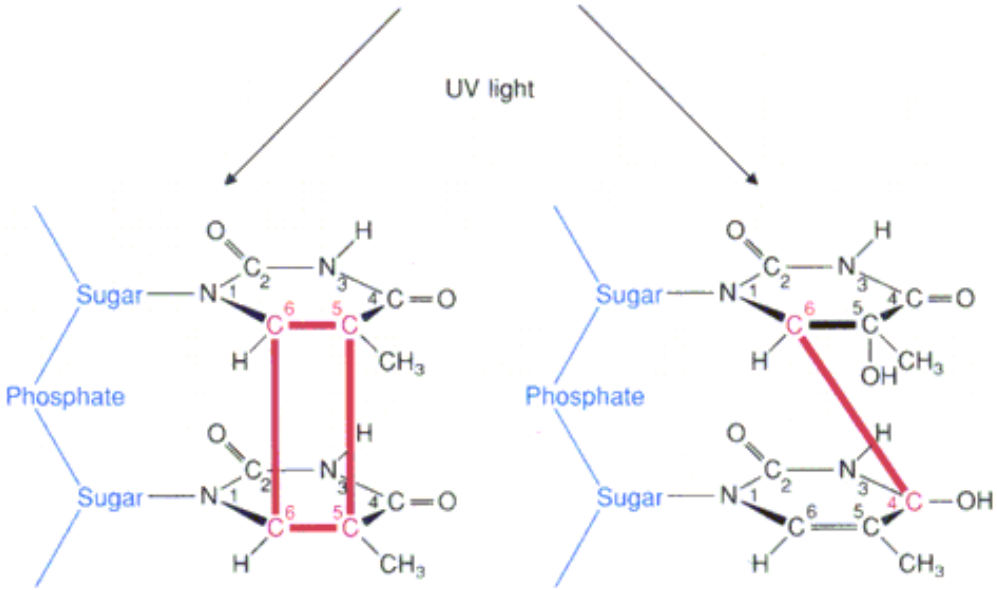
Formación de dímeros de timina en el ADN inducido por la radiación UV

Dímero de pirimidina



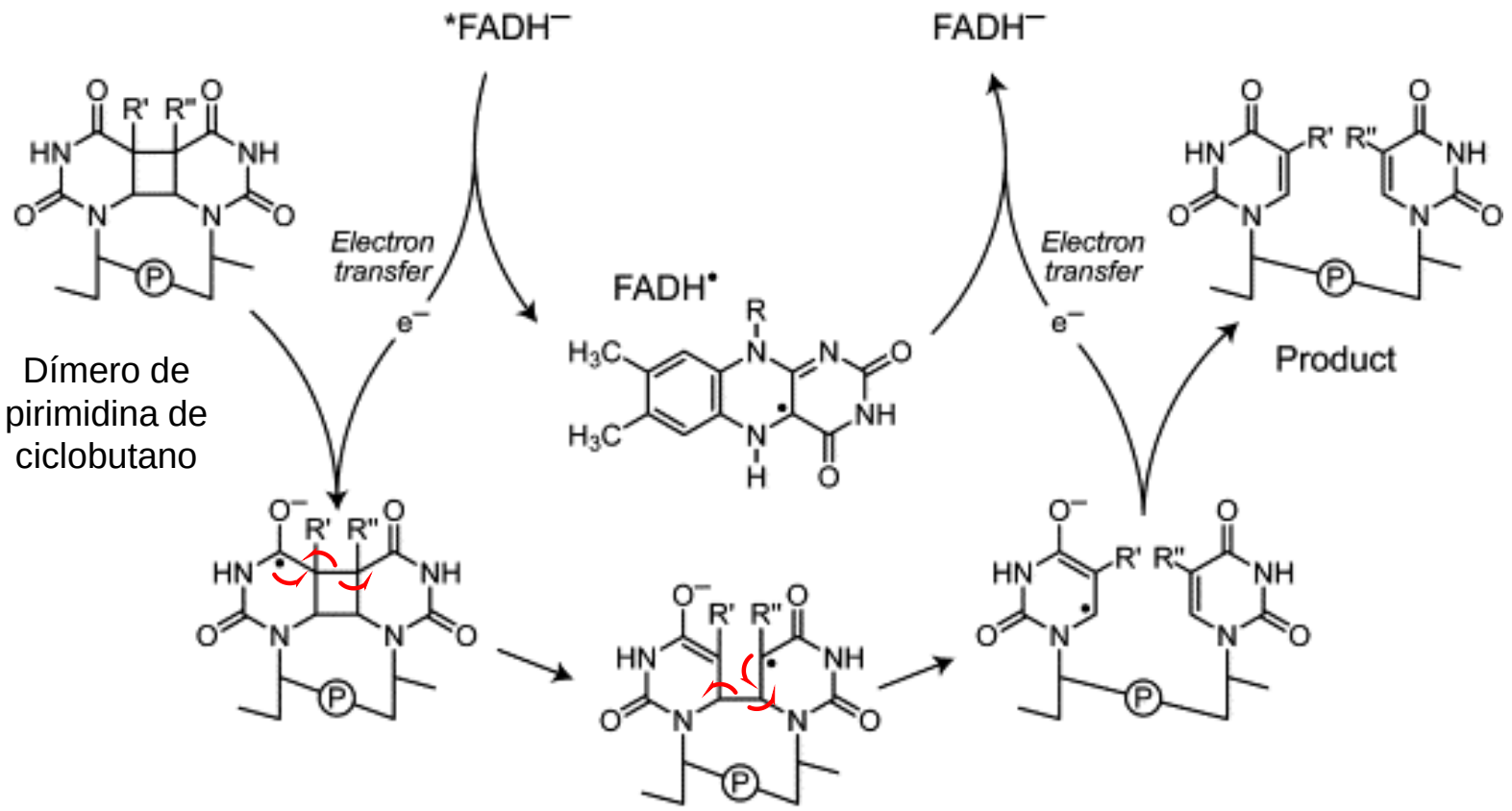


Residuos de timina adyacentes



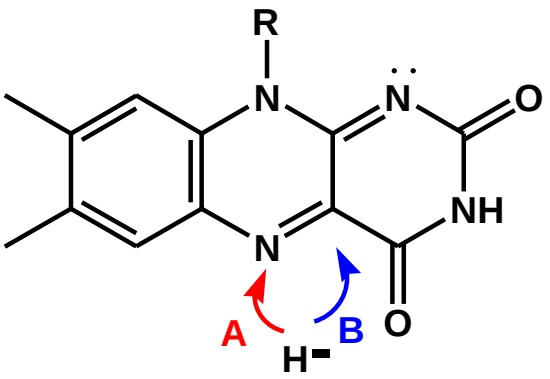
(a) Cyclobutane thymine dimer (b) 6–4 photoproduct

Fotorreactivación (reparación) del ADN catalizada por fotoliasa dependiente de FAD

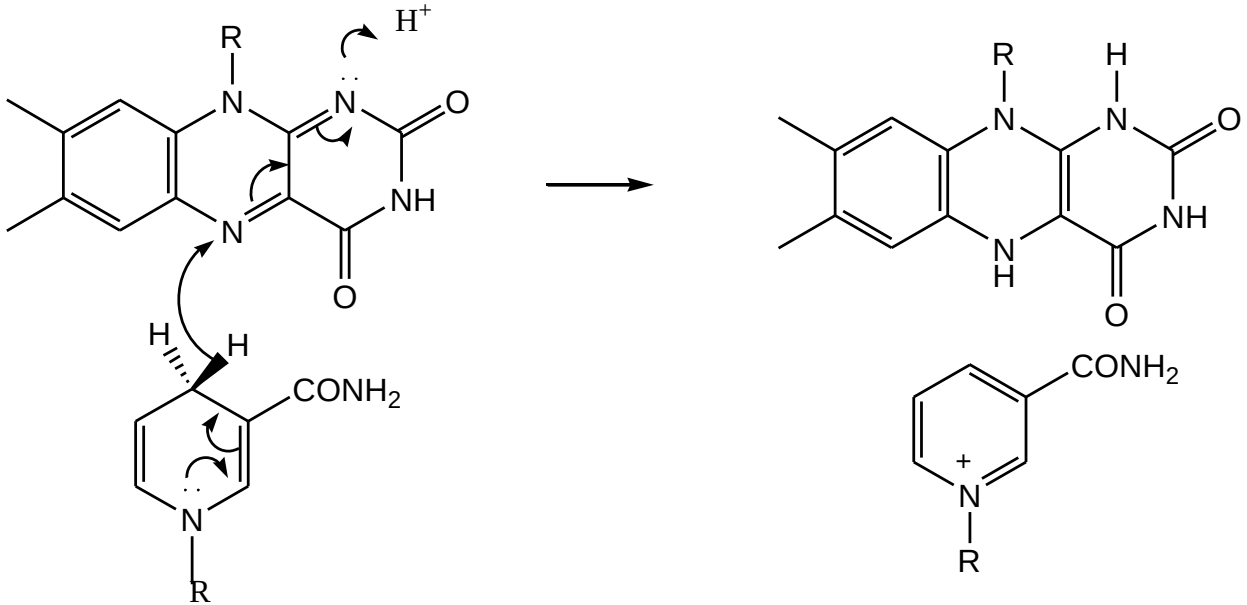


El FAD es reducido por NAD(P)H

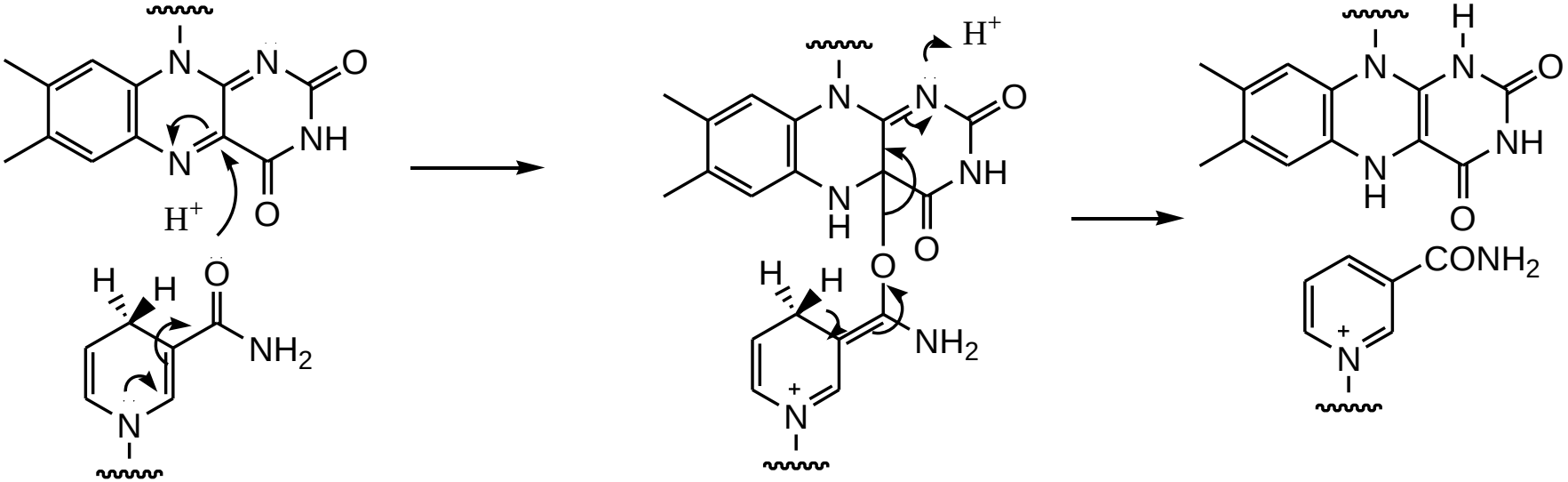
$$NAD(P)H + FAD \overset{H^+}{\rightleftharpoons} NAD(P)^+ + FADH_2$$



A)Mecanismo con transferencia directa de hidruro



B)Mecanismo sin transferencia directa de hidruro



Mono-oxigenasas

Monooxigenasas (EC 1.13. y 1.14.x.x)

- **con grupo Hemo**
  - **citocromo P450 (EC 1.14.x.x)**
- **sin grupo Hemo**
  - **Flavina dependientes (FMO)**
  - **Pteri(di)na dependientes**

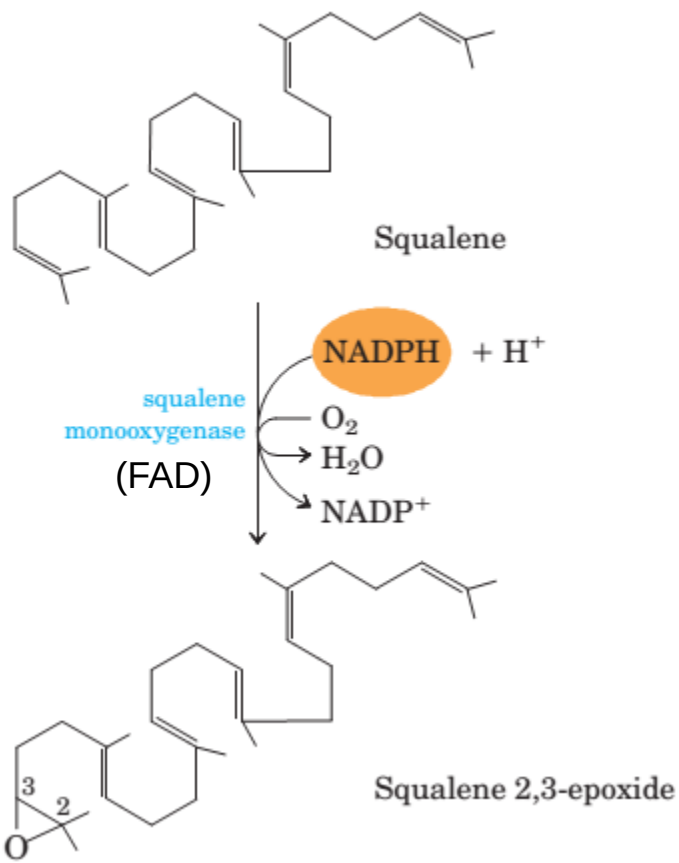
Cofactores: NAD(P)H

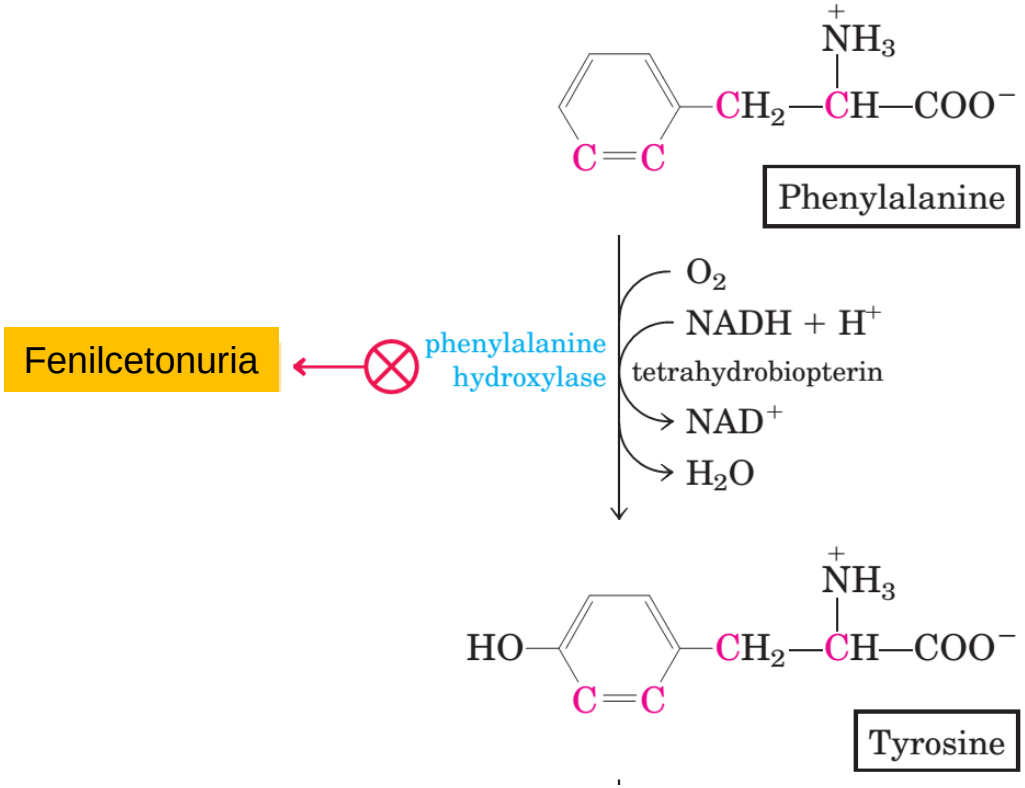
EC 1.13. Actúan sobre un único sustrato con incorporación de oxígeno molecular (oxigenasas)
EC 1.14. Actúan sobre un par de sustratos con incorporación o reducción de oxígeno molecular

Reacciones catalizadas por monooxigenasas

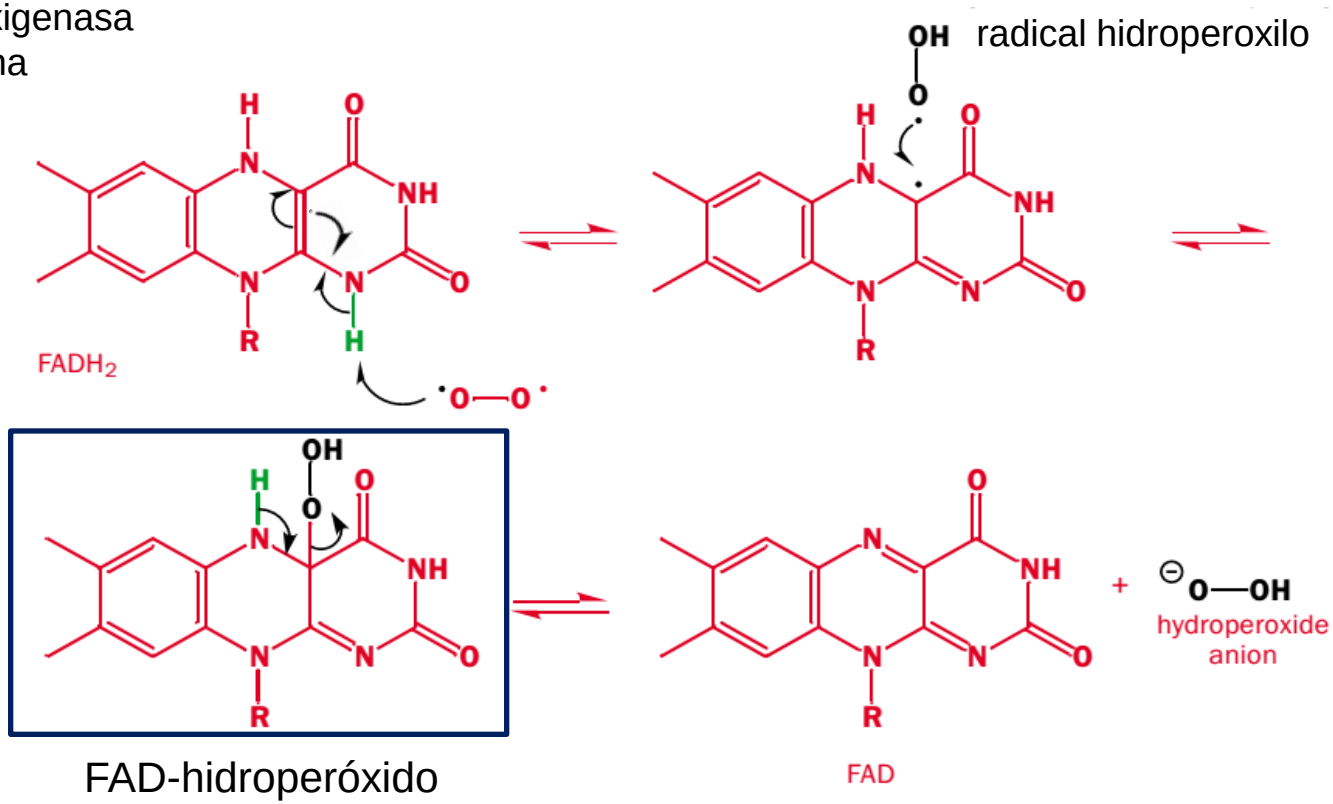
Sustrato	**Producto**	**Tipo de reacción**
Alcano	Alcohol	Hidroxilación
Alqueno	Epóxido	Epoxidación
Aromático	Fenol	Hidroxilación
Heteroátomo (N, S, P, Se)	Óxido del heteroátomo	Oxidación de heteroátomo
Cetona	Éster o lactona	Baeyer-Villiger

Ejemplos de reacciones catalizadas por monooxigenasas

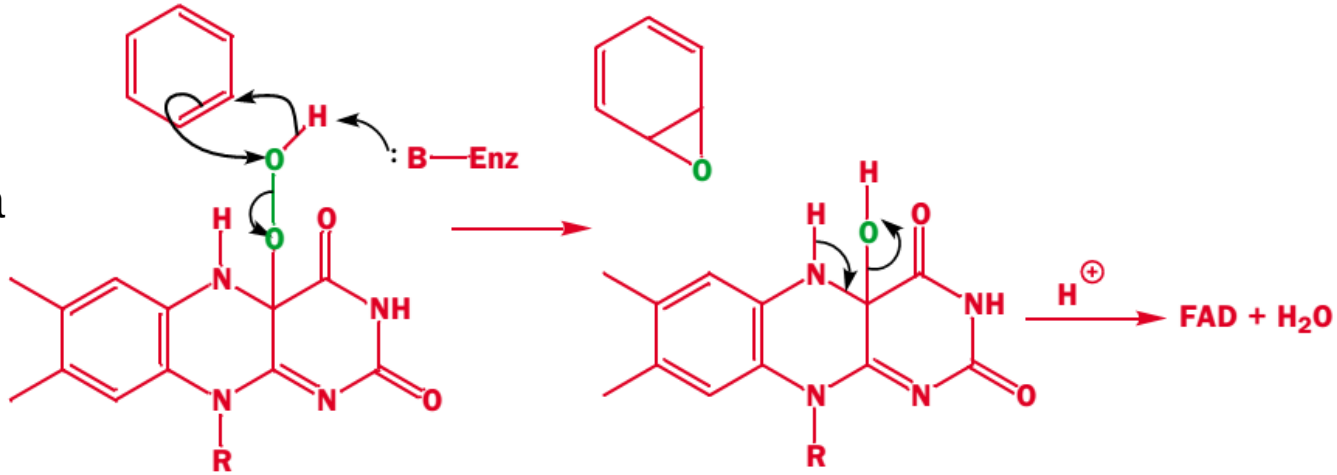




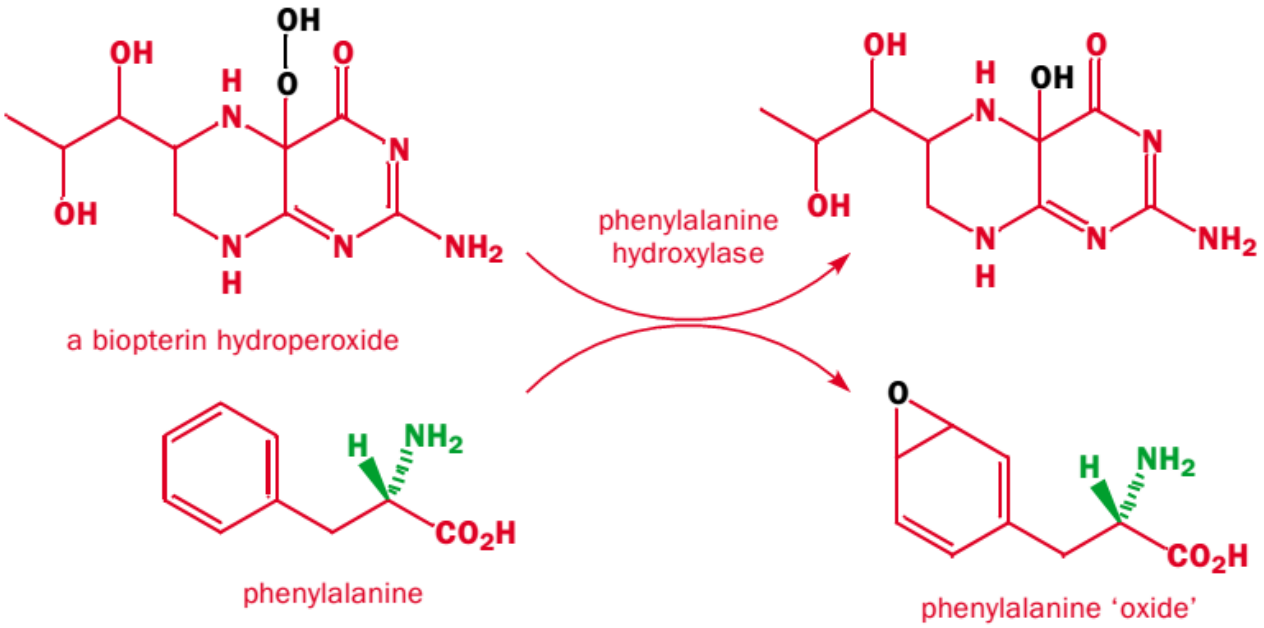
Ejemplo de epoxidación de arenos mediada por monooxigenasa dependiente de flavina

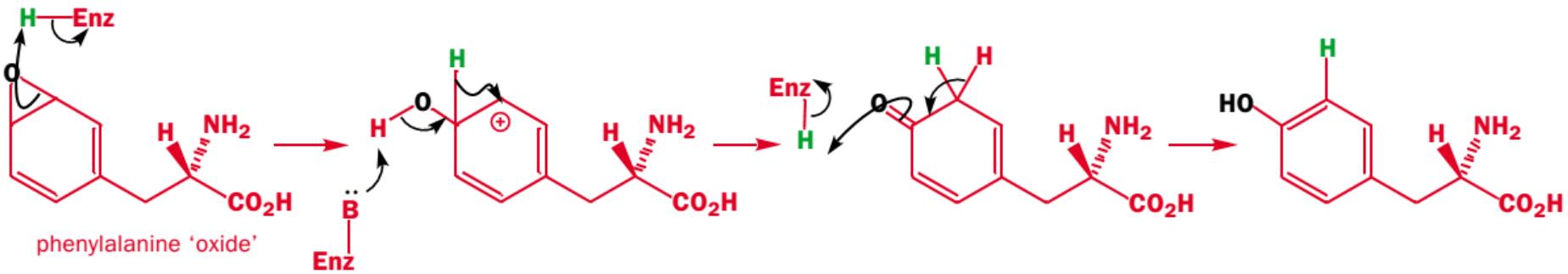


monooxigenasa

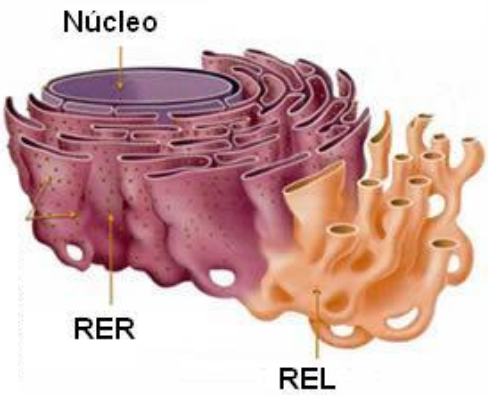


Hidroxilación de fenilalanina mediada por tetrahidrobiopterina





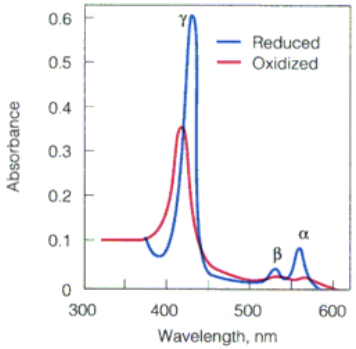
Desplazamiento NIH (National Institute of Health) es la migración del H de C-4 a C-3



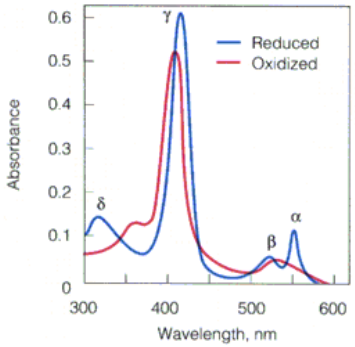
Citocromos

Citocromos

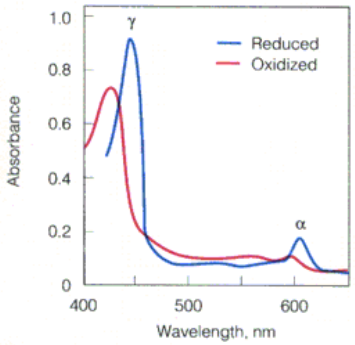
Espectros de absorción de citocromos



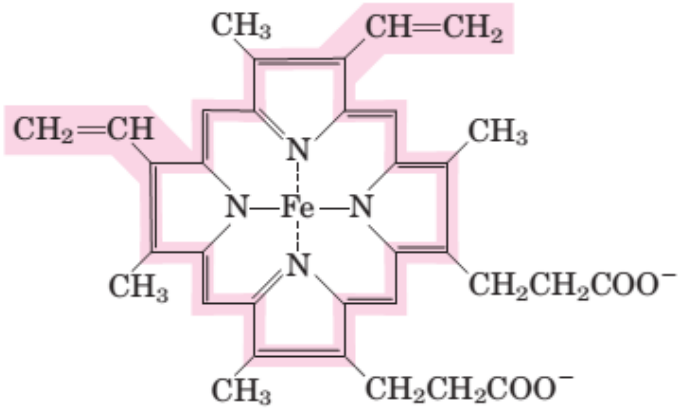
(a) Cytochrome *b*



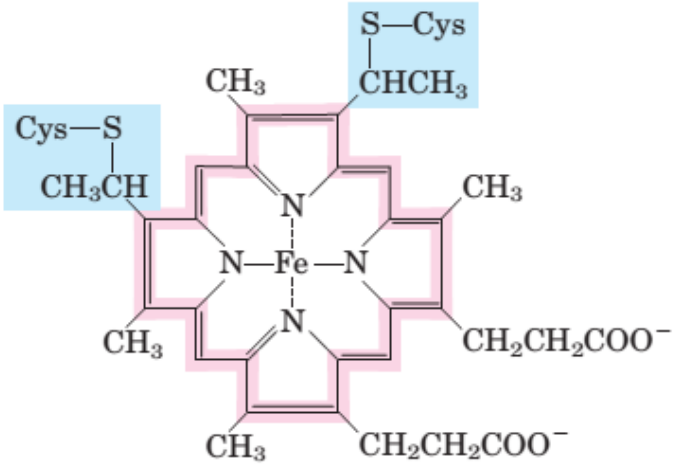
(b) Cytochrome *c*



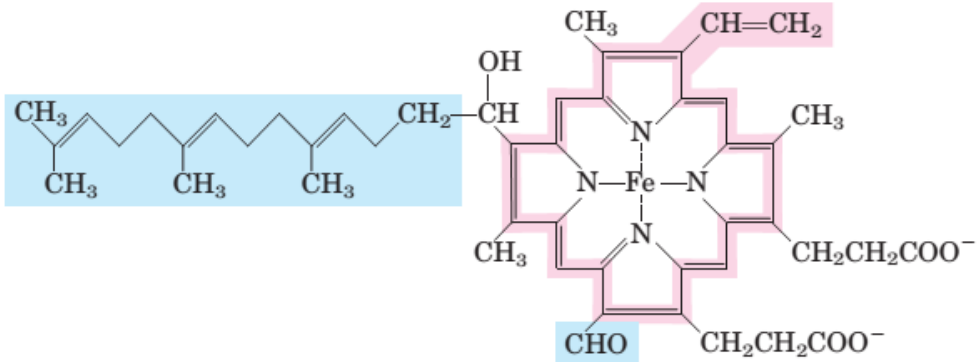
(c) Cytochromes *a* and a_3



Iron protoporphyrin IX
(in *b*-type cytochromes)

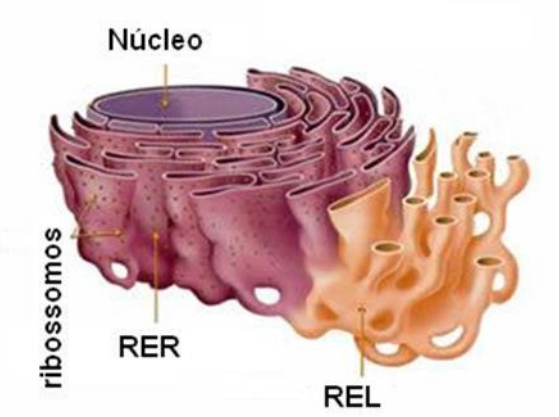


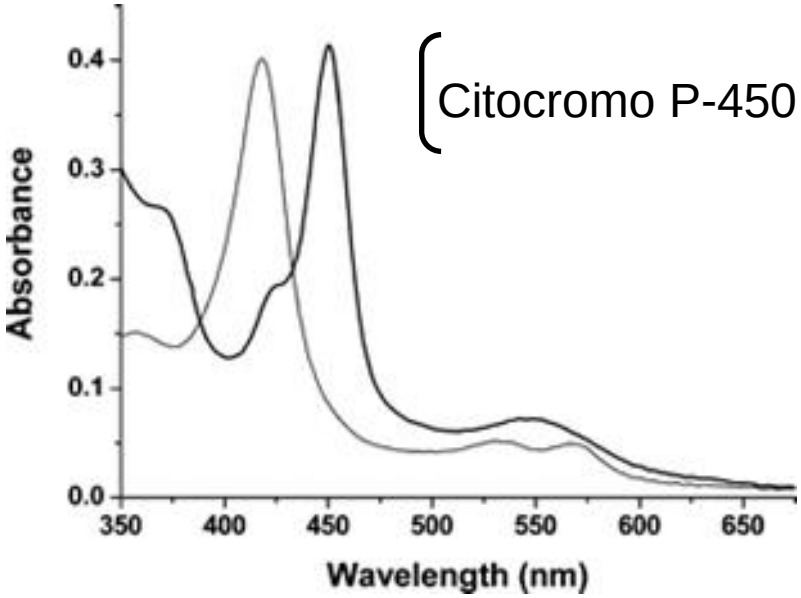
Heme C
(in *c*-type cytochromes)



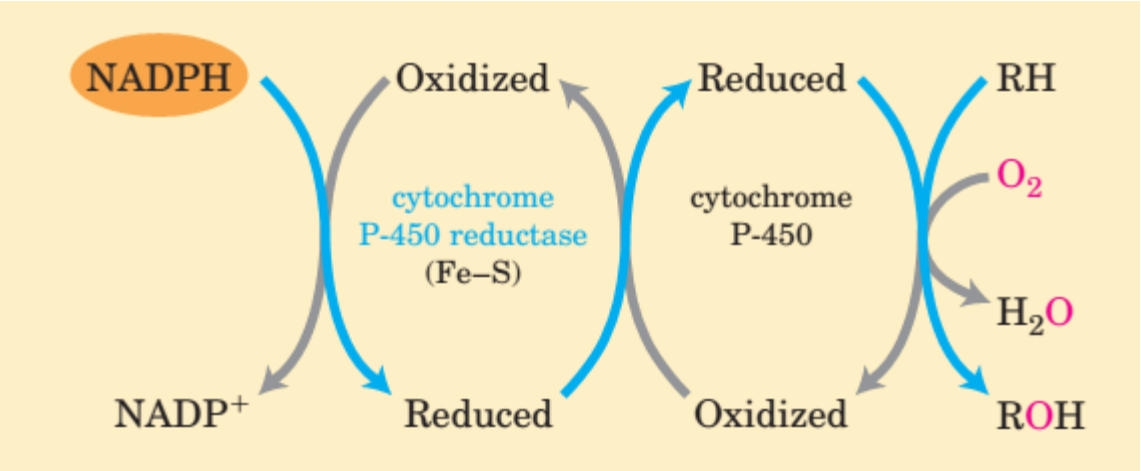
Heme A
(in *a*-type cytochromes)

Citocromos P-450 (CYP)



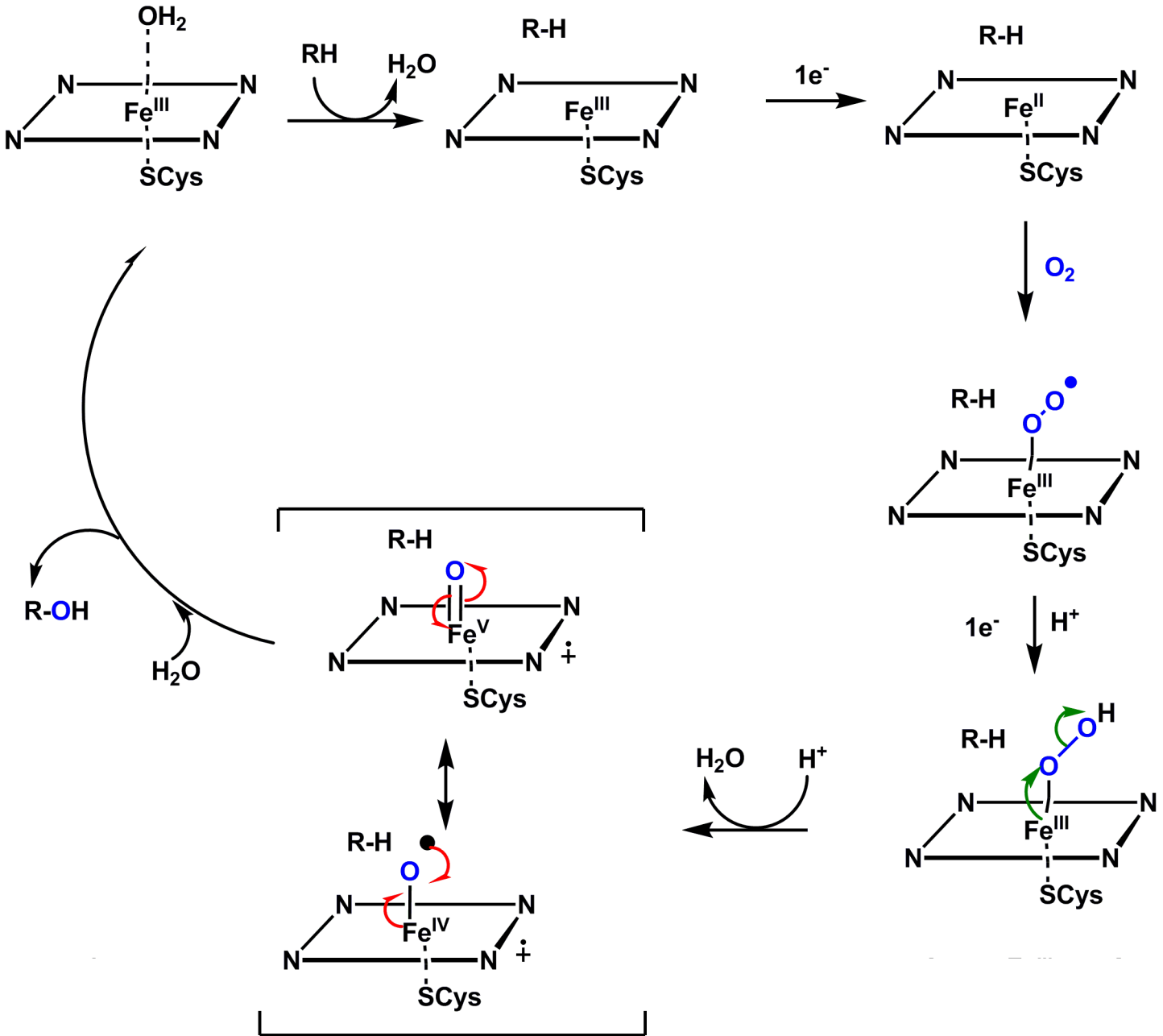


(Citocromo P-450 --- CO)

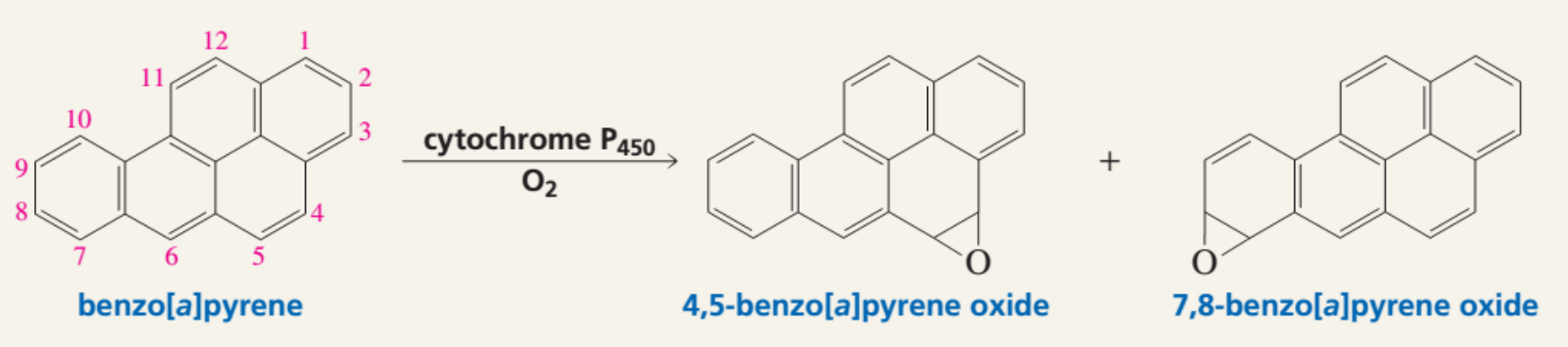


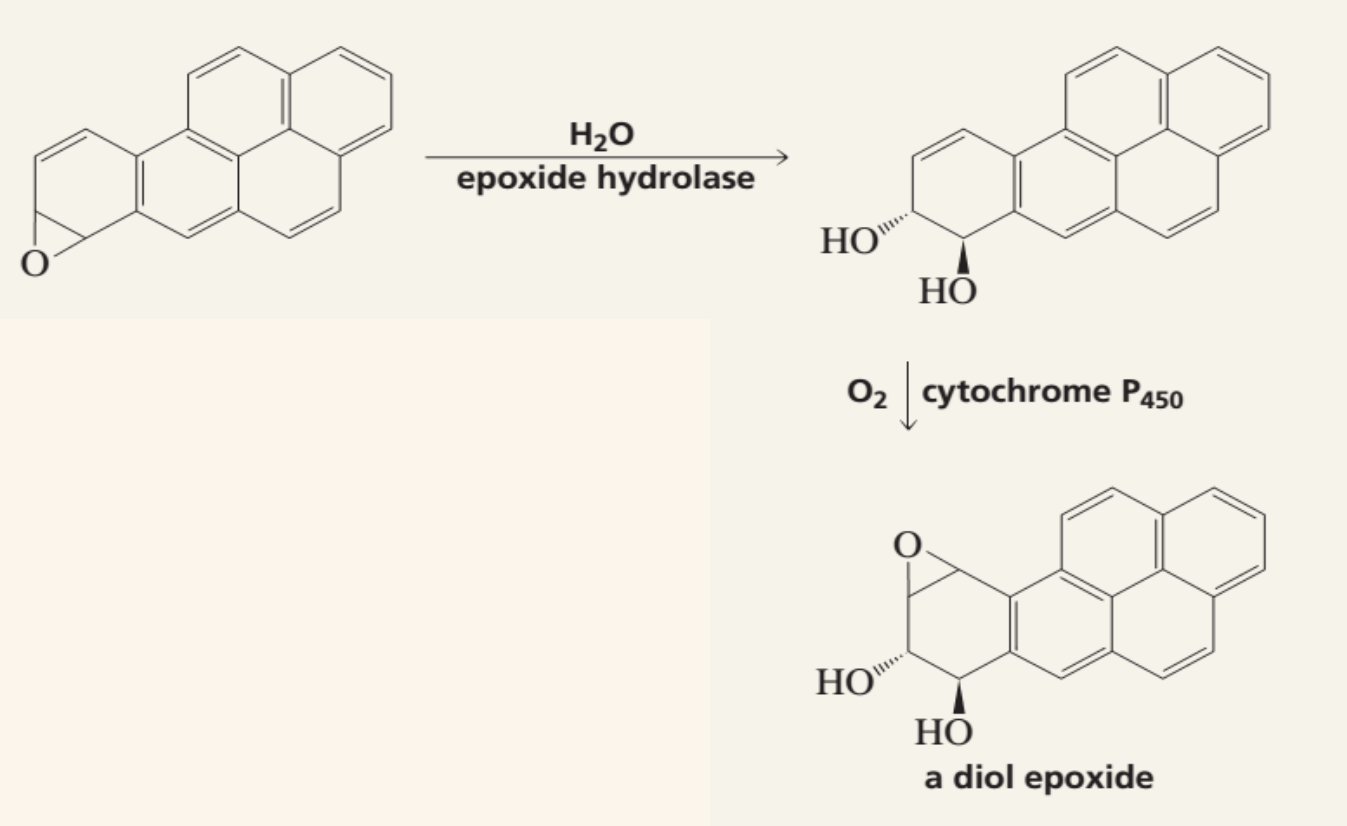
Sustratos:
Esteroides
Fármacos
Xenobióticos
Alcaloides

Ciclo catalítico de monooxigenasas dependientes del Citocromo P450



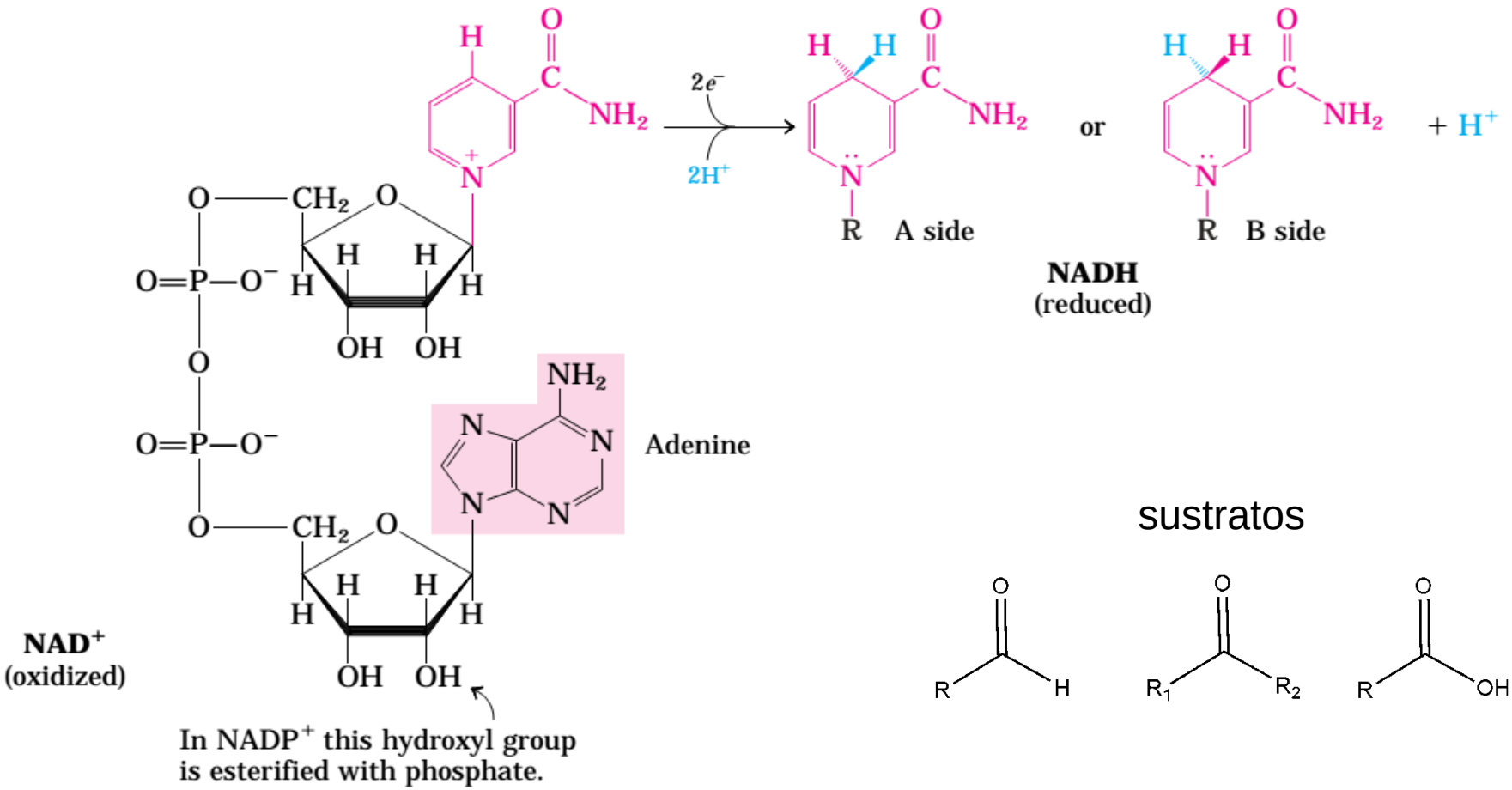
Ejemplo de epoxidación catalizada por CYP



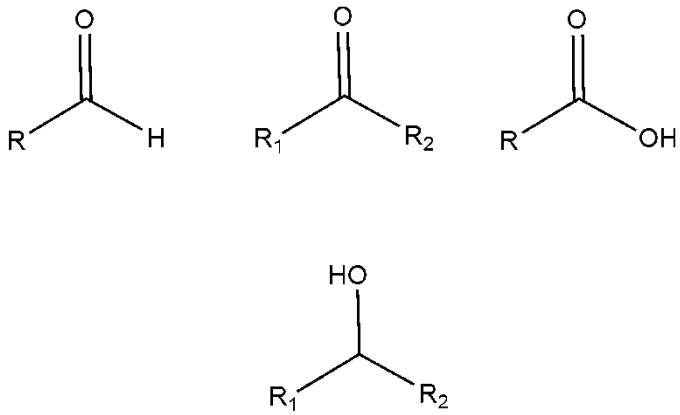


En resumen:

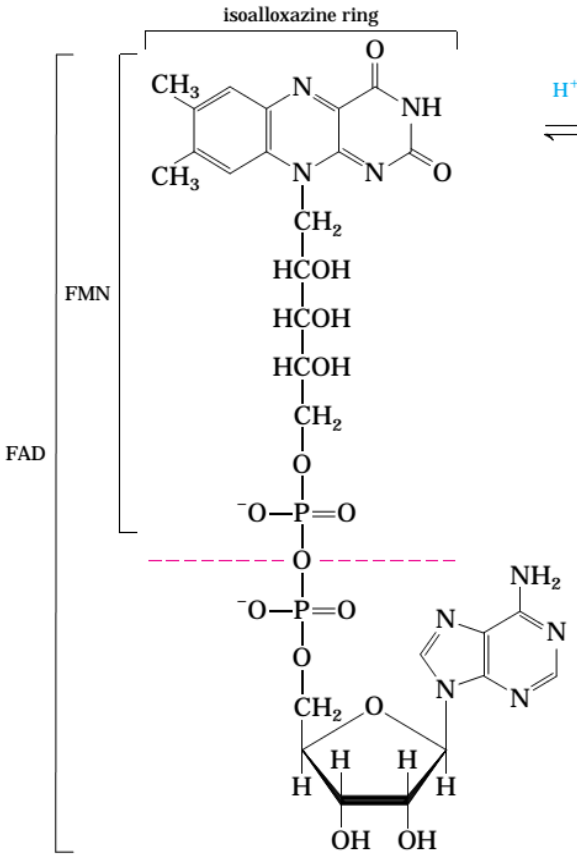
Nucleótidos de nicotinamida



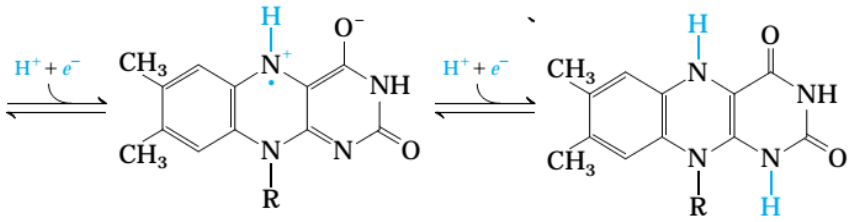
sustratos



Nucleótidos de flavina

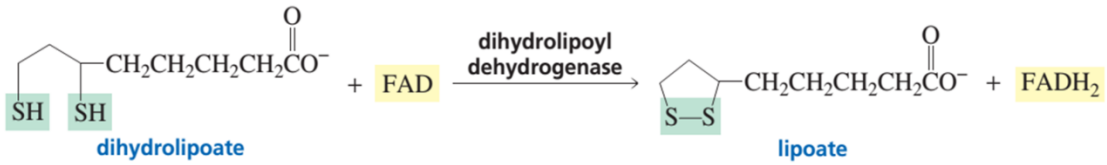


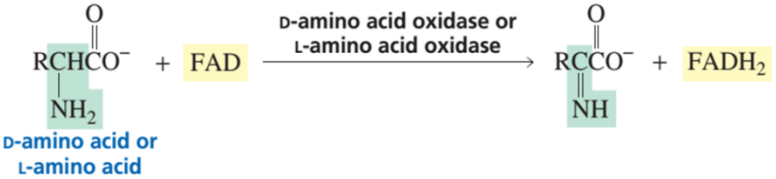
Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)

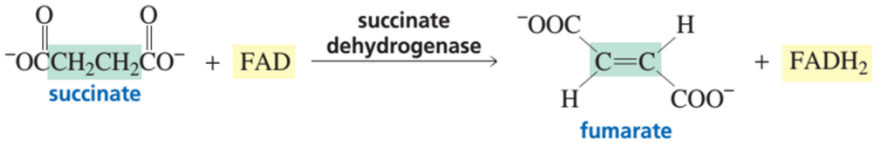


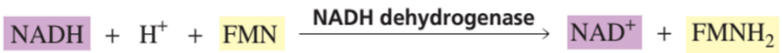
FADH• (FMNH•) (semiquinone)

FADH₂ (FMNH₂) (fully reduced)



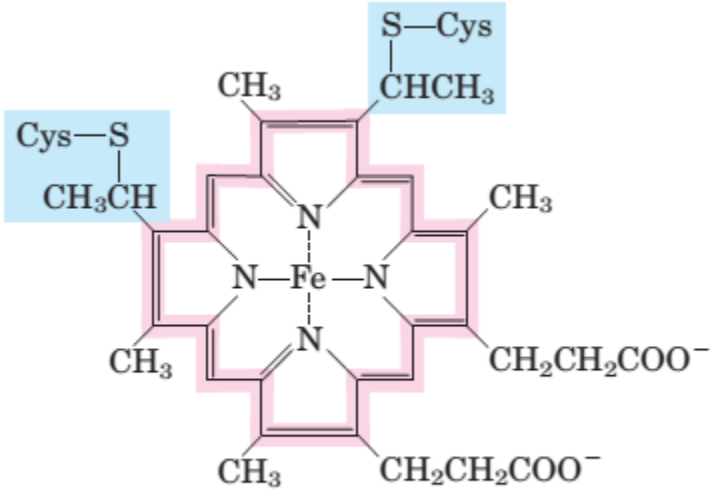




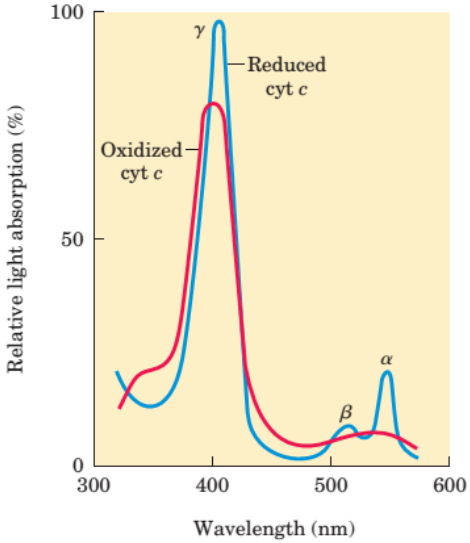


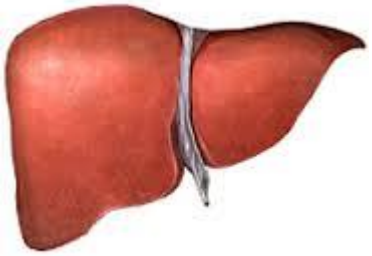
Citocromos

$$Q \longrightarrow \text{Cyt } b \longrightarrow \text{Cyt } c_1 \longrightarrow \text{Cyt } c \longrightarrow \text{Cyt } (a + a_3) \longrightarrow O_2$$



Heme C
(in *c*-type cytochromes)





Cit P450

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