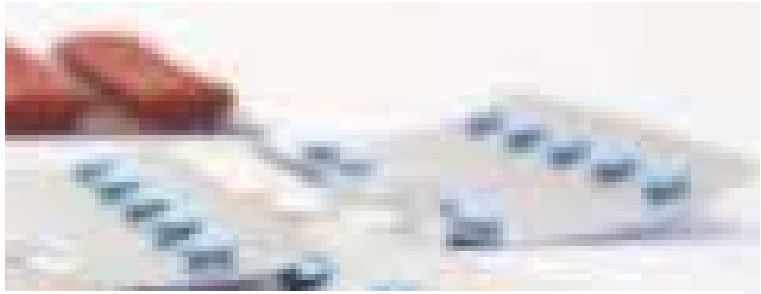
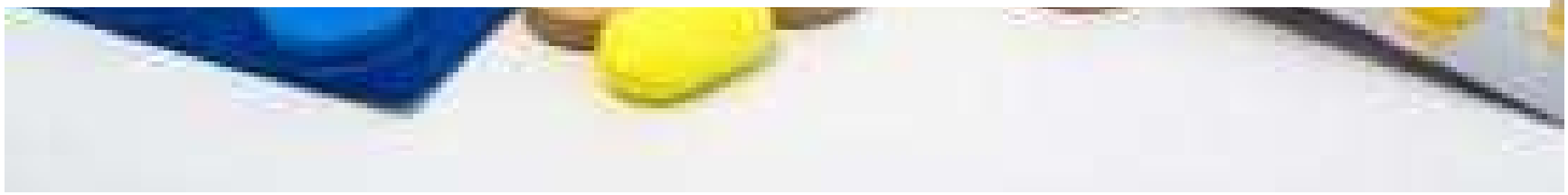


PRINCIPI DI FARMACOCINETICA

METABOLISMO

ESCREZIONE

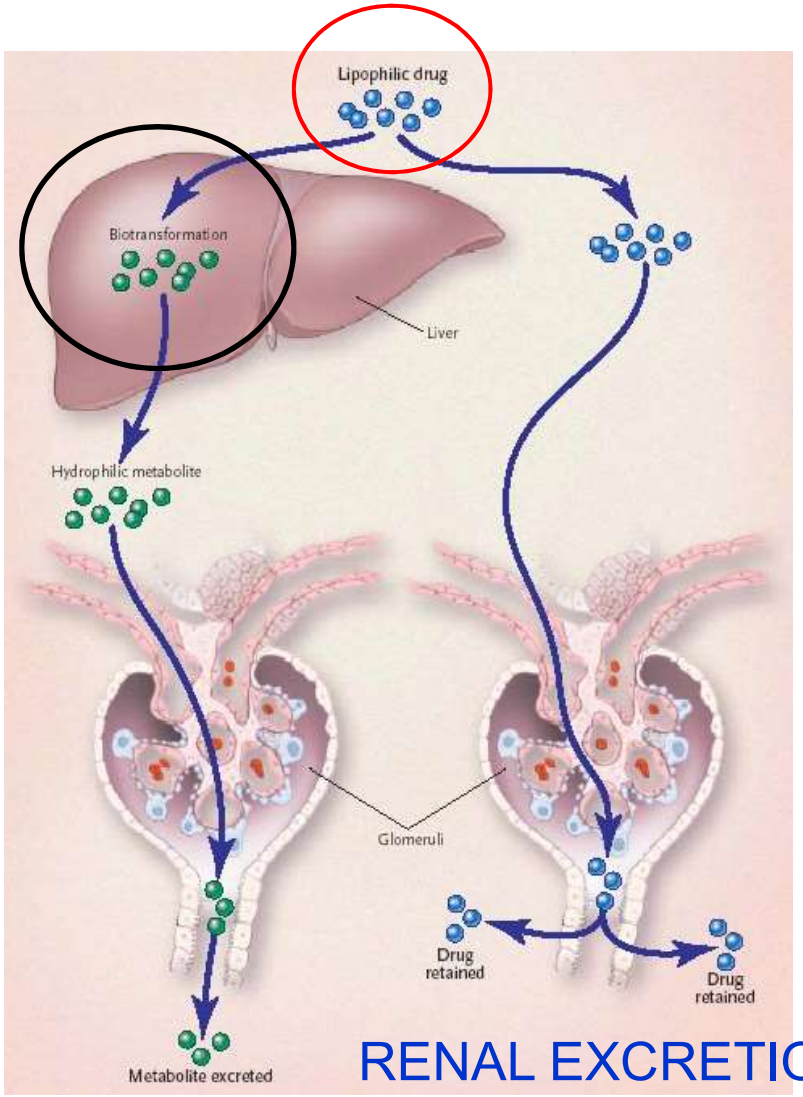


DRUG METABOLISM

BIOTRASFORMATION INTO POLAR METABOLITES

MAINLY in the LIVER

Gut, lungs, skin, kidneys and brain



RENAL EXCRETION

Drug Metabolism: pharmacological background

Phase I Reactions-Functionalization

-Introduction of a polar group

(-OH, -SH, -NH_2,-COOH)

-Demasking polar group

Phase II Reactions-Conjugation

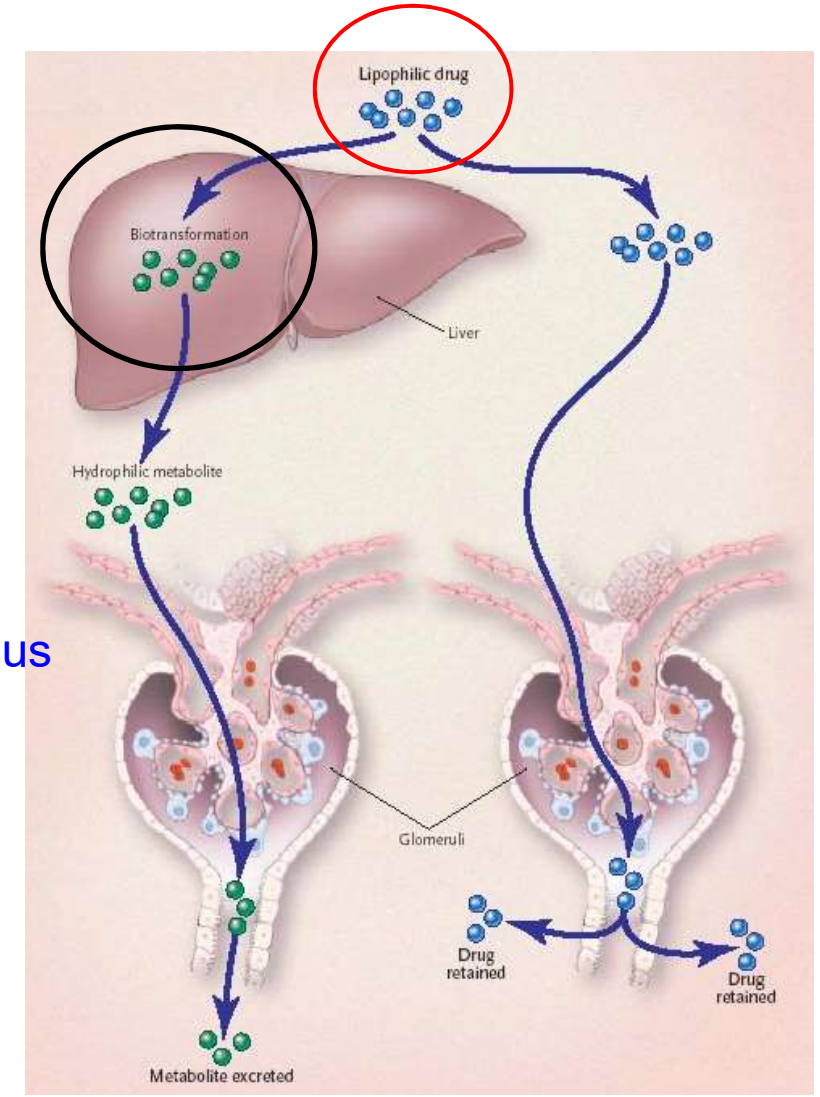
Parent drug/ Phase I metabolites/Endogenous

-Glucuronic acid

-Sulfate

-Glutathione

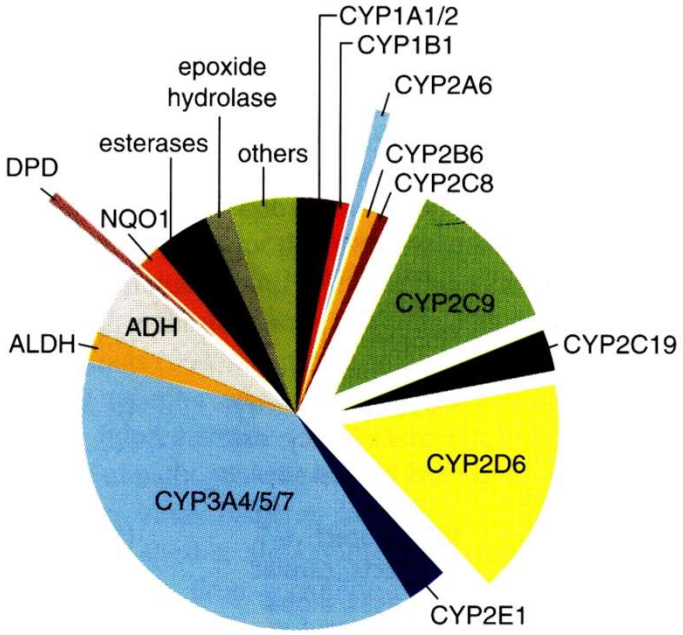
-Others (metilation, acetilation,...)



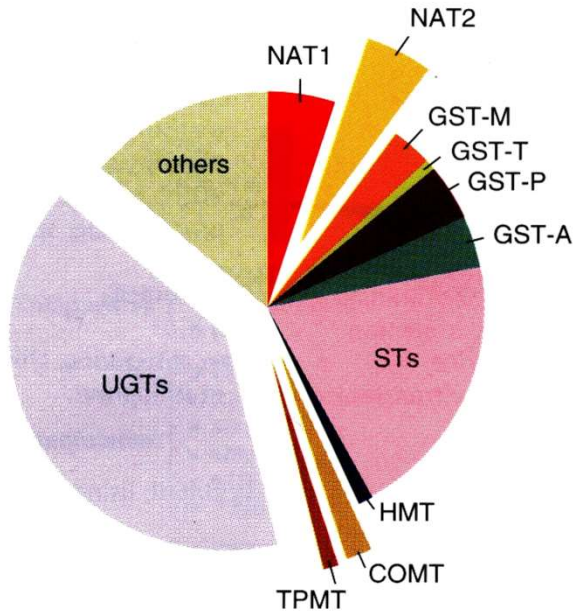
Excretion

Drug Metabolism: pharmacological background

Phase I Enzymes



Phase II Enzymes



Evans & Relling, Science 286: 487, 1999

CYP450

Oxidation

Others

Reduction

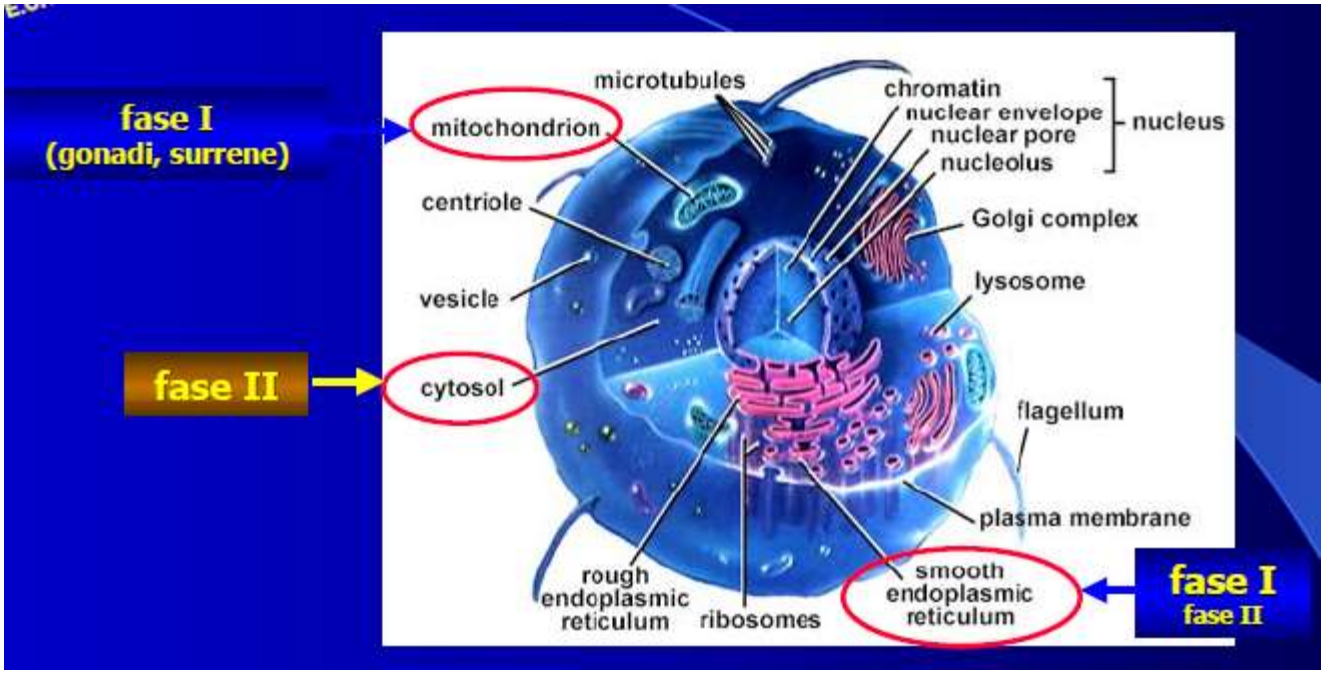
Hydrolysis

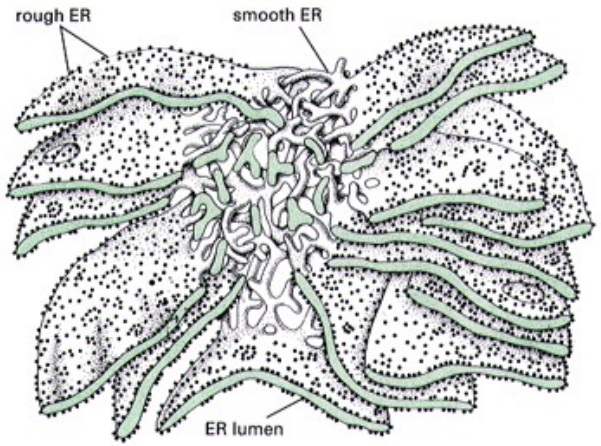
Others (isomerization,...)

Transferases

Conjugation

Drug Metabolism: pharmacological background





Membrane	Epatocita	Cellula esocrina pancreatica
Cell membrane	2 %	5 %
Rough RE	35 %	60 %
Smooth RE	**16 %**	**<1 %**
Golgi	7 %	10 %
Mitochondria, external membrane	7 %	4 %
Mitochondria, internal membrane	32 %	17 %

Phase I enzymes

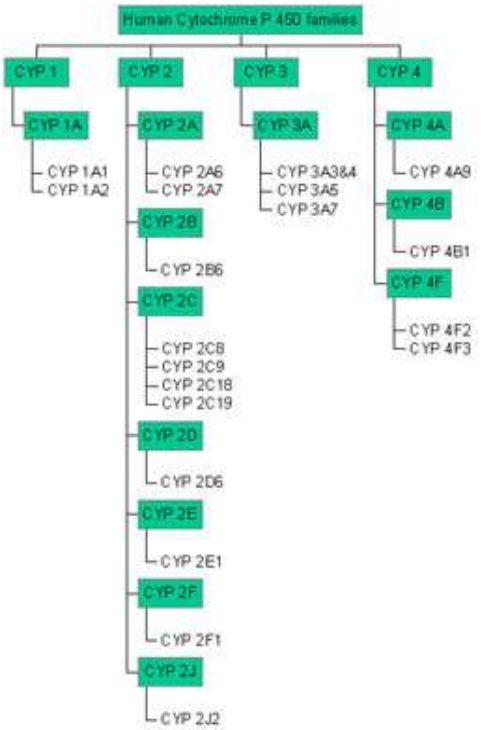
Cytochrome P450 Superfamily

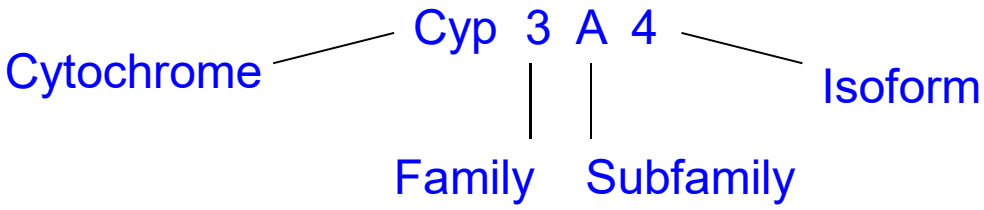
Human genome

57 genes (~ 57 pseudogenes)
~ 150 members

74 families (40% identity)
subfamiles (55% identity)

CYP1 (3 subfamilies, 3 genes, 1 pseudogene)
CYP2 (13 subfamilies, 16 genes, 16 pseudogenes)
CYP3 (1 subfamily, 4 genes, 2 pseudogenes)
CYP4 (6 subfamilies, 12 genes, 10 pseudogenes)
CYP5 (1 subfamily, 1 gene)
CYP7A (1 subfamily member)
CYP7B (1 subfamily member)
CYP8A (1 subfamily member)
CYP8B (1 subfamily member)
CYP11 (2 subfamilies, 3 genes)
CYP17 (1 subfamily, 1 gene)
CYP19 (1 subfamily, 1 gene)
CYP20 (1 subfamily, 1 gene)
CYP21 (1 subfamily, 1 gene, 1 pseudogene)
CYP24 (1 subfamily, 1 gene)
CYP26A (1 subfamily member)
CYP26B (1 subfamily member)
CYP26C (1 subfamily member)
CYP27A (1 subfamily member)
CYP27B (1 subfamily member)
CYP27C (1 subfamily member)
CYP39 7 (1 subfamily member)
CYP46 (1 subfamily member)
CYP51 (1 subfamily, 1 gene, 3 pseudogenes)





Cytochrome P450: heme-proteins
monooxygenases- Mixed function oxidase

Substrate binding

.

First reduction

$Fe^{3+} \rightarrow Fe^{2+}$ by an e^- transfered from NAD(P)H via an electron transfer chain.

Oxygen binding

An O_2 molecule binds rapidly to the Fe^{2+}

Slow conversion to a more stable complex Fe^{3+}-O_2. (evidence)

Second reduction

O_2 cleavage

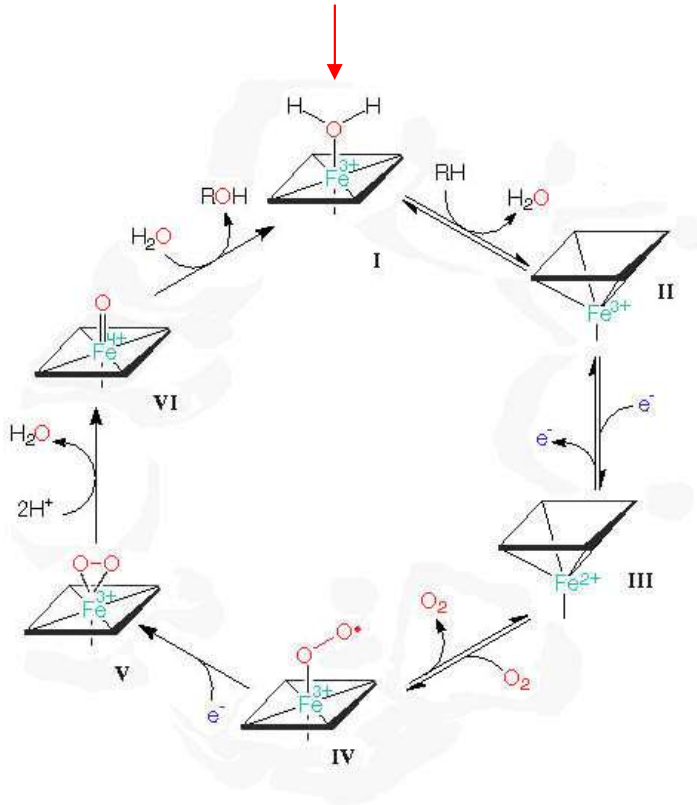
O_2^{2-} reacts with $2H^+$ from the surrounding solvent, breaking the O-O bond, forming H_2O and leaving an $(Fe\text{-}O)^{3+}$complex.

Product formation

Transfer of the Fe-ligated O atom to the substrate forming an hydroxylated form of the substrate.

Product release

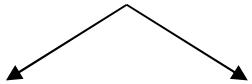
Enzyme returns to its initial state.



$Substrate\text{-}H + NADPH(H^+) + O_2 \rightarrow S\text{-}OH + NADP^+ + H_2O$

Cytochrome P450: Biological Function

Intra-cellular Metabolism



- Endogenous compounds
  - Steroids
  - Fatty acids
  - Prostaglandins
  - Other
- Xenobiotics/drug compounds
  - Phase I biotrasformations

Cytochrome P450 metabolism

- Endogenous compounds

CYP4 arachidonic acid or fatty acid metabolism
CYP5 Thromboxane A2 synthase
CYP7A bile acid biosynthesis 7-alpha hydroxylase of steroid nucleus
CYP7B brain specific form of 7-alpha hydroxylase
CYP8A prostacyclin synthase
CYP8B bile acid biosynthesis
CYP11 steroid biosynthesis
CYP17 steroid biosynthesis, 17-alpha hydroxylase
CYP19 steroid biosynthesis, aromatase forms estrogen
CYP20 Unknown function
CYP21 steroid biosynthesis
CYP24 vitamin D degradation
CYP26A retinoic acid hydroxylase important in development
CYP26B retinoic acid hydroxylase
CYP26C retinoic acid hydroxylase important in development
CYP27A bile acid biosynthesis (1 subfamily member)
CYP27B Vitamin D3 1-alpha hydroxylase activates vitamin D3
CYP27C Unknown function
CYP39 7 alpha hydroxylation of 24 hydroxy cholesterol
CYP46 cholesterol 24-hydroxylase
CYP51 cholesterol biosynthesis, lanosterol 14-alpha demethylase

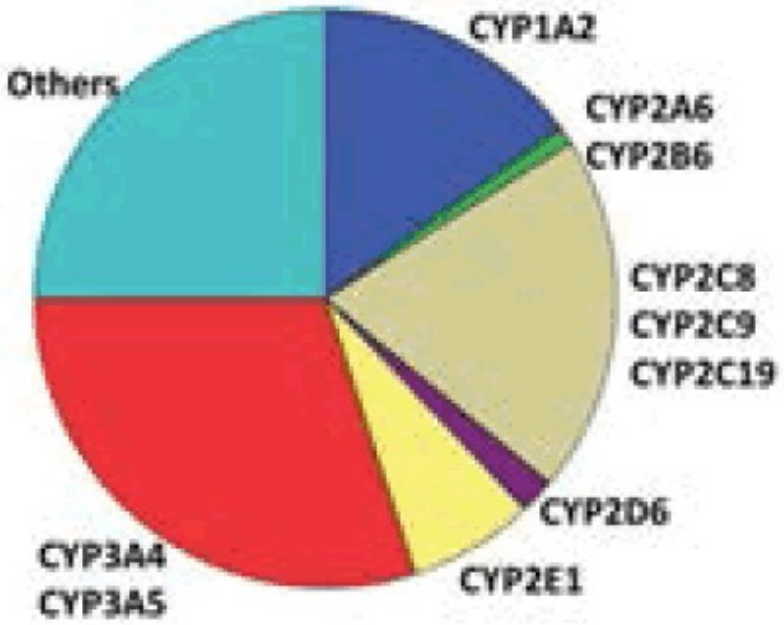
Biosynthesis/Metabolism of
Endogenous lypophilic compounds

- Xenobiotics/drug compounds

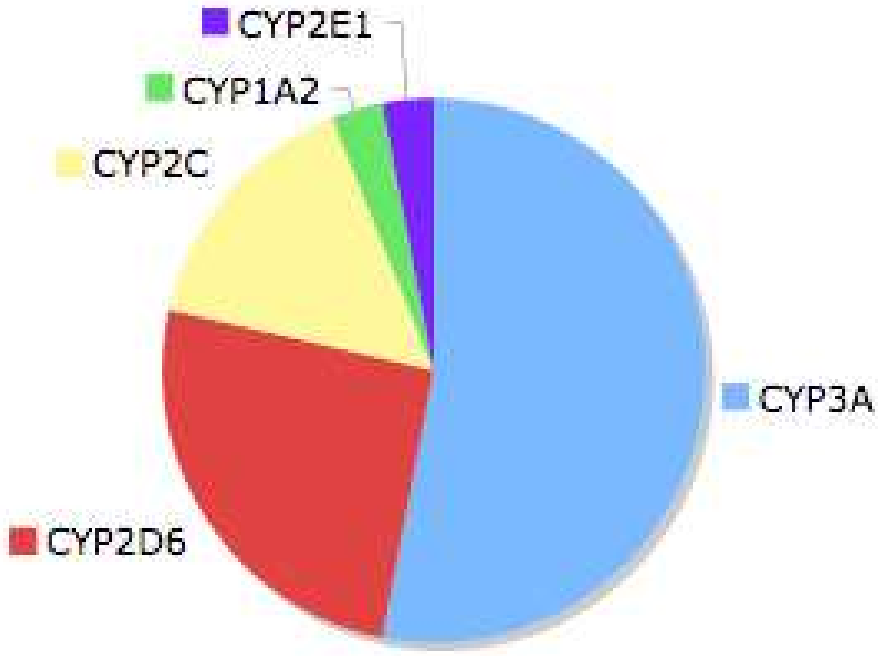
CYP**1** drug metabolism
CYP**2** drug and steroid metabolism
CYP**3** drug metabolism

Broad overlapping
substrate specificities

Cytochrome P450 drug metabolism



Distribution of the main
CYP isoforms in the liver



Relative importance of
CYP isoforms in drug metabolism

Phase I reactions

Xenobiotics → Phase I metabolite

Inactive

Equally active →

More active →

Toxic

Activation of "prodrug"

Farmaco	Metabolita	Reazione di biotrasformazione
Allopurinolo	Ossipurinolo	Idrossilazione
Cloralio idrato	Tricloroetanolo	Riduzione aldeide
Clorazepato	Nordiazepam	Conversione spontanea
Codeina	Morfina	O-demetilazione
Digitossina	Digossina	Idrossilazione
Fenilbutazone	Ossifenilbutazone (antiinfiammatorio) γ-idrossifenilbutazone (uricosurico)	Idrossilazione aromatica Idrossilazione alifatica
Imipramina	Desipramina	n-demetilazione
Propranololo	3-idrossipropranololo	Idrossilazione

Phase I reactions

Xenobiotics → Phase I metabolite

Inactive

Equally active

More active

Toxic →

Activation of "prodrug"

Farmaco	Metabolita	Reazione di biotrasformazione
Amine aromatiche	Idrossilamine (cancerogene)	N-idrossilazione
Amobarbitale	Idrossiamobarbitale	Idrossilazione
Anilina	N-idrossianilina (induce metaemoglobinemia)	N-idrossilazione
Fenitoina	Derivato epossidico (teratogeno)	Epossidazione
Idrocarburi aromatici policiclici	Derivati epossidici (teratogeni)	Epossidazione
Isoniazide	Acetilidrazina (epatotossica)	Acetilazione e idrolisi amidica
Metanolo	Formaldeide (retinopatia)	Ossidazione alcolica
Metossiflurano	Ione fluoruro (nefrotossico)	Ossidazione
Paracetamolo	N-idrossiacetaminofene	N-idrossilazione

Phase I reactions

Xenobiotics → Phase I metabolite

Inactive

Equally active

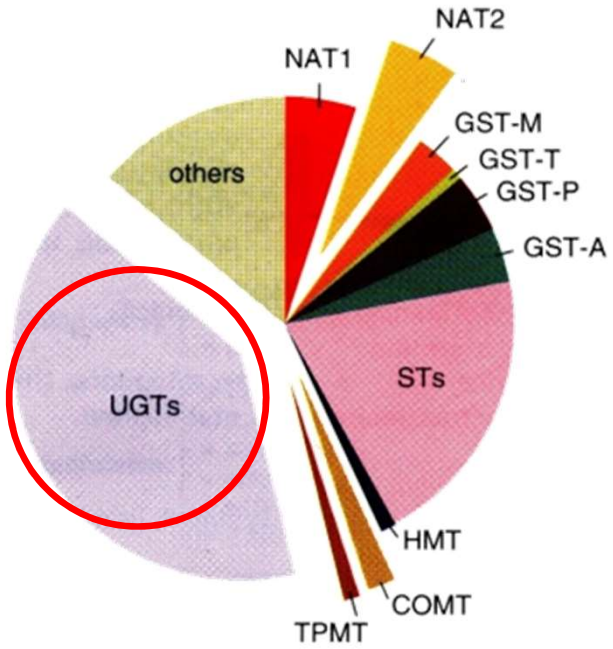
More active

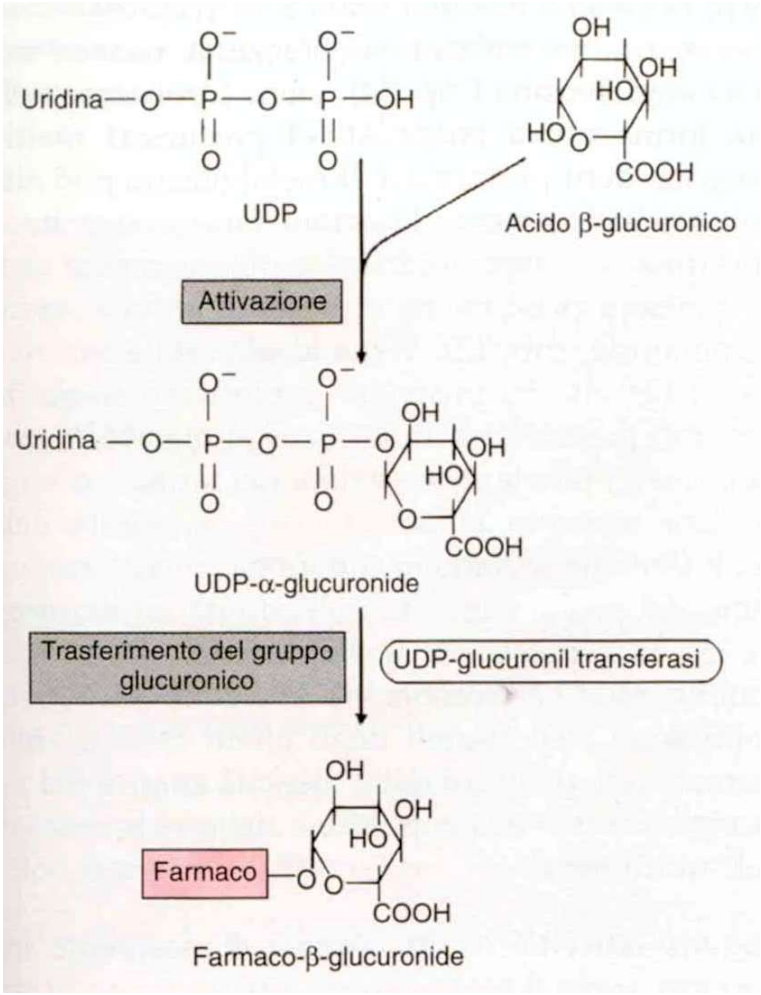
Toxic

Activation of "prodrug" →

Profarmaco	Farmaco attivo	Reazione di biotrasformazione
Prednisone	Prednisolone	Riduzione gruppo chetonico
Cortisone	Cortisolo	Riduzione gruppo chetonico
Cloramfenicolo esteri	Cloramfenicolo	Idrolisi esterea
Levodopa	Dopamina	Decarbossilazione
Mercaptopurina	Mercaptopurina-ribosio-5'-fosfato	Pirofosforilazione
Metildopa	α-metilnoradrenalina	Decarbossilazione e β-idrossilazione
Parathion	Paraoxon	Desulfurazione
Enalapril	Composto attivo	
Vitamina D_3	1,25-diidrossi-colecalciferolo	idrossilazione

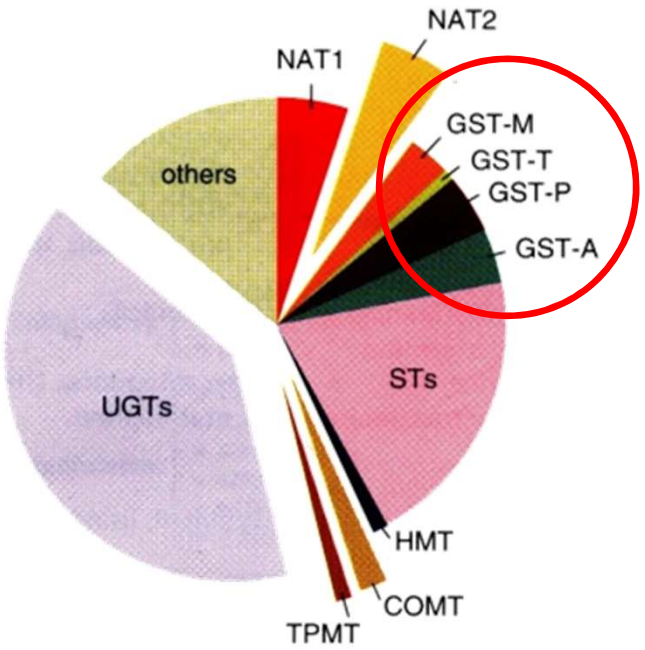
Phase II reactions

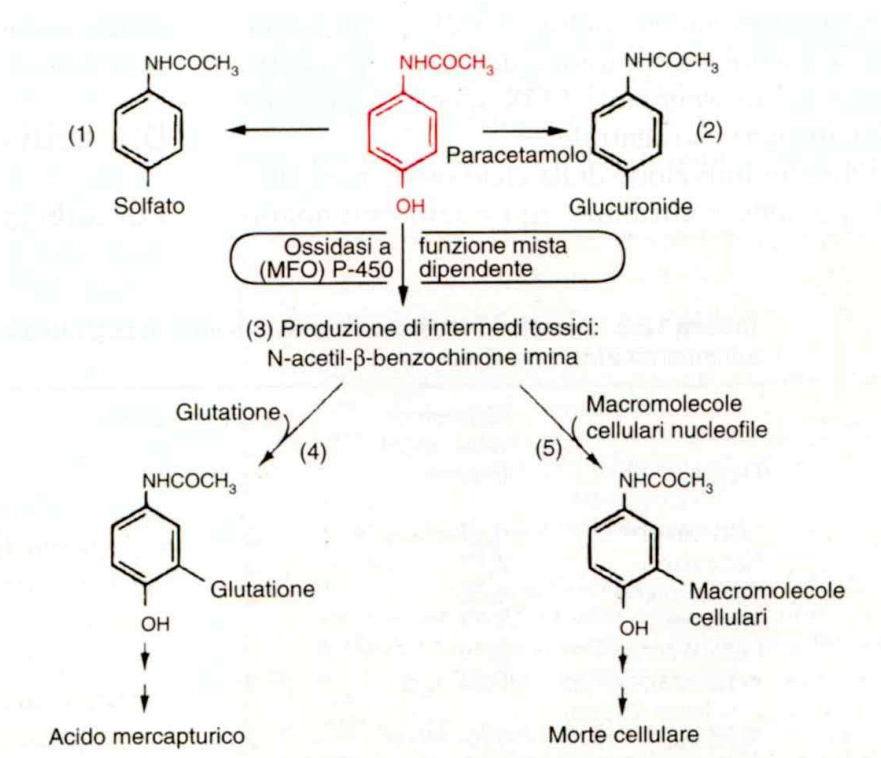




Reazione	Esempi di substrato
Glucuronidazione	Morfina, paracetamolo, nitrofenolo, diazepam, meprobamato,bilirubina, acido benzoico

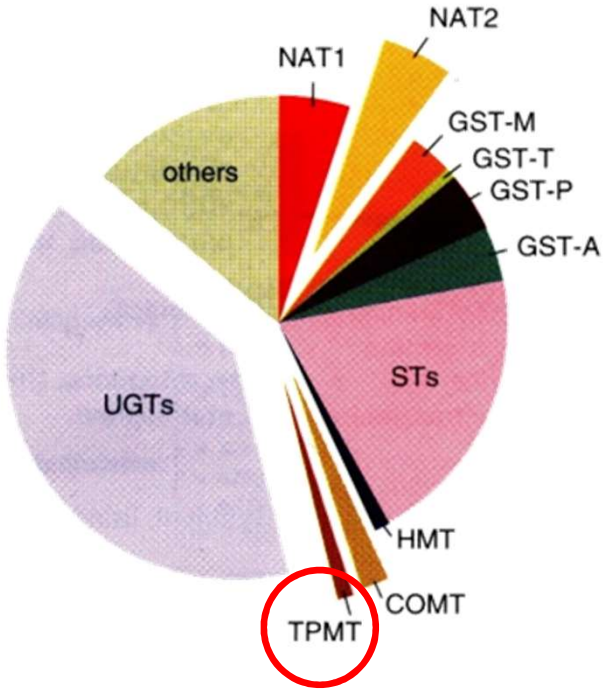
Phase II reactions





Reazione	**Esempi di substrato**
Coniugazione con glutatione	Acido etacrinico, bifenili policlorurati, naftalene, caffeina, paracetamolo

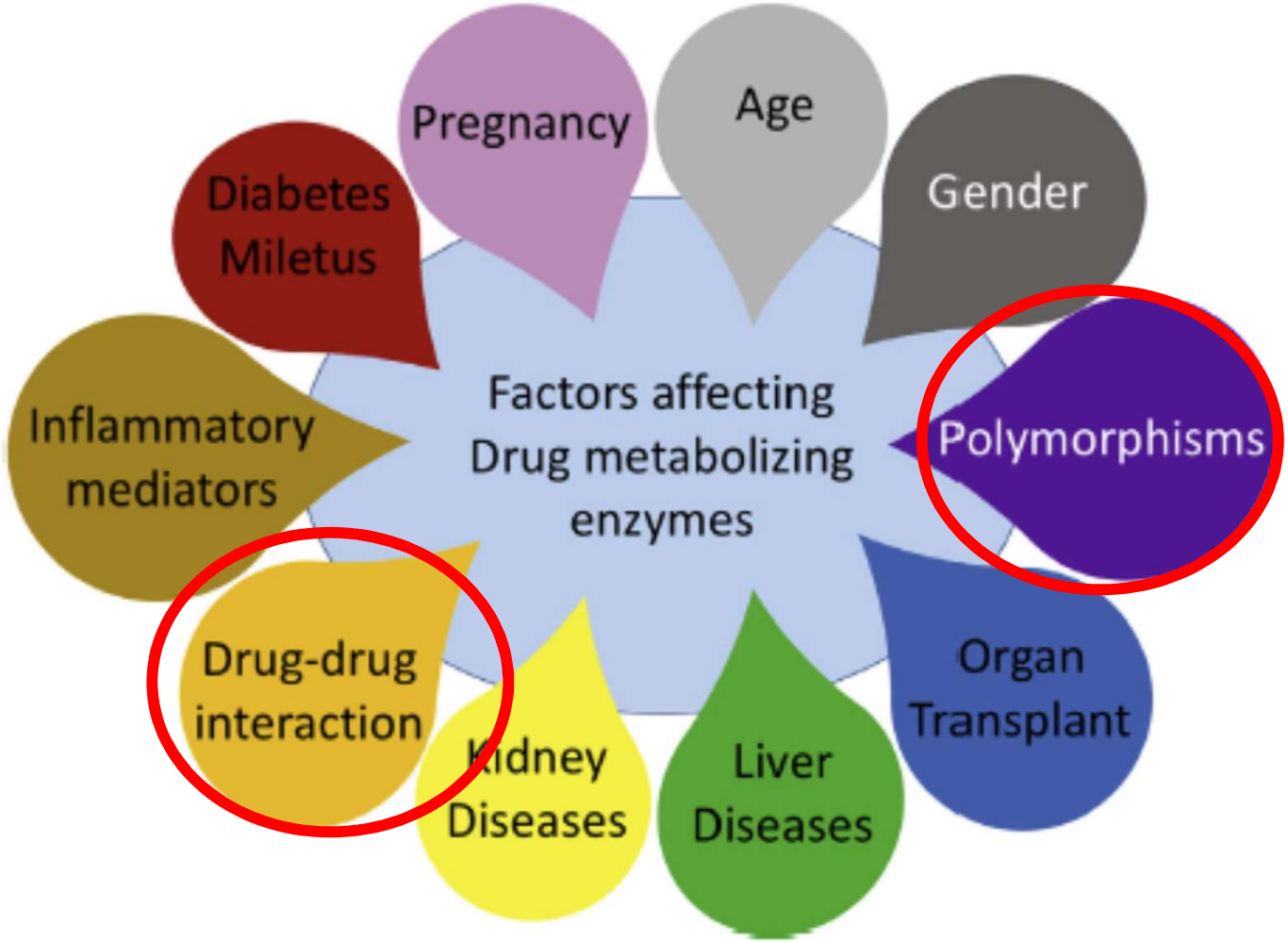
Phase II reactions



Reazione	Esempi di substrato
N-acetilazioni	Sulfamidici, idralazina, isoniazide, clorazepam, acido para-amino-salicilico, dapsone
N-metilazioni	Istamina, noradrenalina, normorfina, chinolina, triptamina, nicotina, nicotinamide
O-metilazioni	Catecolamine
S-metilazione	Tiouracile, mercaptoetanolo
Coniugazione con solfato	Estrone, anilina, fenolo, paracetamolo, metil-dopa, salicilamide
Coniugazione con aminoacidi	Acido benzoico, acido salicilico, acido nicotinico
Ribonucleosidazione	Mercaptopurina

METABOLISM

FATTORI CHE INFLUENZANO l'ESPRESSIONE E LA FUNZIONE DEGLI ENZIMI EPATICI



Almazroo et al., Clin Liver Dis. 2017 Feb;21(1):1-20.

METABOLISM and DRUG-DRUG INTERACTION

CLINICAL IMPORTANT ASPECTS
(mainly of cytochrome P450)

- INDUCTION
- INHIBITION

DRUG-DRUG INTERACTION
DRUG-DIET INTERACTION

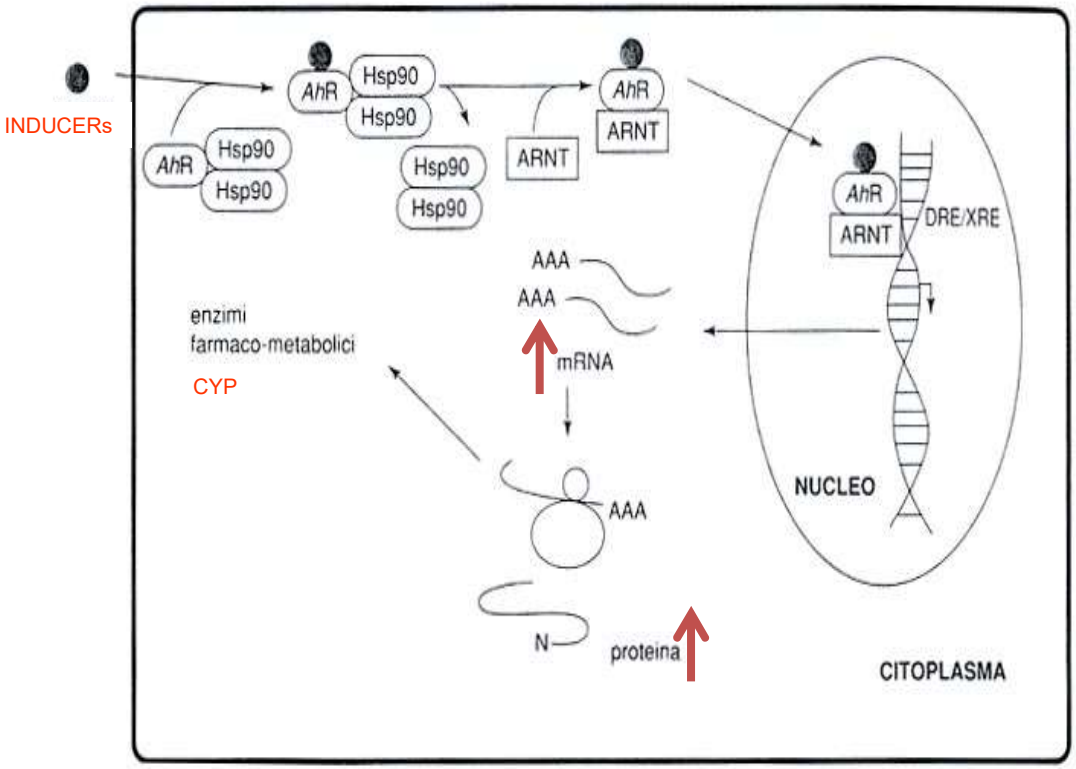
- GENETIC POLYMORPHISMS

Cytochrome P450 induction

- Reversible increase in CYP enzymes concentration
- Induction by chemicals (inducers)
  - Endocrine controls (ACTH induction of steroid biosynthetic P450s)
  - Xenobiotics
- Time dipendent effect
- Possible alteration in the metabolism of substrates taken concurrently or later on

  ↓ ↑ therapeutic effect
  ↓ ↑ drug toxicity

Cytochrome P450 induction mechanisms



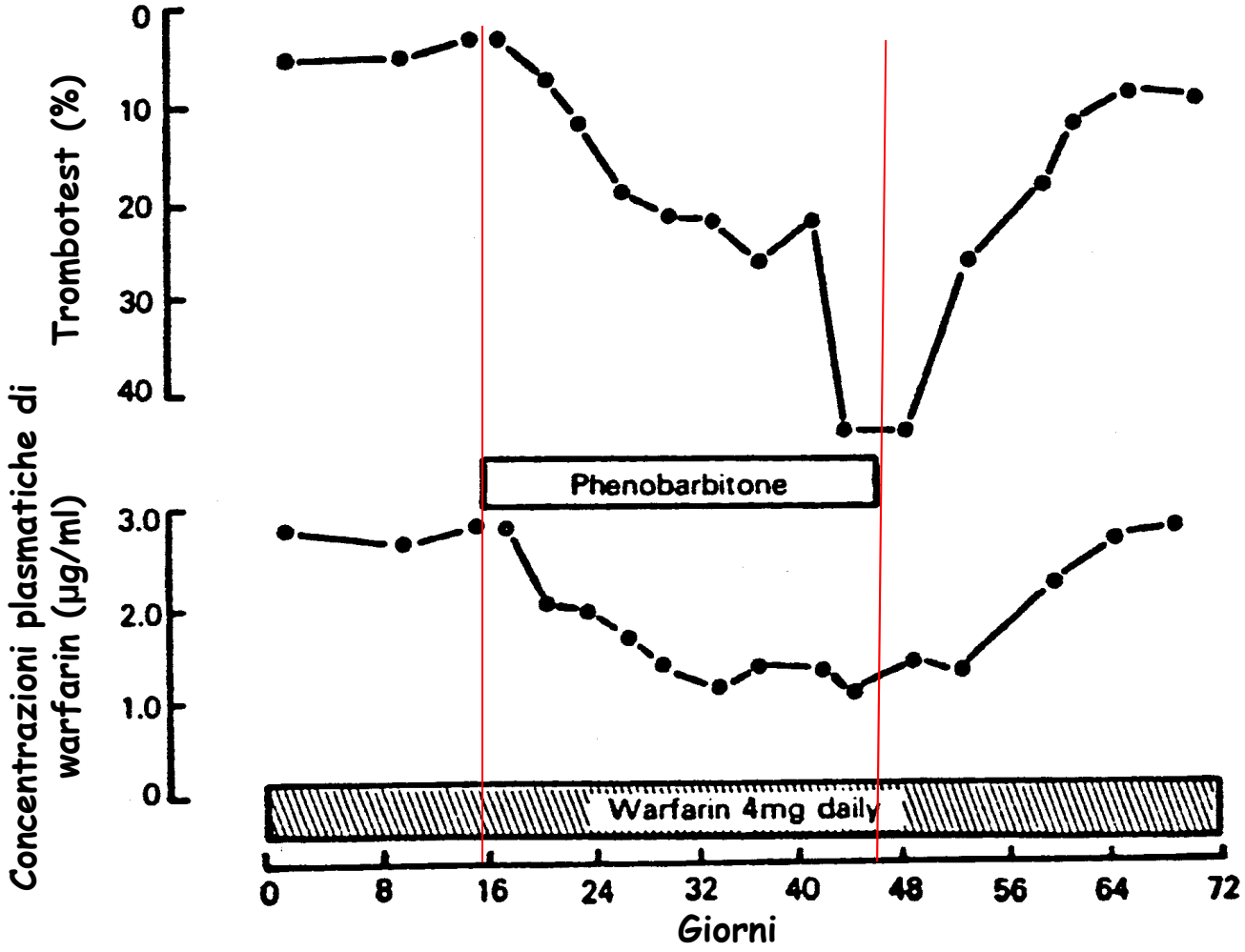
CYP	Inducers	Mechanism
1A2	3-metilcolantrene	mRNA stabilization
3A1	Desametasone	Transcription control
2B1/2B2	Fenobarbital	Transcription control
2E1	Etanolo,acetone, isoniazide	Protein stabilization

Cytochrome P450 induction

CLINICAL Examples

Inducers can decrease the therapeutic levels of drugs

Phenobarbital effect on warfarin plasma concentration



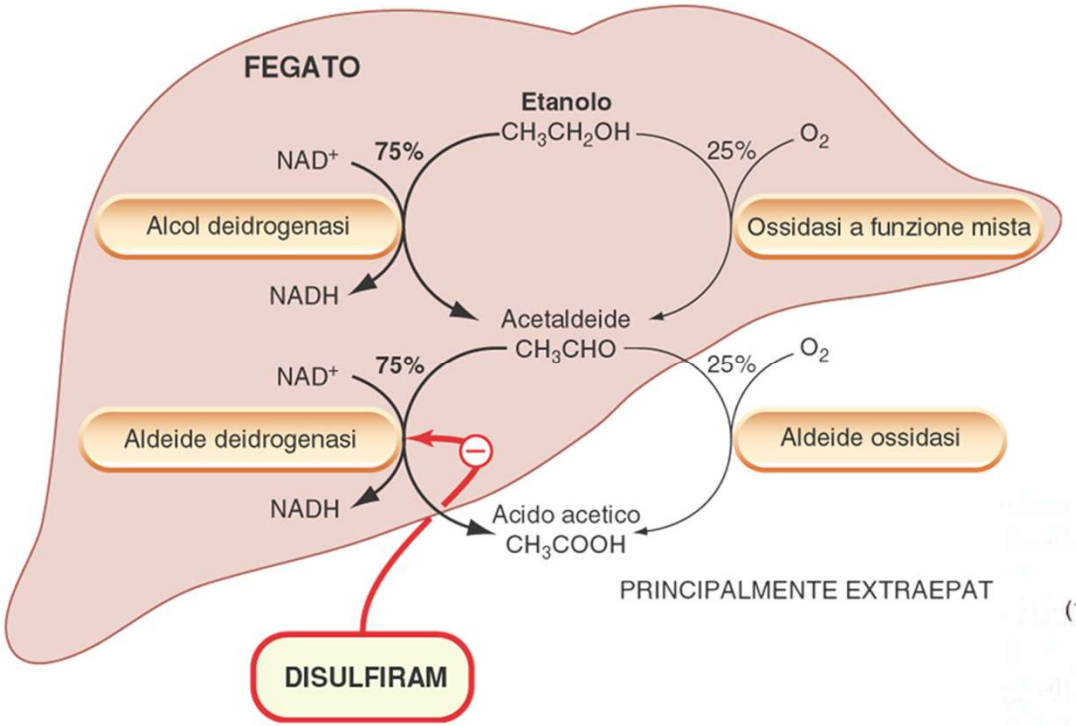
Cytochrome P450 induction

CLINICAL Examples

Inducers can decrease the therapeutic levels of drugs

Inducing agents	CYP affected	Substrates (Drug)		Potential outcome
Carbamazepine	CYP3A4	Cyclosporin	↑ inactivation	↑ risk of transplant reject
Rifampicin	CYP2C9	Warfarin	↑ inactivation ↓INR	↑ risk of Thrombosis
Hypericum	CYP3A4 (↑)	Protease inhibitors	↑ inactivation ↓HIV cocktail effectiveness	↑ HIV viral load
Cigarette Smoking	CYP1A2	Theophylline	↑ inactivation	↑ risk of Asthma attack
Ethanol	CYP2E1	Acetaminophen Ethanol		Epatotoxicity

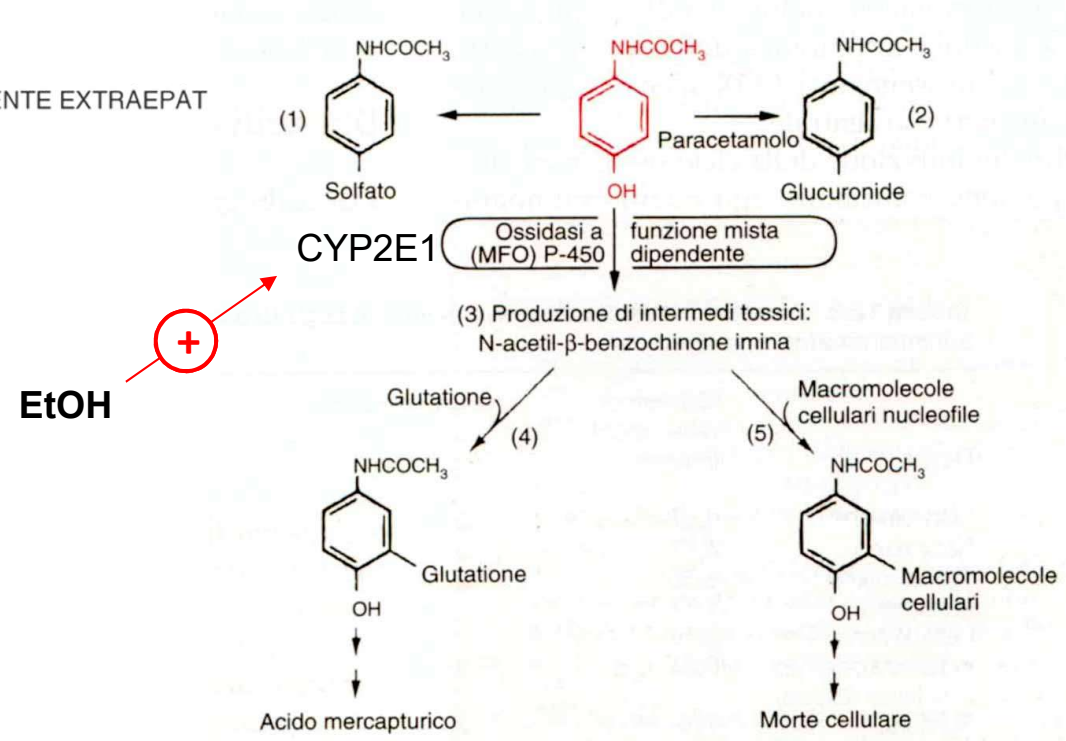
Cytochrome P450 induction



EtOH

CYP2E1

TOLERANCE
(Pharmacokinetic)



Cytochrome P450 inhibition

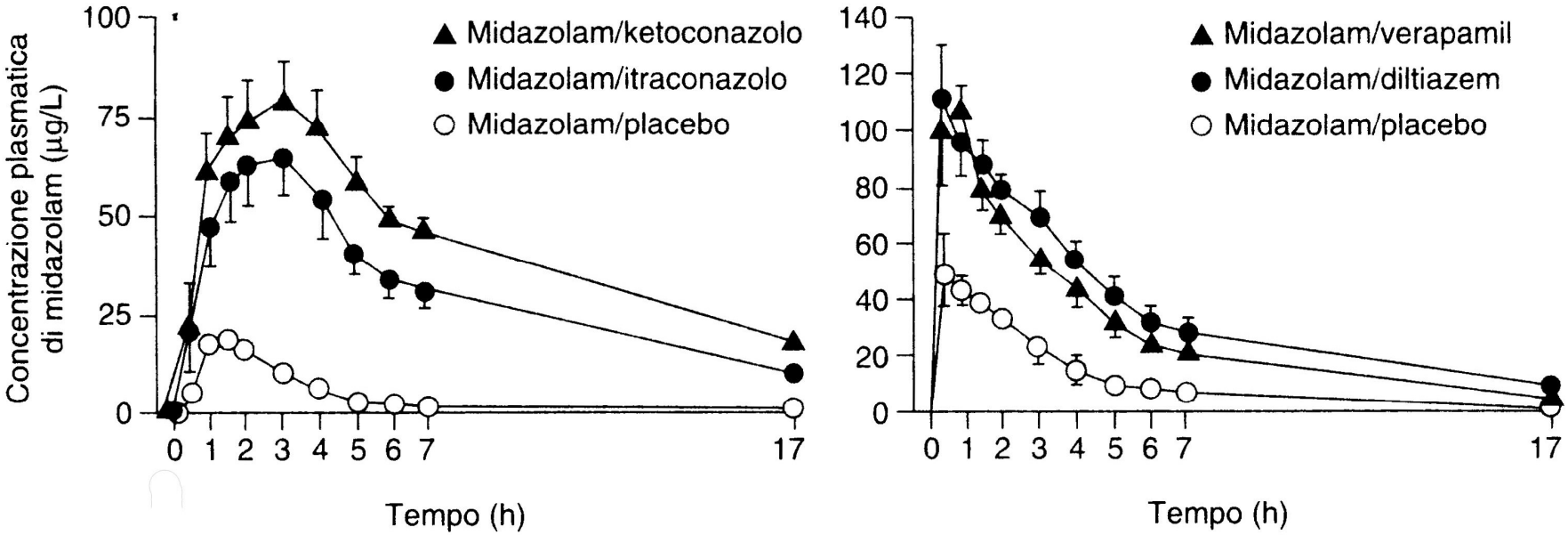
- CYP enzymes inhibition by competitive or non competitive mechanisms
- Reversible binding
- First-dose effect
- Possible increase in the serum concentration of a drug taken concurrently
- Possible toxicity

CLINICAL Examples

Inhibitors can increase the serum levels of substrates → Toxicity

Inhibitors	CYP affected	Substrates (Drug)	Potential outcome
Metronidazole	CYP2C9	Warfarin	Hemorrage
Ketoconazolo	CYP3A4	Cyclosporin	↑ immunosupression
Fluoxetina	CYP2D6	Dextromethorphan	CNS depression
Grapefruit Juice (6'-7'-dihydroxybergamottin)	CYP3A4	Cyclosporin	

Cytochrome P450 inhibition



Bacman et al., Pharmacol Toxicol 85:157, 1999

Transplantation Proceedings, 35, 215–216 (2003)

ELSEVIER

Efficacy and Safety of Low-Dose Ketoconazole (50 mg) to Reduce the Cost of Cyclosporine in Renal Allograft Recipients

M.A. Abraham, P.P. Thomas, G.T. John, V. Job, V. Shankar, and C.K. Jacob

POLYMORPHISMS

A **polymorphism** is a genetic variant that appears in at least 1% of a population

Single nucleotide polymorphism (SNP)
Transition (pur→pur, pir→pir)
Transversion (pur↔ pir)

Deletion
insertion
Sequence repeat

Microsatellite
Mini satellite
Copy number variation
Translocation
Inversion

Inter-individual and inter-ethnic differences in drug metabolism

METABOLISM and POLYMORPHISMS

Polymorphisms in phase I/II encoding genes

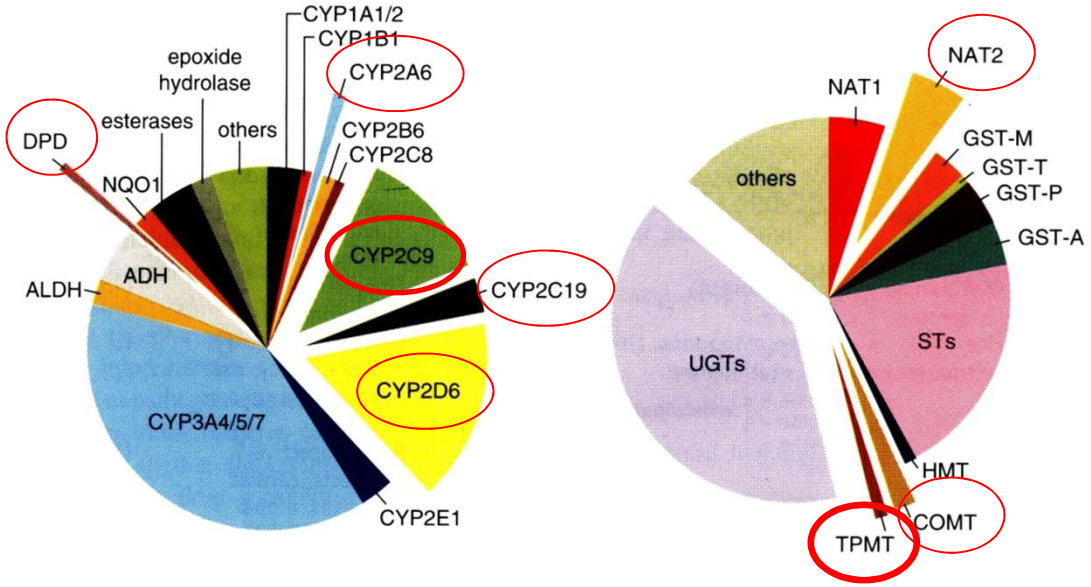
Poor metabolizers

Rapid metabolizers

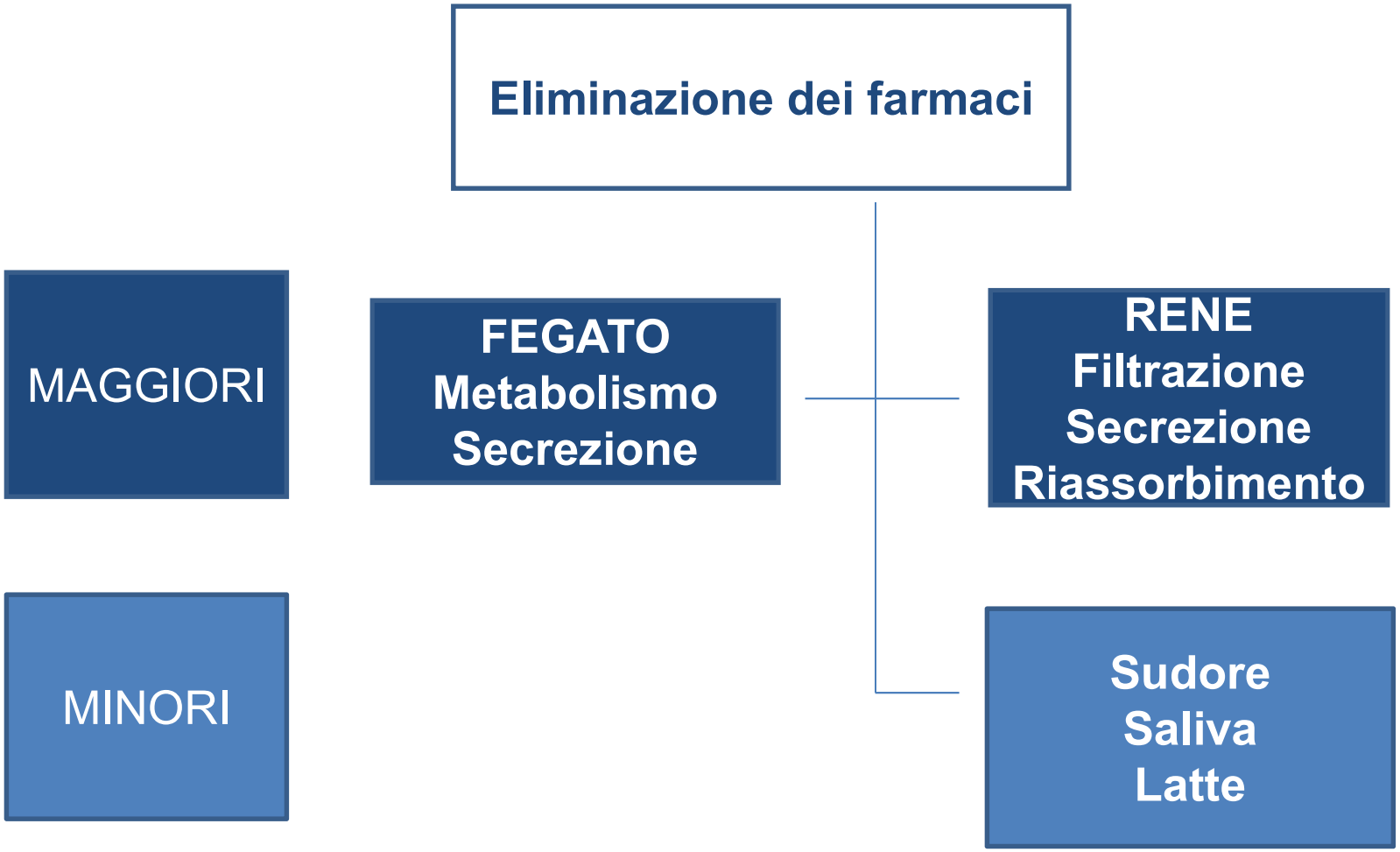
Ultrarapid metabolizers

↑ ↓Toxicity

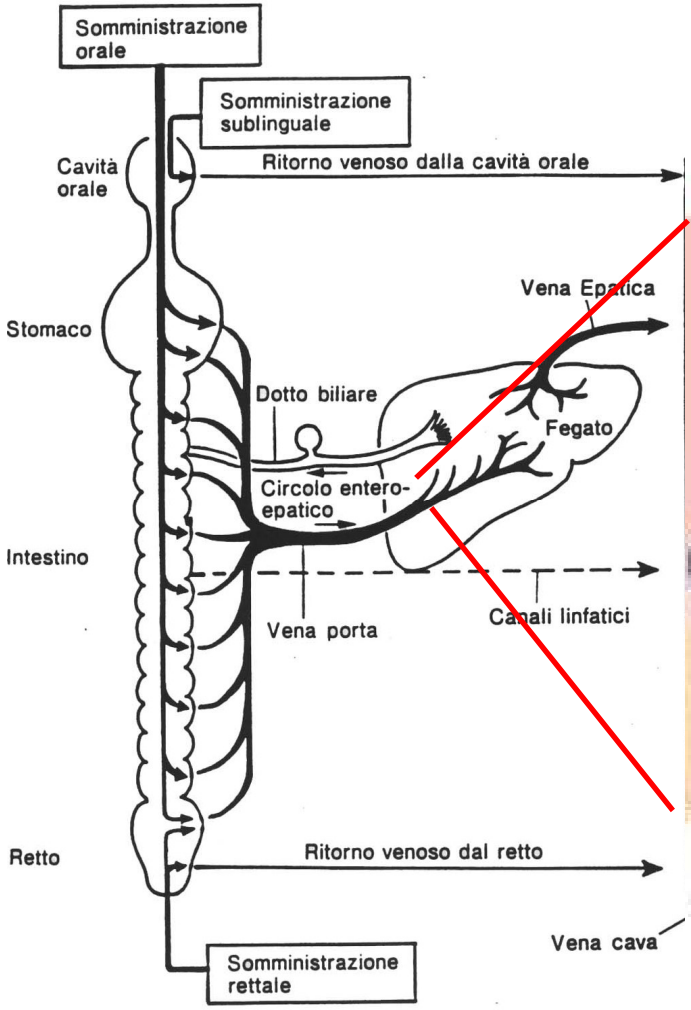
↑ ↓ Therapeutic responce

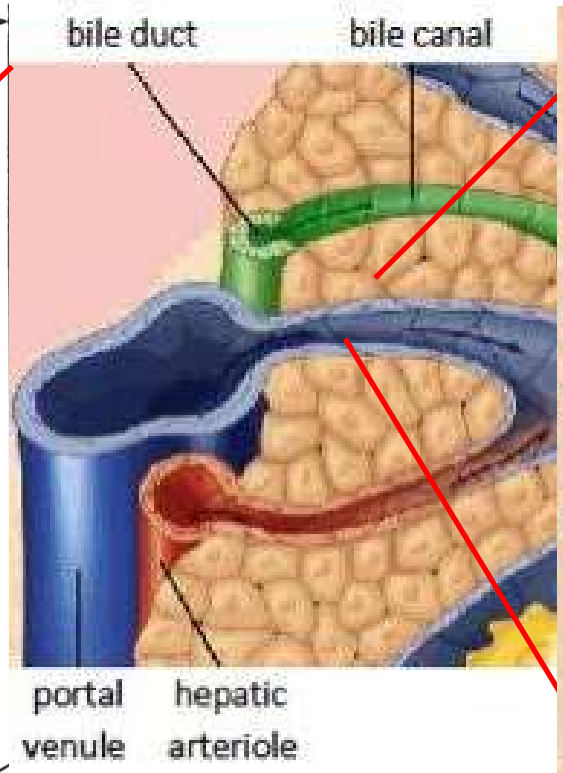


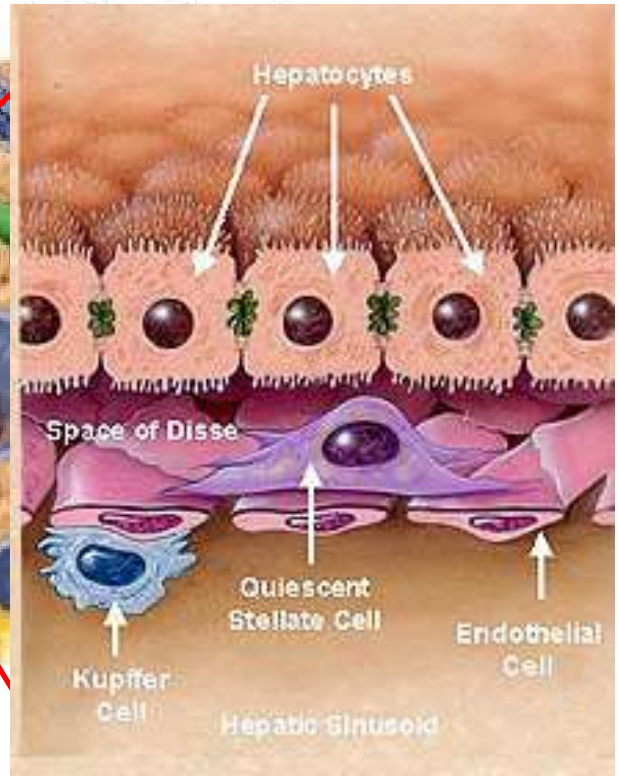
ELIMINAZIONE



ESCREZIONE BILIARE





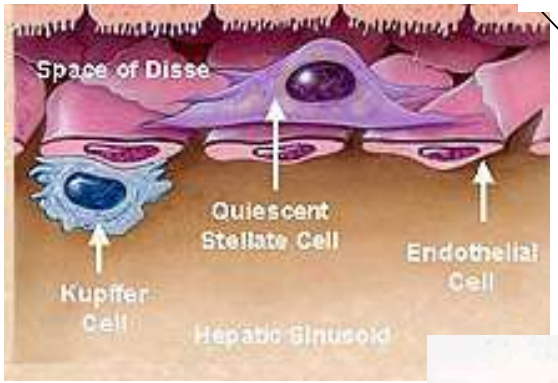


Vena porta

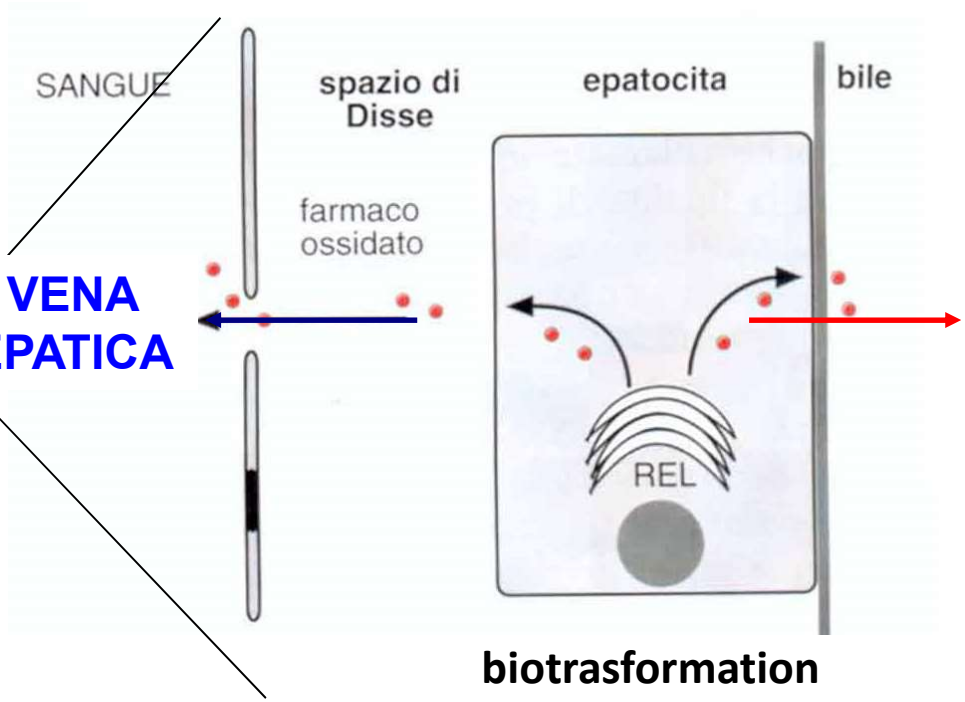
Vena epatica

ENTEROHEPATIC RECIRCULATION and GLUCORONIDED DRUGS

EFFETTO di PRIMO PASSAGGIO

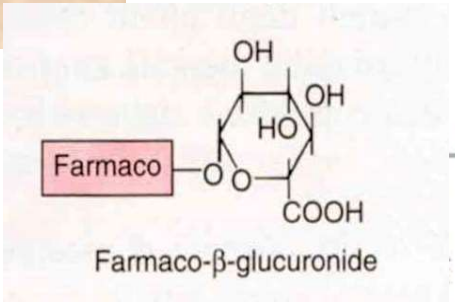


VENA EPATICA



INTESTINO

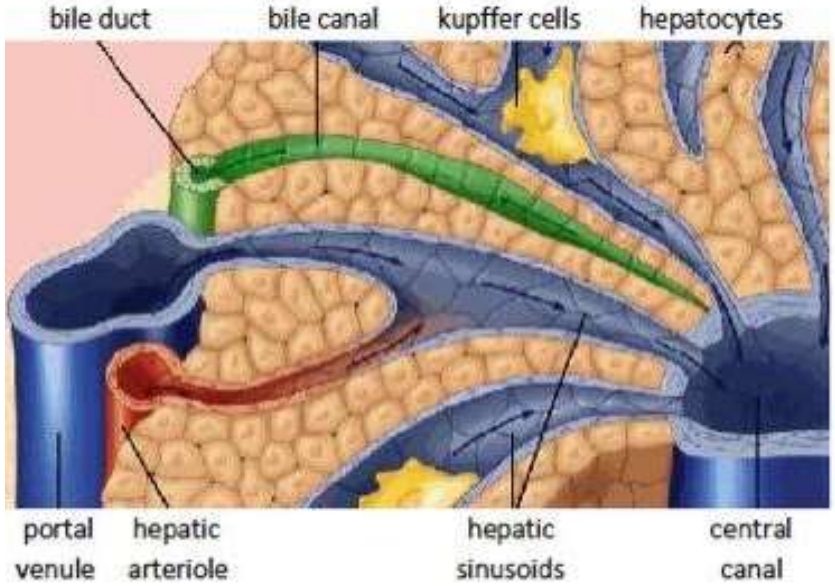
biotrasformation



→ **substrato per le β–glucuronidasi nella flora intestinale**

FEGATO → **BILE** → **INTESTINO** → **FLORA BATTERICA**

ESCREZIONE BILIARE



- ***Epatociti → Bile → Intestino***
- ***Farmaci e metaboliti coniugati***
- ***Effetto di primo passaggio o eliminazione presistemica epatica***
- ***Circolo enteroepatico (20% del farmaco)***

Secrezione: 4 sistemi di trasporto attivo

- **Acidi**
- **Basi**
- **Composti organici neutri**
- **Acidi bilari**

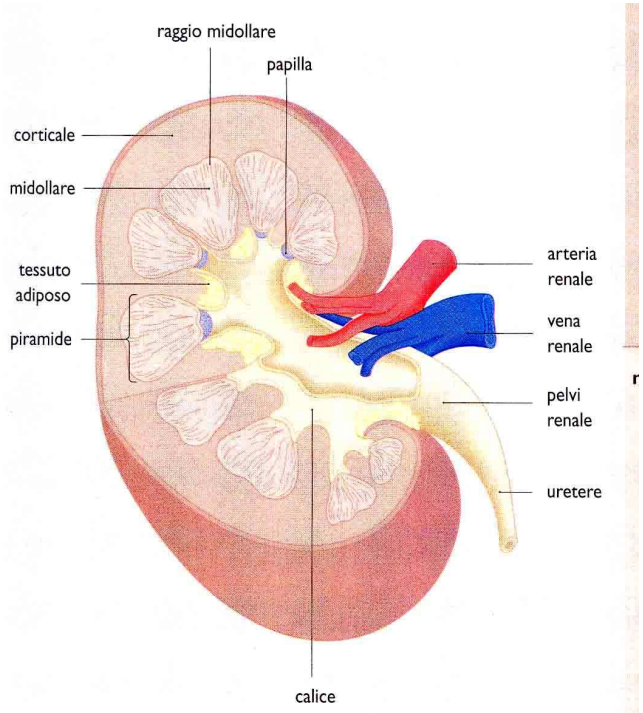
- *Contro gradiente di concentrazione*
  - *Distinti*
  - *Inibizione per competizione*
  - *Saturabili (velocità massima)*

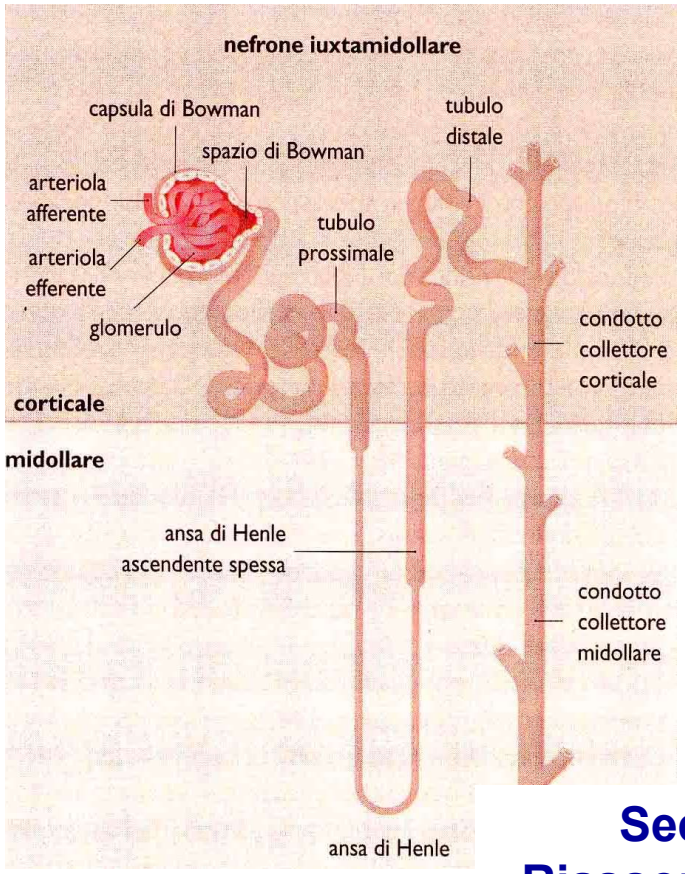
Composti polari

- **P.M. < 250 → eliminazione renale**
- **P.M. > 500 → eliminazione biliare**

ESCREZIONE RENALE

- Meccanismo principale con cui i farmaci vengono allontanati dall'organismo.
- Richiede che i farmaci o i loro metaboliti abbiamo delle caratteristiche idrofiliche.





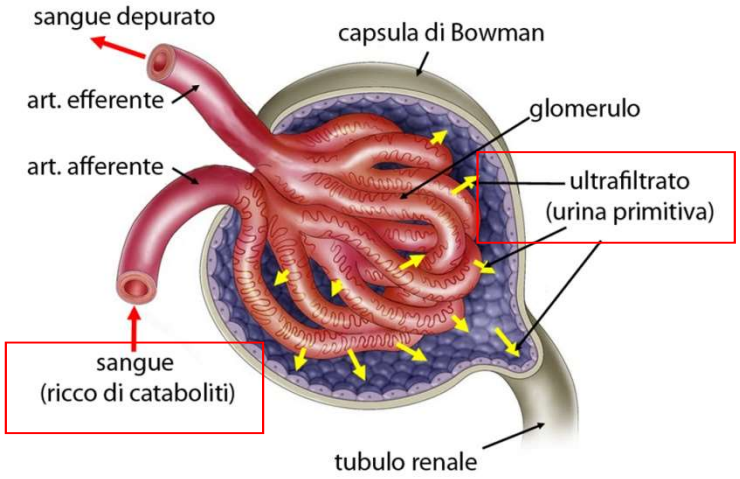
Filtrazione glomerulare

Secrezione tubulare
Riassorbimento passivo

ESCREZIONE RENALE: FILTRAZIONE

in 24 ore

~850 l di sangue ultrafiltrato (50x i liquidi extracellulari, 15 l)



Passano attraverso il filtro glomerulare

- -farmaci <20000 Da
- -farmaci non legati alle proteine plasmatiche

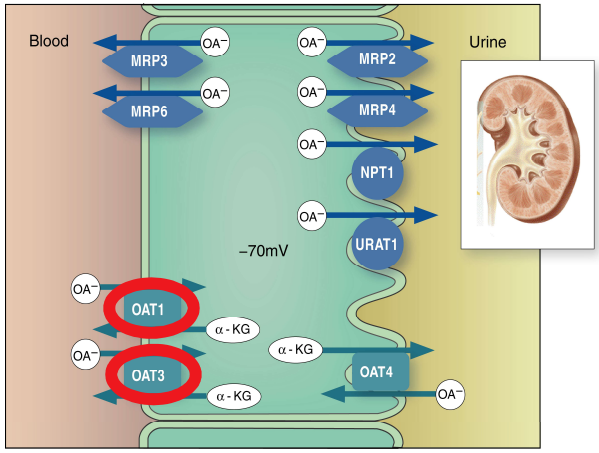
~170 l di preurina (~20% del sangue ultrafiltrato)

~65% nel tubulo contorto prossimale, 15% nella branca discendente dell'ansa di Henle, 19% nel tubulo contorto distale e nel dotto collettore

~ 1% dell'ultrafiltrato viene escreto nelle urine (1.7 l)

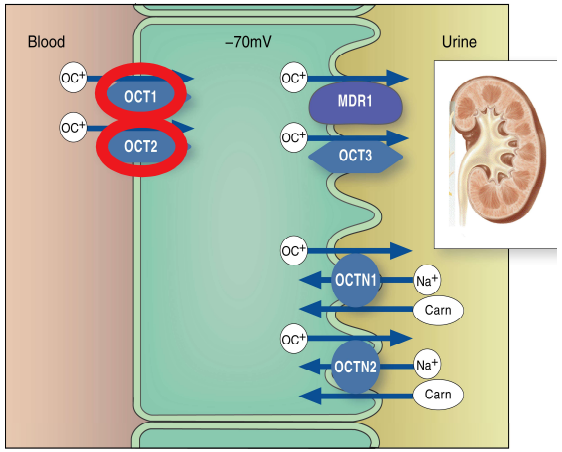
ESCREZIONE RENALE: SECREZIONE

OAT: trasportore degli anioni organici (farmaci acidi)



Contro gradiente di concentrazione
Sistema specifico
Alta velocità
Saturabile
Capacità massima

OCT: trasportore dei cationi organici (farmaci basici)



Composti endogeni
Sali biliari, bilirubina, cAMP, cGMP, acidi grassi, ossalati, urati, prostaglandine
Farmaci e metaboliti
Aciclovir, cefalosporine, penicilline, sulfamidici, probenecid, captopril, chinolonici, tiazidici, furosemide, acido etacrinico, ibuprofene, indometacina, salicilati Glucuroconiugati, coniugati con glutatione, coniugati con glicina, sulfoconiugati, sulfamidici acetilati

Composti endogeni
Acetilcolina, creatinina, catecolamine
Farmaci
Amiloride, atropina, cimetidina, ranitidina, ß-bloccanti, procainamide, chinidina, chinina, triamterene, trimetoprim

ESCREZIONE RENALE: RIASSORBIMENTO

- ***Tubulo contorto prossimale***
  - ***Molecole endogene***
    - ***glucosio, vitamine, aa***
  - ***Carriers***

- ***Tubulo distale/ dotto collettore***
  - ***Farmaci***
  - ***passivo, secondo gradiente***
  - ***Lipofilia***
    - Farmaci liposolubili (alta permeabilità tubulare)→eliminazione lenta.
    - Farmaci (o metaboliti) idrosolubili (bassa permeabilità tubulare) →eliminazione veloce
  - ***pKa***
    - Farmaci acidi → escreti più facilmente in ambiente alcalino
    - Farmaci basici → più facilmente escreto in ambiente acido
  - ***Flusso urinario***
    - Volume maggiore
    - diluizione → diminuisce il gradiente di concentrazione e il tempo di contatto

CLEARANCE

Parametro farmacocinetico,
misura quantitativamente l'eliminazione

CLEARANCE è il volume VIRTUALE di sangue depurato dal farmaco dopo passaggio attraverso un organo per unità di tempo (ml/min/kg).

E' determinata prevalentemente da una componente renale (Cl_R) ed una epatica (Cl_H)

$$CL = Q \frac{(C_1 - C_2)}{C_1}$$

Q= flusso ematico

C_1= concentrazione plasmatica del farmaco nel sangue arterioso in entrata all'organo

C_2= concentrazione plasmatica del farmaco nel sangue venoso in uscita dall'organo

CLEARANCE RENALE

CLEARANCE RENALE è il volume di sangue (ml) depurato dal farmaco dal rene in 1 minuto

$$C_R = \frac{U \times V}{P}$$

U= concentrazione nell'urina (mg/ml)

V= volume di urina (ml) in un minuto

P= concentrazione plasmatica

$C_R = 0$ → Viene completamente riassorbito (glucosio)

$C_R = Cl_{INULINA}$ =volume di plasma ultrafiltrato (110-142 ml/min uomini; 100-130 ml/min donne)

=velocità di filtrazione glomerulare

Non si lega alle proteine, non subisce riassorbimento né secrezione

$C_R < Cl_{INULINA}$ → Viene in parte riassorbito

$C_R > Cl_{INULINA}$ → Viene in parte secreto

$C_R = Cl_{PAI}$ = flusso plasmatico renale totale (700 ml/min)

Filtrazione glomerulare e secrezione, non riassorbito

Clearance

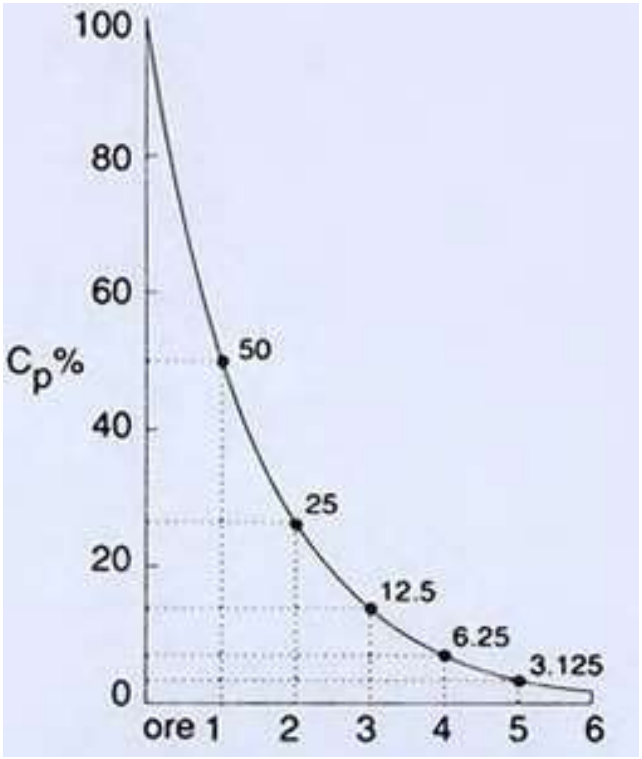
- Eliminazione completa di un farmaco indipendentemente dalla via di somministrazione
- Cl = volume di liquido depurato dal farmaco nell'unità di tempo (ml/min/kg)
- Dipende dal $t_{1/2}$ e dal Vd

Cl = K x Vd

TEMPO di EMIVITA

Parametro faramcocinetico
TEMPO NECESSARIO A RIDURRE del 50% il FARMACO PRESENTE NELL'ORGANISMO

Cinetica di I° ordine

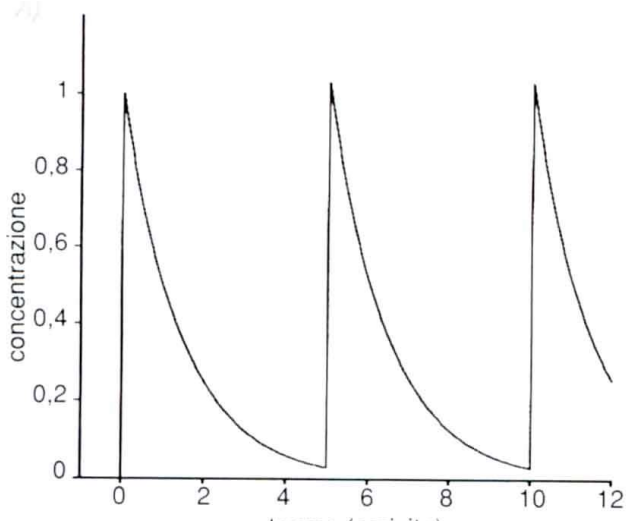


N° di $t_{\frac{1}{2}}$	Frazione di farmaco **rimanente**
0	100%
1	50%
2	25%
3	12.5%
4	6.25% → >94% eliminato
5	3.125
6	1.56%
7	0.78%
8	0.39%
9	0.195%
10	0.0975% → 99.9% eliminato

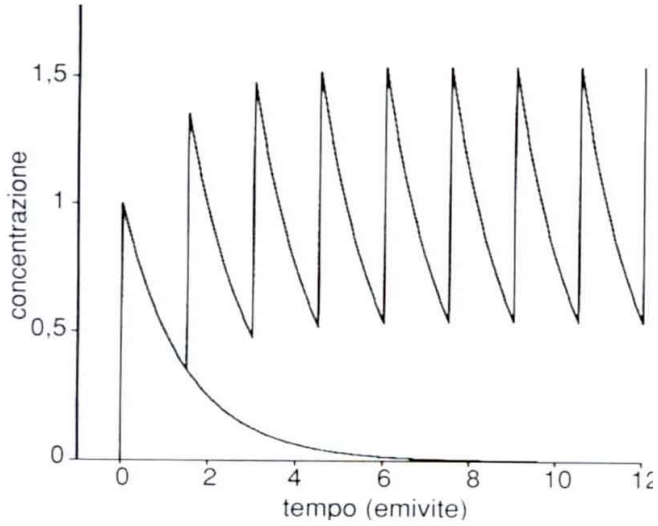
TEMPO di EMIVITA

TEMPO NECESSARIO A RIDURRE del 50% il FARMACO PRESENTE NELL'ORGANISMO

- **<u>Necessario per determinare:</u>**
  - **Durata dell'effetto benefico o tossico**



  - **SOMMINISTRAZIONI RIPETUTE**
    - **Intervalli tra le dosi**
    - **Tempi di sospensione**

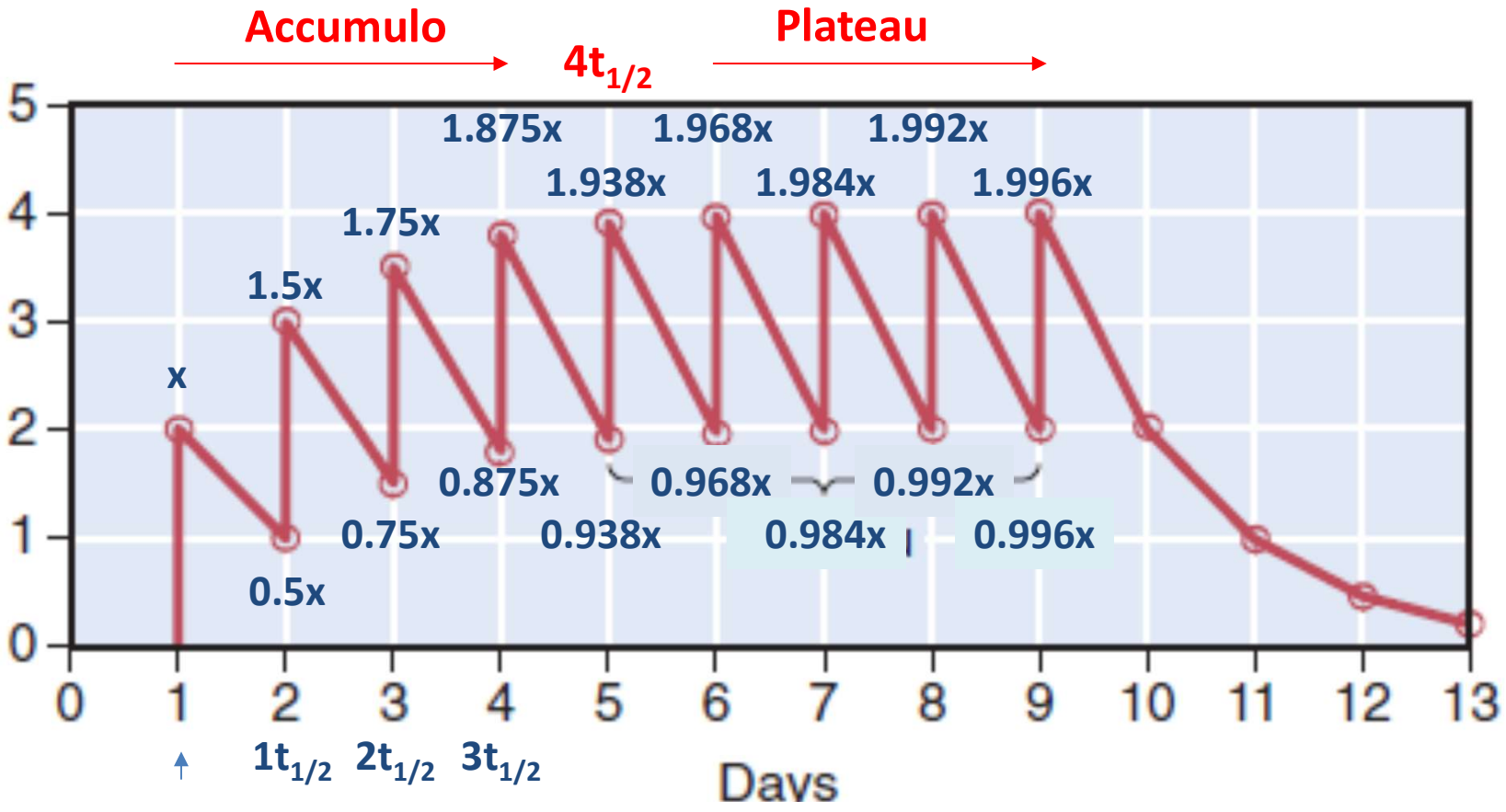


TEMPO di EMIVITA

ESEMPIO: $t_{1/2}$ = 1 giorno

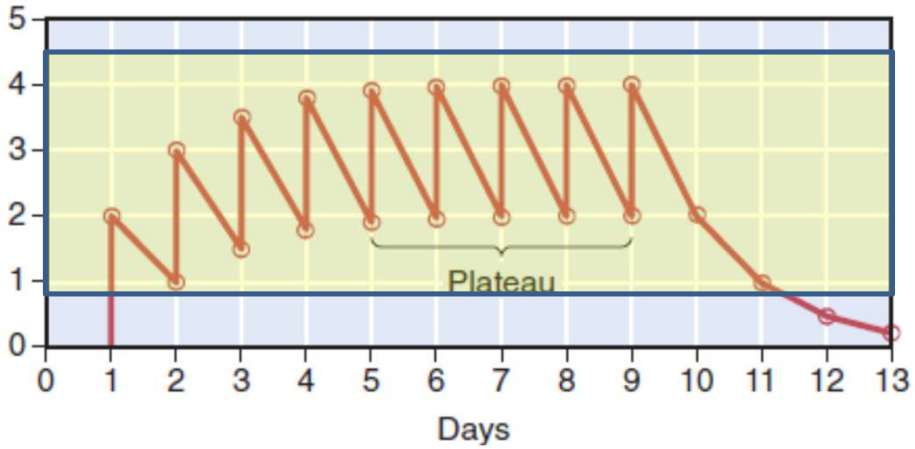
Stessa dose x

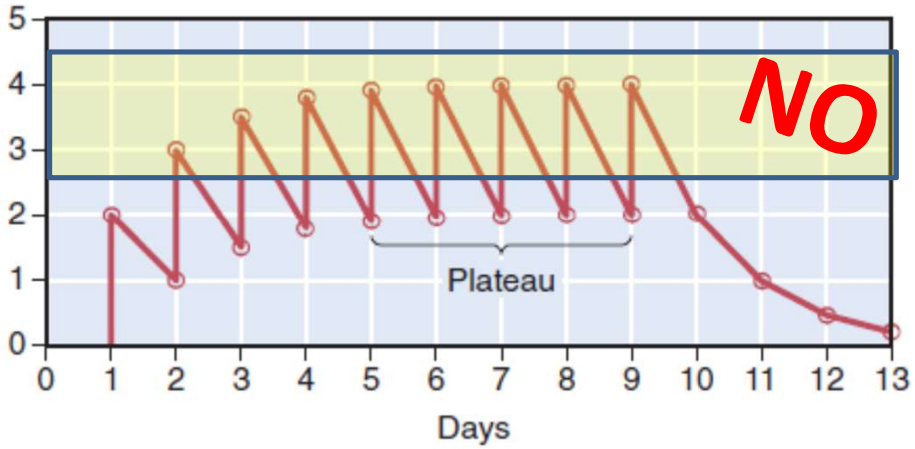
$T_{somministrazione} = t_{1/2}$

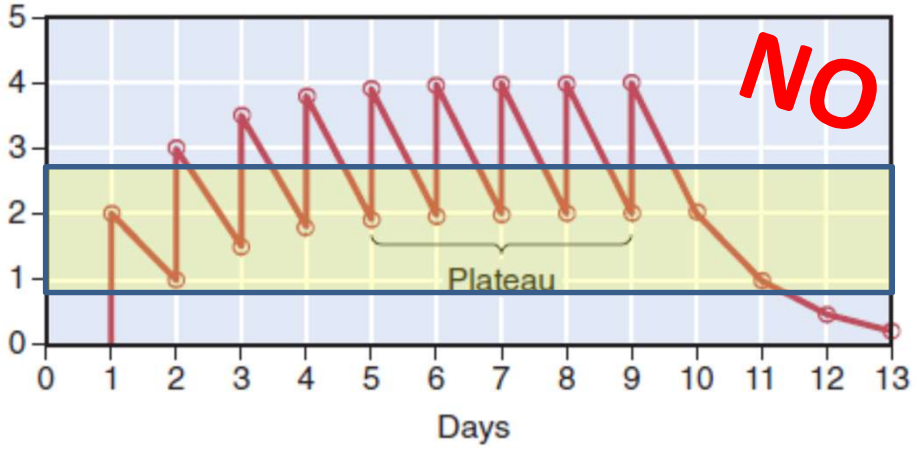


Accumulo di un **farmaco** (**t½: 4h**) nell'organismo
dopo somministrazioni ripetute

Intervallo tra le dosi: 4h			**Intervallo tra le dosi: 8h**		
ore	Quantità di farmaco nell'organismo		ore	Quantità di farmaco nell'organismo	
	Prima della dose	Dopo la dose		Prima della dose	Dopo la dose
0	0	10	0	0	10
4	5	15		5	
8	7.5	17.5	8	2.5	12.5
12	8.75	18.75		6.25	
16	**9.375**	19.37	16	**3.125**	13.125
20	9.69	19.69		6.56	
24	9.84	19.84	24	3.281	13.281
28	9.92	19.92		6.640	
32	9.96	19.96	32	3.320	13.320
∞	10.00	20.00	∞	3.330	13.333







Somministrazioni ripetute

Stessa dose x, $T_{somministrazione} = t_{1/2}$