- **Pharmacokinetic principles**
- **Drug absorption, distribution, metabolism and elimination**

**Jan Juřica, PharmD., Ph.D.**

# Pharmacokinetics

**Occupation theory:** The intensity of pharmacological response (E) is proportional to the conentration of reversible drug-receptor complex

**= Action of a drug requires presence of a certain concentration in the fluid bathing the target tissue.**

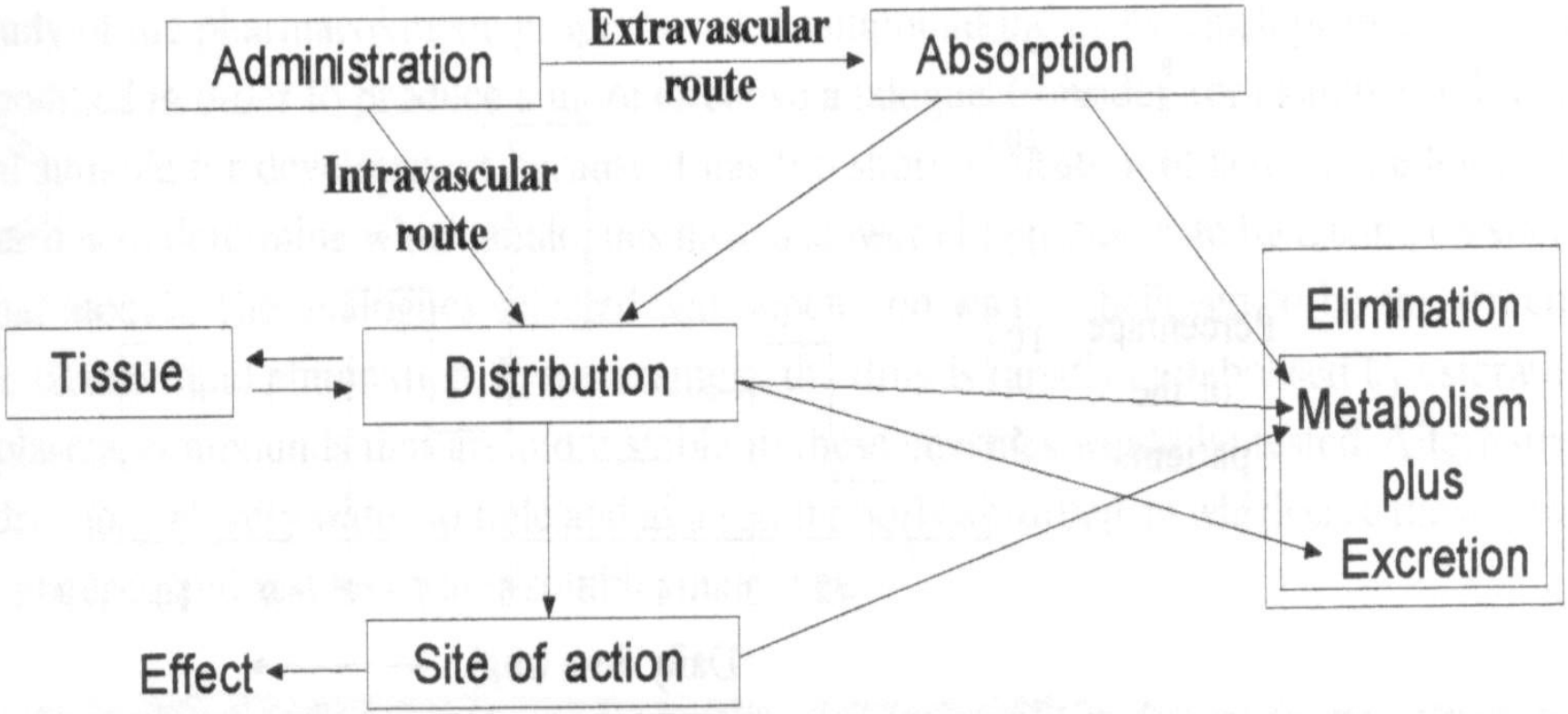
Pharmacokinetics deals with the processes of

absorption, **A**
distribution, **D**
metabolism **M**
excretion of the drug **E**

**invasion**
**elimination**
**"ADME"**

And their relationship with their biological (pharmacological) effect

**„WHAT DOES ORGANISM DO WITH THE DRUG"**

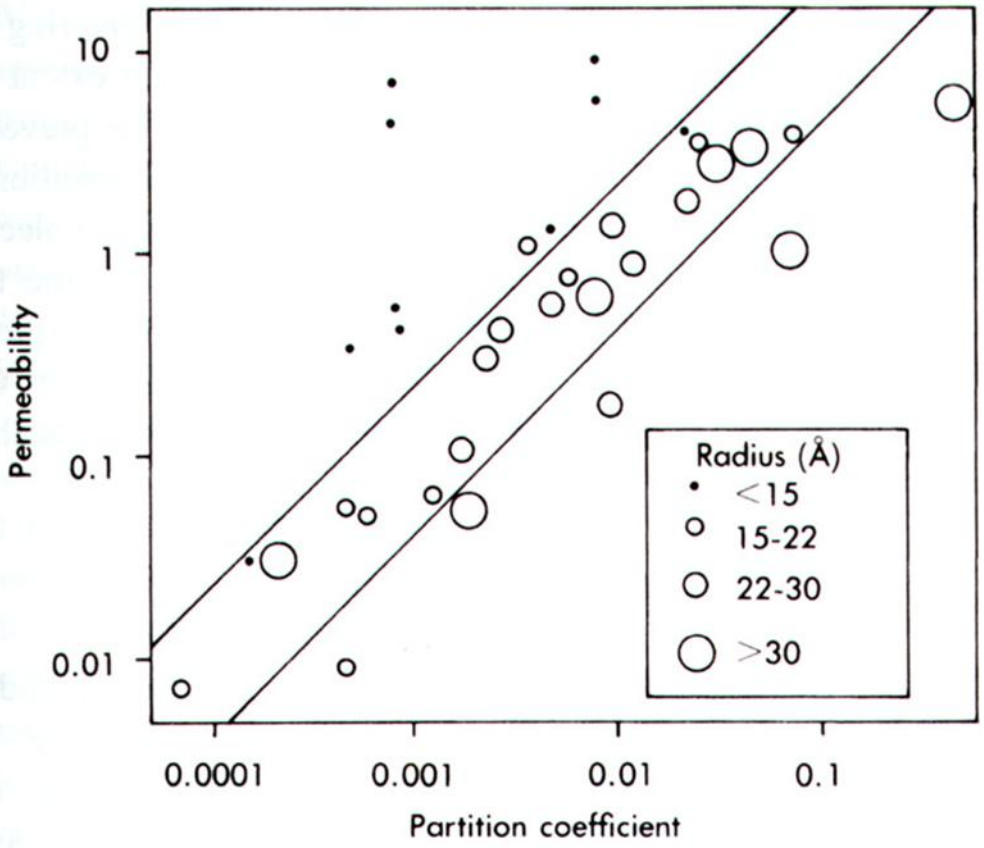



The general stages and their relationships in the life cycle of a drug after administration.

# What does influence the movements of the drug in the body?

## physico-chemical properties

lipophilic/hydrophilic properties, molecule structure, pKa, charge…




Ionized compounds tend to be *less* lipid soluble.

Non-Ionized compounds tend to be *more* lipid soluble.

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**physico-chemical properties**

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**AH ⇆ A- + H+ B + H+ ⇆ BH+**

**permeation across the membranes**

lipophilic – difusion (passive)

hydrophilic – through the pores

active transport

**bonds of the drugs to:**

plasma proteins

blood cells in the circulation

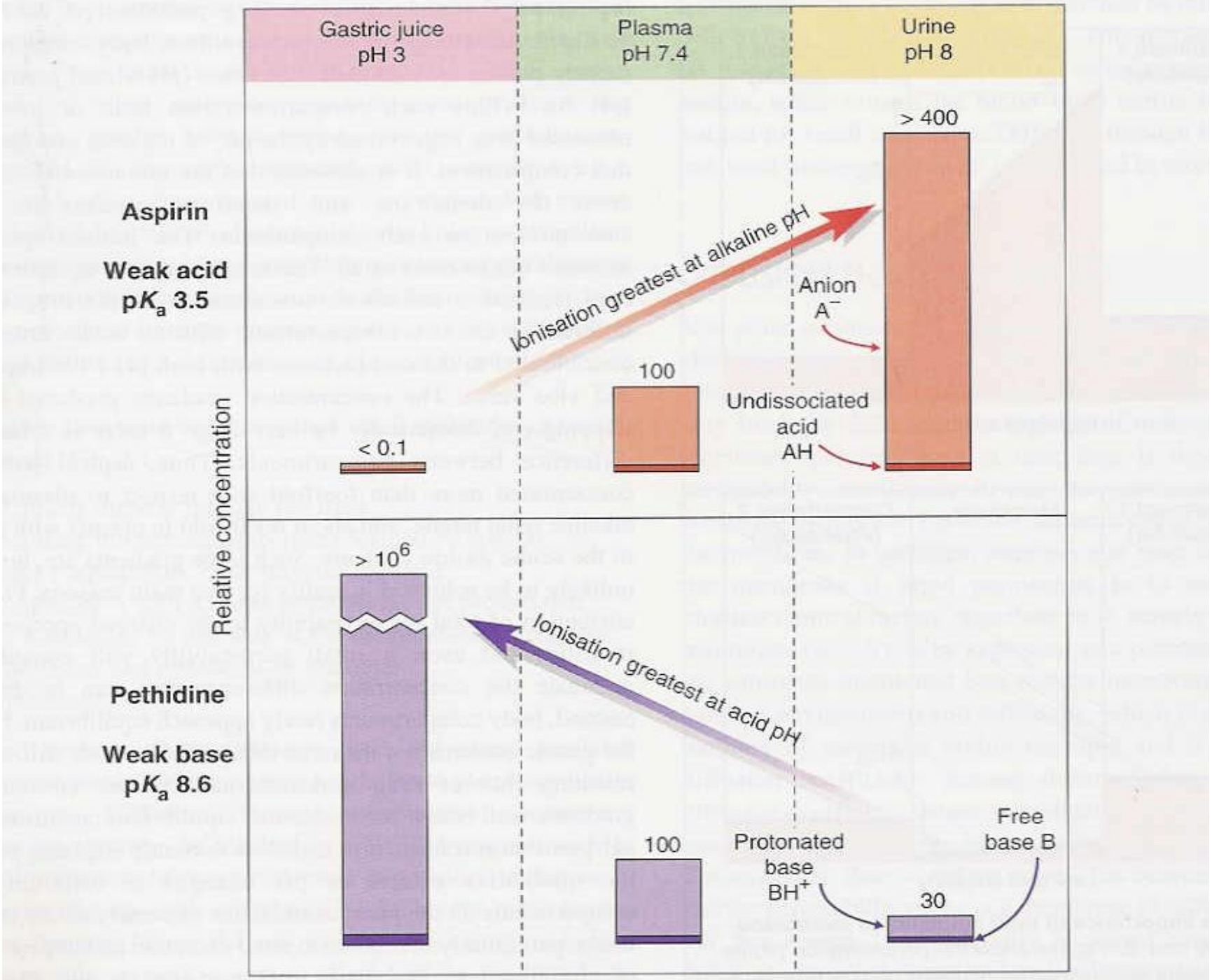
tissue

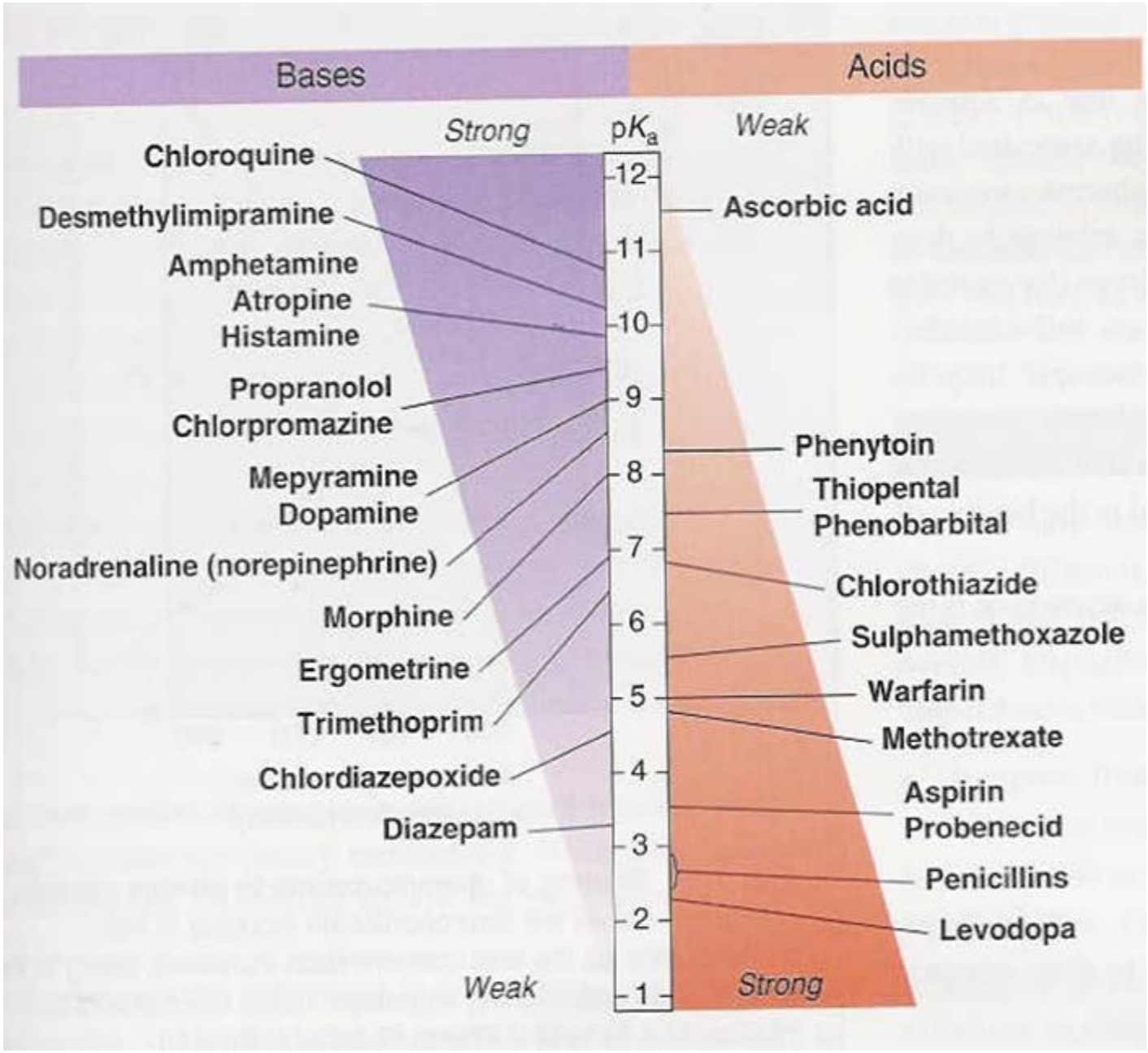
receptors

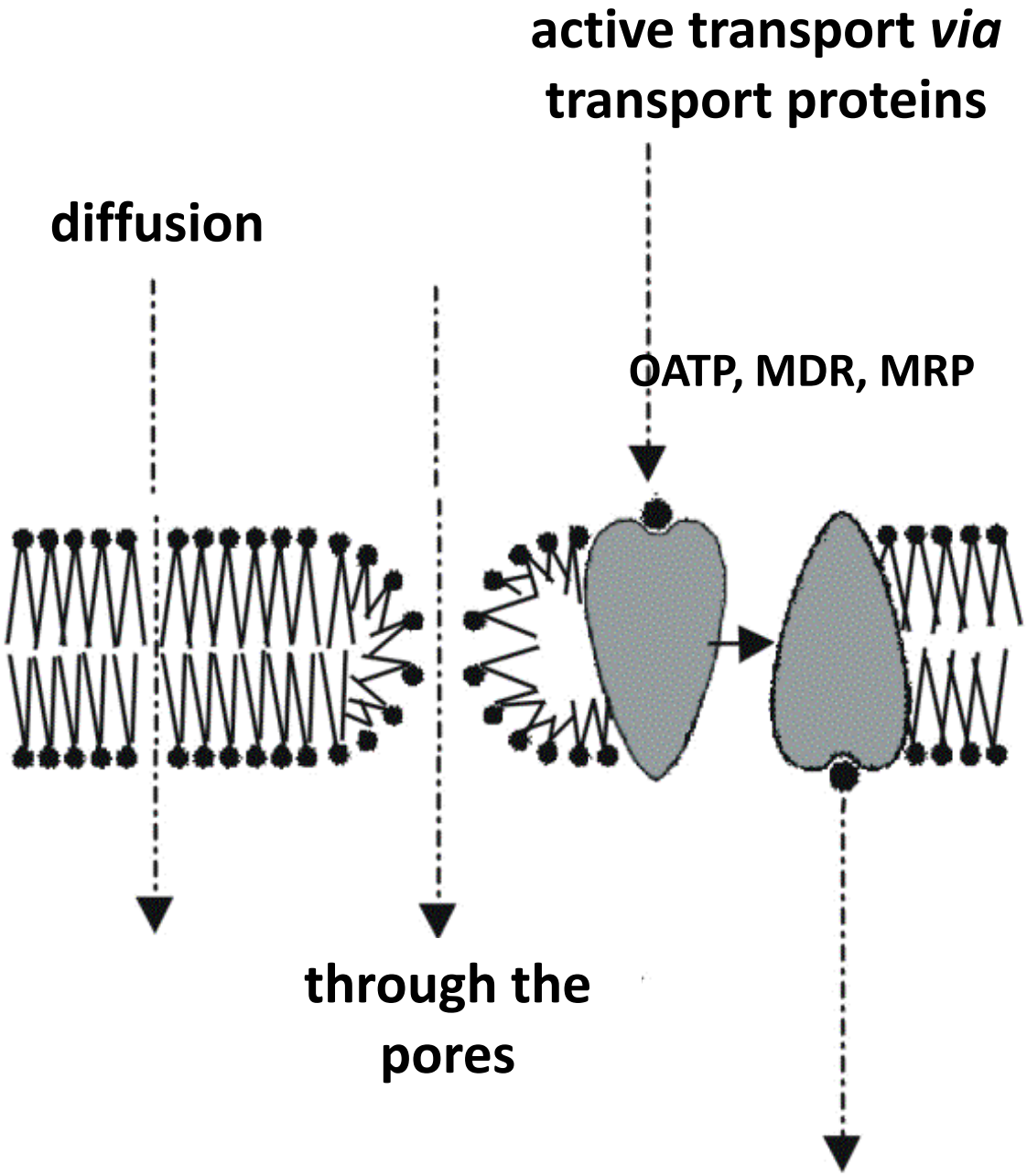
**perfusion of the tissues**

a) brain, heart, liver, kidney

b) fat tissue


Gastric juice
pH 3
Plasma
pH 7.4
Urine
pH 8
Aspirin
Weak acid
p$K_a$ 3.5
> 400
Ionisation greatest at alkaline pH
Anion
$A^-$
100
Undissociated
acid
AH
< 0.1
Relative concentration
> $10^6$
Ionisation greatest at acid pH
Pethidine
Weak base
p$K_a$ 8.6
100
Protonated
base
$BH^+$
Free
base B
30


Bases
Acids
Strong
pKa
Weak
Chloroquine
Desmethylimipramine
Amphetamine
Atropine
Histamine
Propranolol
Chlorpromazine
Mepyramine
Dopamine
Noradrenaline (norepinephrine)
Morphine
Ergometrine
Trimethoprim
Chlordiazepoxide
Diazepam
12
11
10
9
8
7
6
5
4
3
2
1
Ascorbic acid
Phenytoin
Thiopental
Phenobarbital
Chlorothiazide
Sulphamethoxazole
Warfarin
Methotrexate
Aspirin
Probenecid
Penicillins
Levodopa
Weak
Strong


active transport *via*
transport proteins
diffusion
OATP, MDR, MRP
through the
pores

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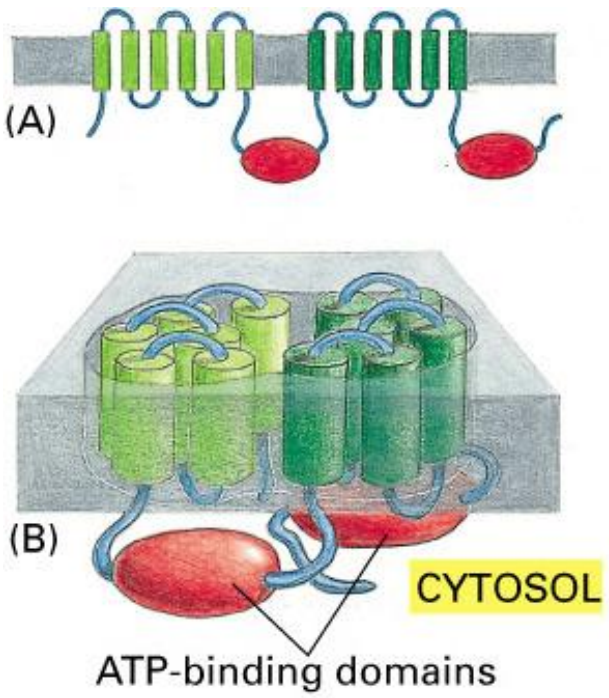
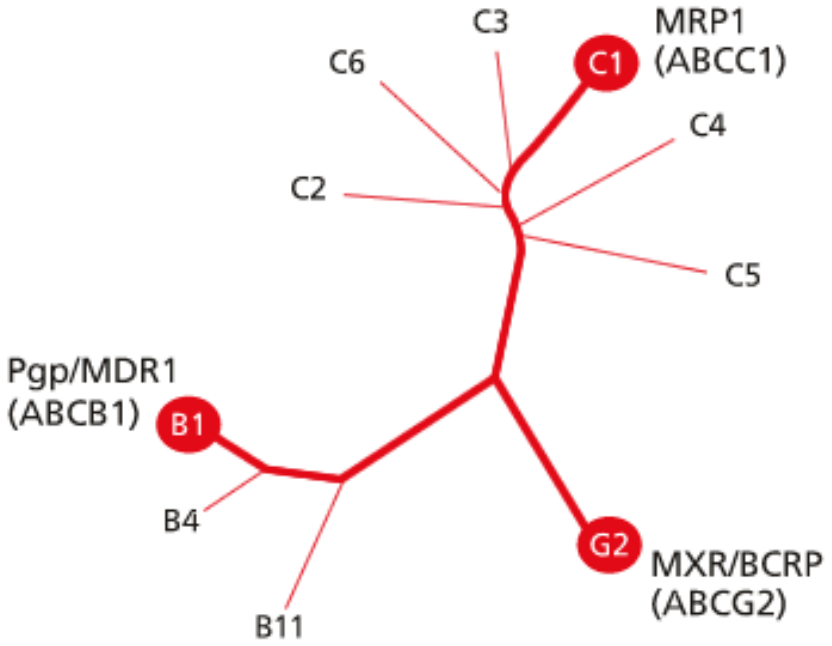
# ABC - ATP-BINDING CASSETTE



**ABC** - **A**TP-**B**INDING **C**ASSETTE

- **MDR - multi drug resistance**
- **MRP - multidrug resistance asociated protein**
- **MXR - mitoxantrone resistance protein**

- **Pgp - P-glycoprotein pump**

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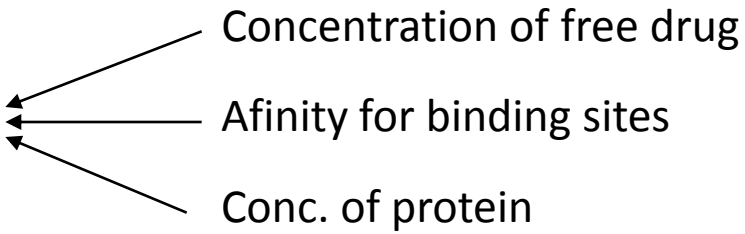


plasma proteins

tissue

blood cells in the circulation

receptors

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- plasma proteins
- tissue
- blood cells in the circulation
- receptors

- most of acidic drugs (at pH of 7.4= anions) are bound on albumin:
  - salicylates, sulfonamides, penicillins

- most of alcalic + neutral drugs (at pH of 7.4= cations) are bound on $\alpha_1$- acidic gylcoprotein and lipoproteins:
  - quinidine, digitoxine, TCA, cyclosporine A

- Bonds with plsama proteins are
  - reversible
  - dynamic
  - competitive

| drug | % bound |
| --- | --- |
| caffein | 10 |
| digoxine | 23 |
| gentamycine | 50 |
| phenytoin | 87 |
| digitoxine | 95 |
| diazepam | 96 |
| warfarin | 98 |
| tolbutamide | 99 |

# A bound drug has no effect!

**Amount bound depends on**:

1) free drug concentration
2) the protein (binding sites) concentration
3) affinity for binding sites

$$\% \text{ bound: } \frac{[\text{bound drug}]}{[\text{bound drug}] + [\text{free drug}]} \times 100$$

## Bonds in peripheral tissues

- specific for some of the drugs
  - tetracycline antibiotics - hydroxyapatit
  - chloramfenicol – skin
  - grisefulvin - skin
  - arsenic – in hair

# ABSORPTION

**Absorption** – permeation of the soluted drug into the body fluids from the site of administration – necessary for the general (systhemic) effect

**topical effect** – on the skin, mucous membranes…
mouth, rectum, vagina

- absorption is fault, can cause difficulties, adverse effects)

(local aenesthetics, corticosteroids)

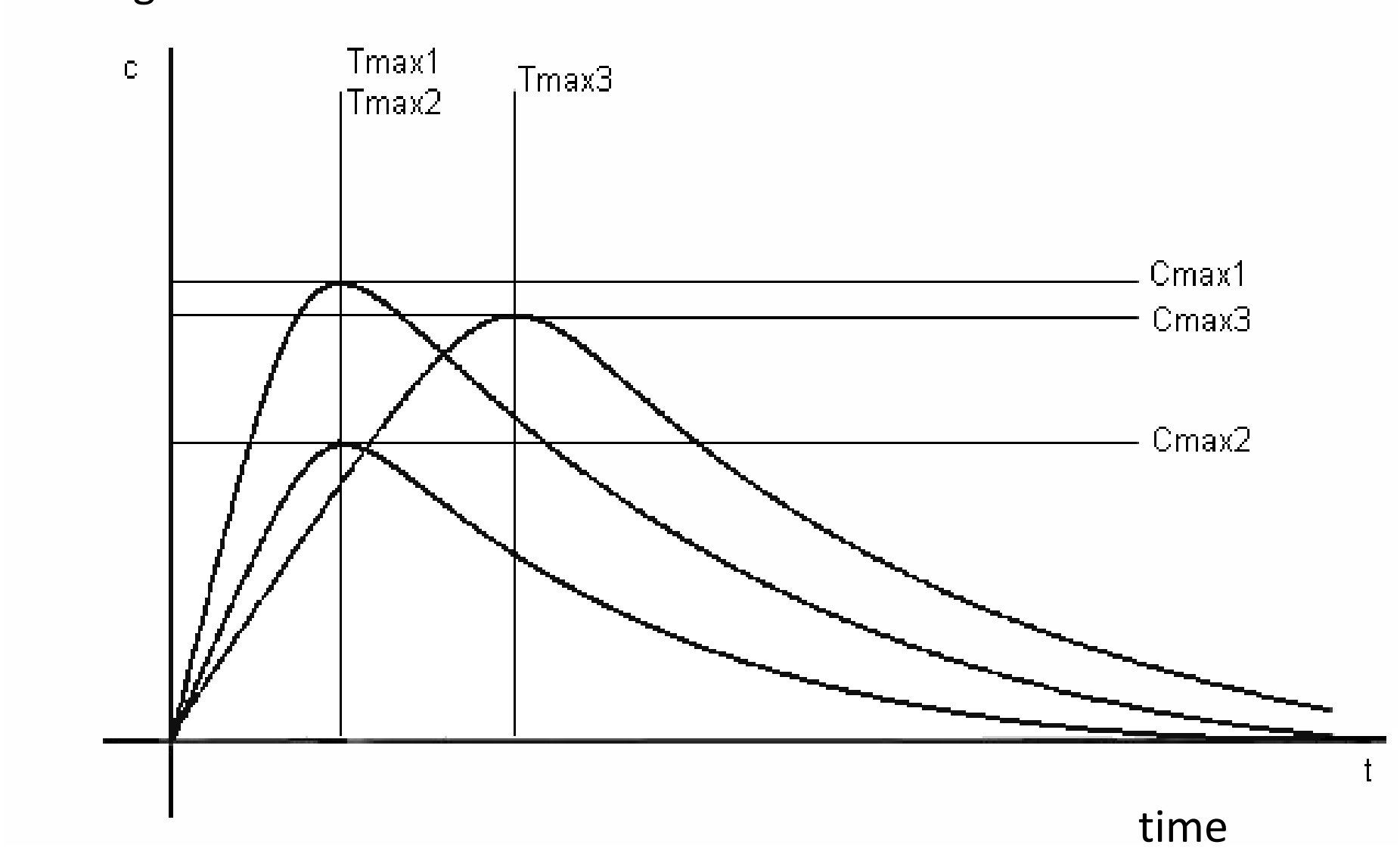
**Rate and extent of absorption are described by the parameters** :

**C max** - max. concentration of the drug in the plasma after single administration

**T max** - time after administration, when is Cmax

**F** - bioavailability (extent of absorption)

Plasmatic concentration of the drug



# Bioavailability

- The fraction of the dose of a drug (F) that enters the general circulatory system,

$$F = \frac{\text{amt. of drug that reach systemic circul.}}{\text{Dose administered}}$$

$$F = AUC_{p.o.} / AUC_{i.v.}$$

# Bioavailability

Extravascular route - 0-100% (resp. 0-1).

Intravenous - 100% = 1

If F is 0-20% = 0-0,2 – not suitable route of administration

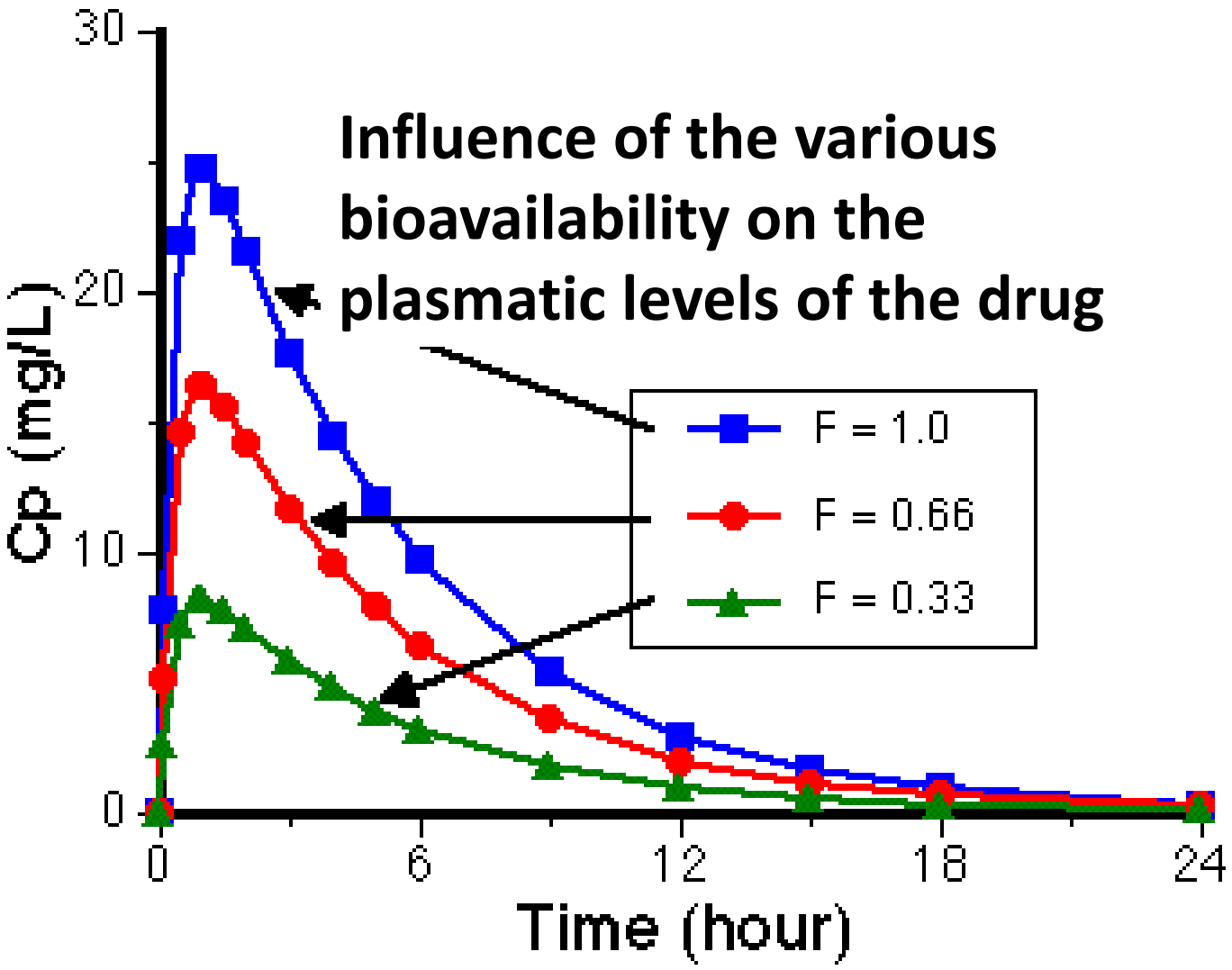
(in spite of that fact, some drugs are administered, even if the F < 2-5 %, such as SET, bisphosphonates).

**F = AUCpo/AUCiv**

**(the same drug, same dose, same patient)**

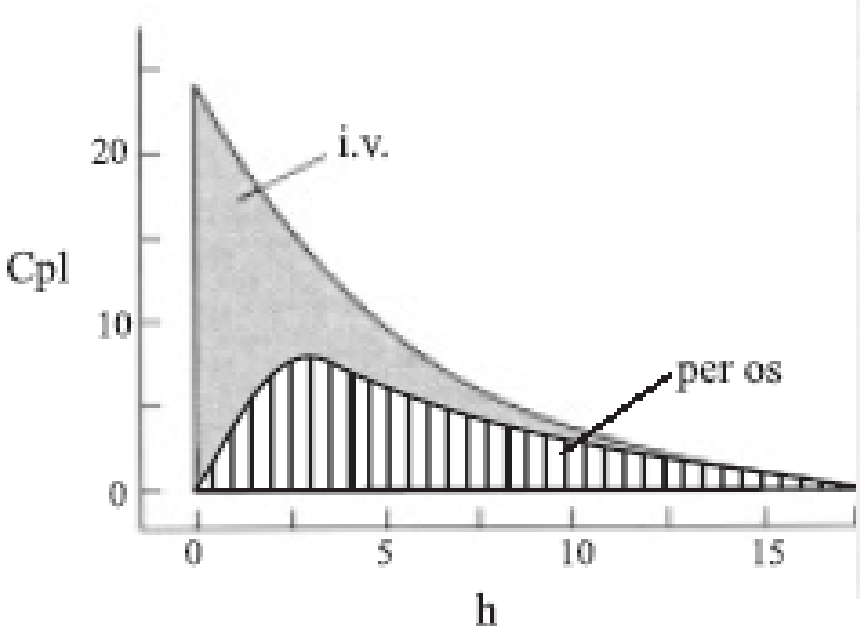
# Bioavailability

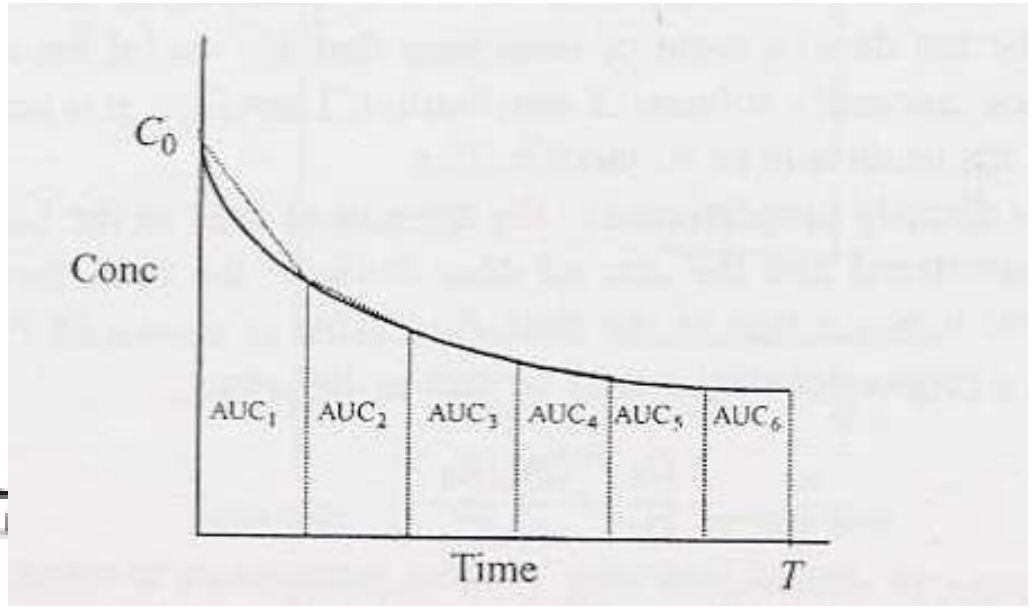
- A concept for oral (extravascular) administration
- Useful to compare two different drugs or different dosage forms of same drug
- depends, in part, on rate of dissolution (which in turn is dependent on chemical structure, pH, partition coefficient, surface area of absorbing region, etc.) Also first-pass metabolism is a determining factor


Influence of the various bioavailability on the plasmatic levels of the drug
F = 1.0
F = 0.66
F = 0.33
Cp (mg/L)
30
20
10
0
0
6
12
18
24
Time (hour)

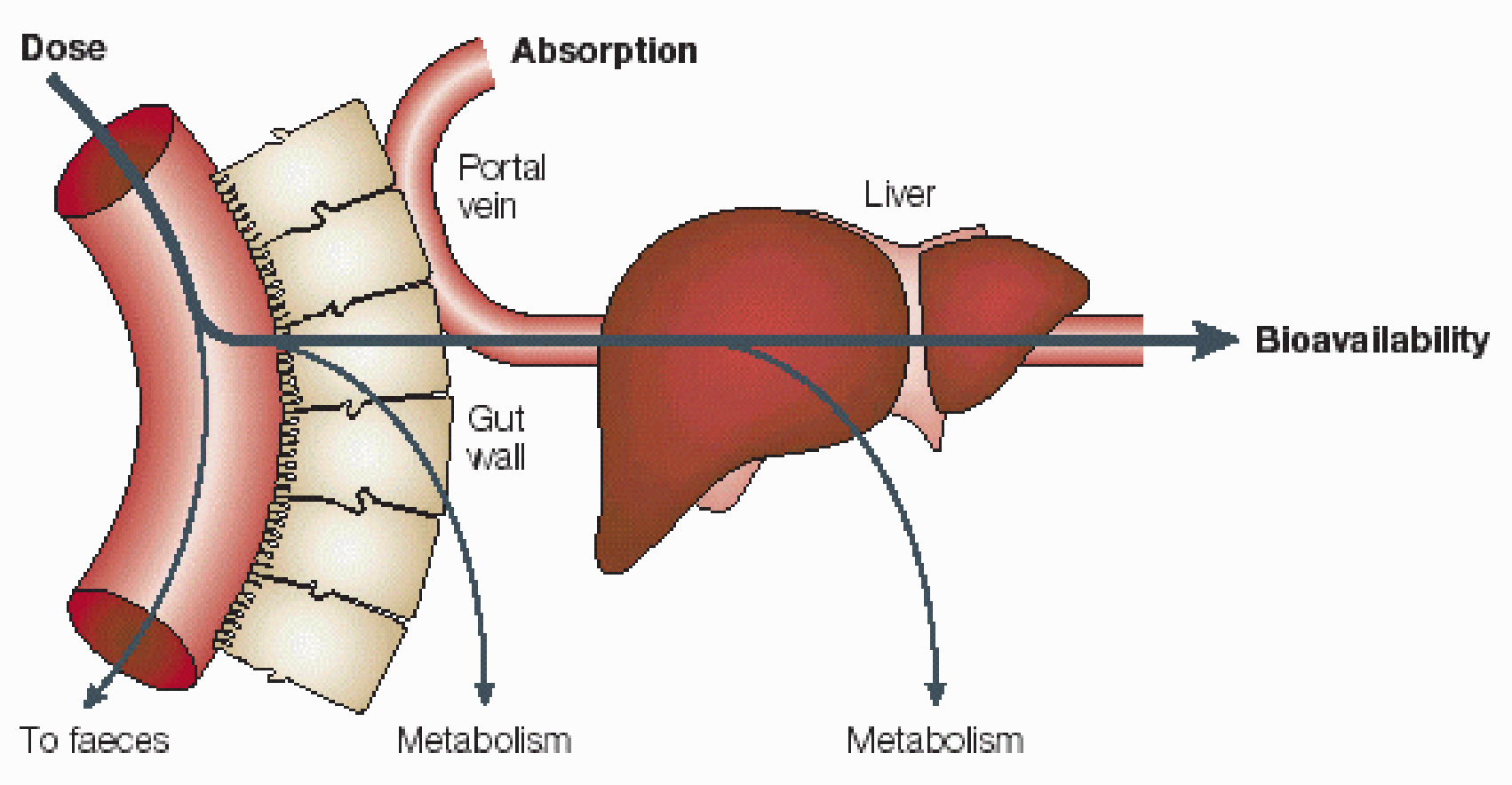
# Area under curve (AUC)

- Is a measure of bioavailability



# First pass effect, presysthemic elimination



# Factors influencing absorption

Drug-dosage form– tbl./ sol./ supp./ TTS/tbl.subling.

Way of administration

Physico-chemical properties of drugs

- absorptive surface area
- concentration gradient
- ionization, lipofility
- interactions

# Other factors influencing the absorption

gender, body weight, plasma volume, gastric amptying rate,

age - pH, bile, enzyme levels and activity

patophysiological state – liver disseases, inflammation

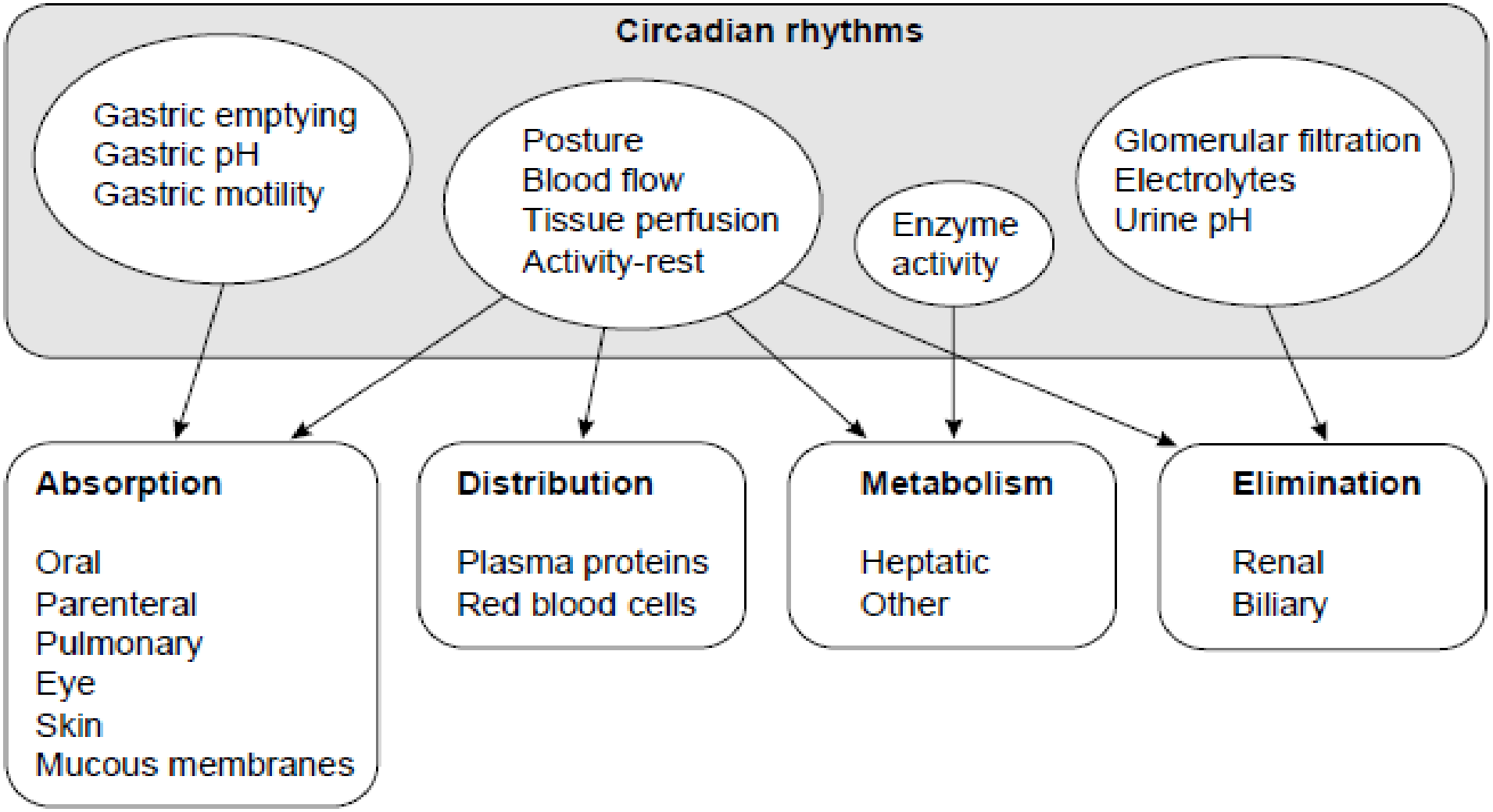
simultaneously eaten meal –

acceleration/decelaration

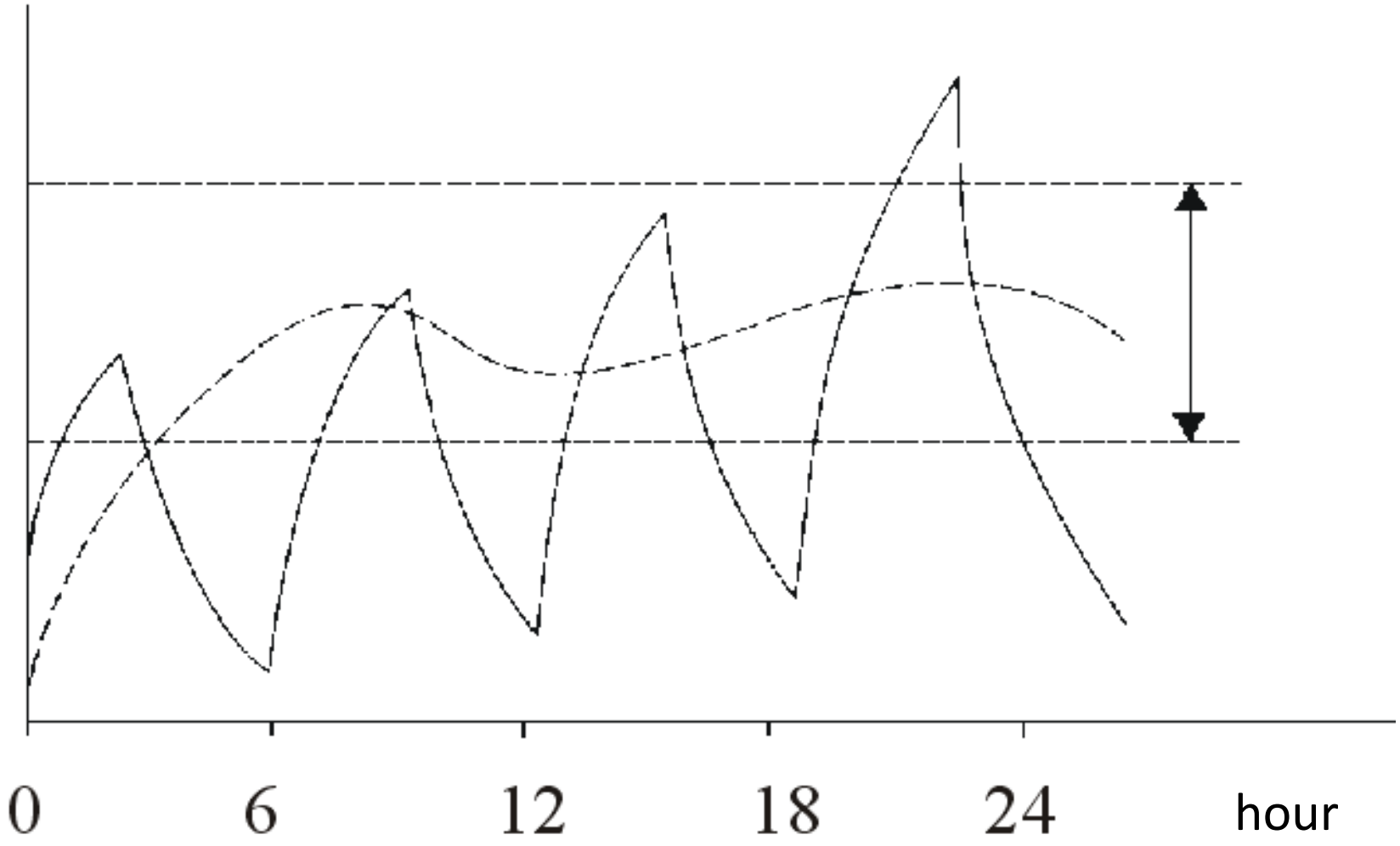
chemical incompatibilities

function of the GIT

# Factors affecting pharmacokinetics



# Drug dosage forms of the 1st and 2 nd generations



# Distribution

= permeation from the body blood to the tissues and site of the action
is dynamic process

**rate** - depends on:
bonds (with the plasmatic proteins…)
permeation across the membrabes
blood perfusion through the organ

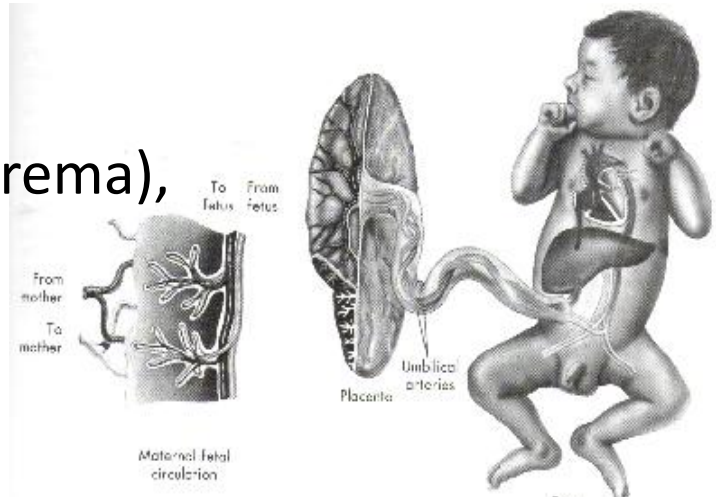
**state** - distribution equilibrium; the the proportion of the free (unbounded) fractions of the drug in the blood and in the tissues are the same

Barriers – the distribution is limited

blood-brain barrier („leaky areas“ – area postrema),

penicilines X aminoglycosides

placental barrier…



# Volume of Distribution

**Volume of distribution – apparent, hypotethical**
the proportion of the quantity of the drug and reached plasmatic concentration

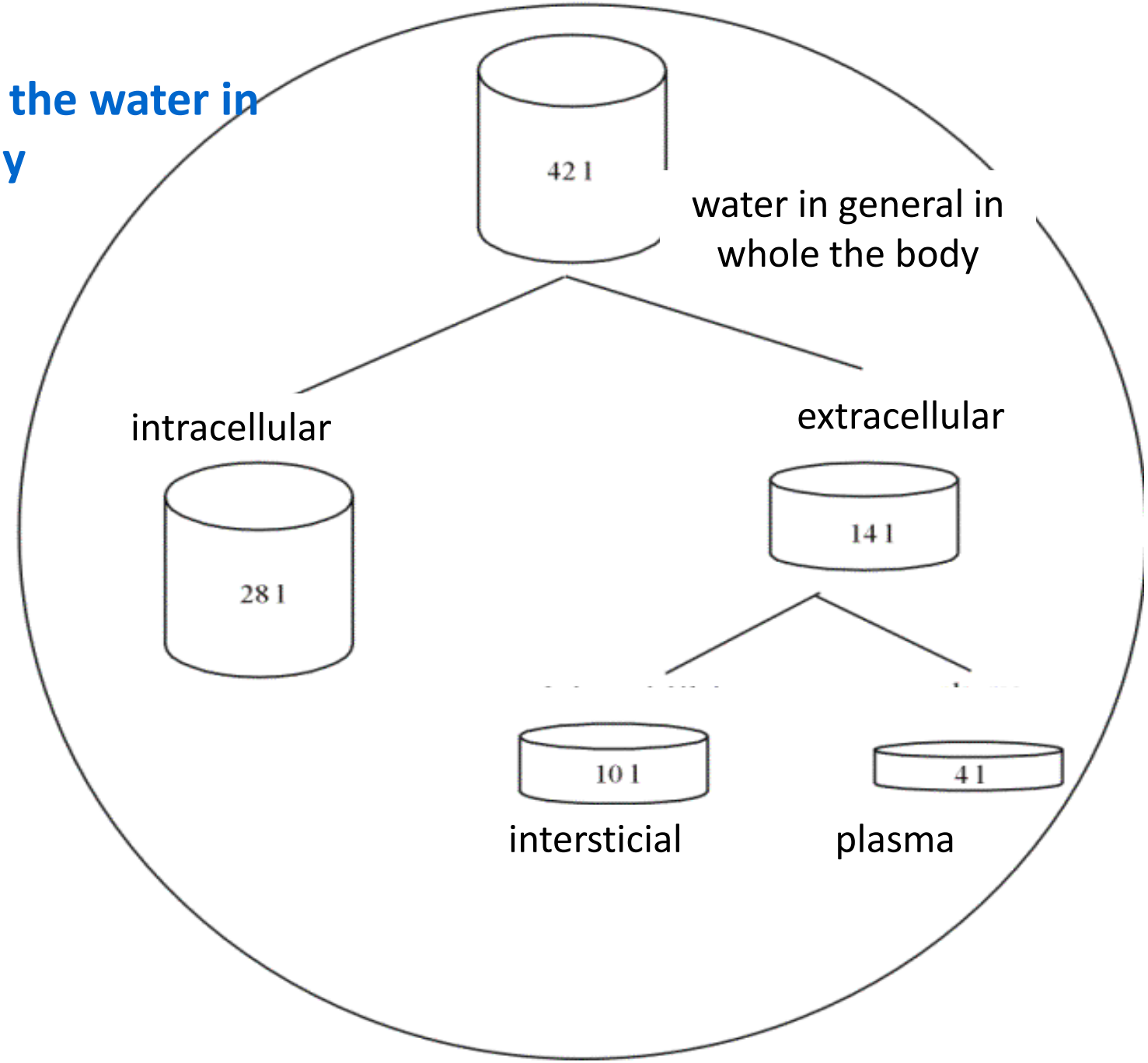
$V_d = D/C$

- $V_d$ is the apparent volume of distribution
- C= Conc of drug in plasma at some time
- D = Total quantity (dose) of drug in system

$V_d$ gives one as estimate of how well the drug is distributed.

Value < 0.071 L/kg indicate the drug is mainly in the circulatory system. Values > 0.071 L/kg indicate the drug has gotten into specific tissues.

# Volumes of the water in human body



## Perfusion through the organs

| organ | perfusion rate (ml/min/g tkáně) | % heart output |
|---|---|---|
| brain | 0.5 | 14 |
| fat | 0.03 | 4 |
| heart | 0.6 | 4 |
| kidney | 4.0 | 22 |
| liver | 0.8 | 27 |
| musculature | 0.025 | 15 |
| skin | 0.024 | 6 |

# ELIMINATION = biotransformation + excretion

- Kinetics of the first order

  = rate of elimination is descending with the descending concentration in the blood

  (linear kinetics)

- Kinetics of the zero order

  = rate of elimination is constant (nonlinear kinetics)

# Types of Kinetics Commonly Seen

- **Zero Order Kinetics**

- Rate = k
- C = Co - kt

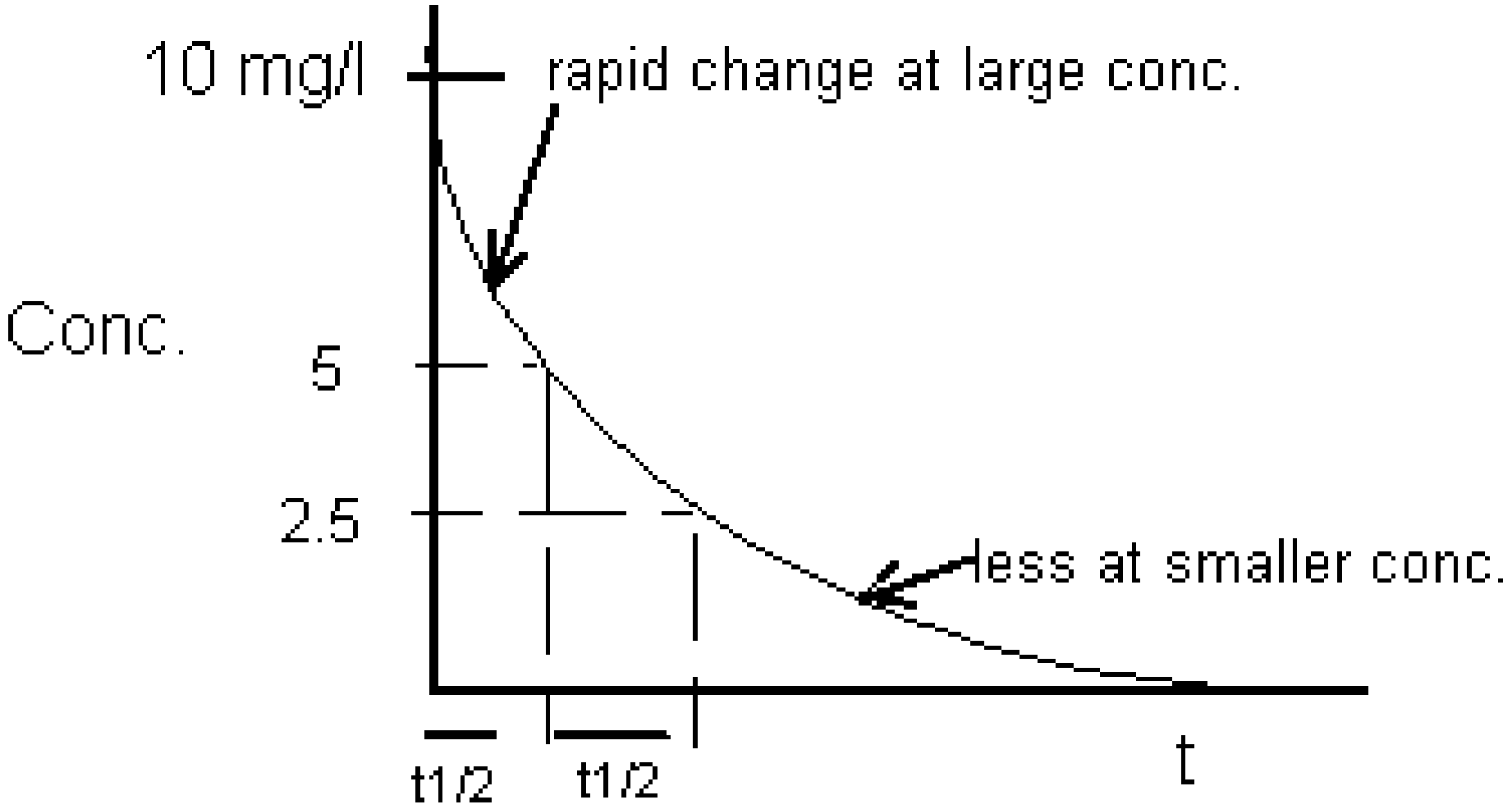
- C vs. t graph is LINEAR

**First Order Kinetics**

- Rate = k C
- $C = C_o e^{-kt}$

- C vs. t graph is NOT linear, decaying exponential.
- Log C vs. time graph is linear.

# First Order Kinetics



# ELIMINATION

## Biotransformation – metabolism

### Sites of biotransformation

anywhere, where the enzymes are present: plasma, kidney, lungh
GIT, brain, but especially **liver**

### Enzymatic

- **biodegradation**
- **bioactivation (prodrug**)

enalapril-enalaprilate

codein-morphine

bromhexin - ambroxol

**1. Phase :** oxidation, hydrolysis

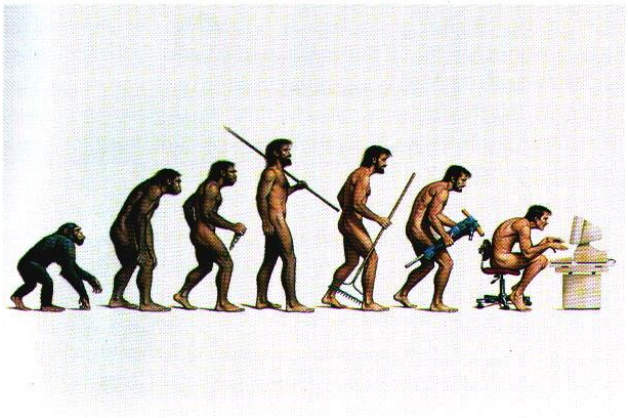
Cytochrom P450, dehydrogenases

**2. Phase :** conjugation – metabolites are more soluble in the water

Metabolite - effective („more / less / in other way“)

- ineffective

- toxic



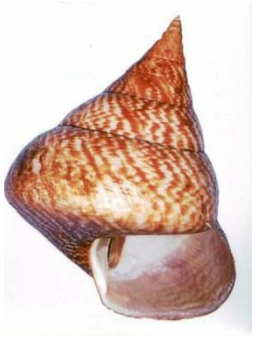
men

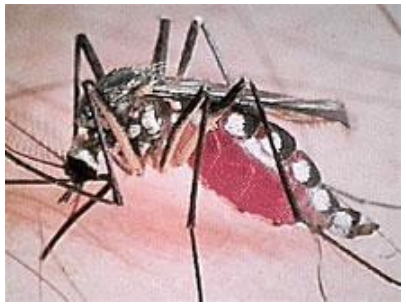
plants

animals

CYP 450

molluscs



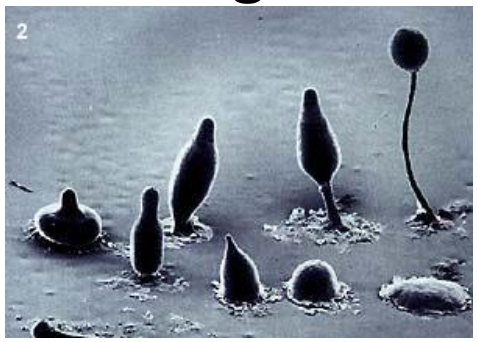


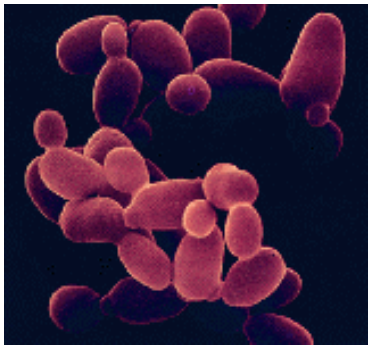
insect

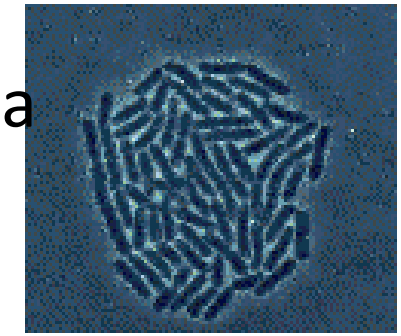
funghi

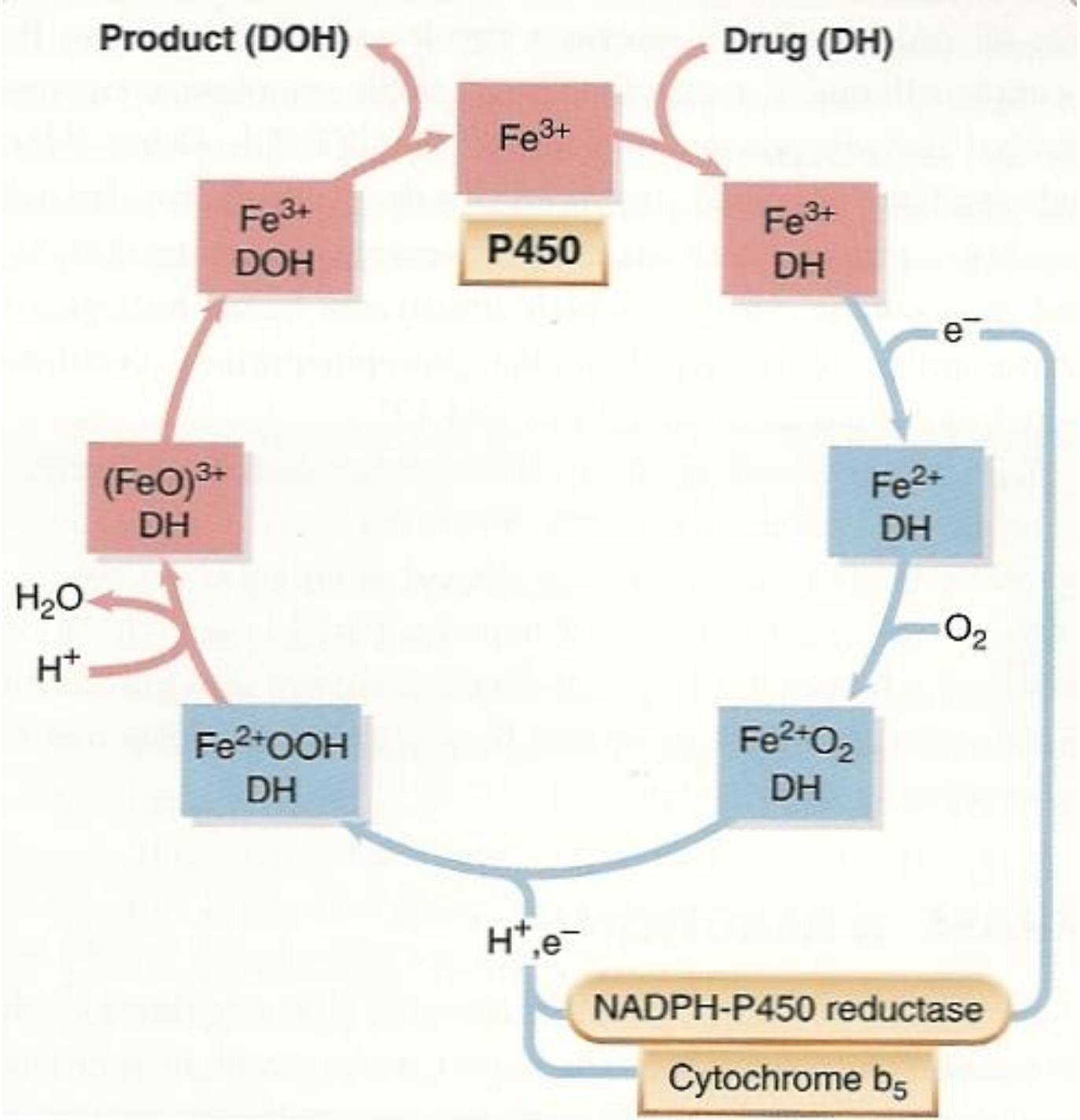
yeast

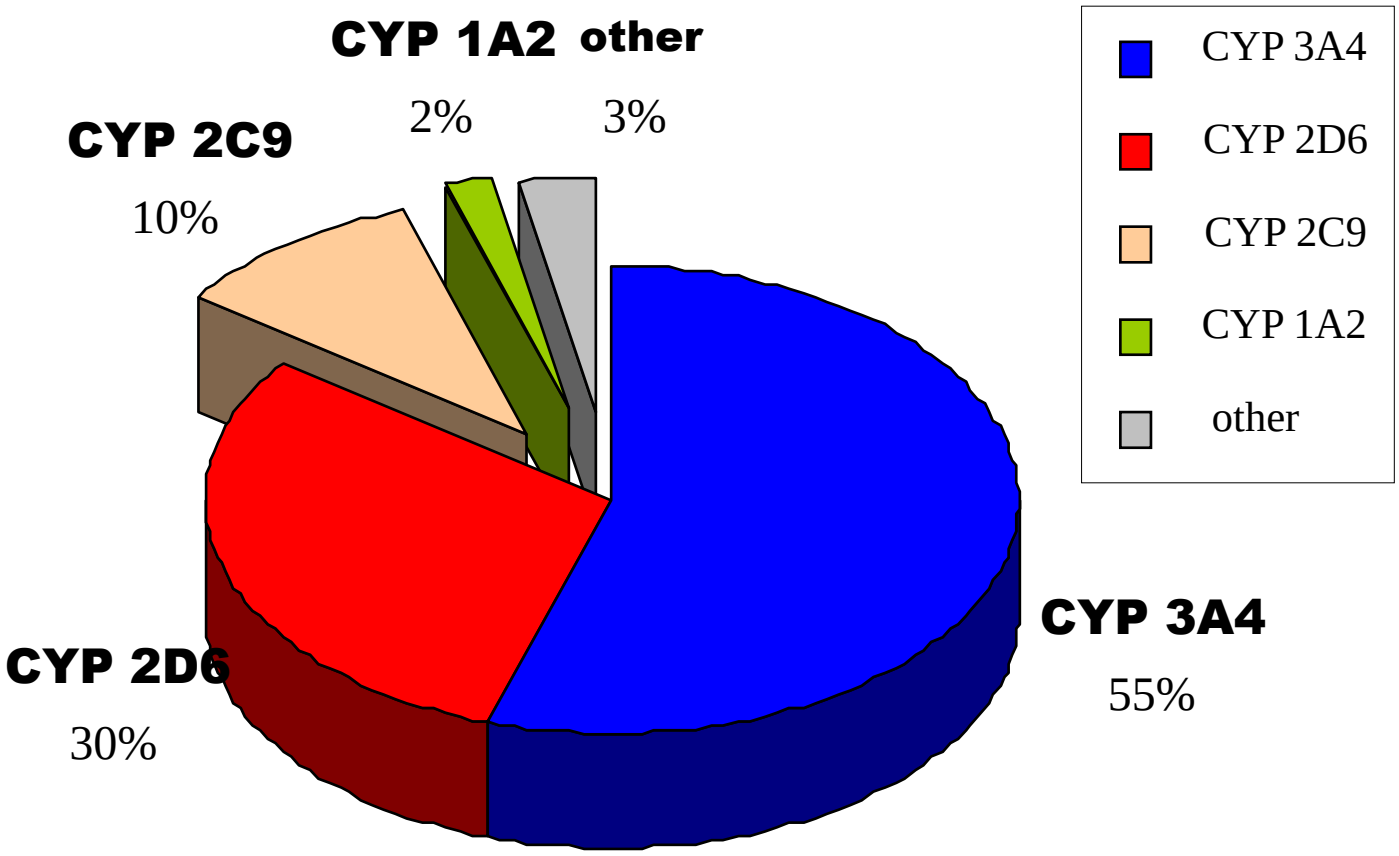
bacteria








Product (DOH)
Drug (DH)
$Fe^{3+}$
P450
$Fe^{3+}$ DOH
$Fe^{3+}$ DH
$e^-$
$Fe^{2+}$ DH
$O_2$
$Fe^{2+}O_2$ DH
$Fe^{2+}OOH$ DH
$(FeO)^{3+}$ DH
$H_2O$
$H^+$
$H^+, e^-$
NADPH-P450 reductase
Cytochrome $b_5$


CYP 1A2
2%
other
3%
CYP 2C9
10%
CYP 3A4
55%
CYP 2D6
30%
CYP 3A4
CYP 2D6
CYP 2C9
CYP 1A2
other

# Genetic polymorphism

Genetic polymorphism = the existence of several
(At least two ) alleles for the gene from which
At least part has a population frequency of at least 1 %

- Pharmacogenetics

focuses on the study of genetically conditioned variability in the response to a drug

- Pharmacogenomics examines the relationship of drug effect on the level of the whole genome, respectively transcriptome

# Genetic polymorphism of biotransformation enzymes

Polymorphism in the gene of ***N - acetyltransferase***

- Inactivation of drugs in the liver : slow x fast acetylators
- Isoniazide , procainamide, hydralazine
- Peripheral neuropathy (prevention - pyridoxine)

Polymorphism of ***thiopurine S - methyltransferase***

- the metabolism of azathioprine
- commercially available genetic test for determining the polymorphisms, prevention of serious adverse reactions

# Genetic polymorphism of CYP2D6

## Null alelles

- encode a nonfunctional protein

- In the homozygous state cause the phenotype PM

SNP (point mutations)

a chromosome deletion

mutation leading to loss of the fction of protein, but the length of protein is maintained

- * 4 allele ( 12-21 % ) in Caucasian populations (most frequent 1846G > A)
- * 5 allele (2-7 % ) causes loss of whole CYP2D6 gene
- allele * 13 and * 16 - 5'- CYP2D7P / CYP2D6-3' hybrid genes , deletion of various parts of CYP2D locus

# GENETIC POLYMORPHISM OF CYP2D6

**The alleles associated with decreased enzymatic function**

**encode an enzyme with reduced activity**

**in the homozygous state or with the null allele in the heterozygous state cause the IM phenotype**

*** 10 (50-70 % Asian, 1-5% of the Caucasian population) 100C > T ( disrupts normal folding of the protein → enzyme is very unstable and has a lowe affinity to a substrate**

# GENETIC POLYMORPHISM OF CYP2D6

**The alleles associated with increased enzyme function**

**- An increase in the number of active gene copy**

**- Alleles * 1 , * 2 , 35 ***

**- The frequency of Caucasians 1-5%**

# Genetic polymorphism of CYP

- **CYP2D6** and antidepressants (especially classical) : significant

PK differences, difficult to adjust dose due to slower onset of effect, long-term drug therapy

- **CYP2C9** and oral antidiabetic drugs - derivatives

sulfonylureas ( e.g. glimepiride, glipizide and tolbutamide)

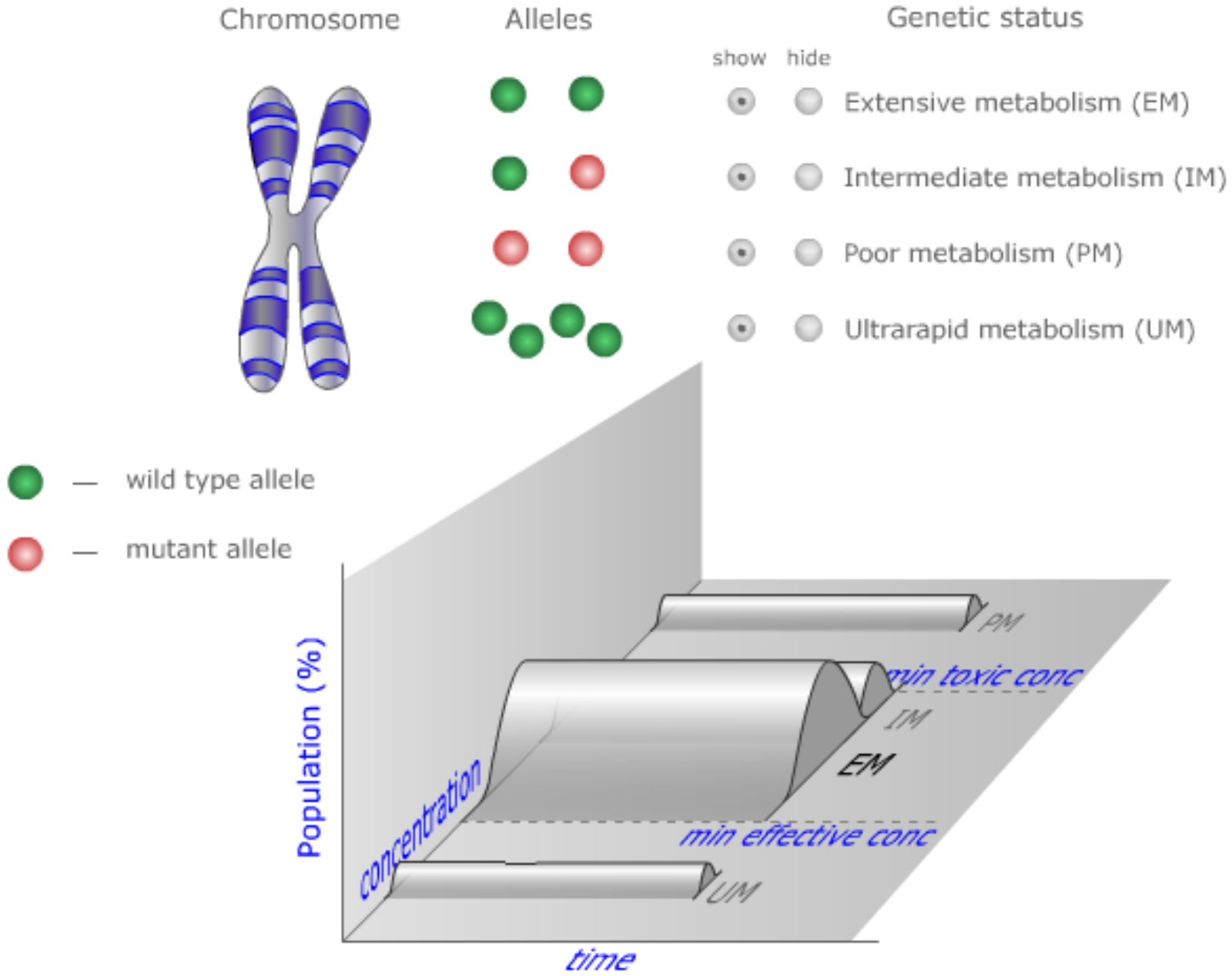
In heterozygotes CYP2C9 * 1 / * 3 , the total clearance of 50 %

homozygotes and CYP2C9 * 3 / 20 * 3 % compared to WT

- **CYP2C9** and anticoagulants - (warfarin) in heterozygous

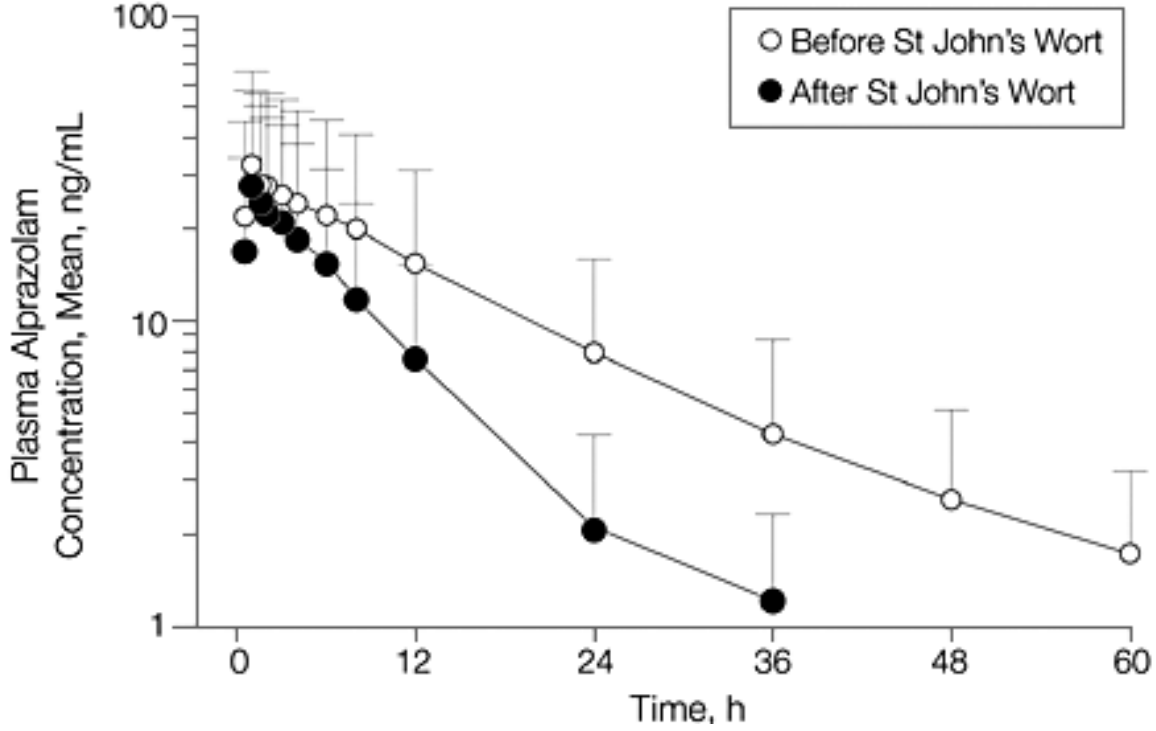
CYP2C9 * 1 / * 3, the total clearance of 70 %, and in

homozygotes CYP2C9 * 3 / 40 * 3 % compared to WT


Chromosome
Alleles
Genetic status
show
hide
Extensive metabolism (EM)
Intermediate metabolism (IM)
Poor metabolism (PM)
Ultrarapid metabolism (UM)
— wild type allele
— mutant allele
Population (%)
concentration
time
PM
min toxic conc
IM
EM
min effective conc
UM

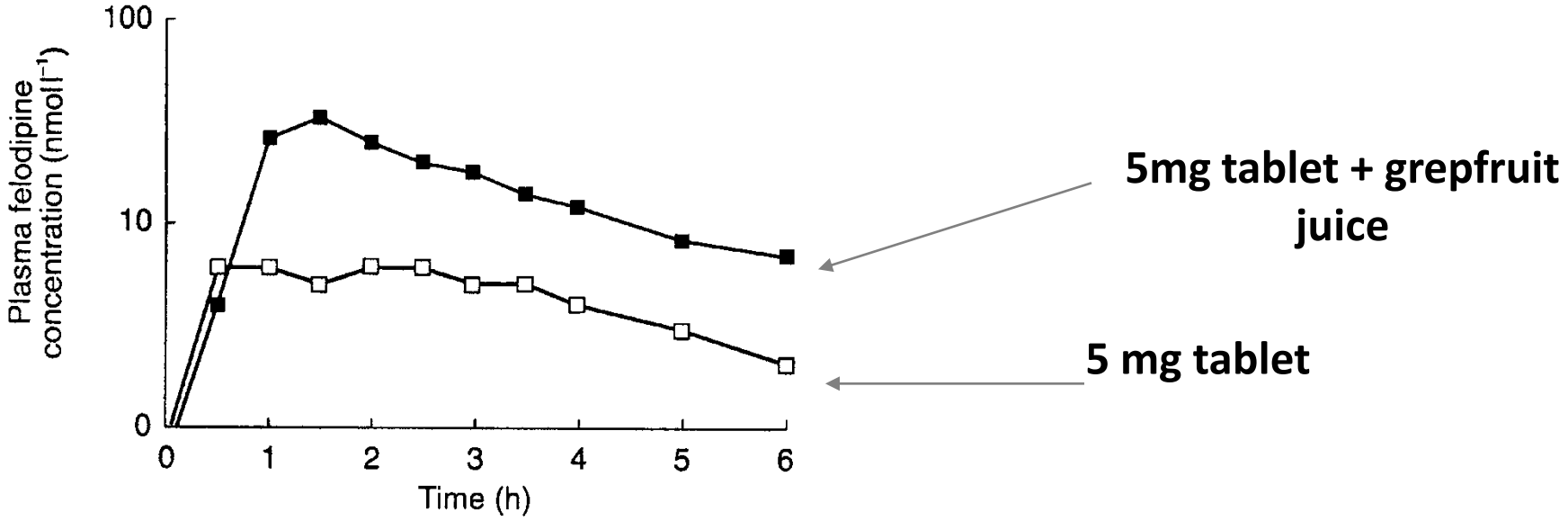
# INDUCERS of CYP 450

- dexamethason
- phenobarbital
- rifampicine
- ph
- St
- *Gi*



# INHIBITORS of CYP 450

- antidepressants (fluoxetine, fluvoxamine, paroxetine)
- quinine, quinidine
- chloramphenicol, erythromycin



# Phase I of biotransformation

hydroxylation $-CH_2CH_3 \rightarrow -CH_2CH_2OH$

oxidation $-CH_2OH \rightarrow -CHO \rightarrow -COOH$

O-dealkylation $-CH_2OHCH_2 \rightarrow -CH_2OH + -CHO$

N-dealkylation $-N(CH_3)_2 \rightarrow -NHCH_3 + CH_3OH$

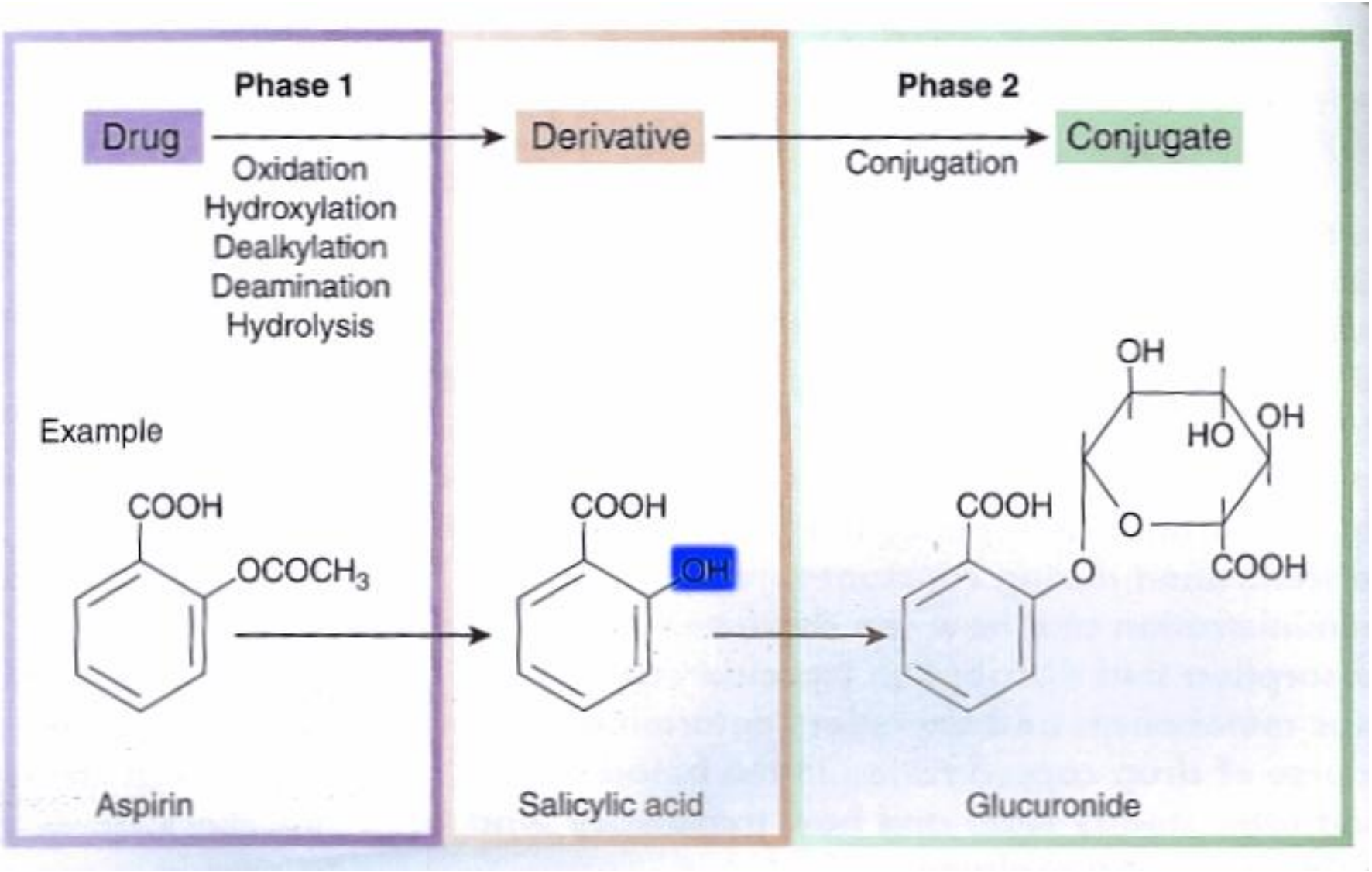
N-oxidation $-NH_2 \rightarrow -NHOH$

oxidative deamination $-CH_2CHCH_3 \rightarrow -CHCOCH_3 + NH_3$

$NH_2$

# Other non-microsomal biotransformations

- hydrolysis of esters in plasma (suxamethonium by cholinesterase)
- dehydrogenation of alcoholic and aldehydic group in cytosol in the liver (ethanol)
- MAO in mitochondria (tyramine, noradrenaline, dopamine, amines)
- xanthinoxidase (6-merkaptopurine, uric acid)
- enzymes with distinct function (tyrosine-hydroxylase, dopa-decarboxylase, etc.)


Phase 1
Drug
Oxidation
Hydroxylation
Dealkylation
Deamination
Hydrolysis
Derivative
Phase 2
Conjugation
Conjugate
Example
COOH
OCOCH3
COOH
OH
OH
HO
OH
COOH
O
O
COOH
Aspirin
Salicylic acid
Glucuronide

# Phase II of biotransformation

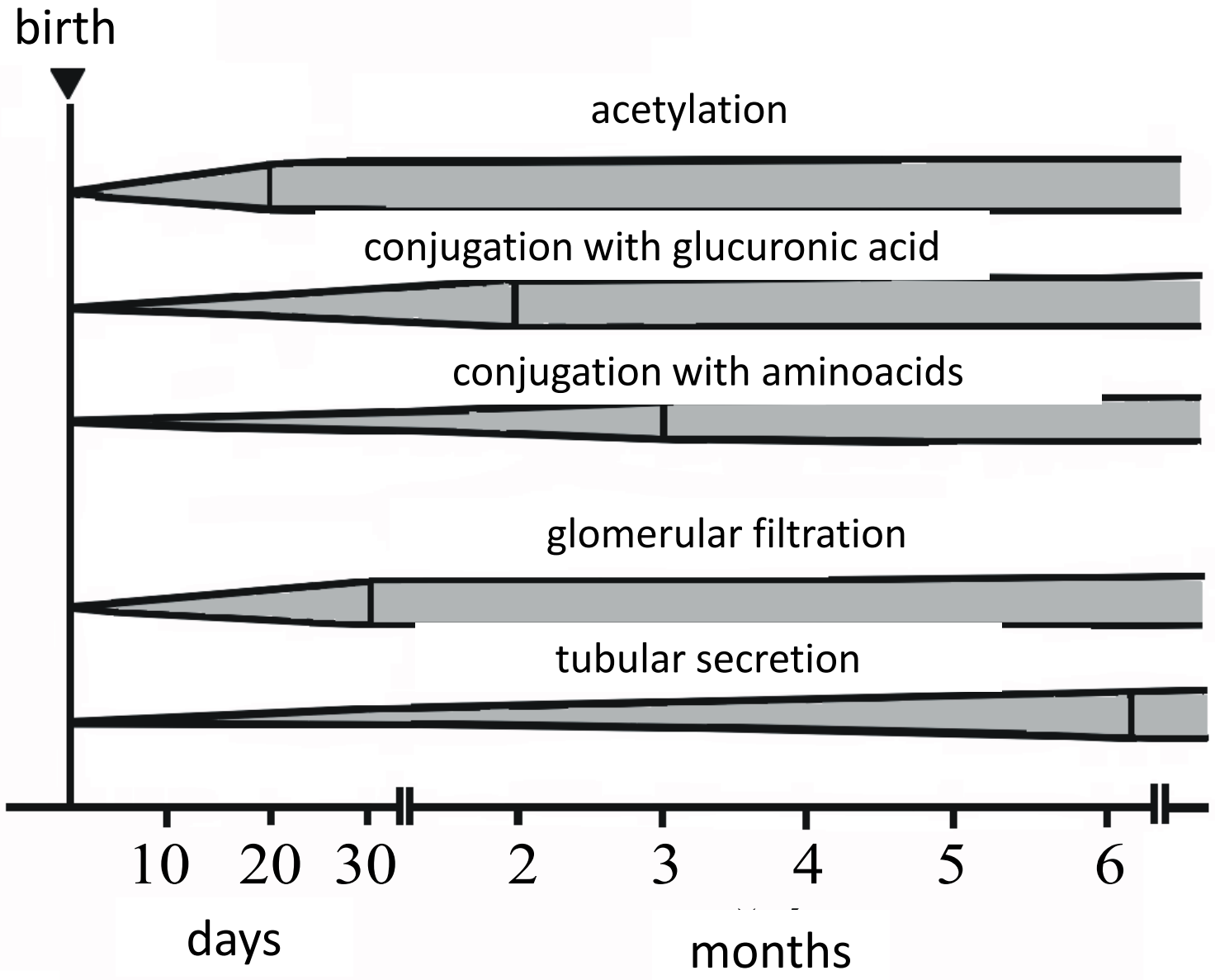
## CONJUGATION

Glucuronides -OH, -SH, -COOH, -CONH wih glucuronyl acid (UDP- GlcUAc)

Sulphates: with -OH functional group

Acetylates: acetyl CoA with $NH_2$, -$CONH_2$, s aminoacid- group

with gluthathion with -halogen- or -nitrate functional groups, epoxides sulphates


birth
acetylation
conjugation with glucuronic acid
conjugation with aminoacids
glomerular filtration
tubular secretion
10
20
30
2
3
4
5
6
days
months

## Excretion

Kidney (urine)

tubular excretion

x tubular reabsorption

liver (bile)

lungh (air)

saliva, skin, hair, breast milk...

## Clearance Cl

- Volume of blood in a defined region of the body that is cleared of a drug in a unit time.
- more useful concept in reality than $k_{el}$ since it takes into account blood flow rate
- Clearance varies with body weight
- Also varies with degree of protein binding

# Kidney

- MW < 60.000 D (MW albumin = 68.000 D)
- tubular secretion
  - organic acids
    - furosemid
    - thiazide diuretics
    - penicilins
    - glucuronides
  - organic bases
    - Morphine
    - Atropine
    - Histamine…
- tubular reabsorption

acidification

acetazolamid (inhibitor of CA)
ammonium chloride

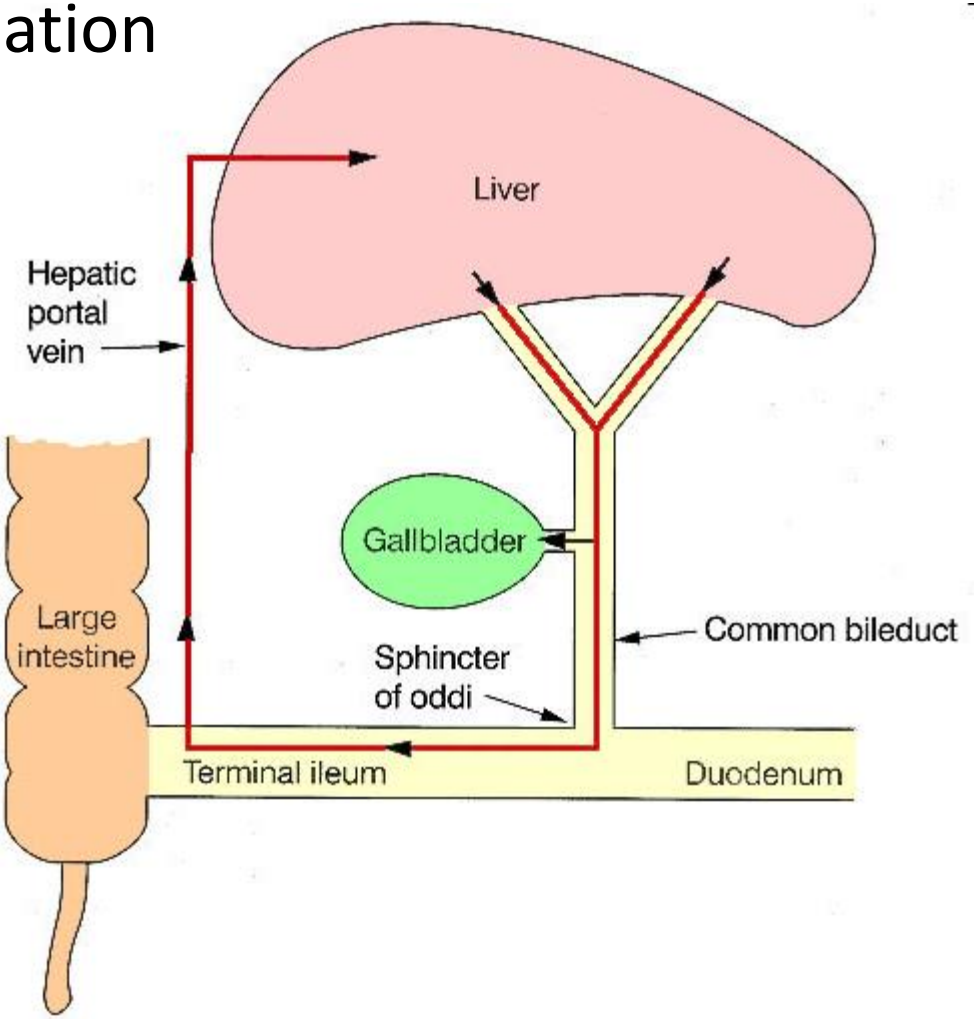
alcalization

sodium bicarbonate

# Liver

Billiar excretion, clearance.

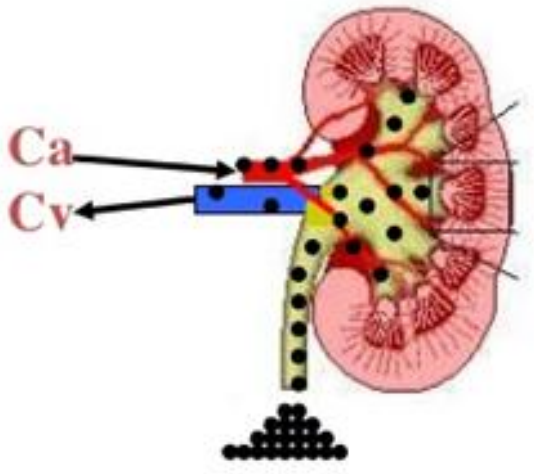
enterohepatic circulation



## Extraction ratio $E_R$

= proportion of the drug removed durring the passage through the organ

$$E_R = c_a\text{-}c_v/c_a$$



## Mean residence time MRT

= The average total time molecules of a given dose spend in the body. Thus, this can only be measured after instantaneous administration.

Non-compartment PK

$$MRT = AUMC/AUC$$

# PHARMACOKINETIC PARAMETERS

PRIMARY

- **Bioavailability (F)**
- **Volume of distribution (Vd)**
- **Clearance (Cl)**

SECONDARY

- elimination half-life ($T_{1/2}$)
- elimination constant (Ke)
- AUC (area under the curve)
- Cumulative index
- Extraction ratio

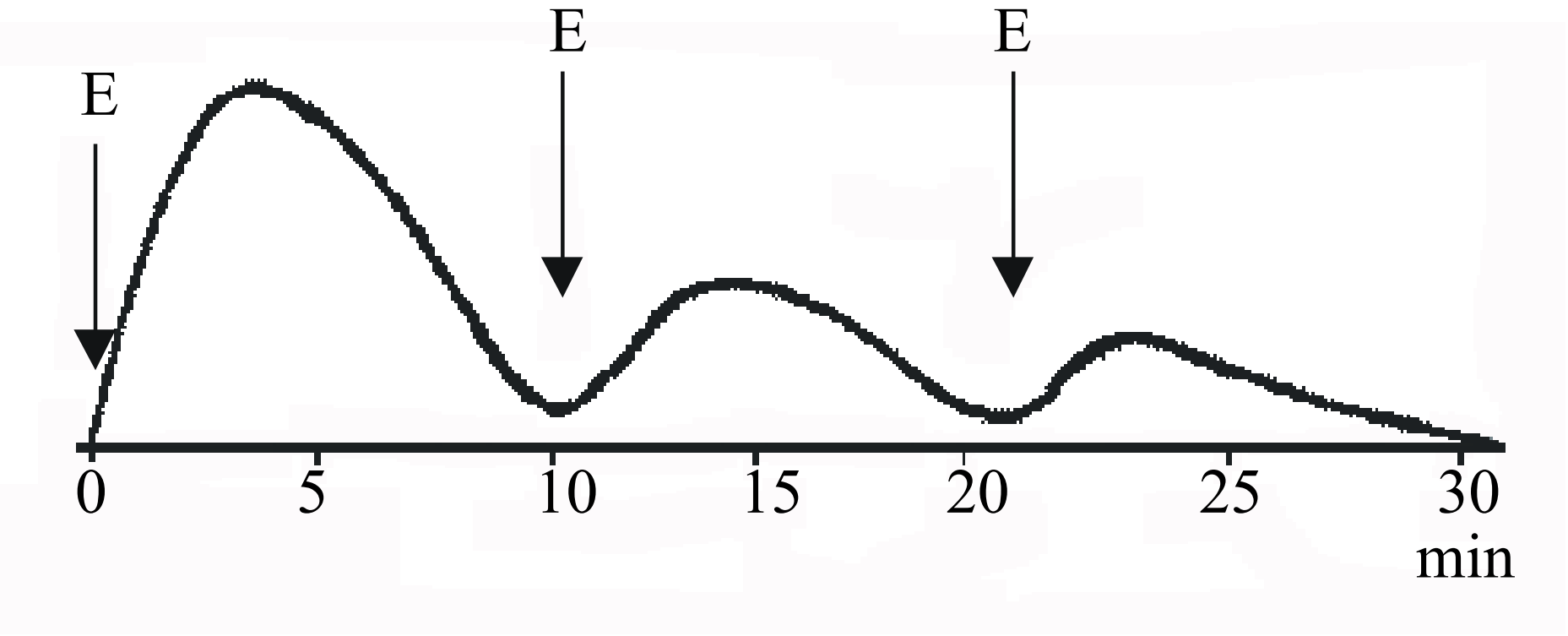
# Repeated administration

- increase in effect – accumulation
  senzitization

- decrease in effect
  - tolerance - changes at the site of receptor
    - chnges in pharmacokinetics
  - tachyphylaxis
  - resistence – „tolerance“ to the drugs inhibiting cell. growth or cytotoxic drugs
    cytostatics, antiinfectives, antiseptics

- drug dependance

# Tachyphylaxis after repeted ephedrine administration (decrease ineffect on blood pressure)




**E = ephedrine administration**

# BASIC PHARMACOKINETIC PARAMETERS

- C ( Co, Cmax ) concentration in plasma
- Tmax time to reach Cmax
- $V_d$ volume of distribution
- Cl clearence ( $Cl_{lungh}$ , $CL_{kid}$ , $Cl_{liv}$ , $Cl_{tot}$ )
- t 0,5 abs half-life of absorption
- $t_{0,5}$ ( $t_{1/2}$ el, $t_{50\%}$ el ) half-life of elimination
- AUC Area Under the Curve

  total amount of the drug during its presence in the body

- F [ % ] bioavailability

# Elimination (first order)

**Elimination constant** $k_e = \ln c_1 - \ln c_2 / t_2 - t_1$

**Half-life of the elimination** – the drug is completely eliminated after 4-5 $t_{0,5}$

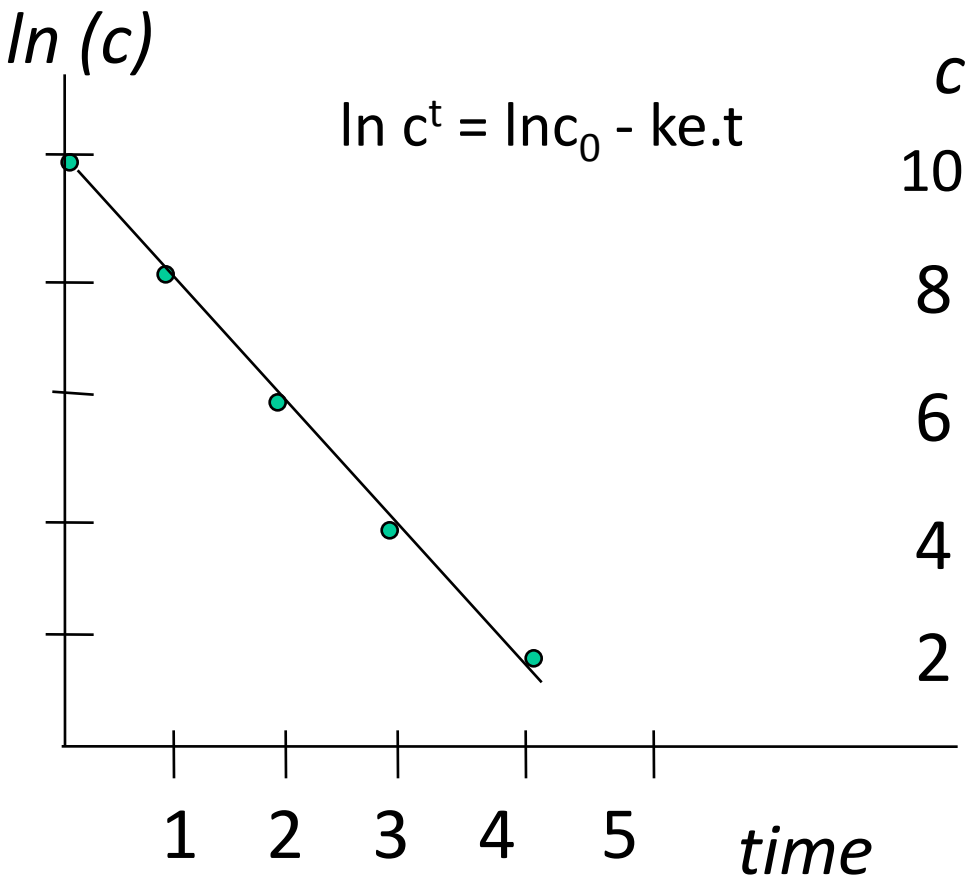
$t_{0,5} = \ln 2 / k^e = 0,7 / k_e$

**clearance** Volume of the blood in a defined region of the body that is cleared of a drug in a unit time

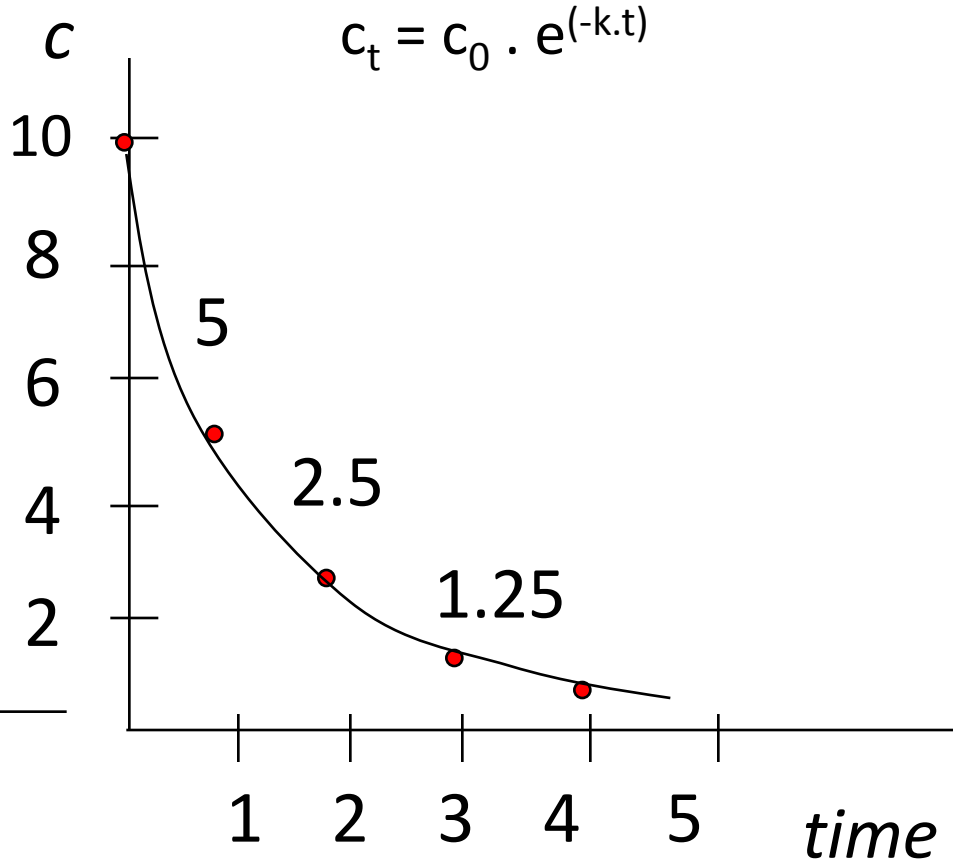
$Cl_{TOT} = D/AUC = k_e Vd$

# Kinetics of elimination of the 1st order –

semilog plot
(i.v. admin)




Normal plot
(i.v. admin)



# Half-Life

- $C = C_o \ e^{-kt}$
- $C/Co = 1/2$ in the time of 1 half-life

- Thus: $0.5 = e^{-k\,t}$
- $\ln 0.50 = -k\ t_{½}$
- $-0.693 = -k\ t_{½}$

- **$t_{1/2} = 0.693 / k$**

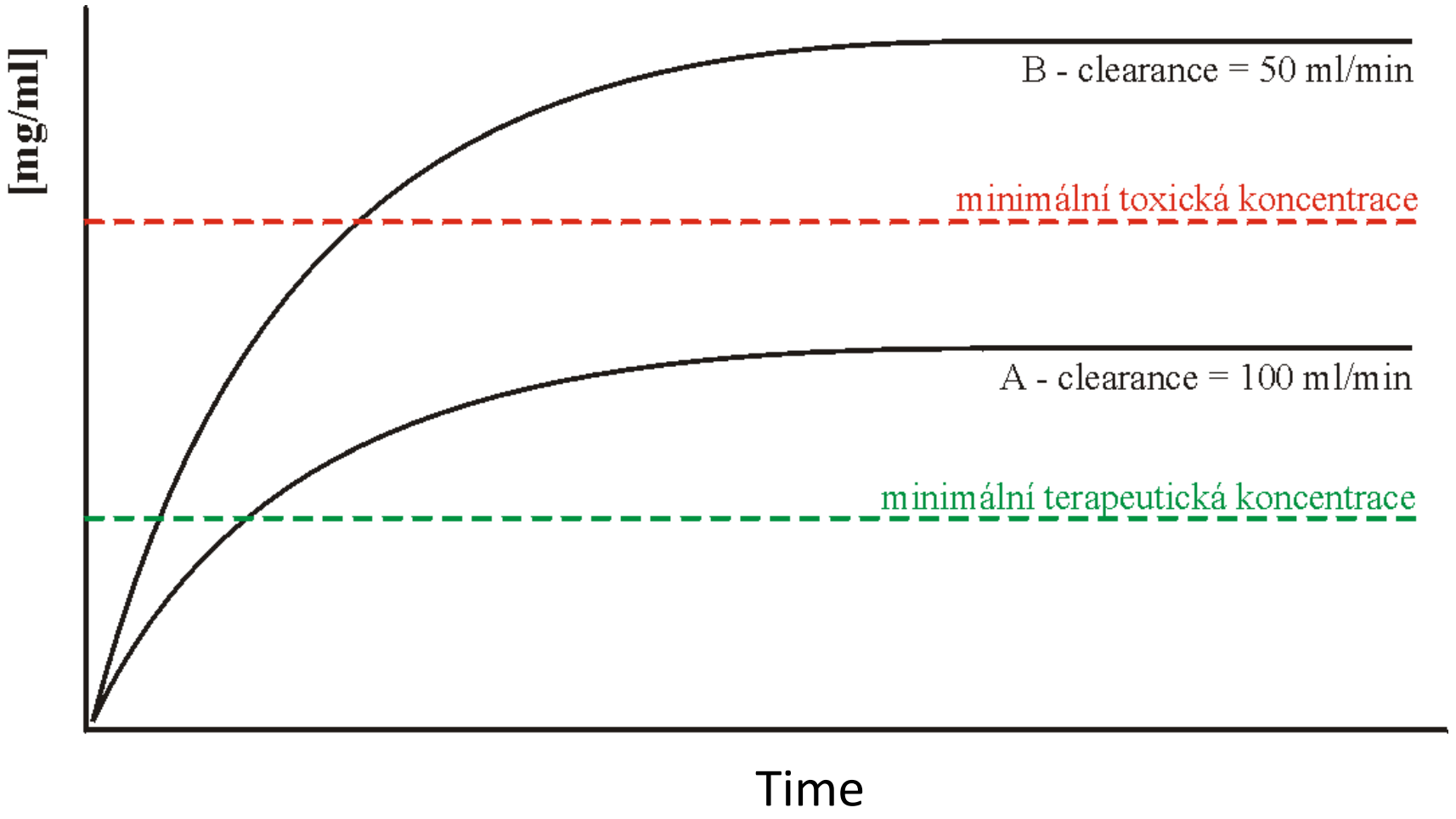
# i.v. infusion

- **Continual drug administration**
- **Administration of the drug e.g. by the infusion pump**

-the plasma concentration increases until the elimination rate become equal to the drug intake –

→ plasmatic concentration is steady - the plateau state (Css).

# I.v. infusion


[mg/ml]
B - clearance = 50 ml/min
minimální toxická koncentrace
A - clearance = 100 ml/min
minimální terapeutická koncentrace
Time

# The Compartment Model

Body = a series of interconnected well-stirred compartments within which the [drug] remains fairly constant.

Movement BETWEEN compartments is important in determining when and for how long a drug will be present in body.

# 2- compartment model