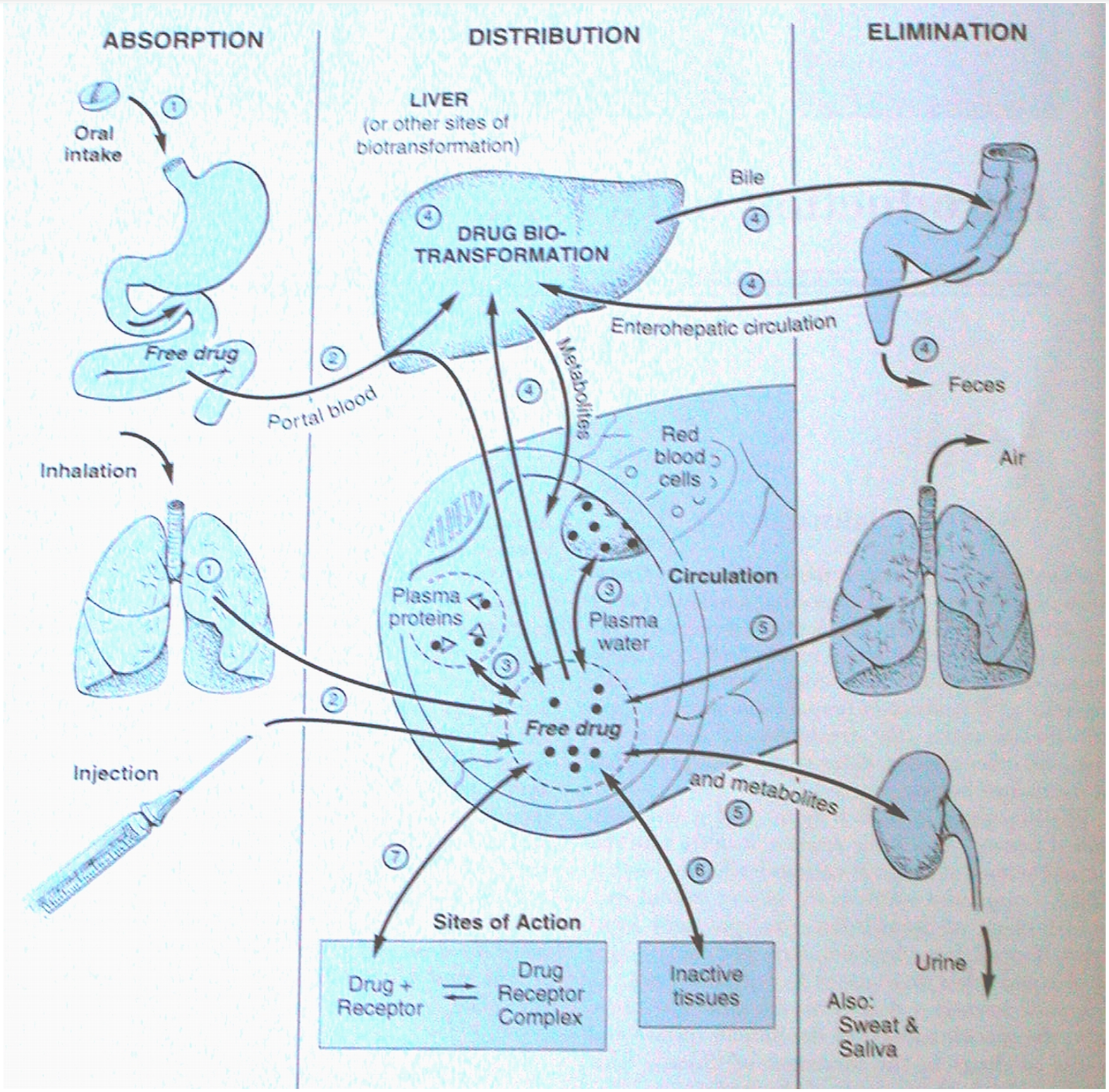
ADME: Metabolism



Metabolism (Biotransformation)

The conversion of a drug from one form to another by the actions of enzymes

- ***Phase I (Non-synthetic) reactions:*** **introduction or unmasking of functional group by oxidation, reduction or hydrolysis**

- ***Phase II (Synthetic) reactions:*** **functional group or metabolite formed by phase I is conjugated with endogenous constituent as glucuronic acid, glutathione, sulphate, glycine or methyl group**

Metabolism (Biotransformation)

Phase I (non-synthetic) reactions may result in:

1- Drug inactivation (most of drugs)

2- Conversion of inactive drug into active metabolite (prodrugs, cortisone→ cortisol)

3- Conversion of active drug into active metabolite (phenacetin→ paracetamol)

4- Conversion to toxic metabolite (methanol → formaldehyde)

Metabolism (Biotransformation)

Phase II (synthetic) reactions:

1- usually result in drug inactivation with few exceptions (morphine-6- conjugate is active)

2- Metabolites formed in synthetic reactions are more polar and thus more readily excreted by the kidneys (in urine) and the liver (in bile)

Most of drugs pass through phase I only or phase II only or phase I then phase II (phase numbers reflect functional rather than sequential classification: isoniazid passes first through phase II then phase I

The same drug can undergo different phase I or phase II reactions

Metabolism of flunitrazepam

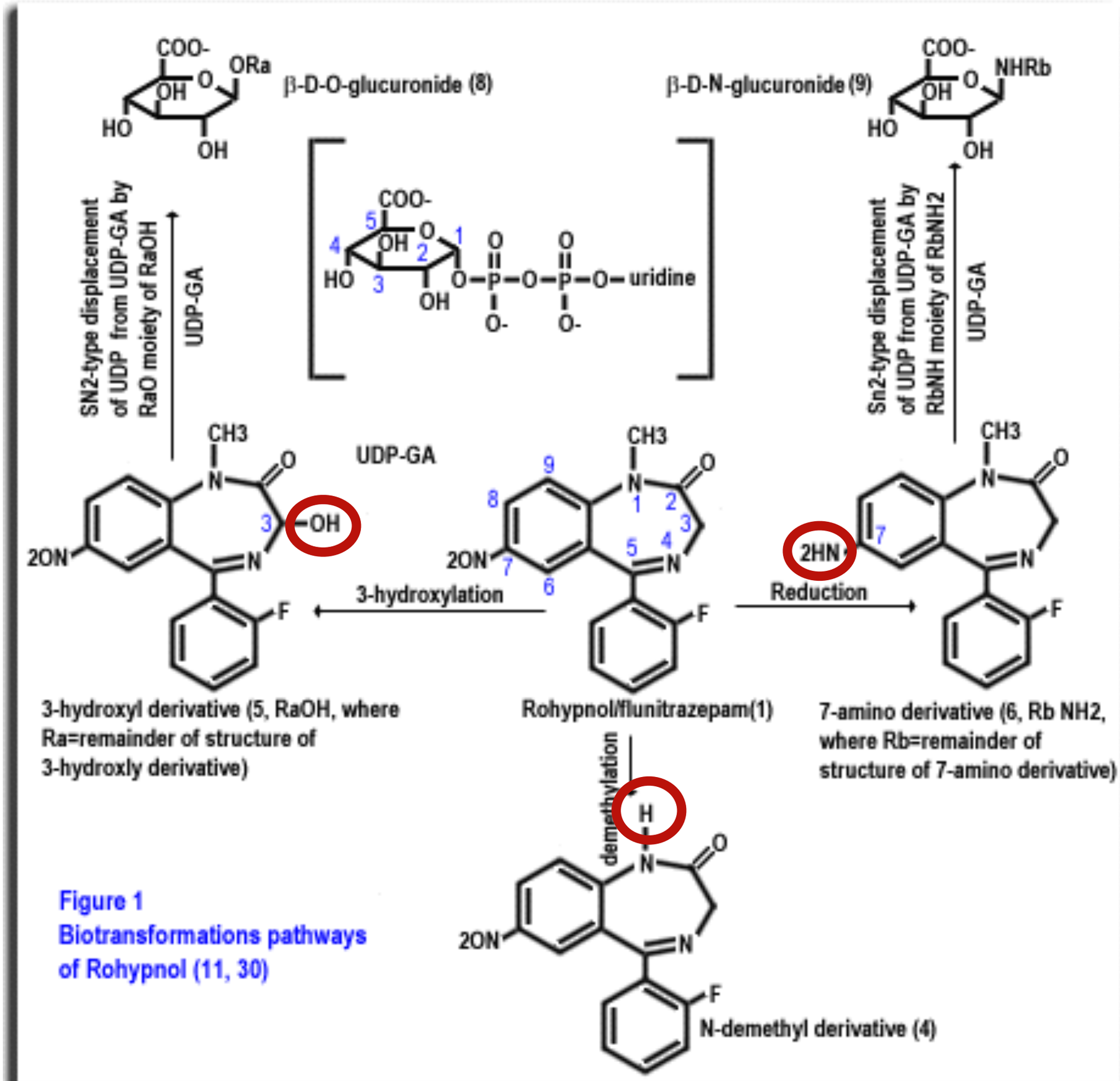


Figure 1
Biotransformations pathways of Rohypnol (11, 30)

Metabolism (Biotransformation)

Microsomal enzymes:

present in smooth endoplasmic reticulum of cells, especially liver

Catalyze

Glucuronide conjugation,

Oxidation by microsomal cytochrome P450 enzymes (CYP450)

Hydroxylation

Reduction

Hydrolysis

❑ **They are affected by drugs and age**

Non-Microsomal enzymes:

present in liver, kidney, plasma, skin, gastrointestinal tract (GIT) …etc

Catalyze

Conjugations other than glucuronic acid, Oxidation by soluble enzymes in cytosol or mitochondria (e.g. MAO and ADH)

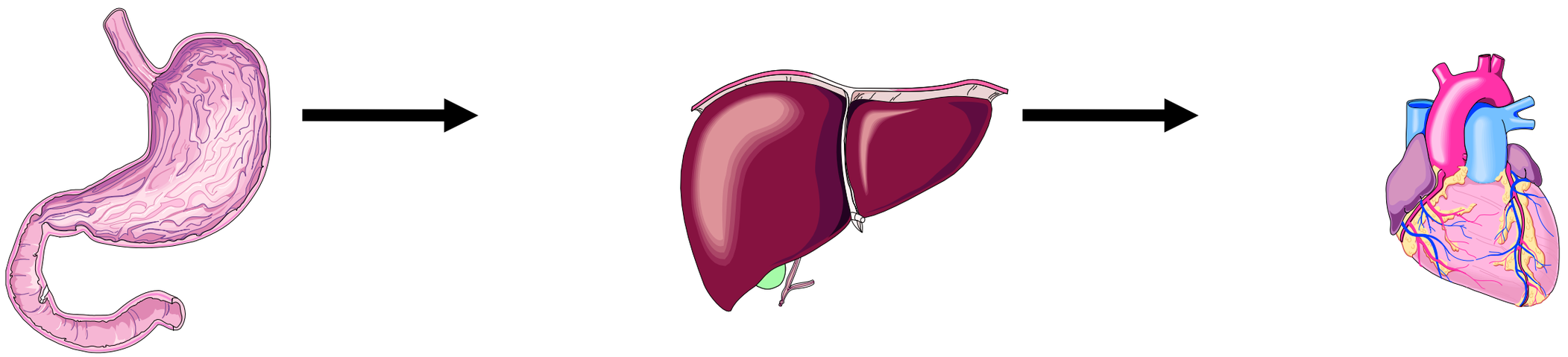
Reduction

Hydrolysis

❑ **Their activity is stable throughout life**

Hepatic ‘First-Pass’ Metabolism

- Affects orally administered drugs
- Metabolism of drug by liver before drug reaches systemic circulation
- Drug absorbed into portal circulation, must pass through liver to reach systemic circulation
- May reduce availability of drug



Cytochrome P450 (CYP 450)

Superfamily of heme enzymes, is the most important enzyme of phase I

Can catalyze many reaction types, mainly hydroxylation

Expressed in all tissues, the highest levels found in the liver

Responsible of the biosynthesis or degradation of endogenous compouds (steroid hormones, TXA2, PGI2, liposolule vitamins, fatty acids, etc.)

It metabolizes a great number of xenobiotics
and gives origin to inactive metabolites or toxic compounds

Can be induced and inhibited (drug interaction)

Exhibit genetic polymorphism (inter individual variability)

Human CYP450 superfamily comprehend 18 families, 43 subfamilies and more than 60 genes and differ for substrate specificity and for sensivity to inducers and inibitors

CYP450 NOMENCLATURE

Based upon Nelson et al. *DNA & Cell Biology* 12:1-51, 1993.

CYP3A4

CYP – abbreviation for cytochrome P450

3 – designates family (≥ 40% sequence identity)

A – designates sub-family (≥ 55% sequence identity)

4 – designates specific gene/enzyme

CYP – designates mRNA or protein

***CYP* – designates gene**

***CYP1A1* – gene that codes for cytochrome P450 1A1**
CYP1A1 – mRNA or protein product of *CYP1A1* gene

Evolution of CYP450 isoenzymes

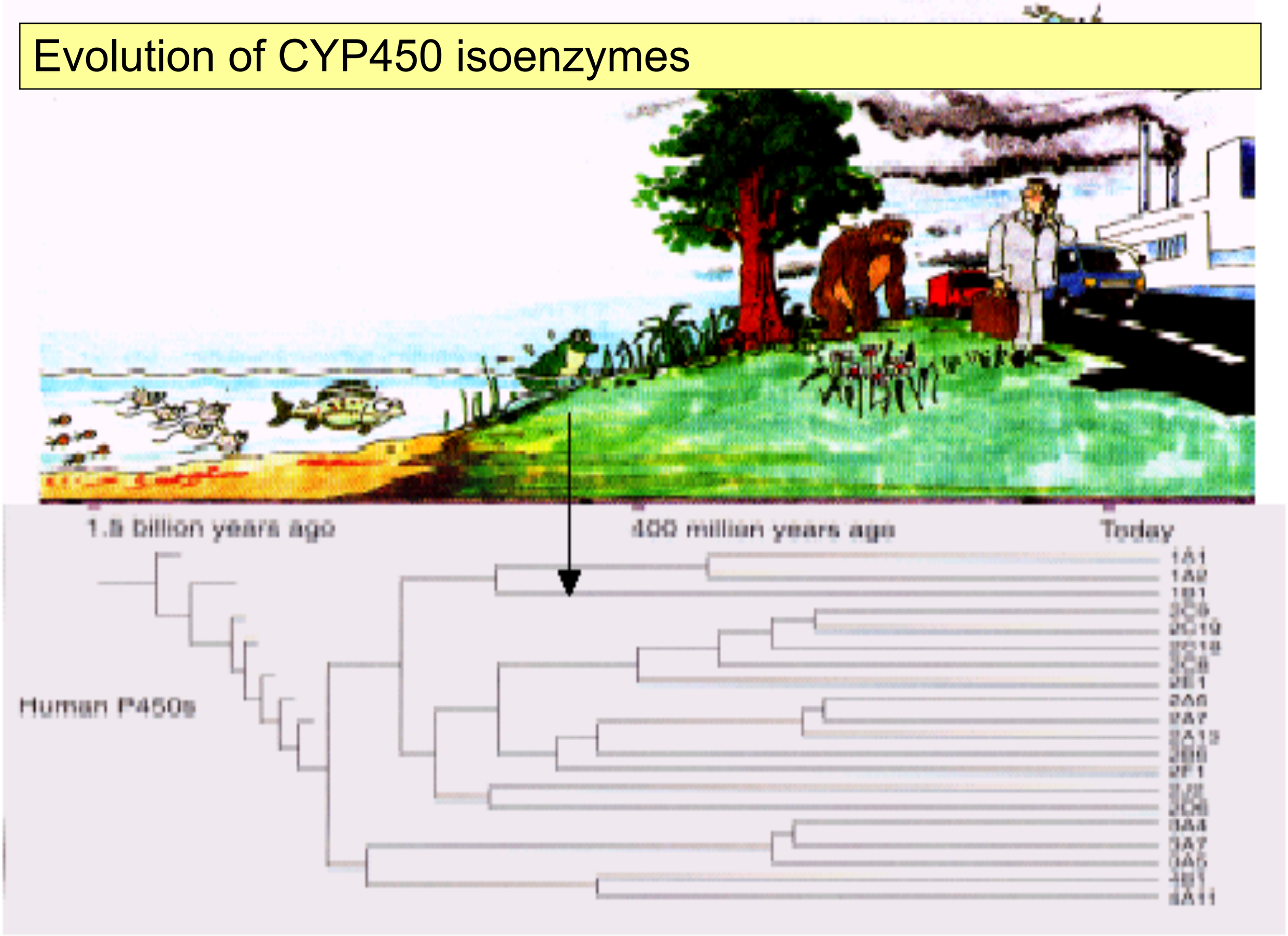
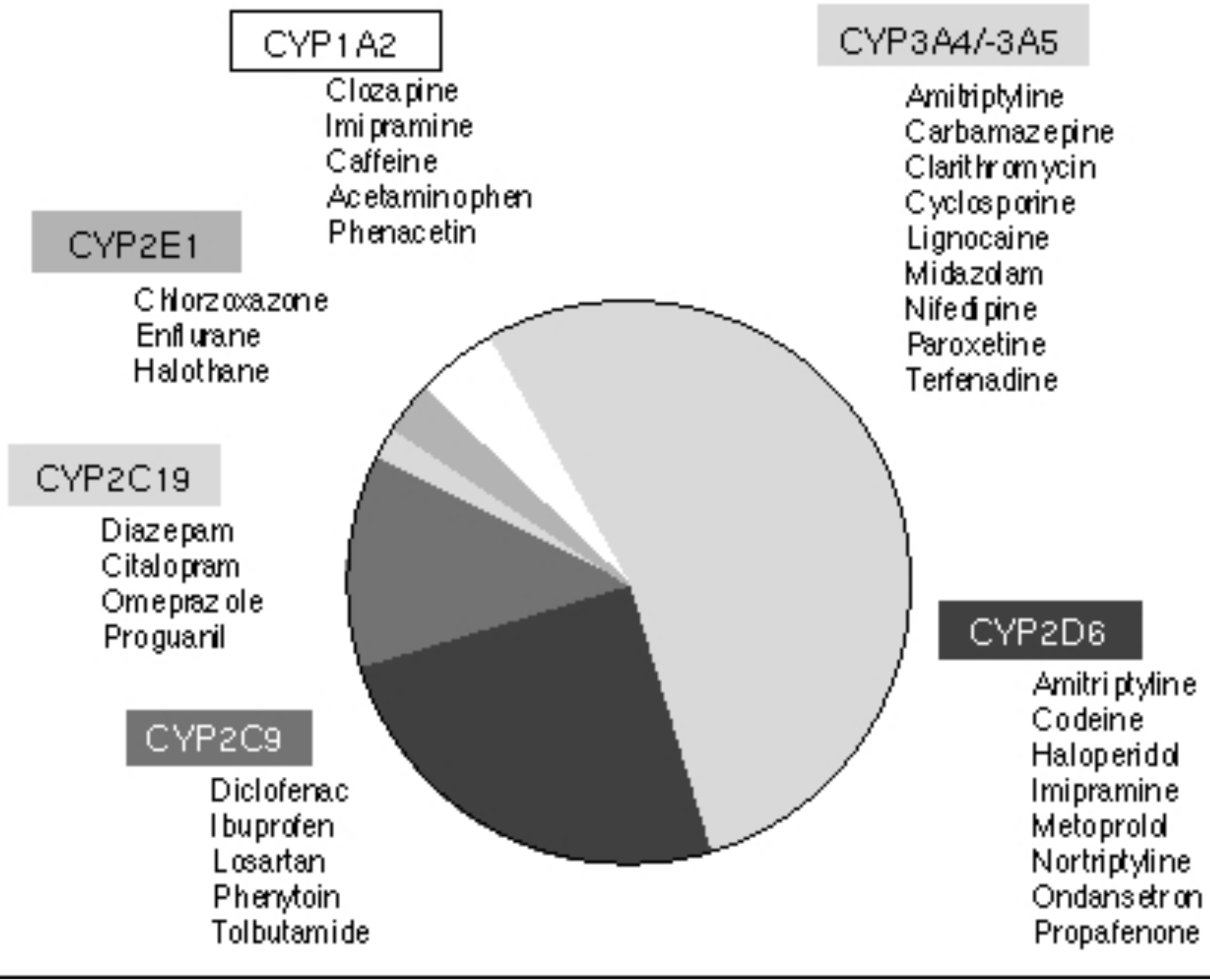


Figure 13: Evolution of P450 and selection pressure by dietary compounds.

THE MAIN FAMILIES OF CYP INVOLVED IN DRUG METABOLISM IN HUMAN LIVER

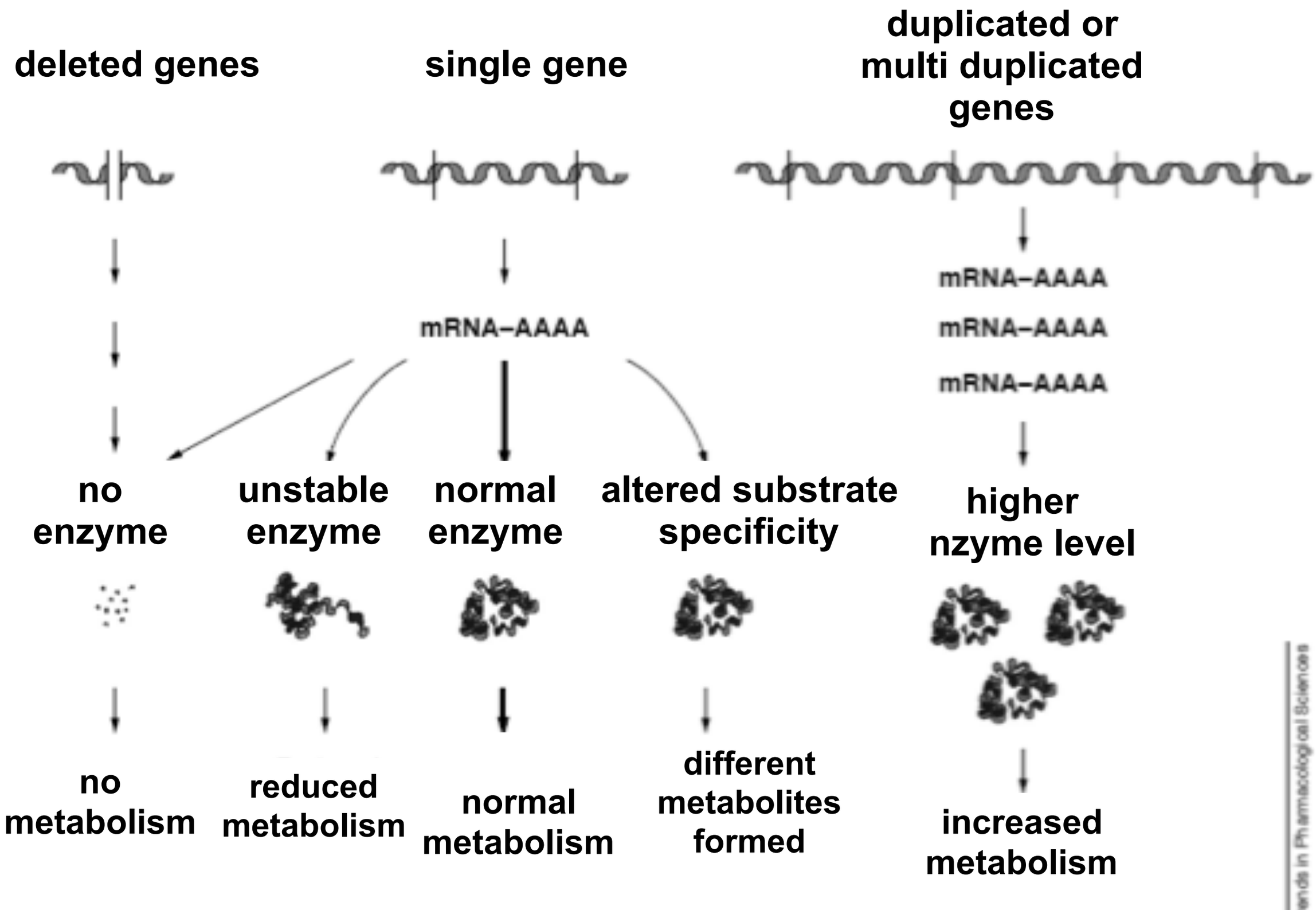


Metabolism (Biotransformation): Factors affecting drug metabolism

1. Genetic (innate)

2. Environmental (acquired)

1. **Genetics variation**: The most important factor is genetically determined polymorphisms



Polymorphisms of cytochrome P450

PMs: Poor Responders

20–30 million subjects have no CYP2D6 enzymes (PMs)

- Too slow drug metabolism
- Too high drug levels at ordinary dosage
- High risk for ADRs
- No response from certain prodrugs (e.g. codeine)

15–20 million subjects have *CYP2D6* gene duplications (UMs)

- Too rapid drug metabolism
- No drug response at ordinary dosage (non-responders)

UMs: Ultrarapid Metabolizers

TRENDS in Pharmacological Sciences

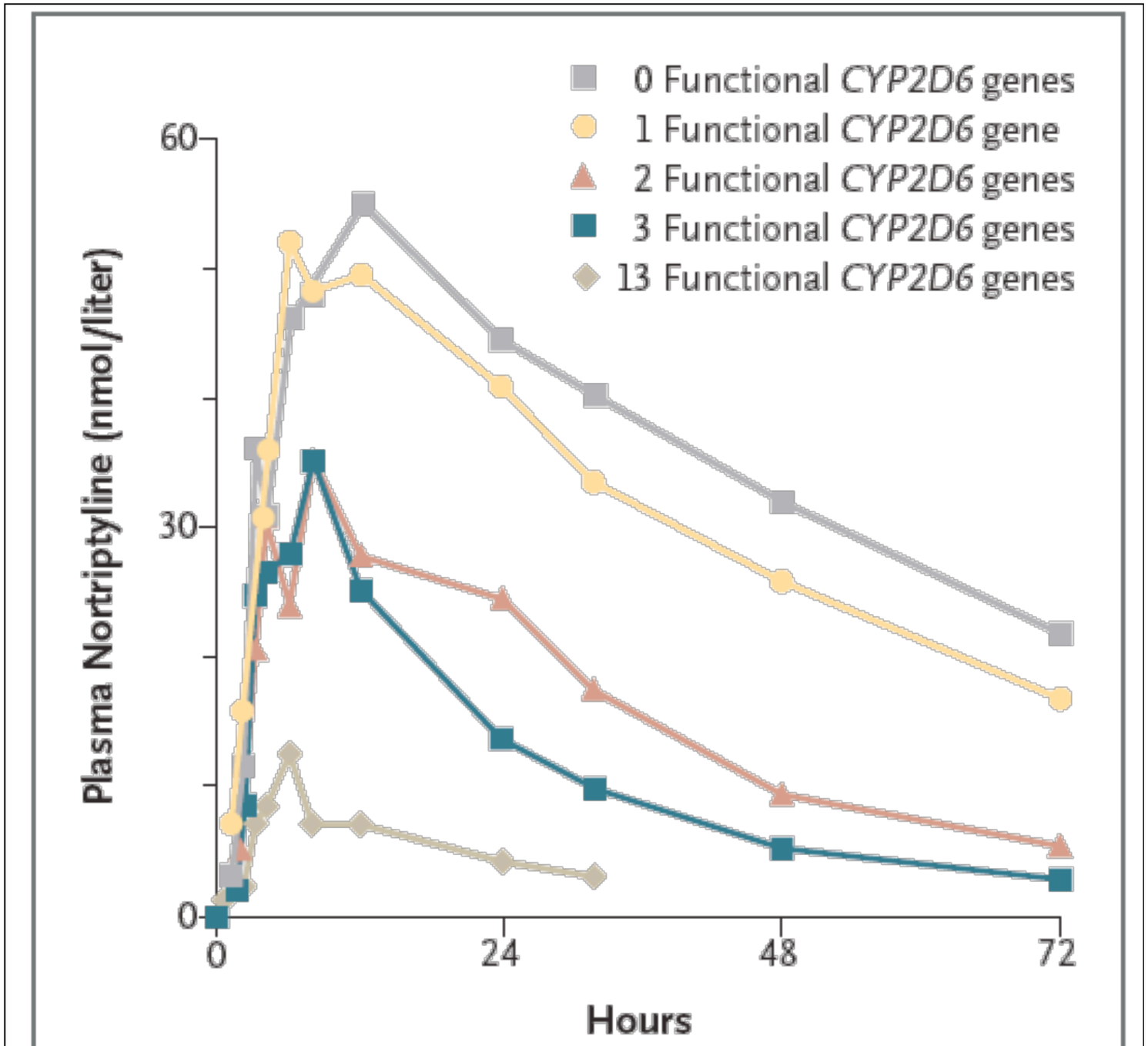


Figure 4. Pharmacogenetics of Nortriptyline.

Mean plasma concentrations of nortriptyline after a single 25-mg oral dose are shown in subjects with 0, 1, 2, 3, or 13 functional *CYP2D6* genes. Modified from Dalén et al.[23] with the permission of the publisher.

Metabolism (Biotransformation): **Factors affecting drug metabolism**

2. **Environmental (acquired)**

Drugs **can stimulate (induce the ex-novo synthesis) or inhibit microsomal metabolizing enzymes**

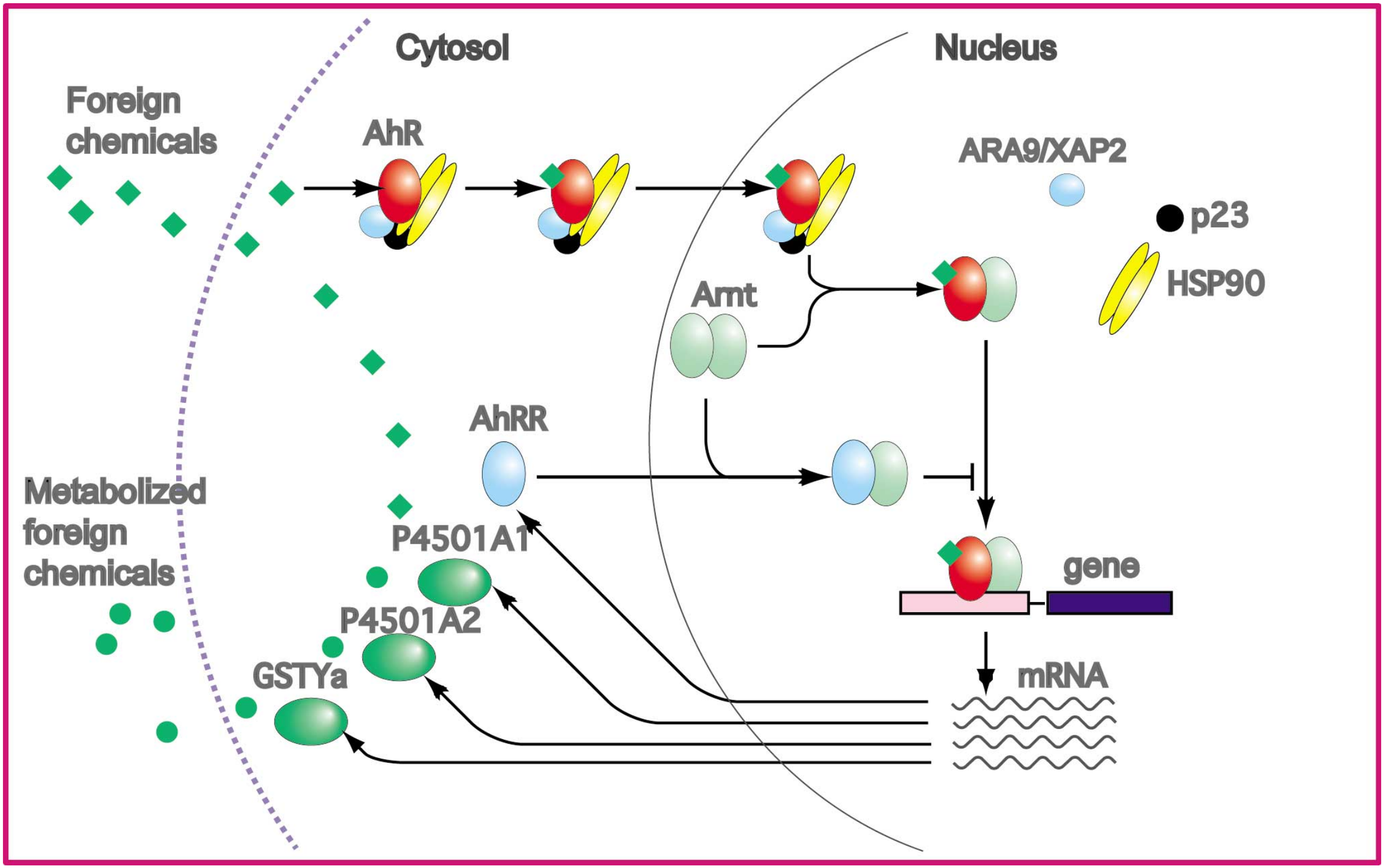
Metabolism (Biotransformation)

Enzyme induction: **Drug-dependent increased synthesis of metabolizing enzymes (example: phenobarbitone, phenytoin, carbamazepine, tobacco smoking, chronic ethyl alcohol)**

Importance of enzyme induction:

- **It is a mechanism of adaptation to environmental pollutants (pollutants induce their own metabolism reducing their toxic effects)**
- **It decreases effect of other drug**
- **Tolerance is sometimes explained by a drug inducing its own metabolism, e.g. ethyl alcohol, phenobarbitone**

MECHANISM OF DRUG-MEDIATED ENZYME INDUCTION MEDIATED BY THE ARYL HYDROCARBON (Ah) RECEPTOR



Note: the Ah receptor is a ligand-activated **transcription factor** involved in the regulation of several genes

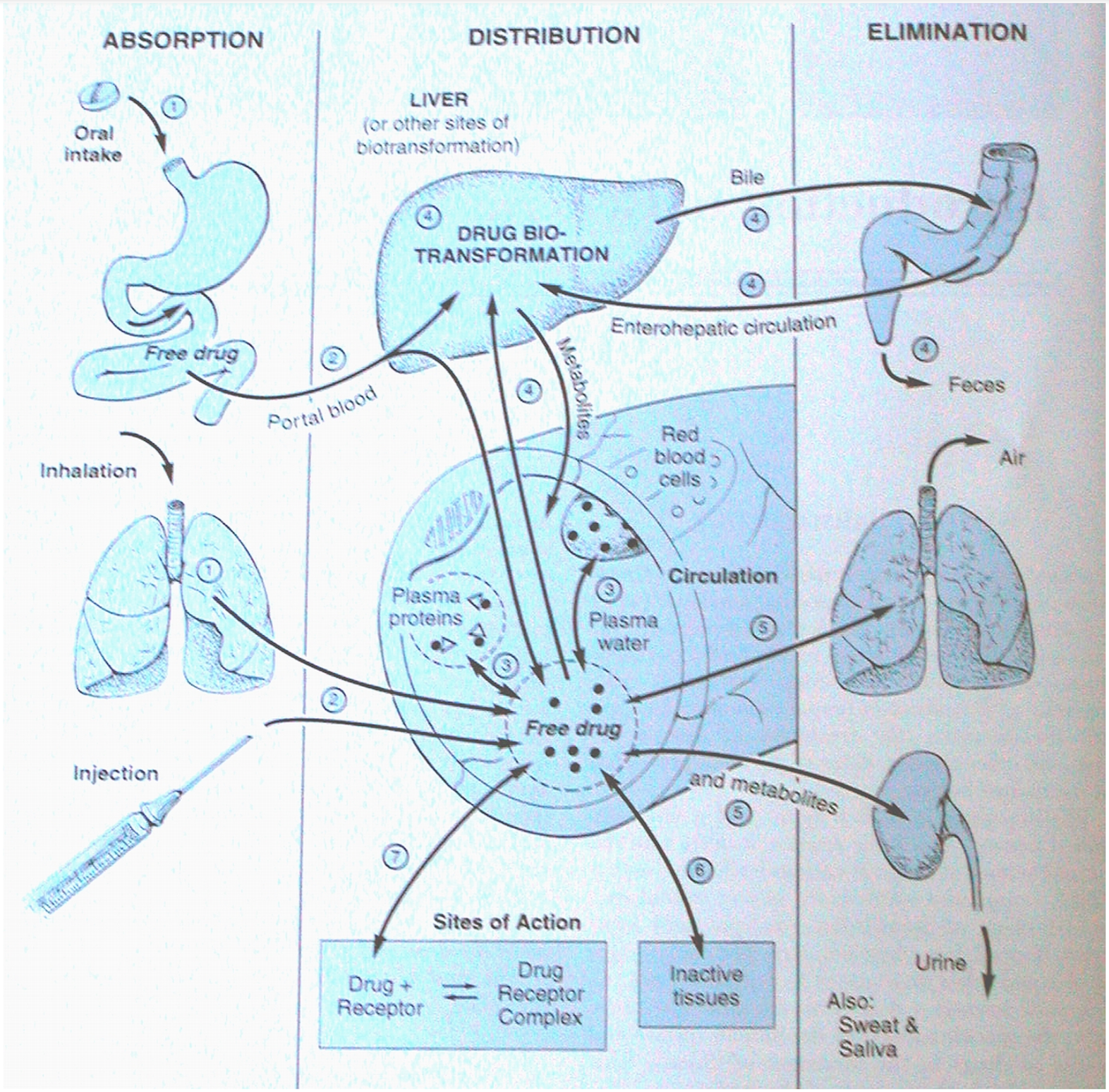
Metabolism (Biotransformation)

<u>Enzyme inhibition:</u> **Drug-dependent reduction of metabolizing enzymes (example: cimetidine, fluvoxamine, paroxetine)**

Importance of enzyme inhibition:

It increases the effect of other drugs that are substrates of the same enzyme (drug interaction)

ADME: Elimination



Drug Elimination

Kidneys
are the primary site
Renal diseases slow drug excretion and prolong drug effects

Gastrointestinal Tract:

a. Salivary glands: e.g., rifampicin and salicylates

b. Stomach: e.g., morphine (free and conjugated)

c. Large intestine: e.g., tetracycline, streptomycin

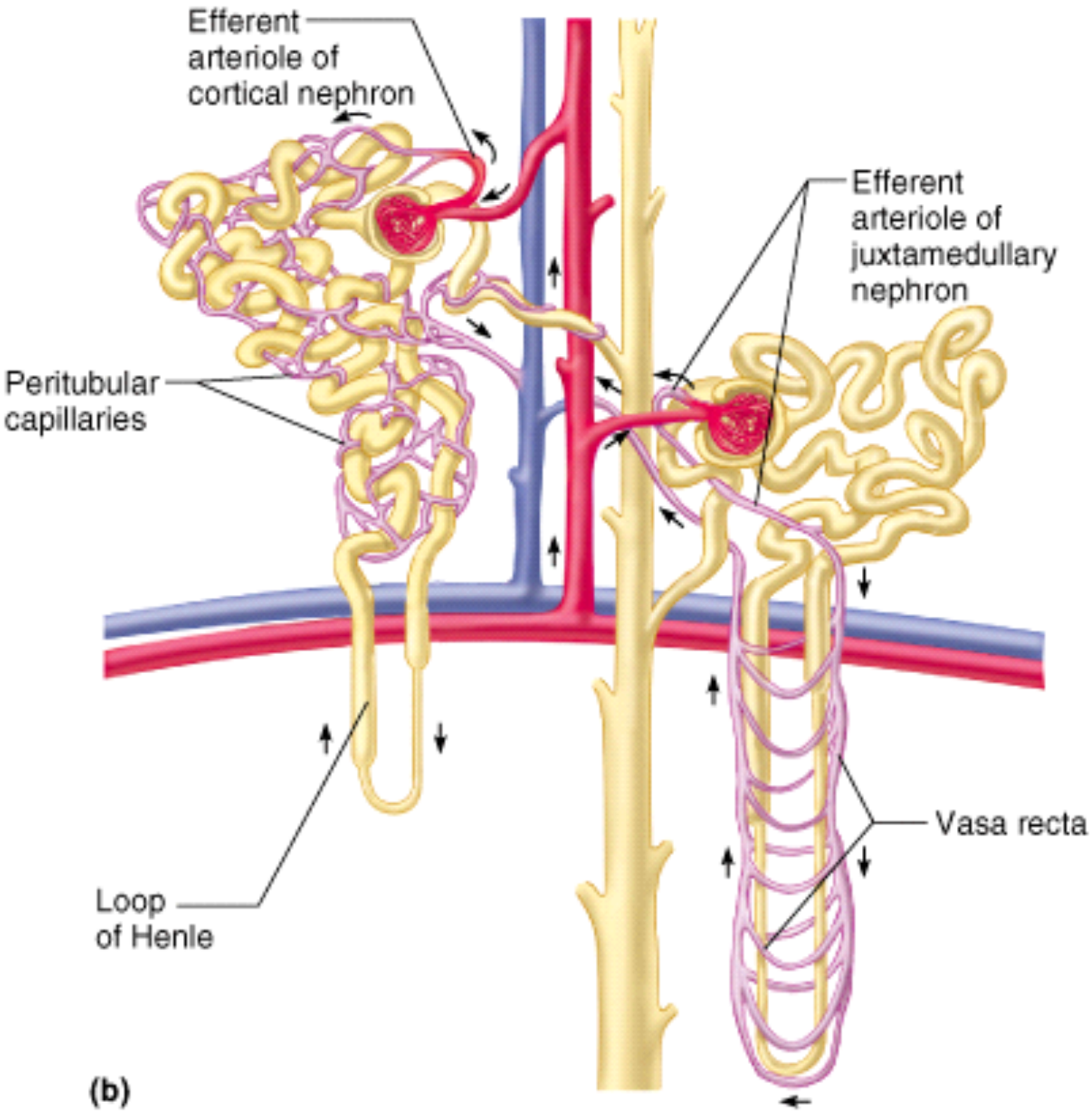
d. Liver through bile, e.g. ampicillin and rifampicin (excreted in active form, can be used in biliary infection)

Sweat: **e.g., rifampicin, vitamin B1.**

Lungs: **e.g., gases and volatile anesthetics**

Milk: **basic drugs are trapped and excreted in acidic milk, e.g., morphine, amphetamine**

Renal Elimination



Renal Elimination

Three general processes determine the composition and volume of urine:

1. **Glomerular filtration of the substance from the blood into the tubular fluid**

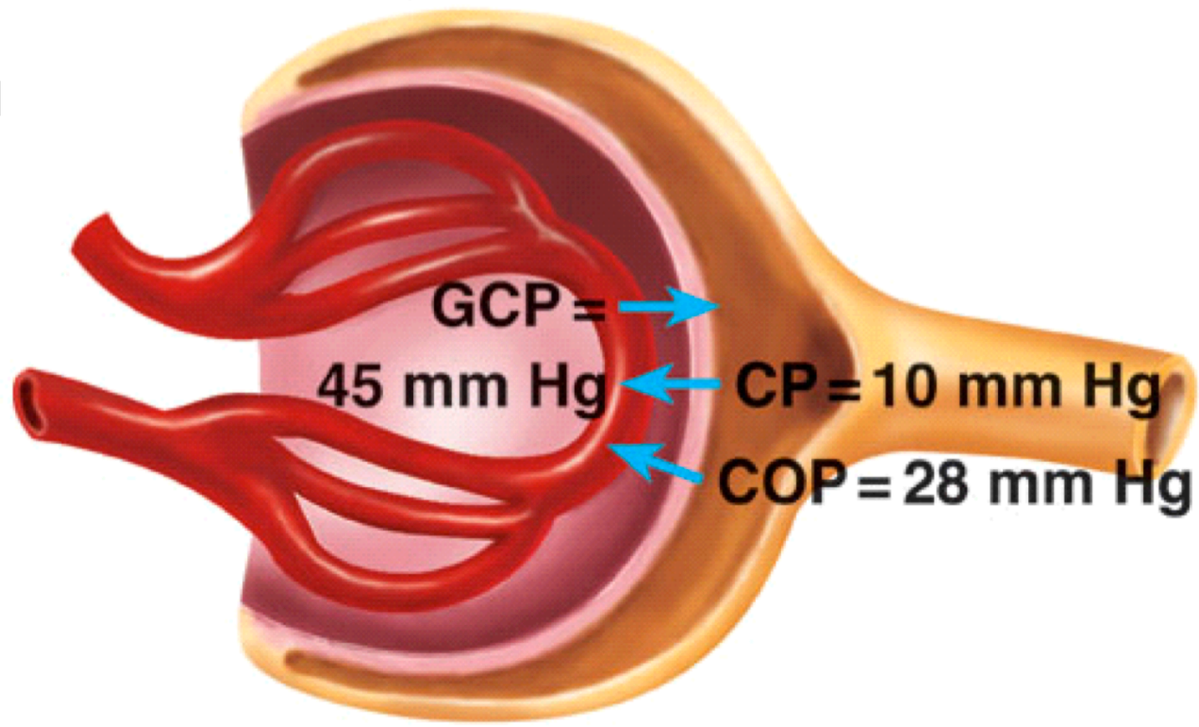
2. **Reabsorption of the substance from the tubular fluid into the blood**

3. **Secretion of the substance from the blood into the tubular fluid**

Glomerular filtration

Depends on:

- **Drug molecular weight**
- **Binding to plasma p**
- **Filtration pressure**



Filtration pressure = GCP – COP – CP

45 mm Hg GCP (glomerular capillary pressure)
–28 mm Hg COP (colloid osmotic pressure)
–10 mm Hg CP (capsule pressure)

7 mm Hg filtration pressure

Tubular Reabsorption

Mostly at the proximal level, depends on:

- **specific transporters**
- **passive diffusion**

along concentration gradient
on the basis of urine pH
AND
of the pKa for week acidic or basic drugs

Alkalinization of urine by NaHCO3 increases excretion of acidic drugs e.g. aspirin

Acidification of urine by NH4CL or vitamin C increases excretion of base drugs e.g amphetamine

Active Tubular Secretion

Mostly at the distal level, is based upon the expression of active transporters

- **saturable**
- **with higher affinity than plasma proteins**
- **competitive**

there is competition among substrates! (uric acid, salicilates)

Renal Elimination

***Amount of Drug Excreted* in Urine is equal to:**

1. The *amount of drug Filtered* through glomeruli into renal proximal tubule

2. Minus the *amount of drug Reabsorbed* into renal vein across renal tubular epithelia

3. Plus the *amount of grug Secreted* into the tubular luminal fluid across the renal tubular epithelia

Pharmacokinetic parameters

- apparent volume of distribution V_d
- clearance Cl
- bioavailability F
- elimination half-life $t_{1/2}$

Clearance (Cl)

- The “clearance” of a solute is the virtual volume of blood that would be totally cleared of a solute in a given time (unit: ml/min)
- Solutes come from blood perfusing kidneys
- Rate at which kidneys excrete solute into urine = rate at which solute disappears from blood plasma
- For a solute (drug) X:

Concentration of X in urine

Volume of urine formed in given time

$$Cl = \frac{U_x \times V}{P_x}$$

Clearance

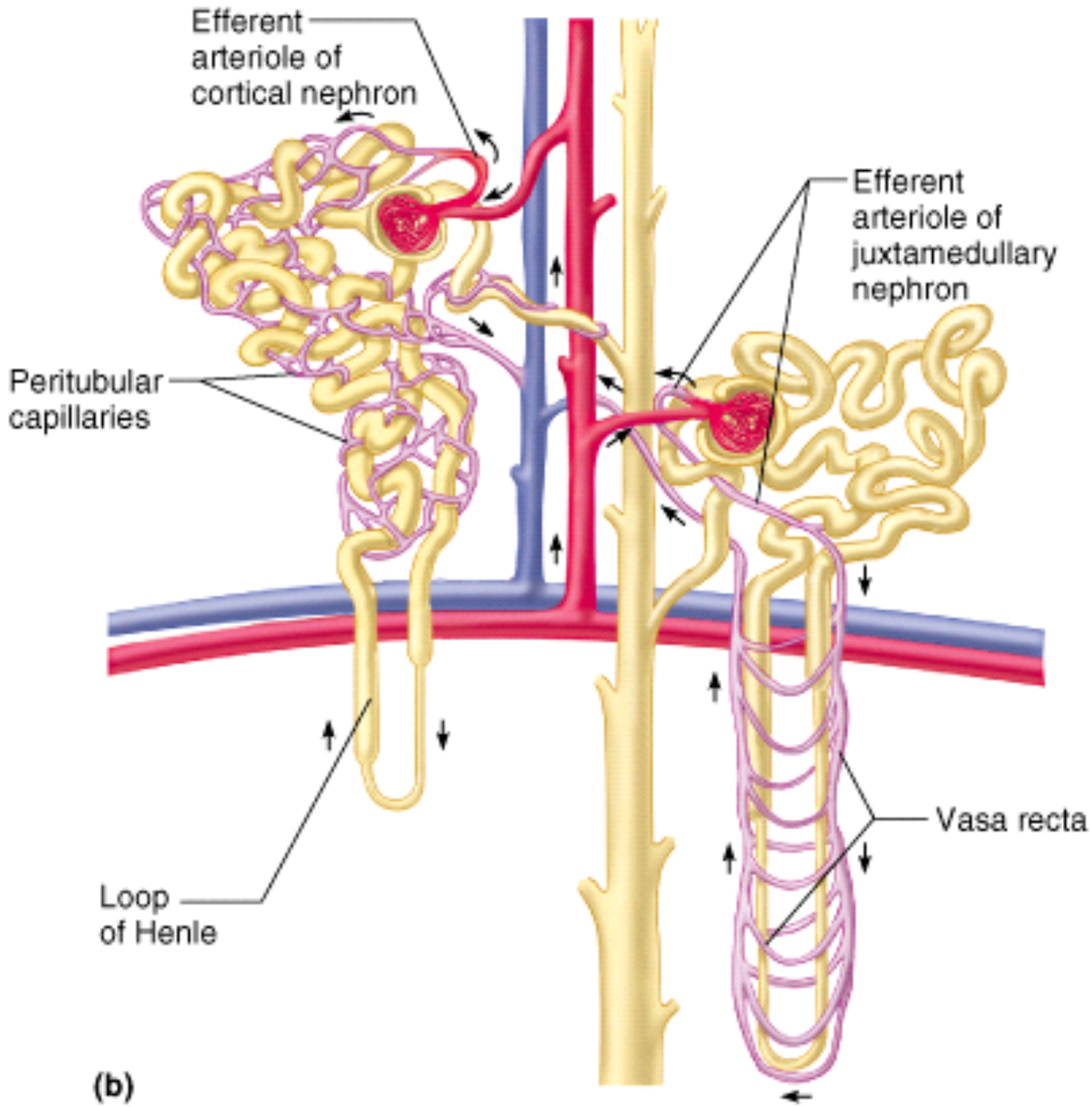
Concentration of X in systemic blood plasma

Clearance

- **~25% of cardiac output (6 L/min) reaches the kidneys**
- **= to ~1.25 L /min**
- **= to ~0,650 L/min plasma water**

- **~20% is filtered by the glomeruli**
- **= to ~0,130 L/min**
- **= to GFR**

- **80 - 85% reaches the tubuli through the peritubular capillaries and the vasa recta**
- **(0,650 - 0,130 L/min)**



CLEARANCE		MECHANISM OF ELIMINATION
Equal to GFR **~ 0,130 L/min**	→	Ultrafiltration no secretion, no reabsorption
Higher then GFR **Between ~ 0,130 and ~0,650 L/min**	→	Tubular secretion
Lower than GFR **Less than 0,130 L/min**	→	Tubular reabsorption

GFR = glomerular filtration rate

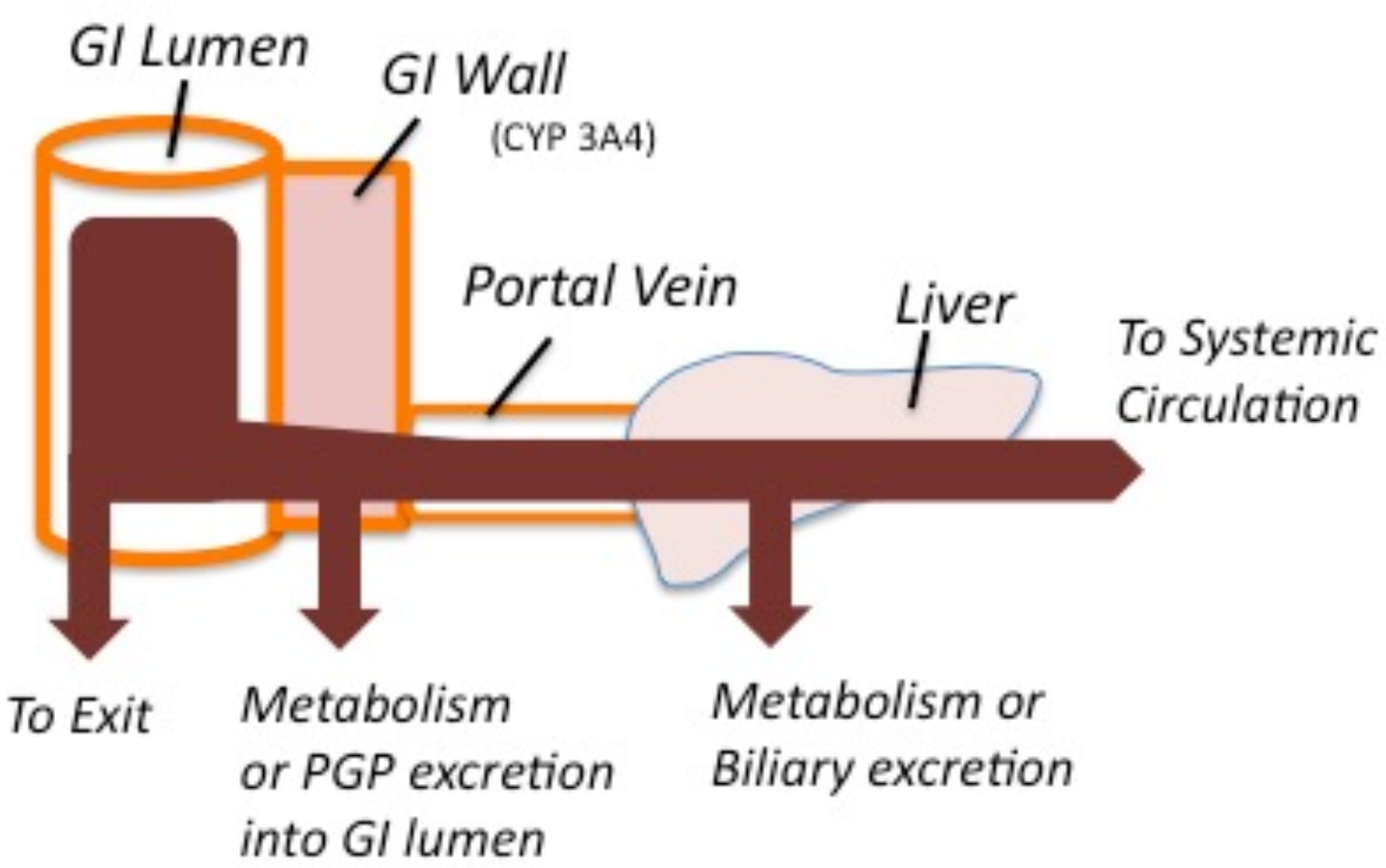
Pharmacokinetic parameters

- apparent volume of distribution V_d
- clearance Cl
- bioavailability F
- elimination half-life $t_{1/2}$

Bioavailability (F)

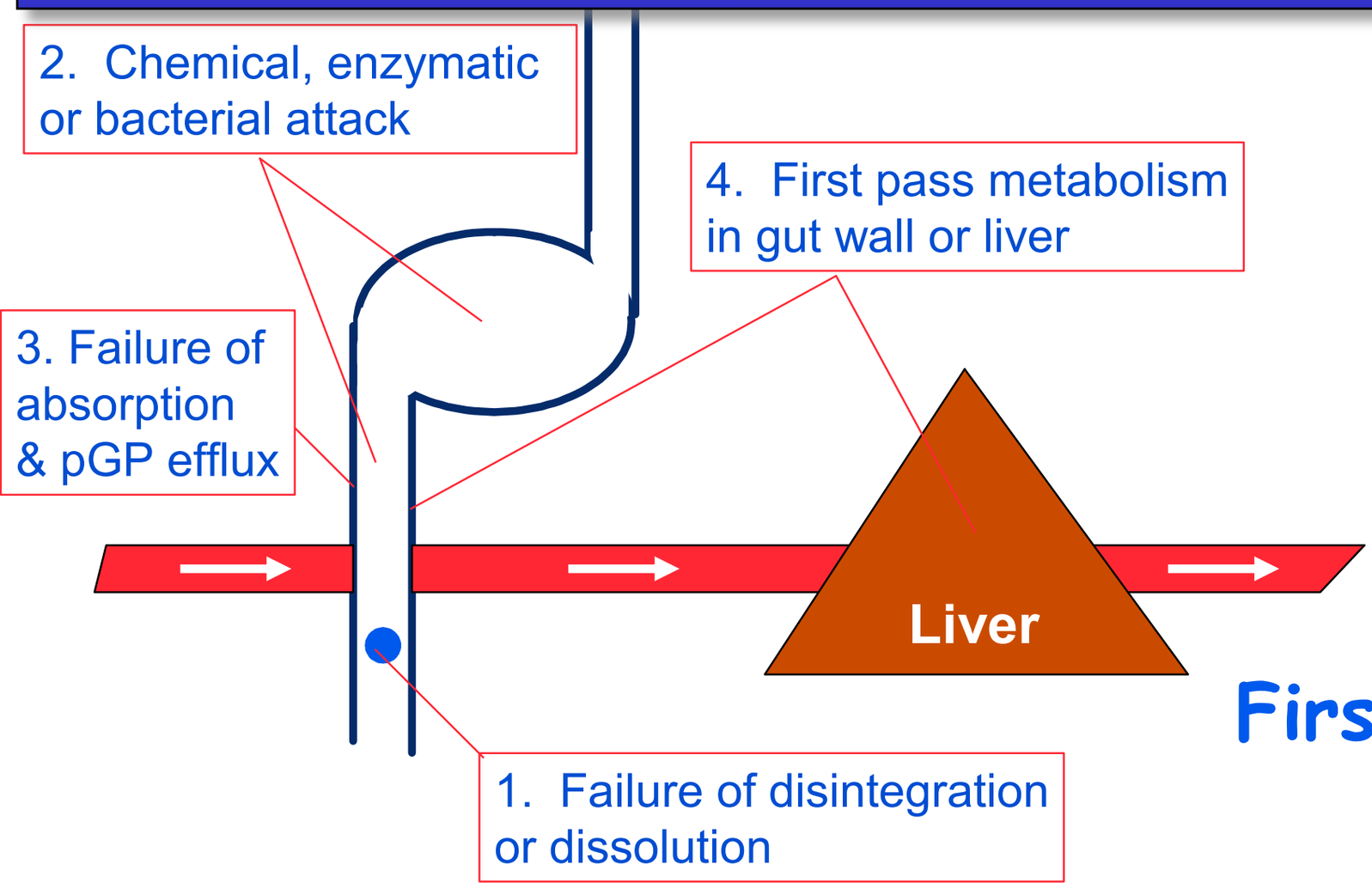
It is the percentage (or fraction) of a drug that reaches the systemic circulation in a chemically unaltered form and becomes available for the pharmacological effect after oral administration.

After intravascular administration, bioavailability is 100%



F = Fractional bioavailability (has no units)

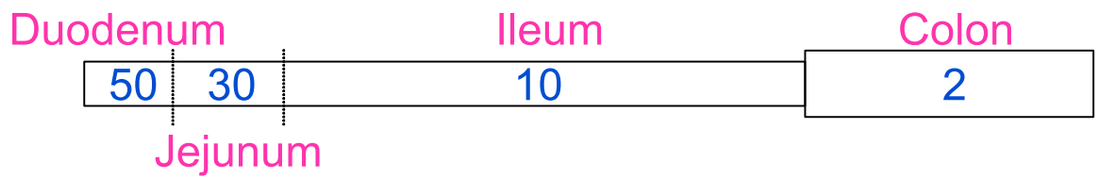
Bioavailability (F)



First pass metabolism in the gut wall

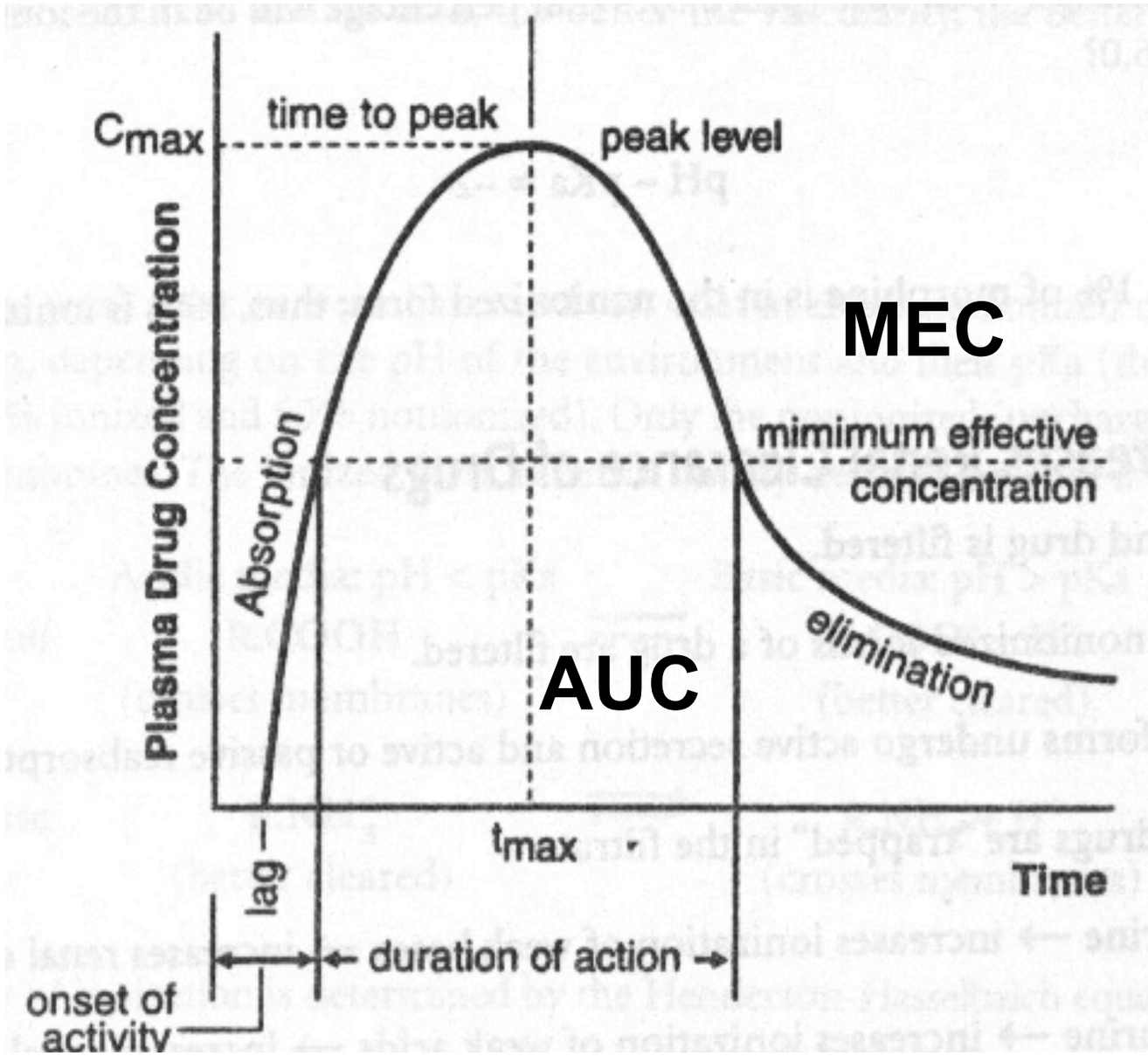
Intestinal epithelium is rich in drug metabolising enzymes. Main Cyt P450 is CYP3A4

Cytochrome P450 activity in intestinal epithelium relative to liver (%)



Plasma level curve after oral administration

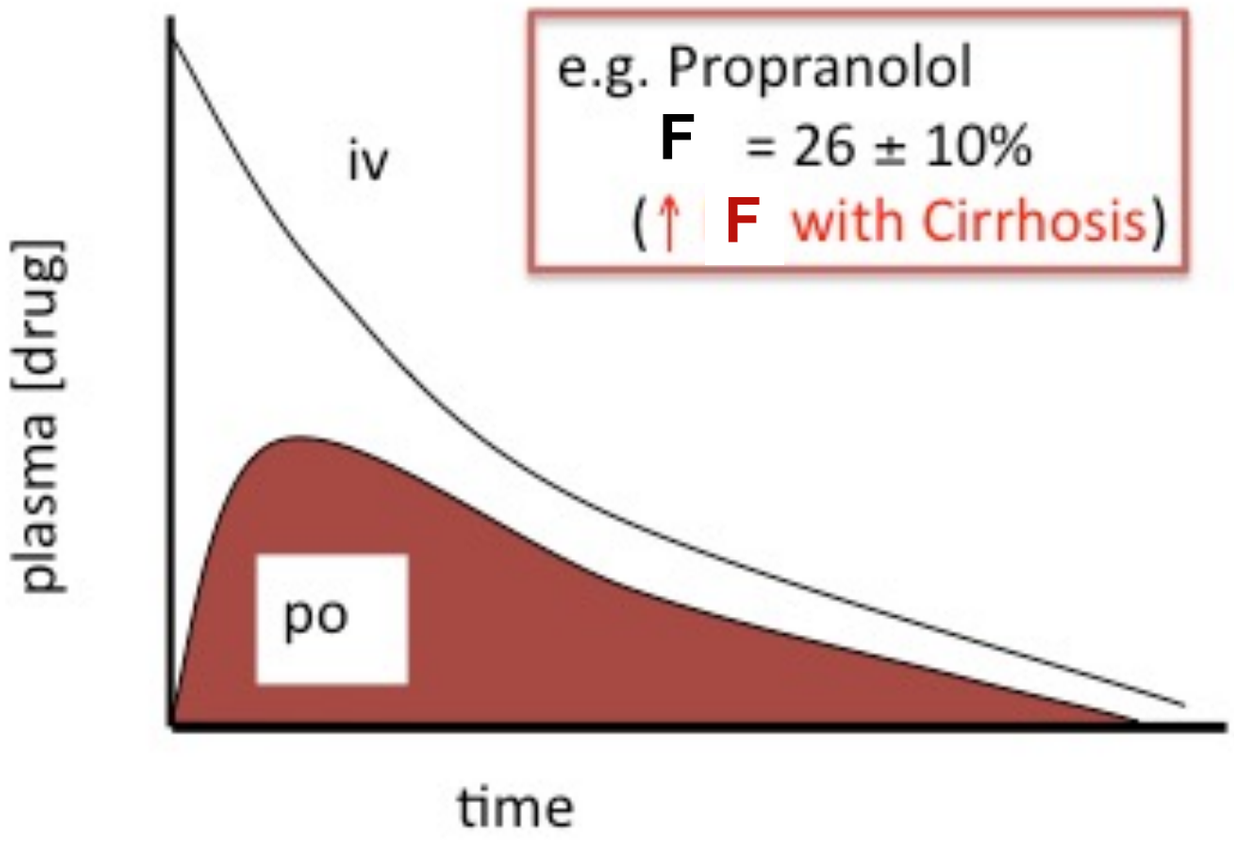
- **Lag time = time from administration to appearance in blood**
- **t_{max} = time at which C_{max} occurs**
- **C_{max} = maximal drug concentration obtained with the dose**
- **Time to peak = time from administration to C_{max}**
- **Onset of activity = time from administration to blood level reaching minimal effective concentration (MEC)**
- **Duration of action = time plasma concentration remains greater than MEC**
- **AUC = Area Under the Curve**



Bioavailability

F is calculated by comparison of the area under the plasma concentration time curve (AUC) after I.V. administration of a drug with that observed when the same drug is given at the same dose by another route e.g. oral

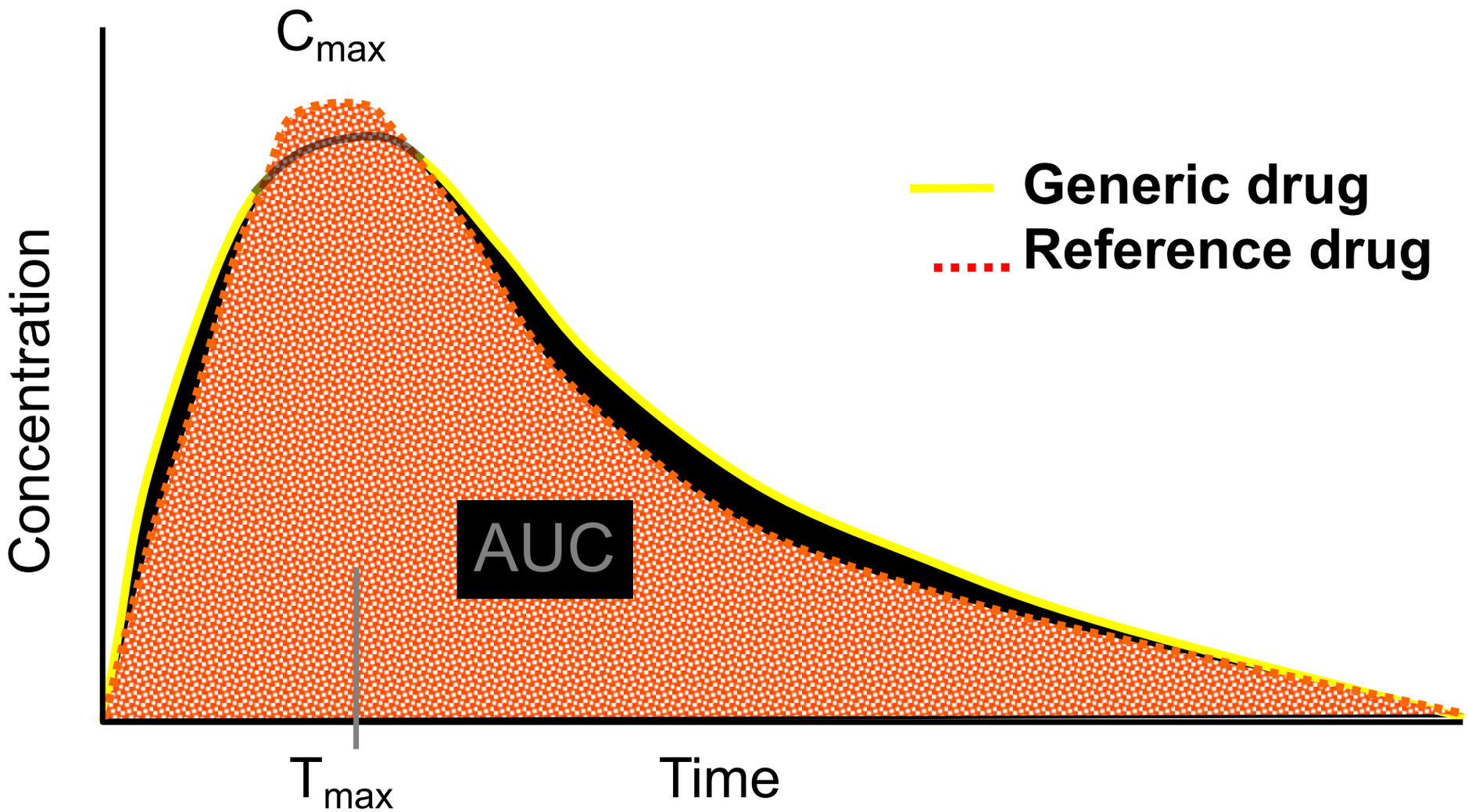
$$F = \frac{AUC_{po}}{AUC_{iv}} \times 100$$



Bioequivalence

Bioequivalence **occurs when two formulations of the same compound have the same bioavailability and the same rate of absorption**

i.e., they have similar AUC, C_{max} and T_{max}



Pharmacokinetic parameters

- apparent volume of distribution V_d
- clearance Cl
- bioavailability F
- elimination half-life $t_{1/2}$

How Vd and Clearance will affect the time of permanence of a drug in the body?

	Vd		
Clearance	Plasma water (3 L)	Extracellular water (12 L)	Total water (42 L)
Partial reabsorbtion (e.g. 30 mL/min)	69 min	277 min	947 min
Glomerular Filtration 130 mL/min	16 min	64 min	219 min
Tubular Secretion 650 mL/min	3 min	13 min	44 min

Elimination half-life ($t_{1/2}$)

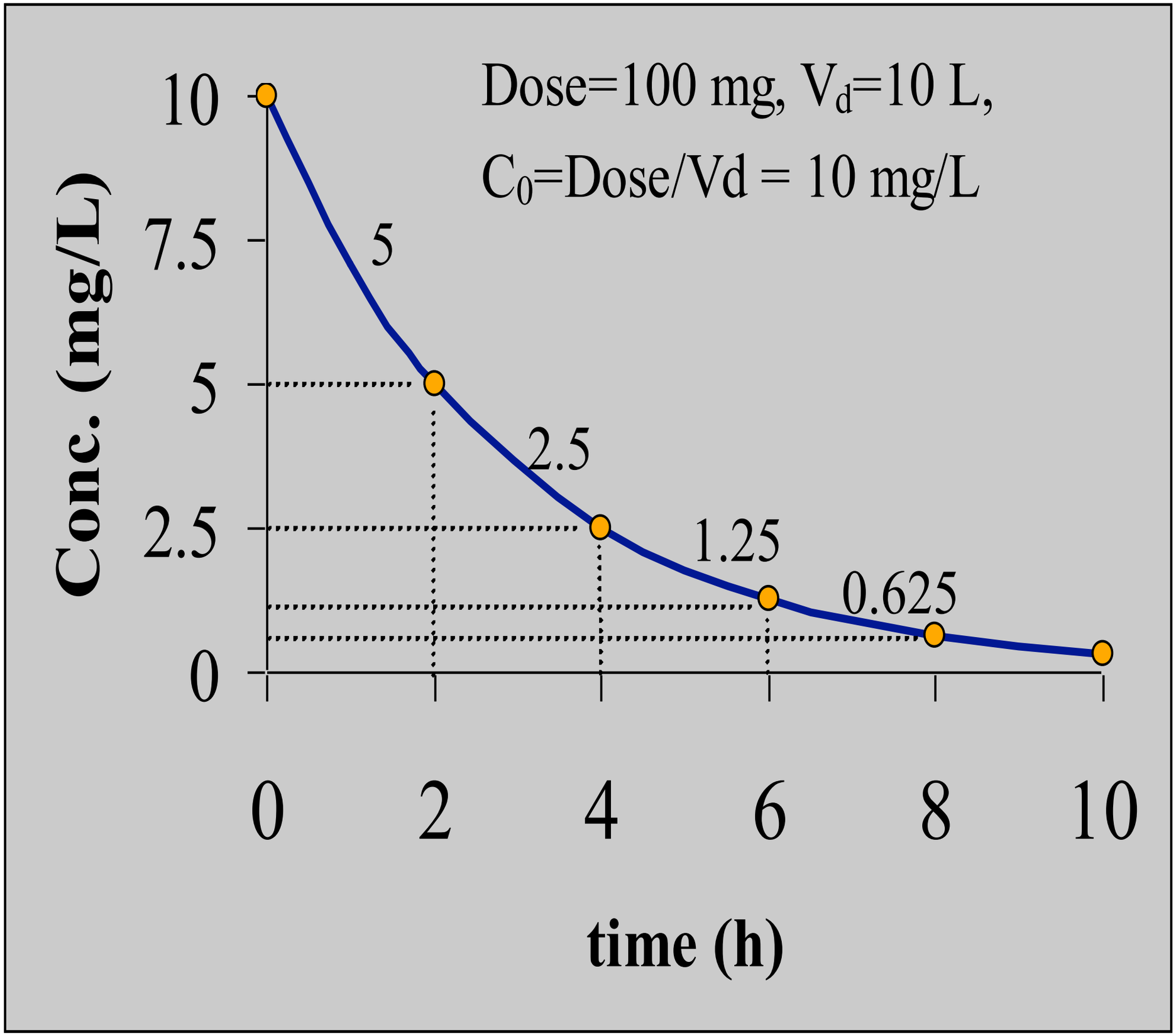
Elimination half-life is the time it takes the drug concentration in the blood to decrease to one half of its initial value after intravascular administration

Unit: time (min, h, day)

Elimination half-life depends on V_D and Clearance values:

$$Cl = k\,Vd \qquad k = \frac{0.693}{T½}$$

Rate of elimination



Rate of elimination

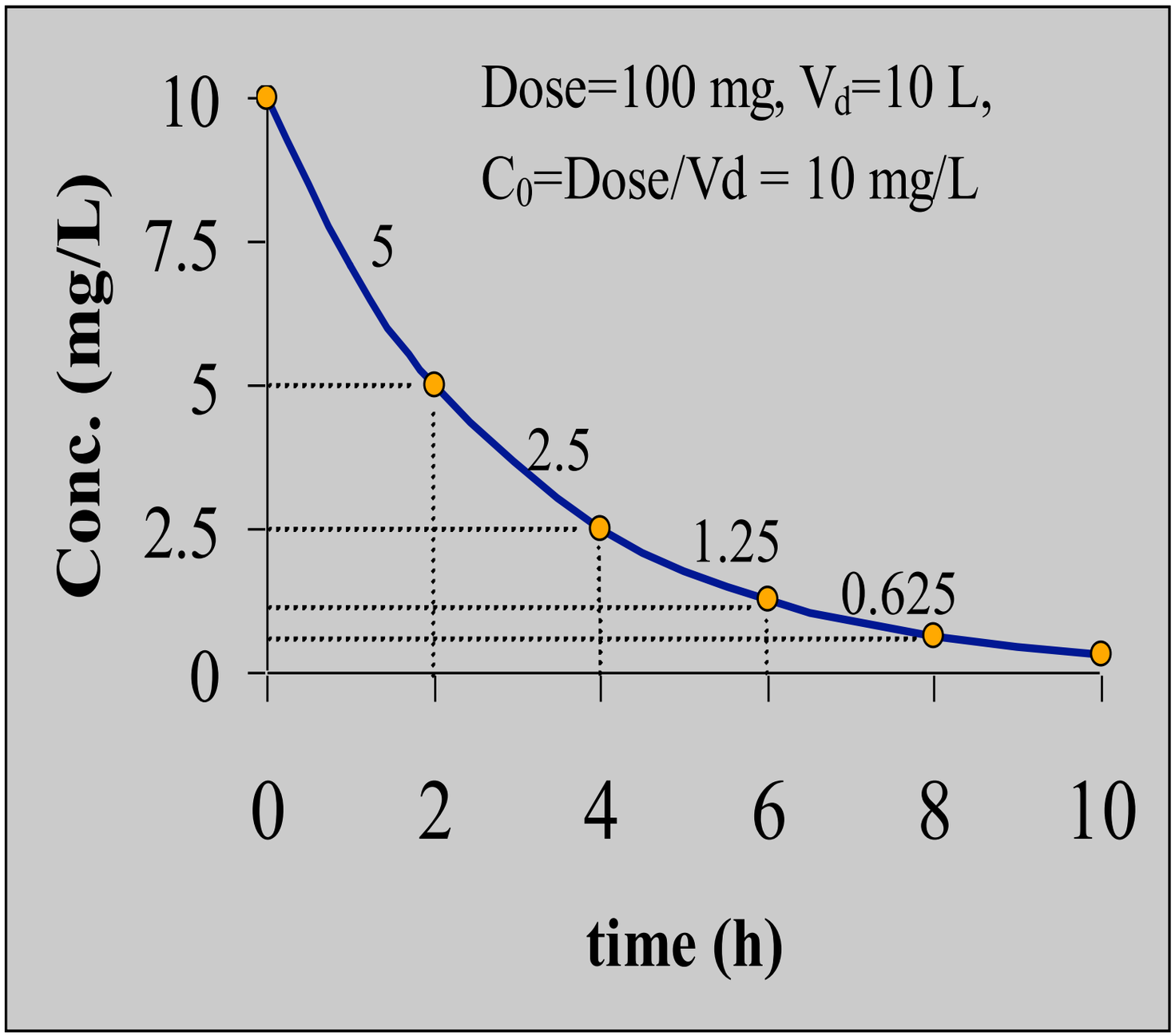
Elimination of most drugs from the body after therapeutically relevant doses follows first-order (linear) kinetics

The drug is given by i.v. bolus injection and blood samples are collected at various times to measure the plasma concentrations of the drug

As the drug is eliminated, the plasma concentration of the drug decreases

Rate of elimination

If the elimination of a drug which follows a first-order kinetic:



the elimination rate is proportional to plasma concentration

and therefore it decreases with time as the plasma concentration of the drug decreases

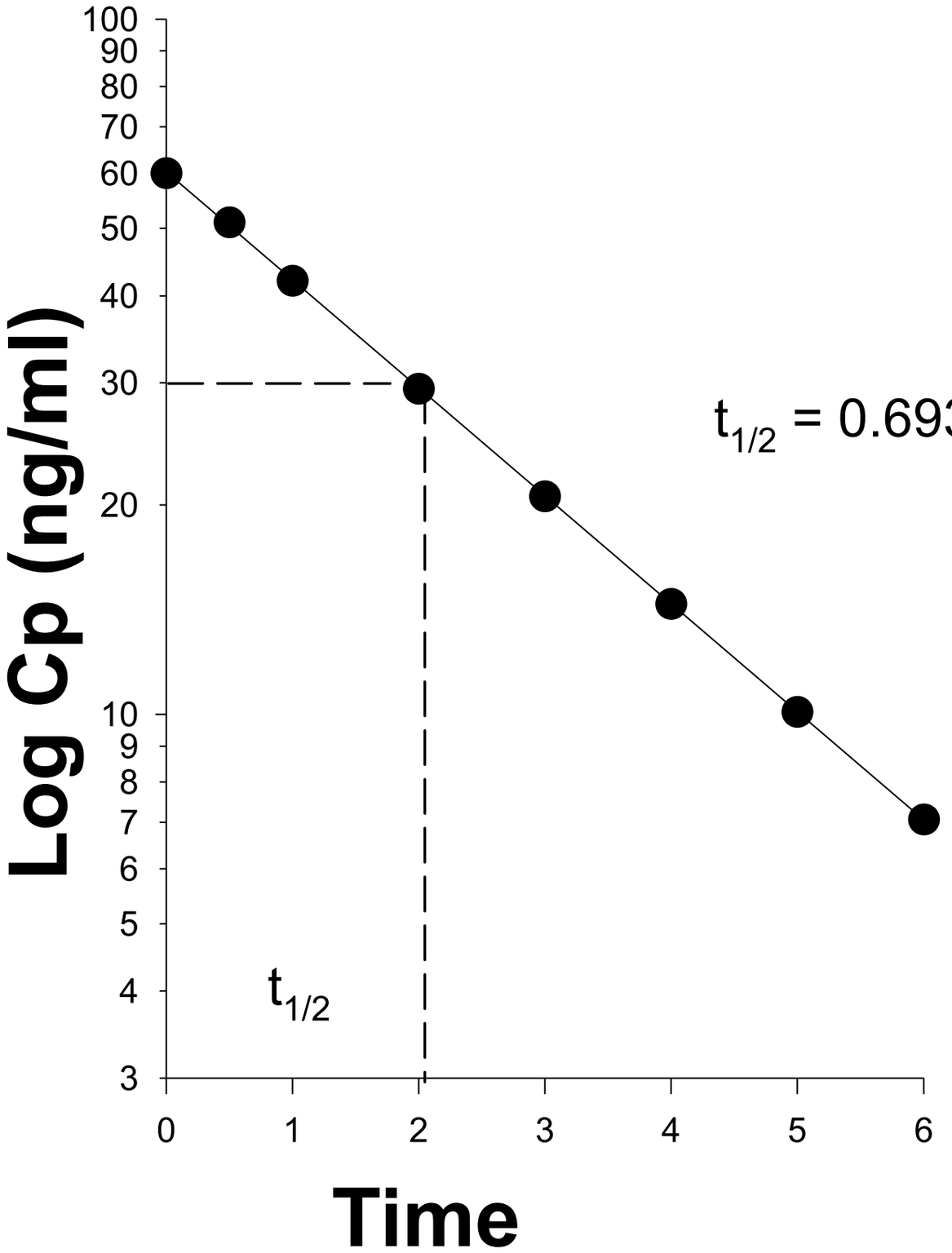
Rate of elimination

If the elimination of a drug which follows a first-order kinetic

in a semi-log graph a straight line is obtained

From the slope of the line the k_{el} can be estimated by means of the linear-regression analysis as well as the $t_{1/2}$:

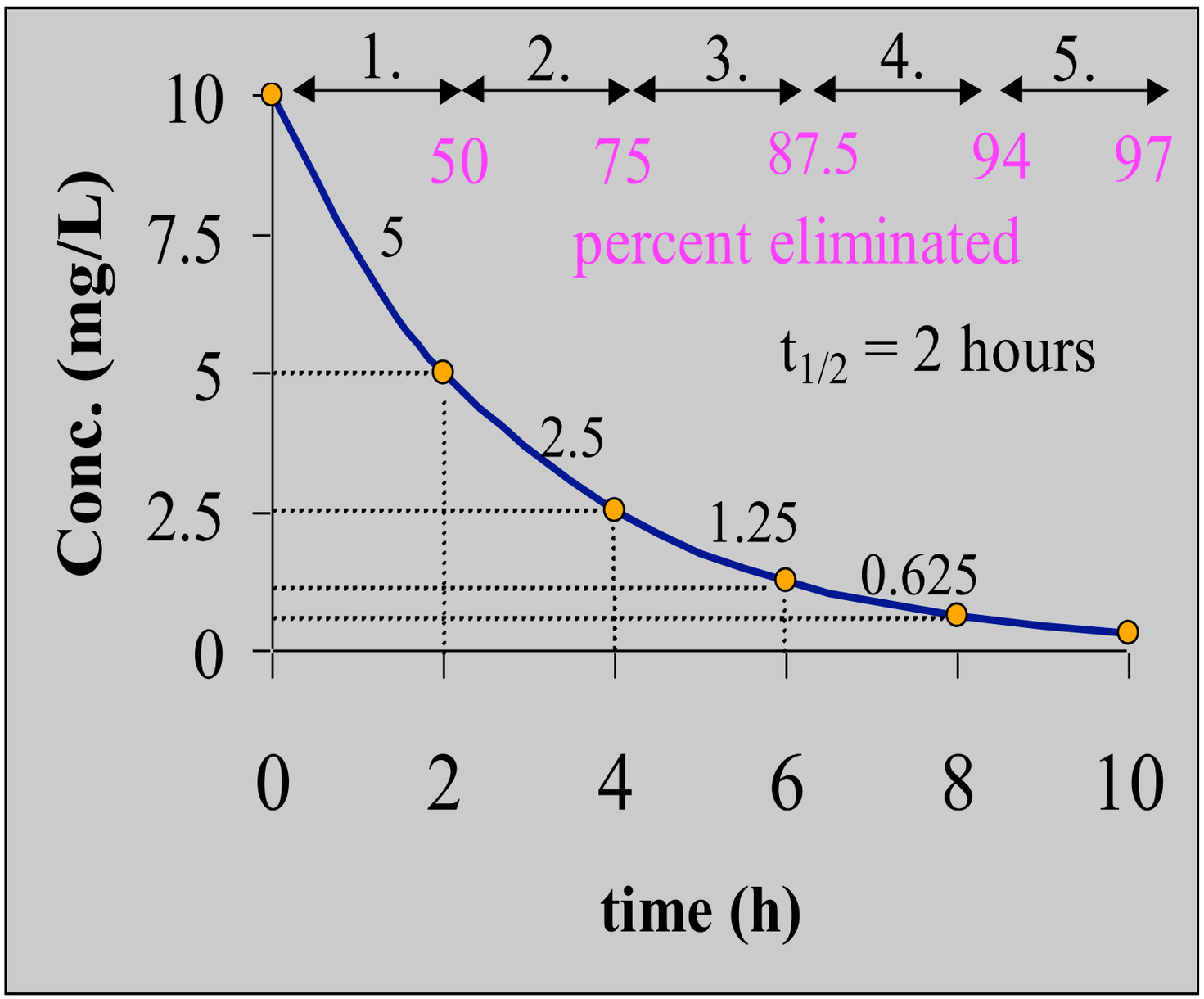
$$t_{1/2} = 0.693 / k_{el}$$



$t_{1/2} = 0.693 / K_{el}$

Use of $t_{1/2}$:

$t_{1/2}$ can be used to predict how long it will take for the drug to be eliminated from plasma



The principle of linear pharmacokinetic

Elimination is not saturable (non-capacity-limited) and the rate of drug elimination is directly proportionate to the plasma concentration of the drug

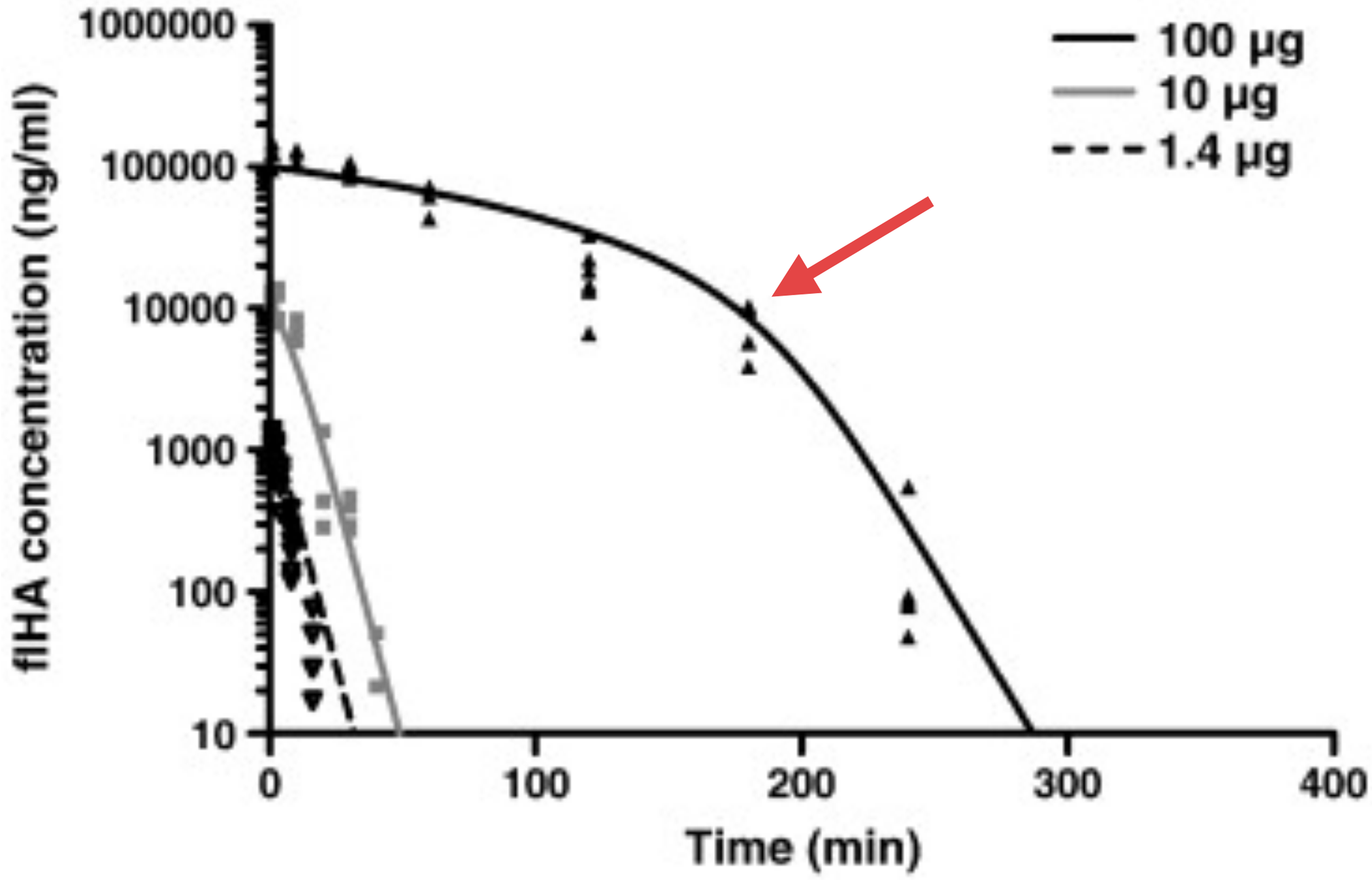
Nonlinear pharmacokinetics

Nonlinear pharmacokinetic is capacity-limited, dose or concentration dependent and saturable
The rate of elimination is constant, irrespective to plasma concentration
No real $t_{1/2}$ can be calculated

$$\text{Rate of elimination} = \frac{V_{max} \cdot C}{K_m + C}$$

Michaelis- Menten
Examples: ethanol, phenytoin, theofylline

Nonlinear pharmacokinetics in a semi-log graph



DOSE-EFFECT RELATIONSHIP: The dose-response curves

The intensity and duration of the effect of drugs are a function of the drug dose and of the drug concentration at the effect site

Dose-Effect Endpoints

Two types of Dose-response curves:

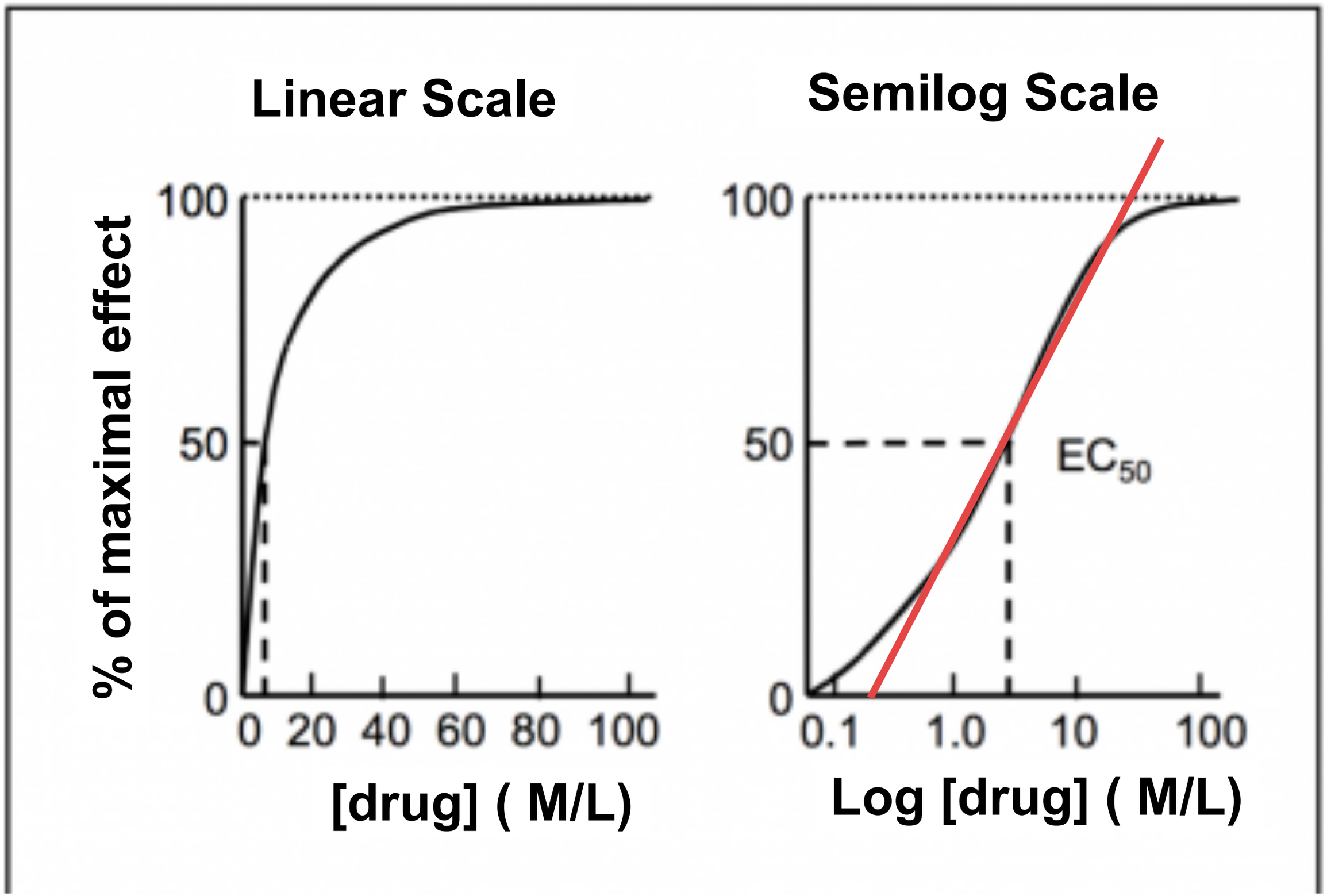
Graded

- **Continuous scale (↑dose , ↑effect)**
- **Measured in a single biologic unit**
- **Relates dose to intensity of effect**

Quantal

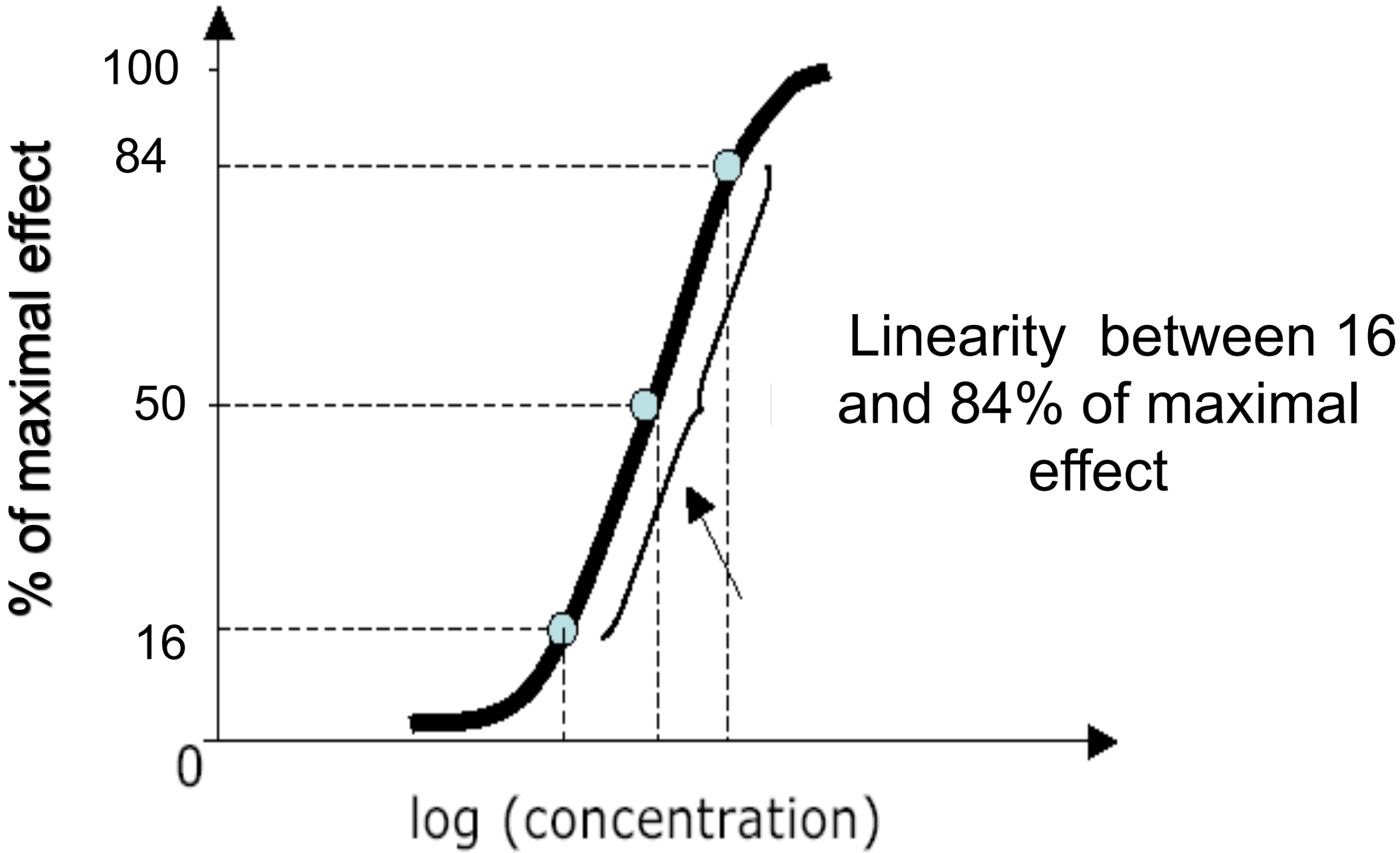
- **All-or-none pharmacologic effect**
- **Population studies**
- **Relates dose to frequency of effect**

Graded Dose-Response Curves



EC50: concentration that gives the half maximal effect

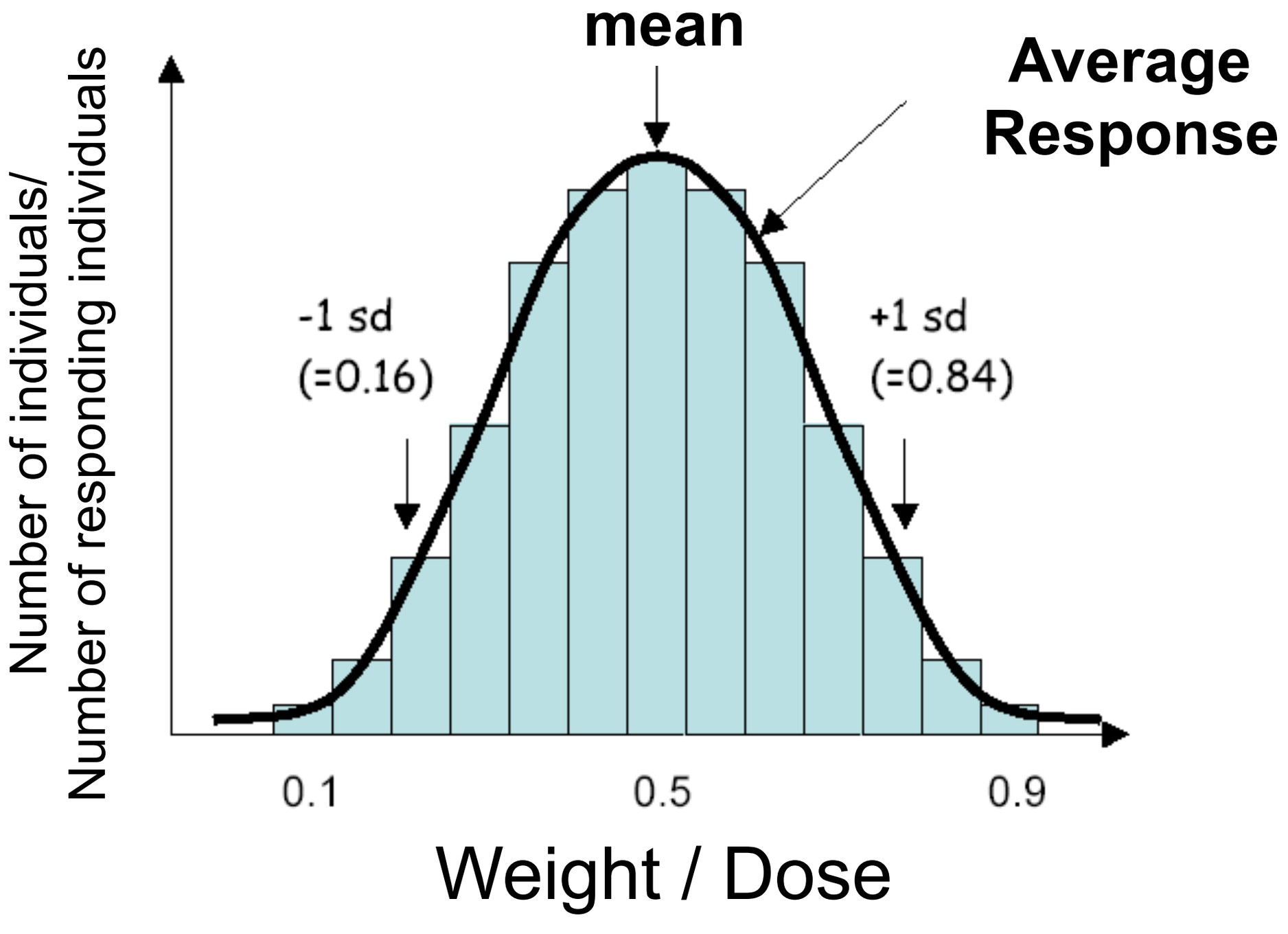
Litchfield-Wilcoxon method for determining EC_{50}



Linearity between 16 and 84% of maximal effect

Quantal Dose-Response Curves

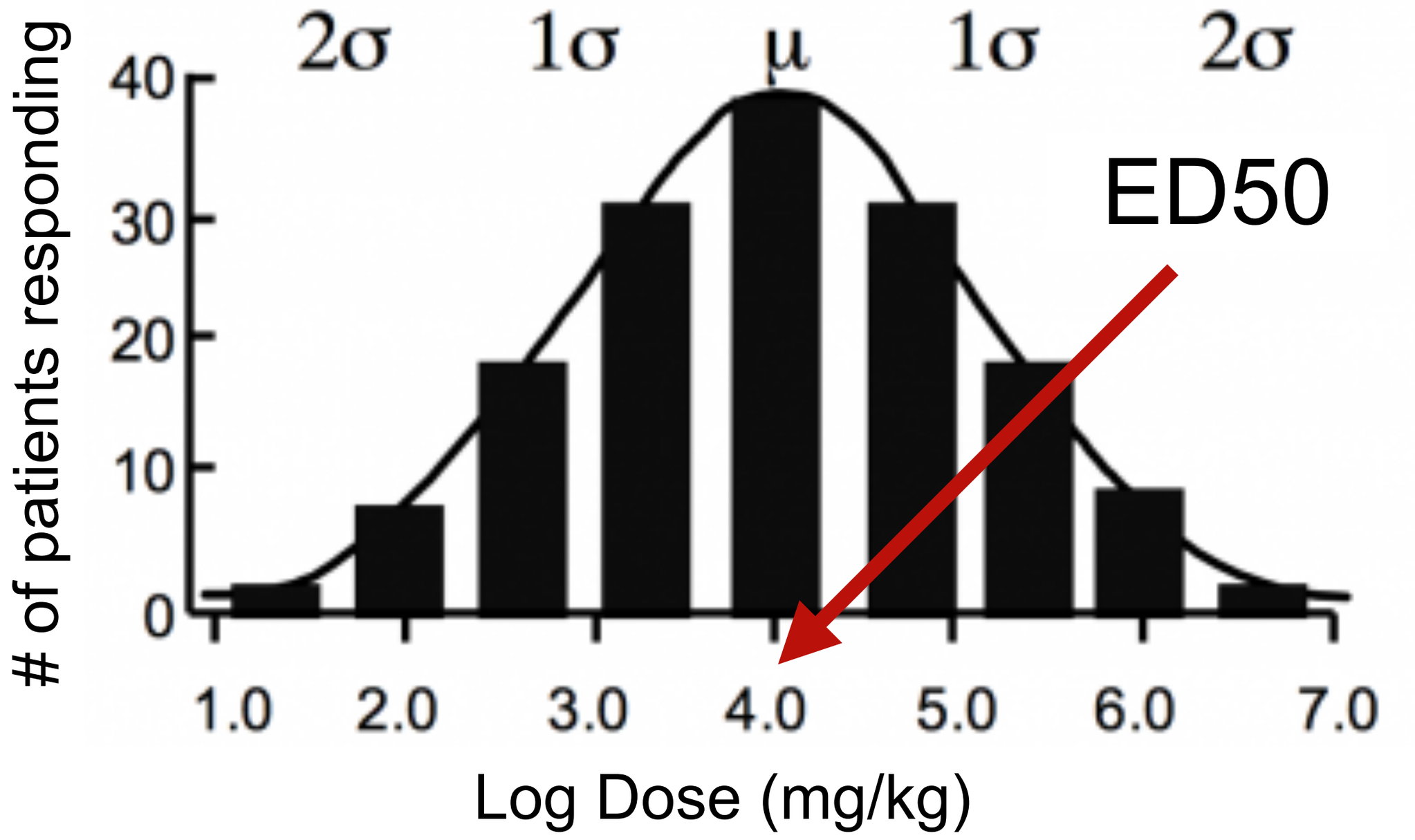
Quantal Dose-response models are based on normal distribution of biological variables



Quantal Dose-Effect Study

Dose	No. of Responding Subjects
1	0
2	1
3	3
4	5
5	7
6	2
7	1
8	1

Quantal Dose-Effect Curve: Frequency distribution

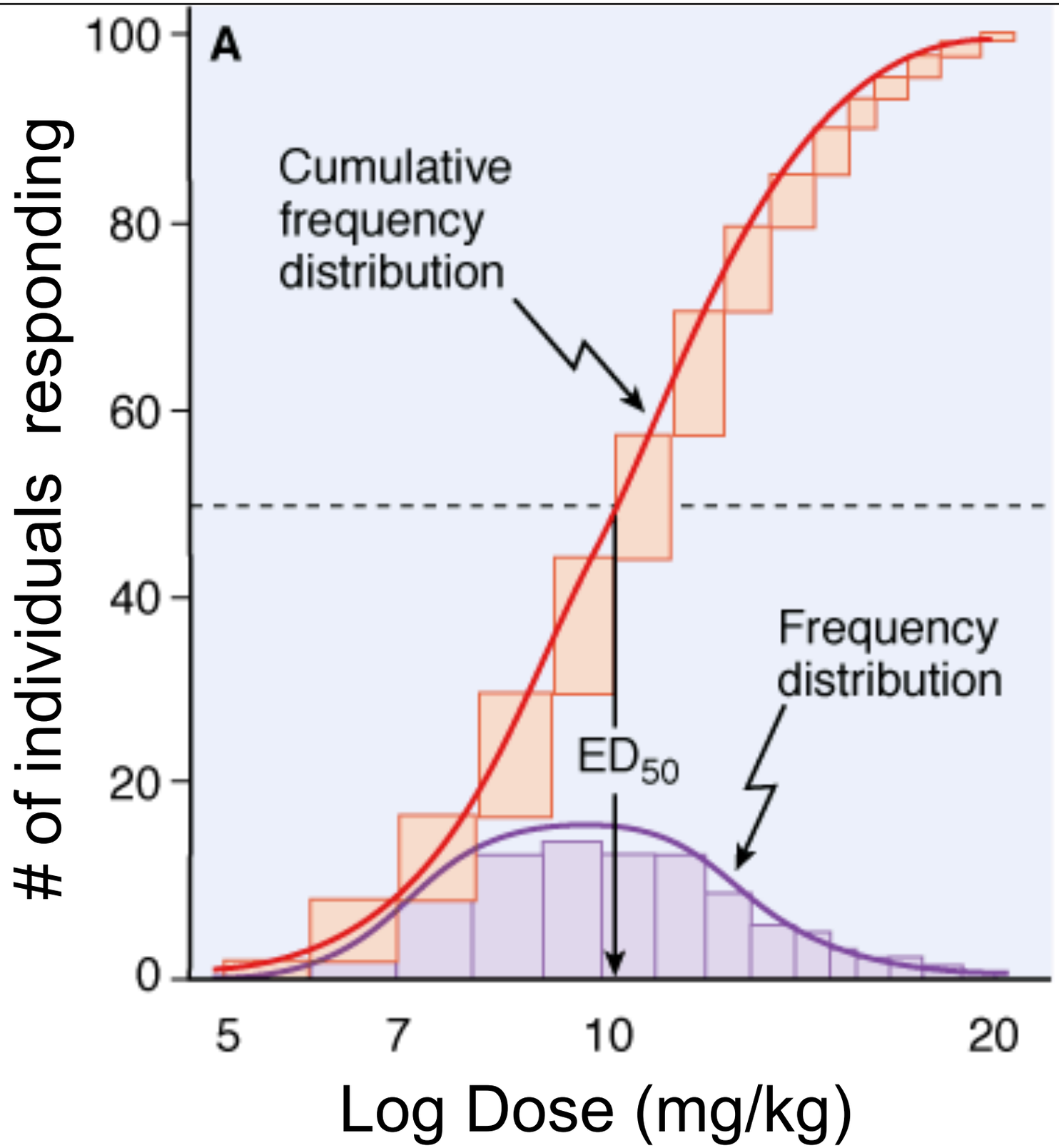


ED50: dose required to produce the therapeutic effect in 50% of the population

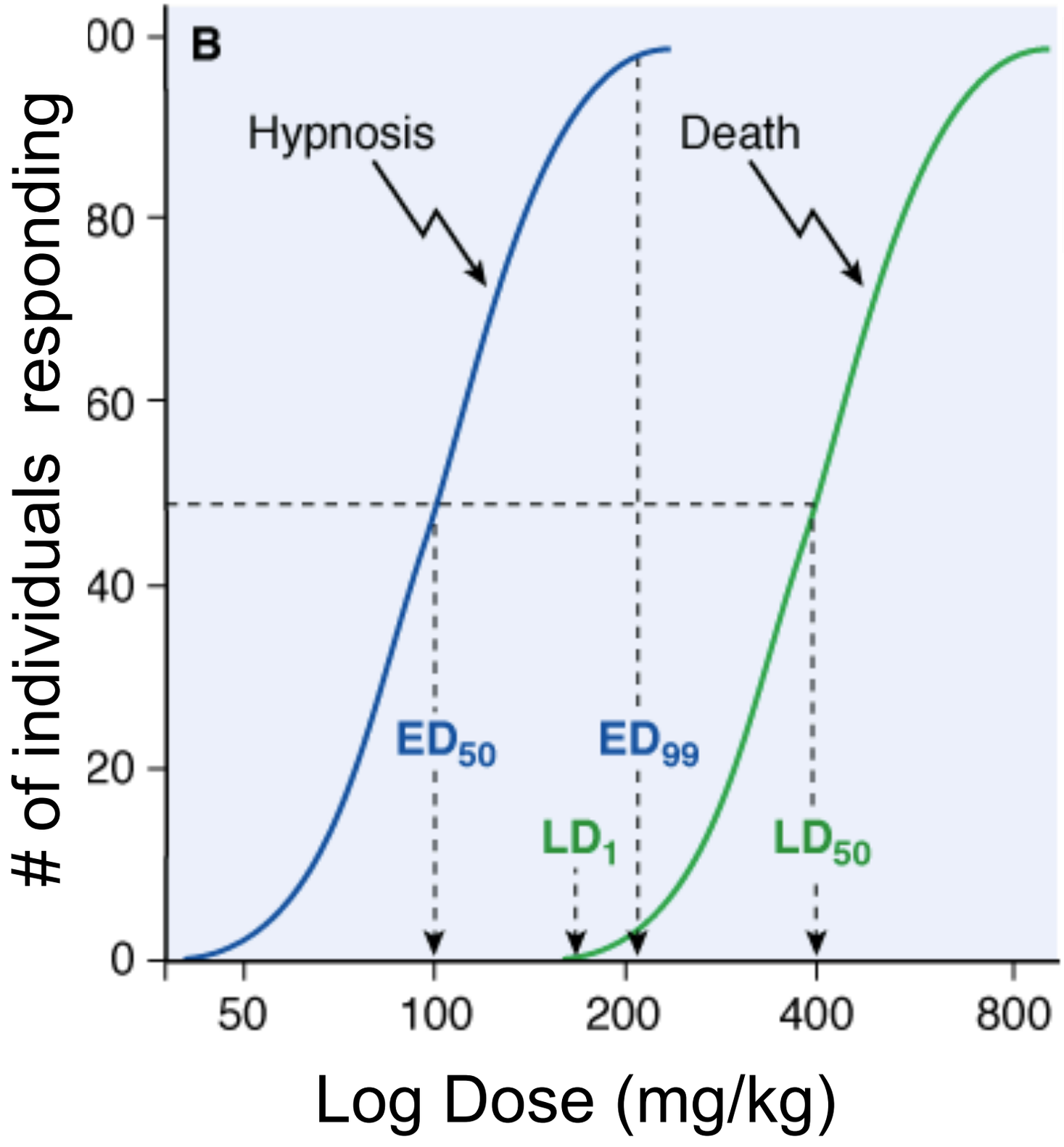
Quantal Dose-Effect Study

Dose	No. of Responding Subjects	Cumulative Response
1	0	0
2	1	1
3	3	4 (3+1)
4	5	9 (5+4)
5	7	16 (9+7)
6	2	18 (16+2)
7	1	19 (18+1)
8	1	20 (19+1)

Quantal Dose-Effect Curve: Cumulative Frequency distribution



Therapeutic Index: $\frac{LD_{50}}{ED_{50}} = \frac{400}{100} = 4$



Margin of Safety: $\frac{LD1}{ED99} = \frac{160}{230} = 0{,}70$

ED50: dose required to produce a therapeutic effect in 50% of the population

LD: letal dose in 50% of the population

The Therapeutic Ratio does not take into account the slope of the dose-response curves

The Safety Factor, ratio of ED99 to TD1, better describes the safety degree of a drug

Drugs A and B have the same TR but A is more safe than B