

EUROPEAN COURSE ON TOXICOLOGIC PATHOLOGY

Second Edition

Organized by The D.E.S.V. in Veterinary Pathology

In partnership with:

**The Society of Toxicologic Pathology
& The French Society of Veterinary Pathology**

**VETERINARY SCHOOL OF NANTES (FRANCE)
APRIL 23–27, 2007**



TECNIPLAST FRANCE





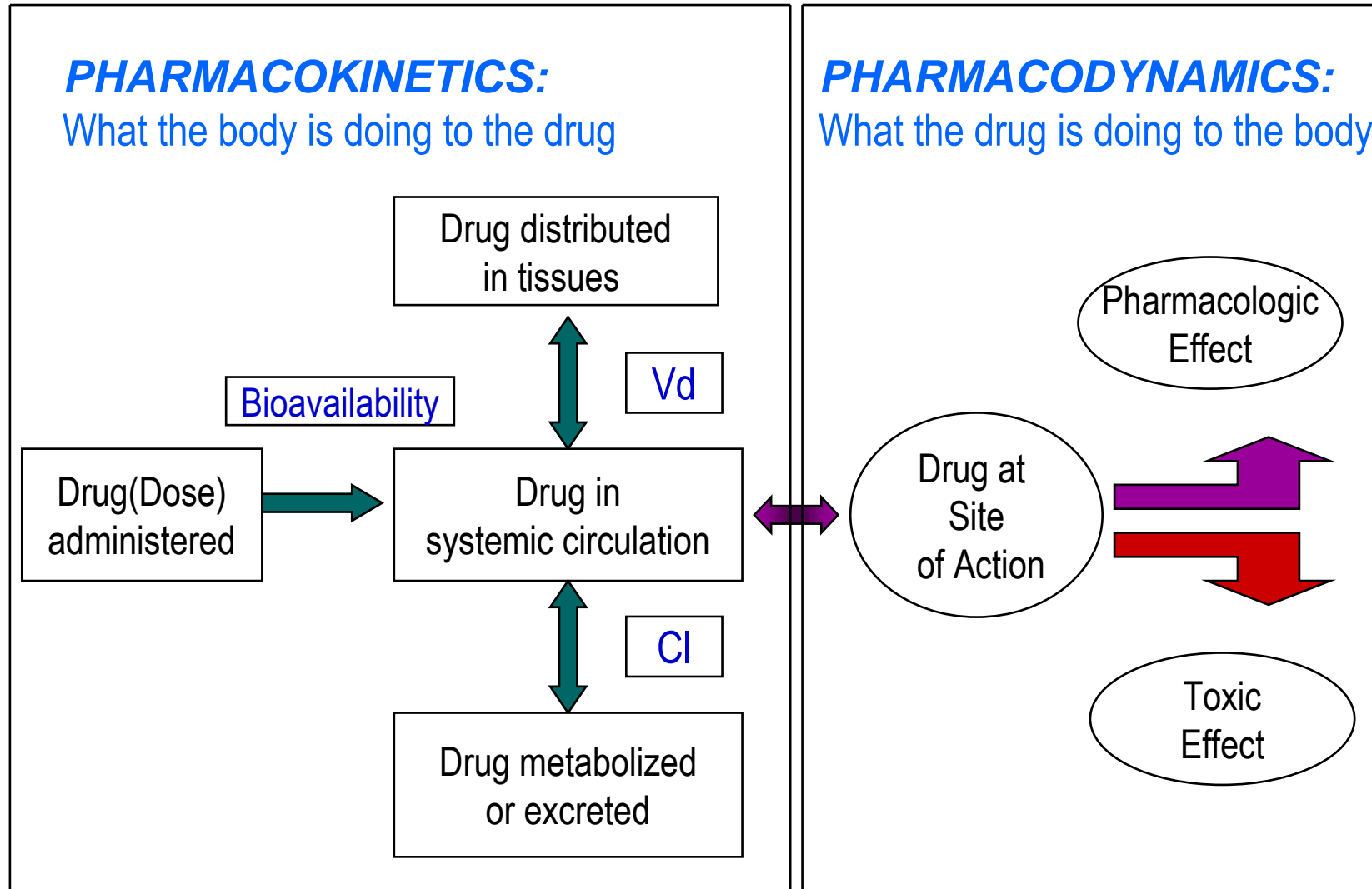
Metabolism & Pharmacokinetics of Xenobiotics

Antoine DESLANDES



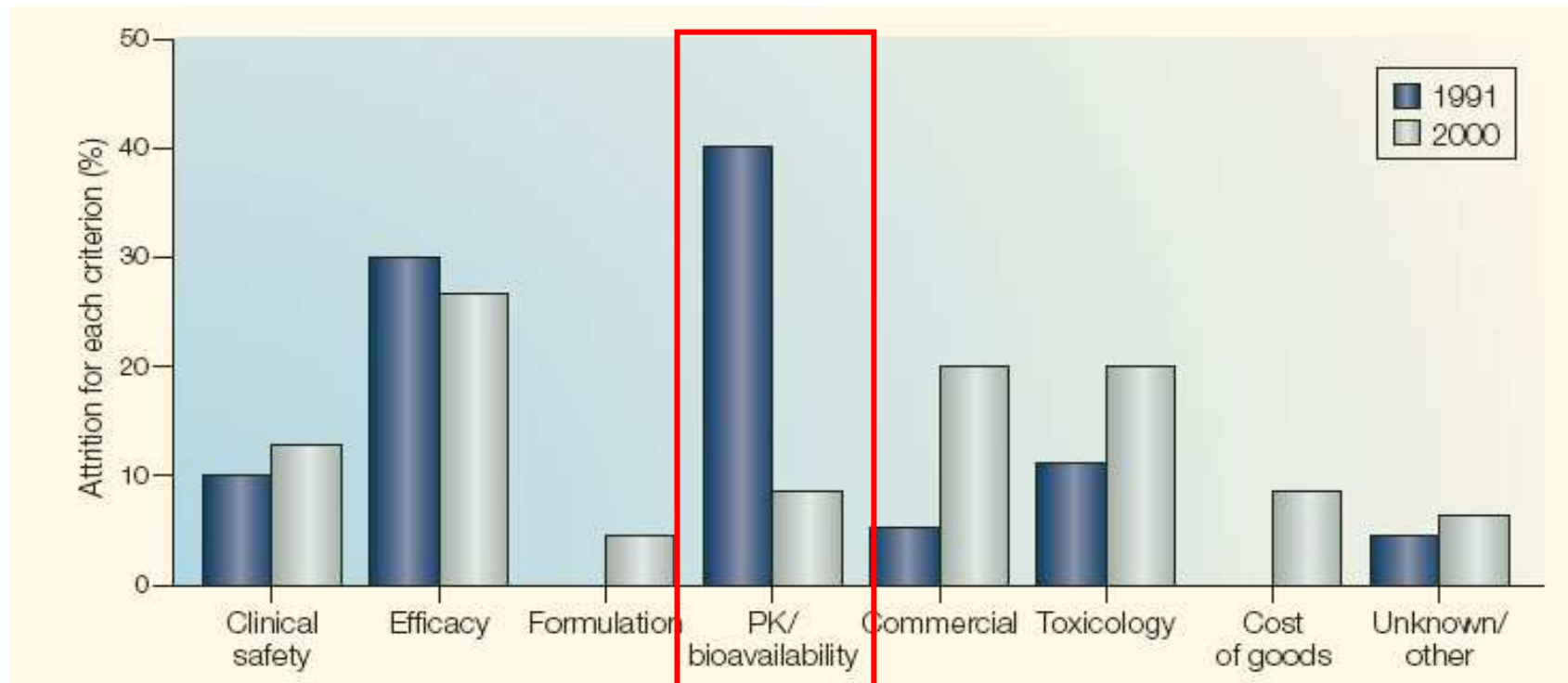


Pharmacokinetics/Pharmacodynamics





Sources of failure in Drug Development

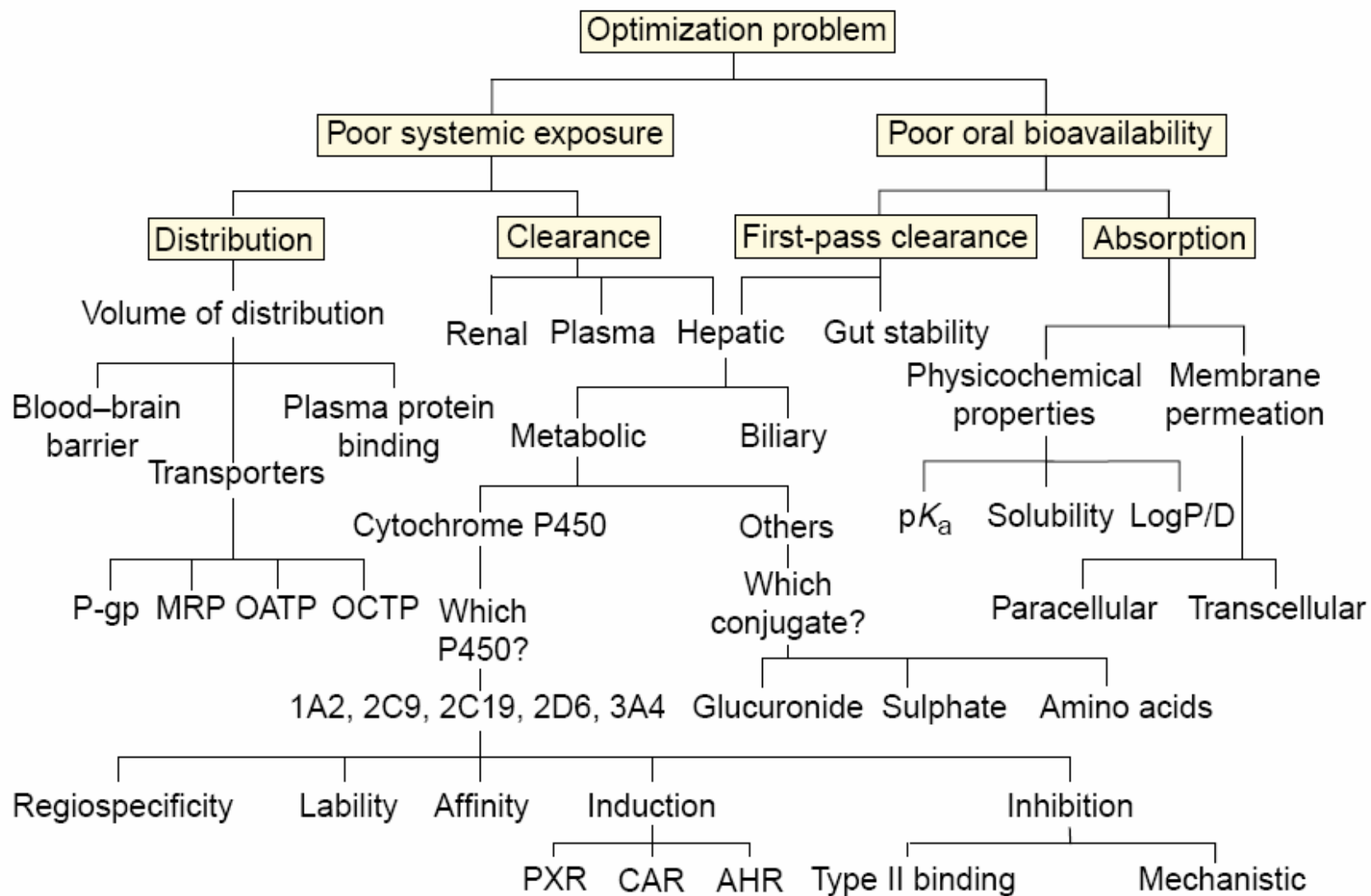


Kola I and Landis J (2004) Can the pharmaceutical industry reduce attrition rates? Nat.Rev.Drug Discov. 3:711-715.





What has been done ?



Selick HE, Beresford AP, and Tarbit MH (2002) The emerging importance of predictive ADME simulation in drug discovery. *Drug Discov. Today* 7:109-116.

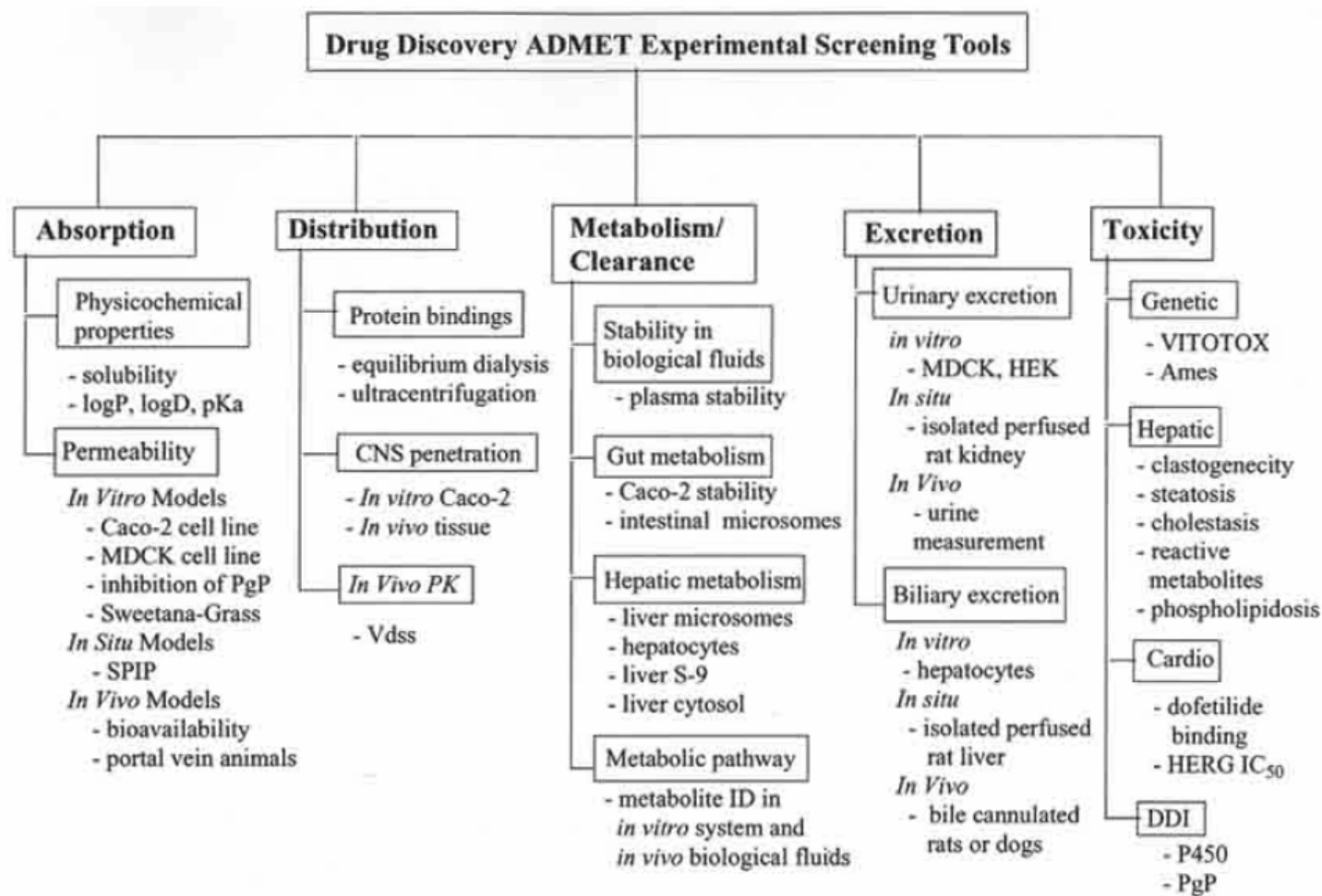
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L'essentiel c'est la santé.



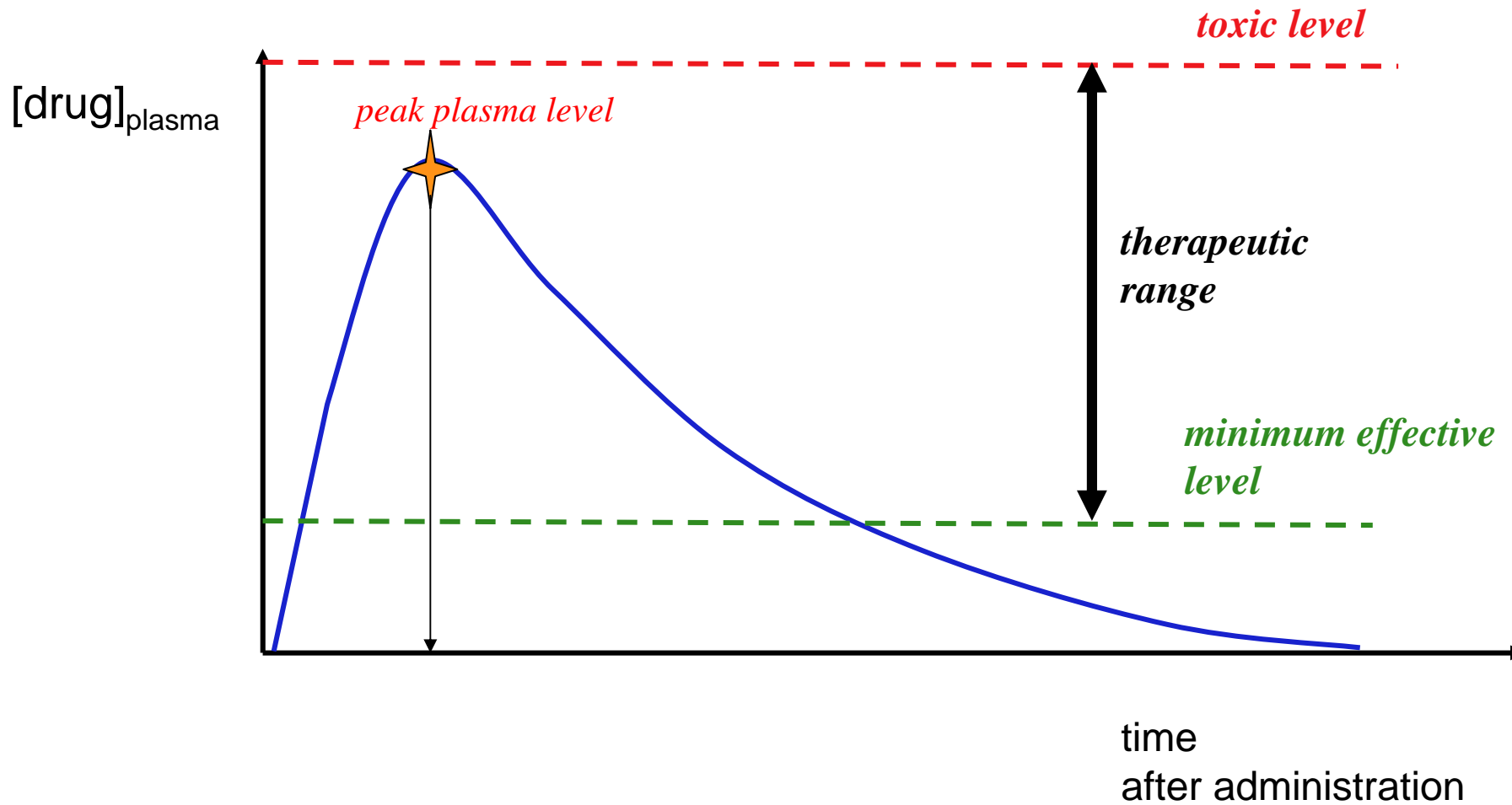


ADMET experimental screening tools for small molecule drug discovery



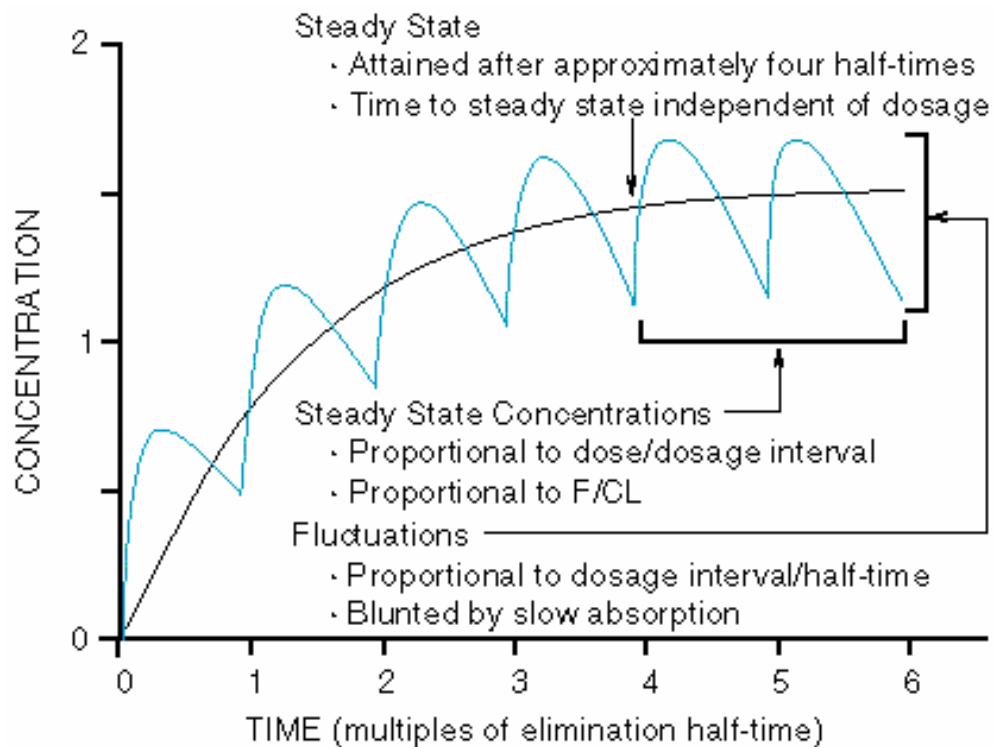


The therapeutic range





PK goal : determine drug dosing to maintain drug levels within a therapeutic range



Therapeutic Range

<i>Drug</i>	<i>Therapeutic Window (μM)</i>
digoxin	0.0008-0.003
propranolol	0.08-0.8
nortriptyline	0.2-0.6
gentamicin	7-21
tobramycin	35-120





How do we obtain PK/TK parameters?

Administer the drug in vivo IV or oral

Follow changes in blood/plasma concentration with time

Mathematical treatment of the data

Parameters :

- Area-under-the-concentration-time curve (AUC)***
- Bioavailability (F)***
- Half-life ($t_{1/2}$)***
- Volume of distribution (V_d)***
- Clearance (CL)***





What are the requirements for a PK/TK study?

- Sensitive, specific and accurate assay for the drug in blood or plasma***
- Relevant species***
- Relevant route of administration***
- Relevant dose level***





Toxicokinetic evaluations in preclinical studies

Safety pharmacology studies

- TK optional
- Cross-reference dose level with exposure in tox study

Single-dose and rising-dose toxicity studies

- TK optional

Repeat-dose toxicity studies

- Support the Phase I clinical study
- TK parameters reported as mean SD
- Information on exposure, dose proportionality, sex- and species-differences, accumulation, inhibition

Reproduction toxicity studies

- TK supports exposure

Genotoxicity studies

- Cross-reference dose level with exposure in tox study

Carcinogenicity studies

- Regulatory expectation for exposure to parent drug and metabolites





TK Control sample analysis

Regulatory guidances require collection and analysis of TK samples from control animals used in GLP toxicology/ toxicokinetic studies

Observation of quantifiable drug levels in the control samples must be followed up to determine the potential impact on the safety evaluations in the study





Bioanalytical Method Validation

Method Validation includes

- **Accuracy**
- **Precision**
- **Sensitivity**
- **Specificity**
- **Recovery**
- **Stability**

FDA Guidance for Industry

Bioanalytical Method Validation (May 2001)

(<http://www.fda.gov/cder/guidance/4252fnl.pdf>)

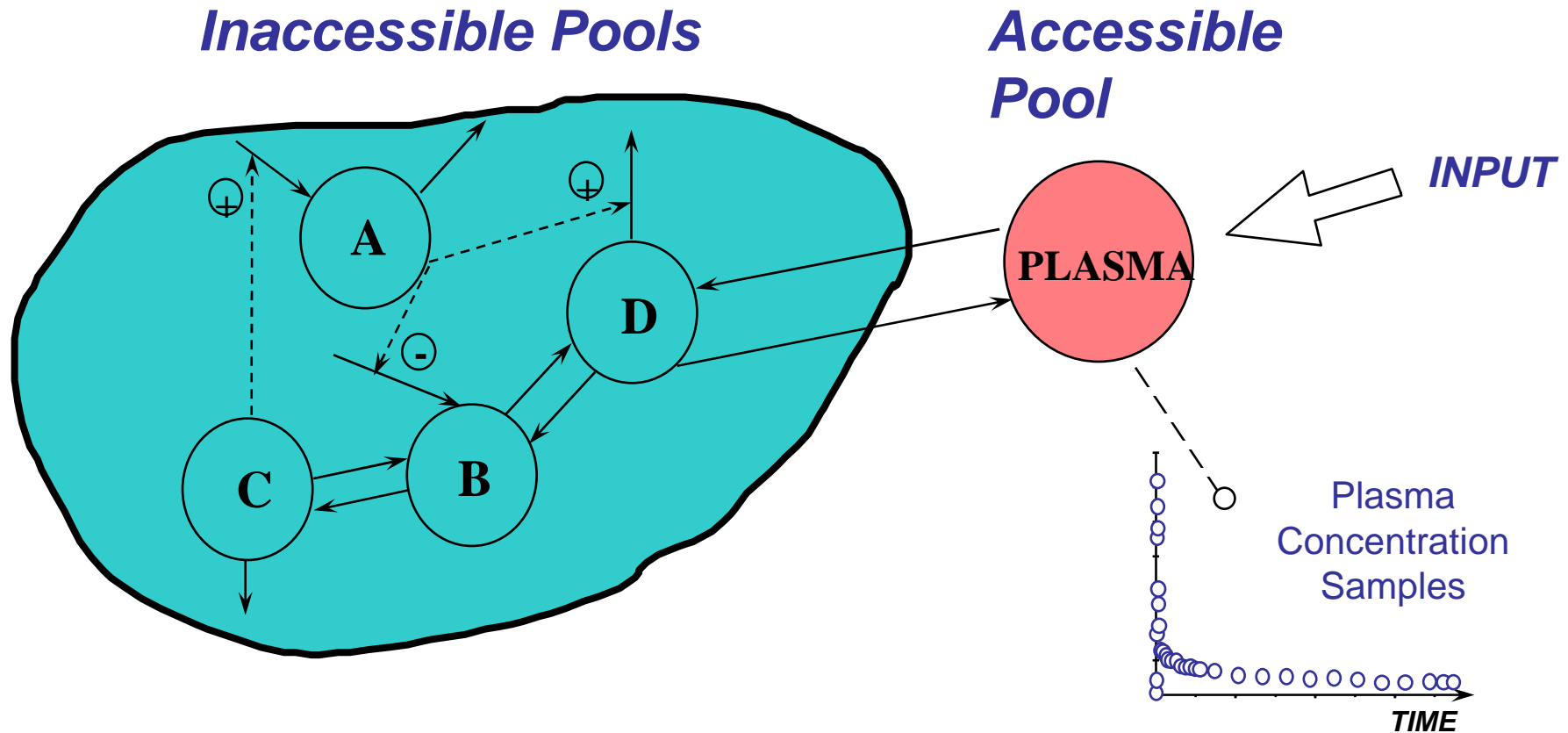
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A Model of the System



Key Concept.

Predict inaccessible features of the system based upon measurements in the accessible pool.





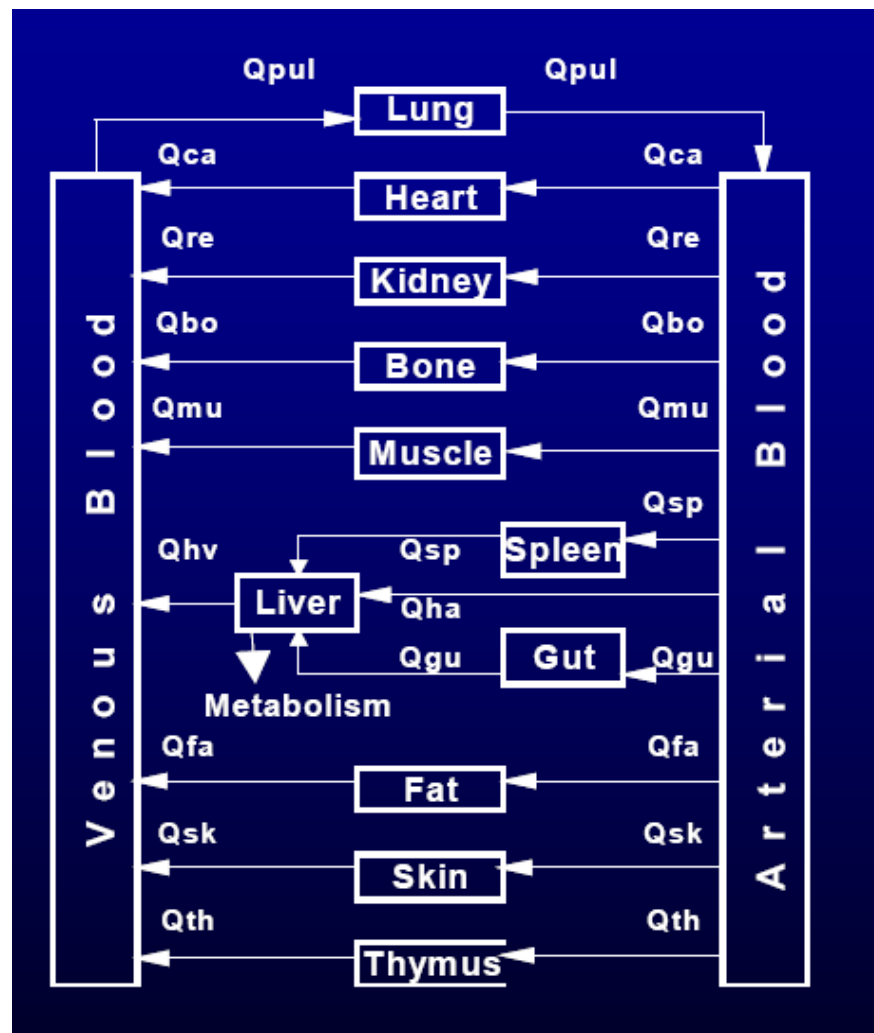
PBPK : physiologically-based PK

Tissue Physiological Parameters :

- Size
- Blood flow
- Composition & Functionality

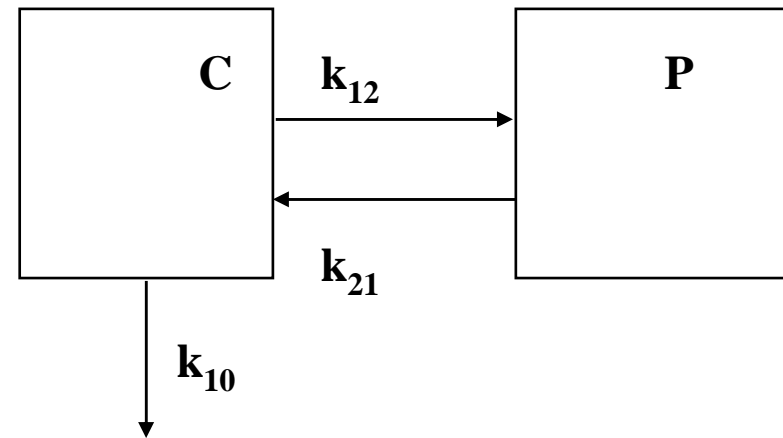
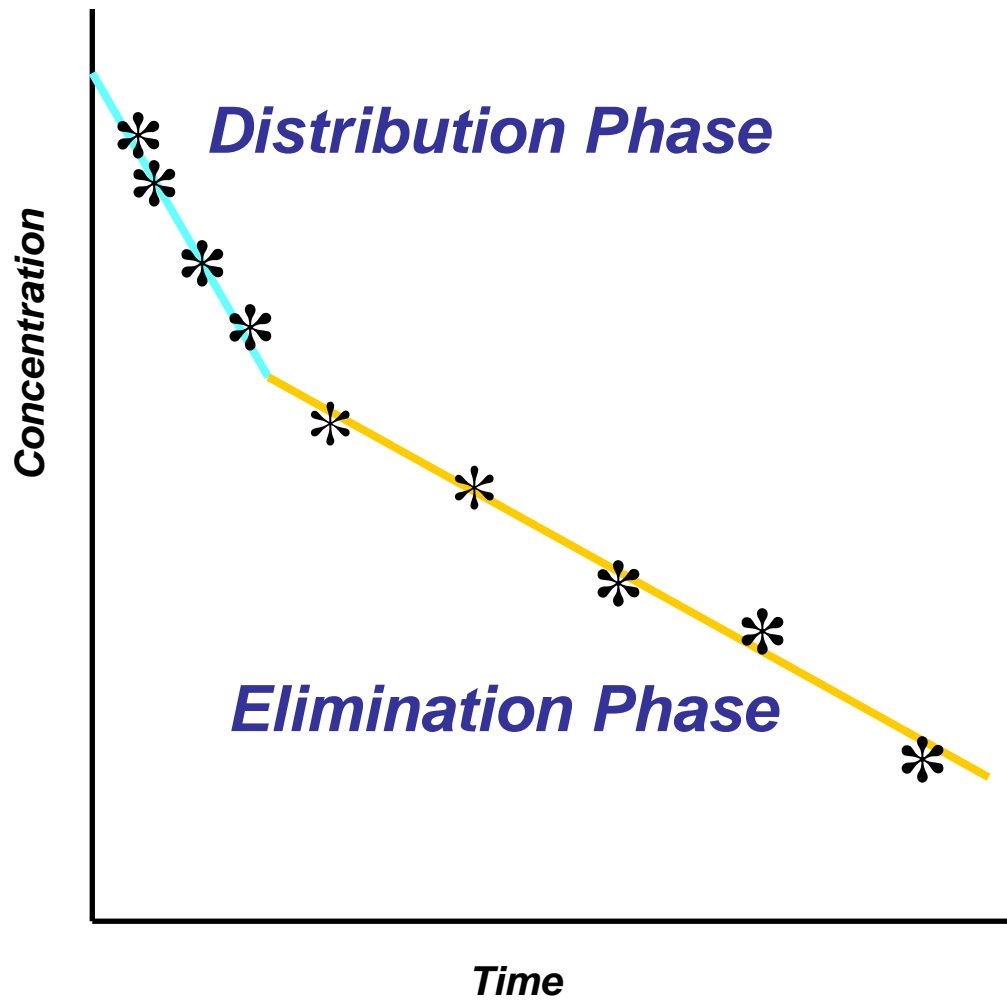
Drug Specific Parameters :

- Binding within blood
- Tissue affinity (Partition Coefficients)
- Membrane permeability
- Enzymatic activity...





Compartmental models : IV administration





Absorption



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Absorption

Definition : the process by which a drug moves from its site of administration to the systemic circulation

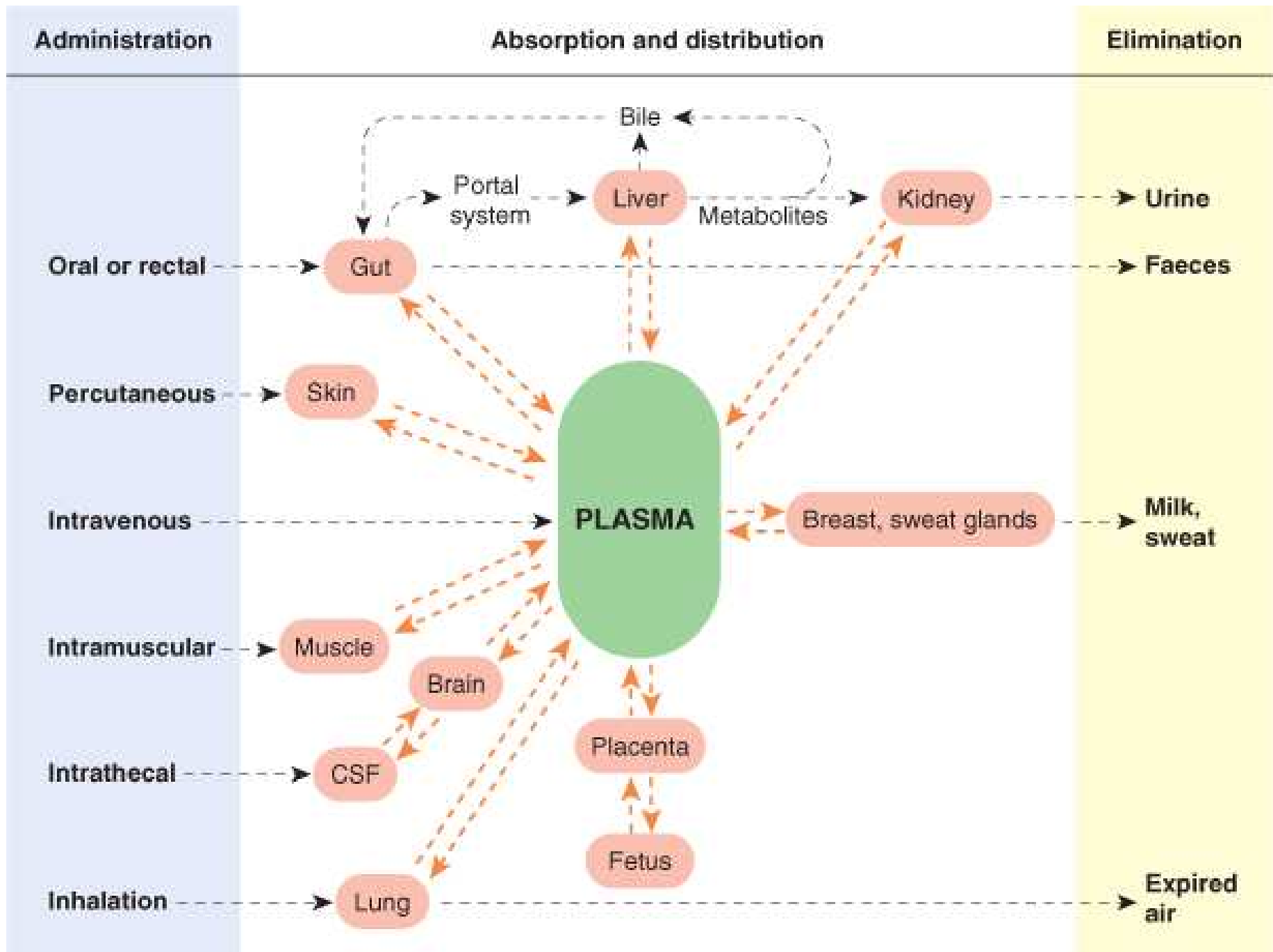
90% of marketed drugs are intended for oral administration

Also, intramuscular, intradermal, subcutaneous, rectal, inhalation, topical etc.

Oral absorption

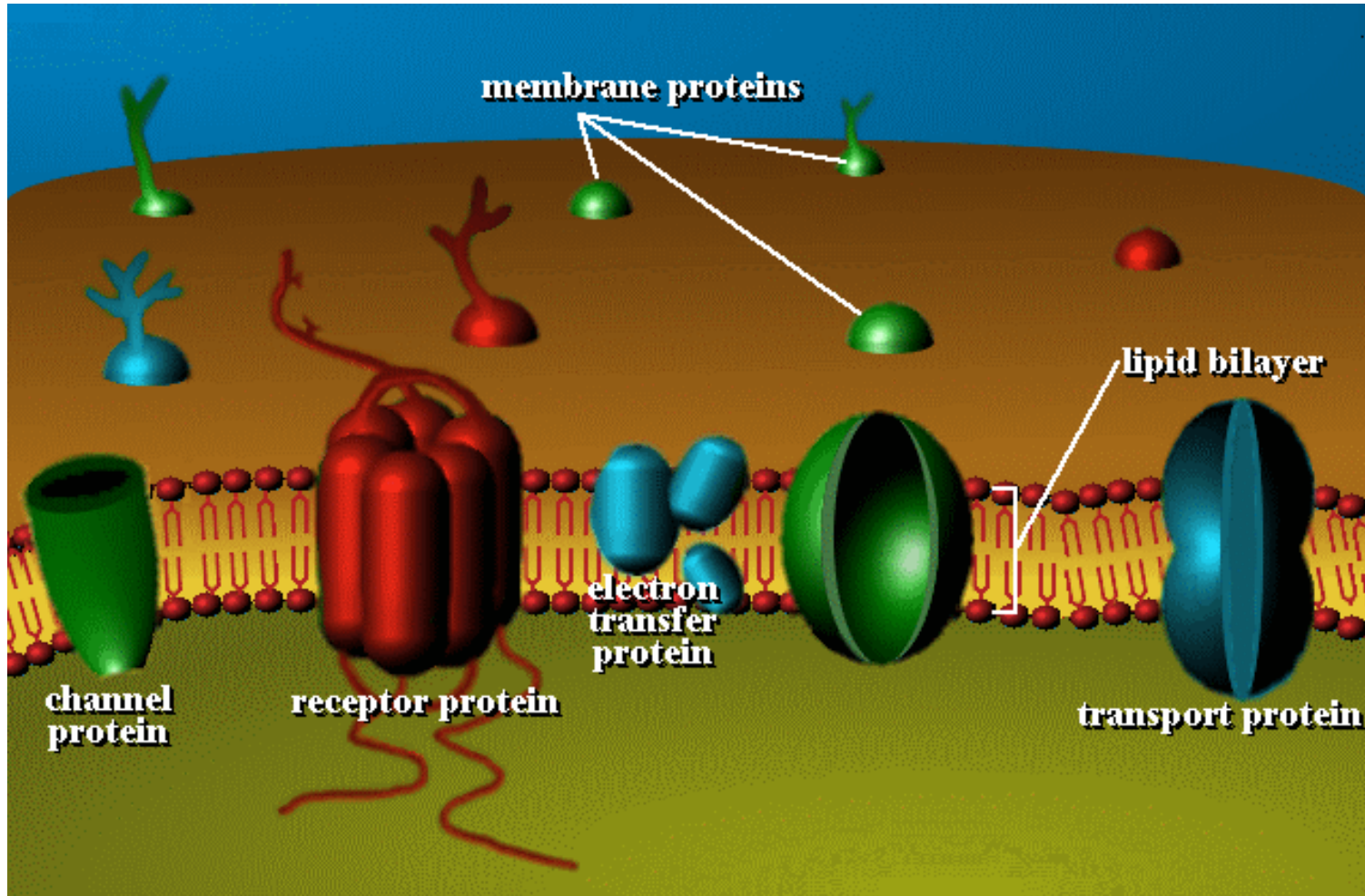
- GI epithelium is the main barrier*
- Large surface area*
- Most important mechanism is : passive diffusion*







Membrane Structure



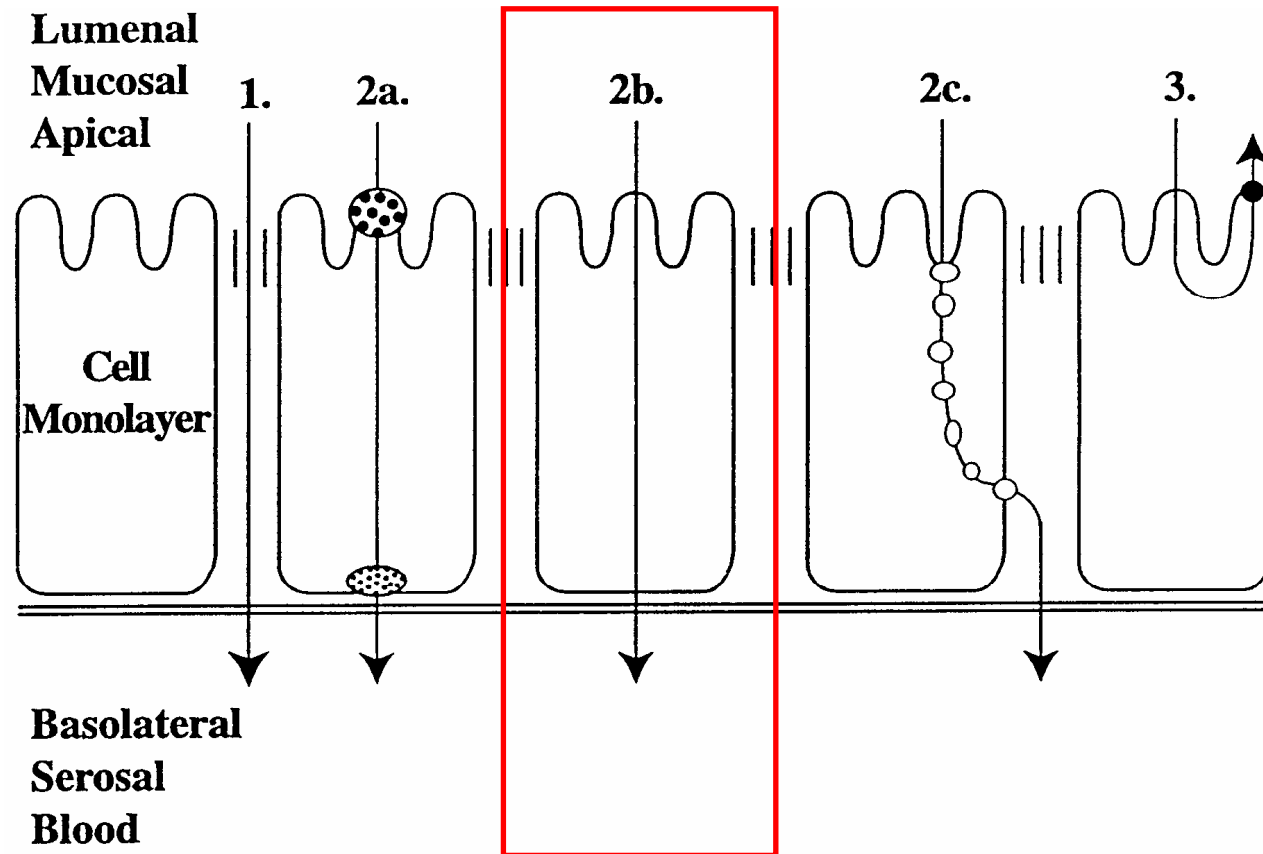
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Parallel Pathways in Intestinal Absorption



1. Paracellular; 2. Transcellular: 2a. Carrier-mediated; 2b. Passive diffusion; 2c. Receptor-mediated endocytosis; 3. Mediated efflux pathway

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Mechanisms of absorption: passive diffusion

To be absorbed, the drug needs to be....

- In solution (at all GI pHs)***
- In an unionised state (pKa)***
- Cross membranes (lipophilicity)***





Oral absorption

Human Intestinal Absorption (HIA)

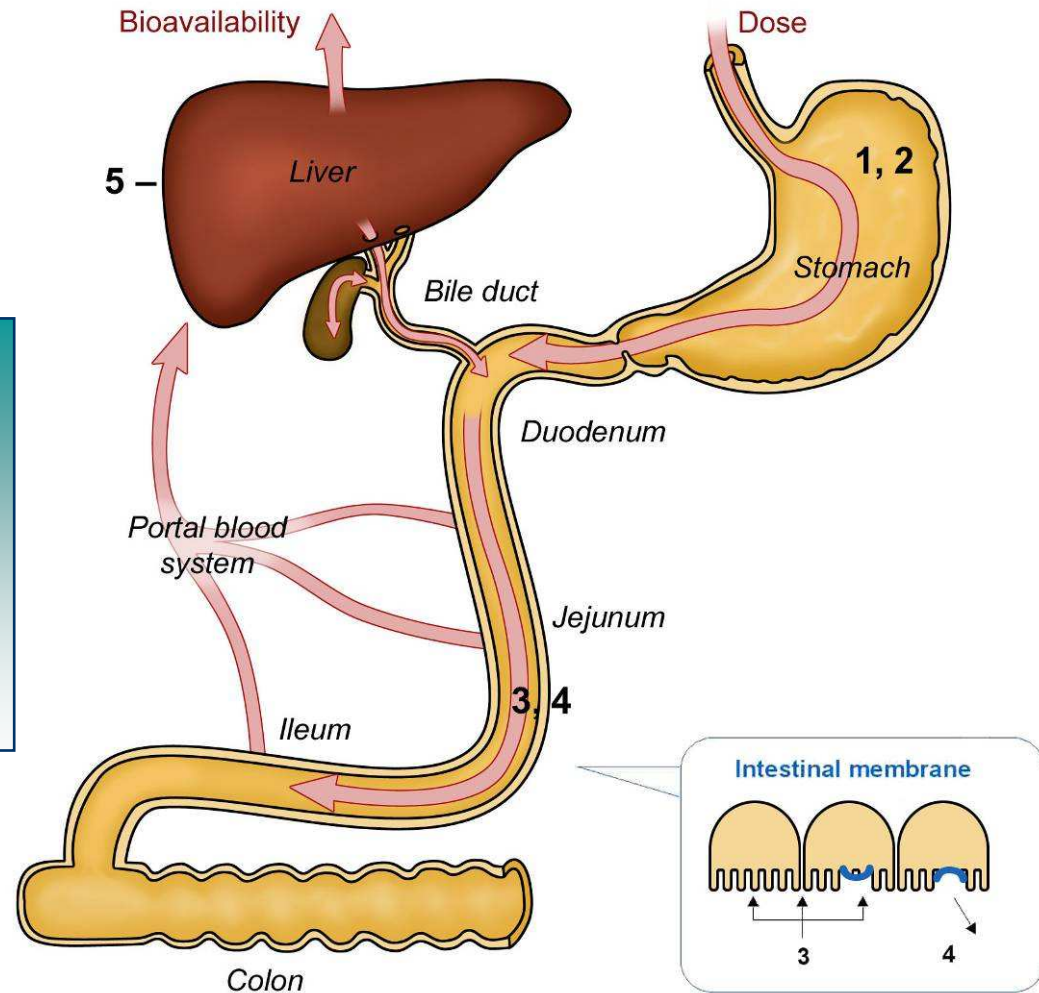
1,2 – Stability + Solubility

3 – Passive + Active Tr.

4 – Pgp efflux + CYP 3A4

5 – 1st Pass in liver

Oral Bioavailability (%F)

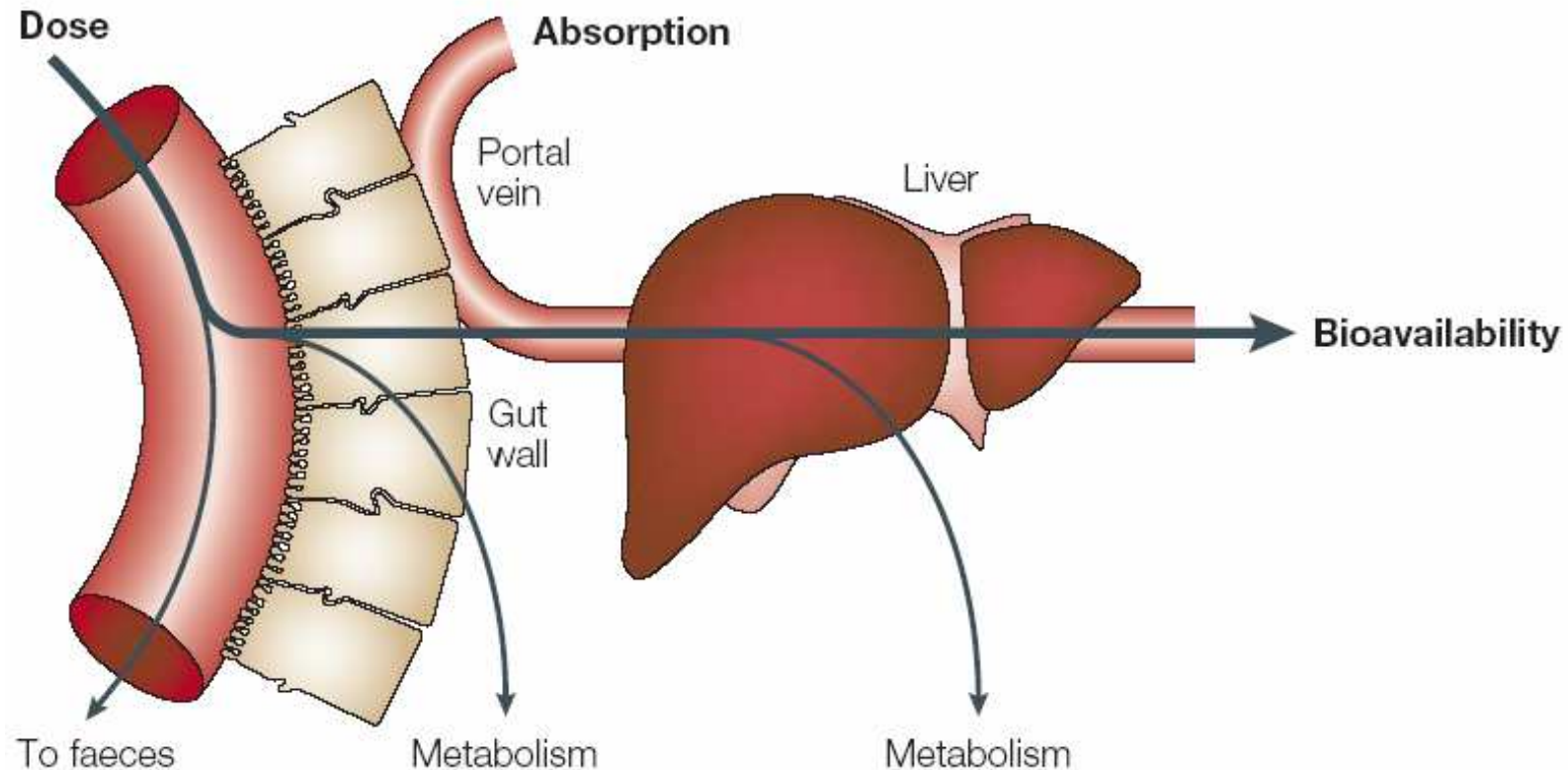


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Oral Bioavailability



Fraction bioavailable = Fraction absorbed - Fraction extracted by First Pass

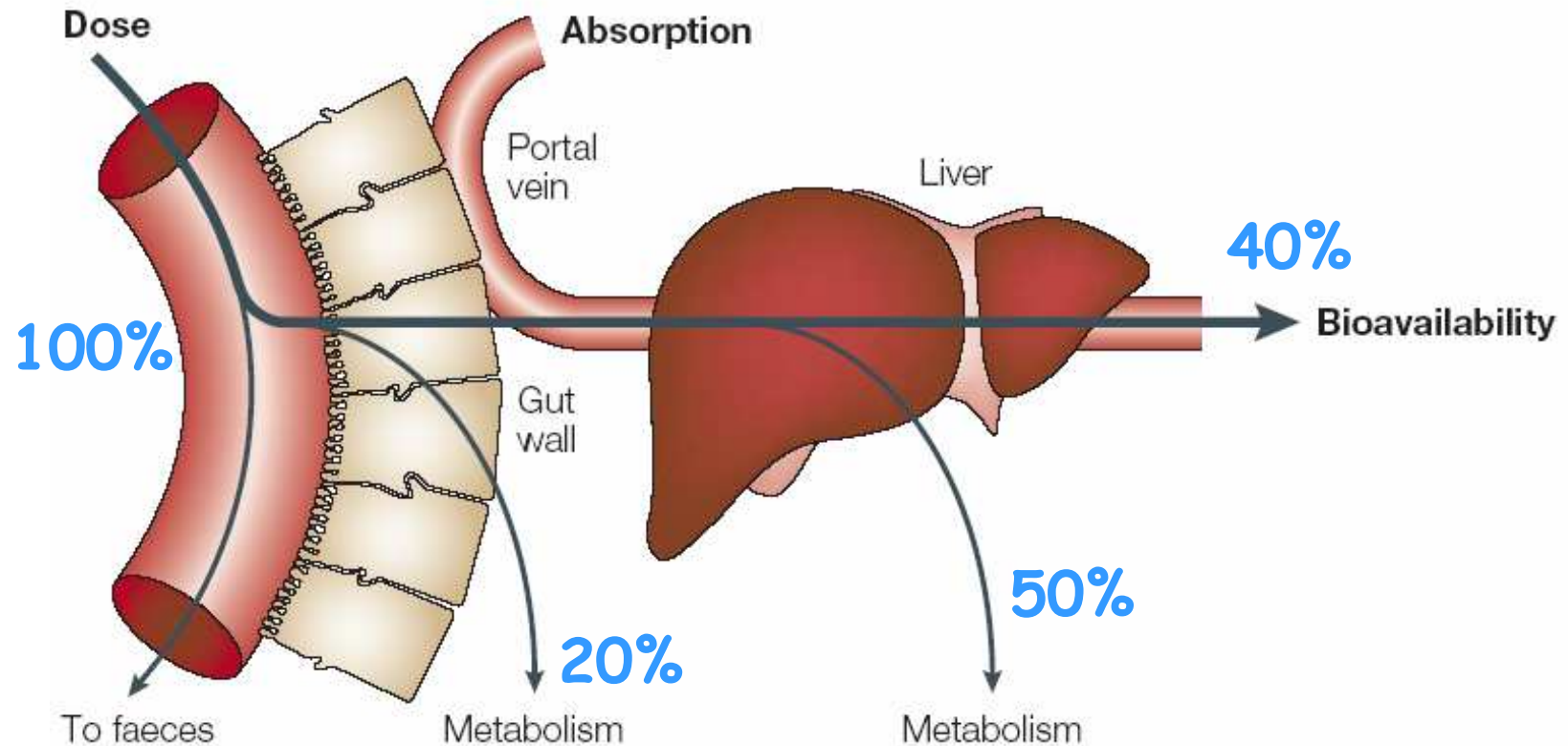
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Oral Bioavailability : Example



Hepatic first-pass extraction is 50%
Overall oral bioavailability (F%) is 40%

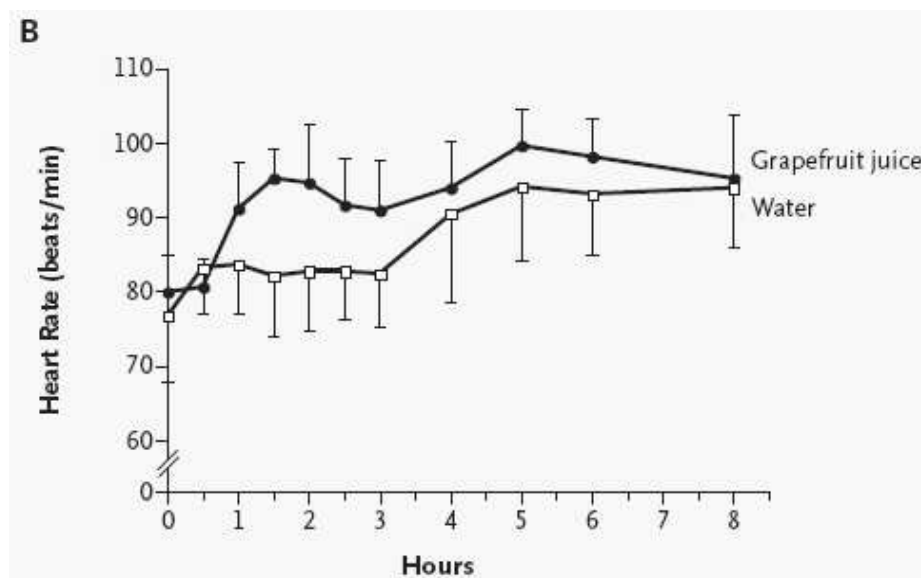
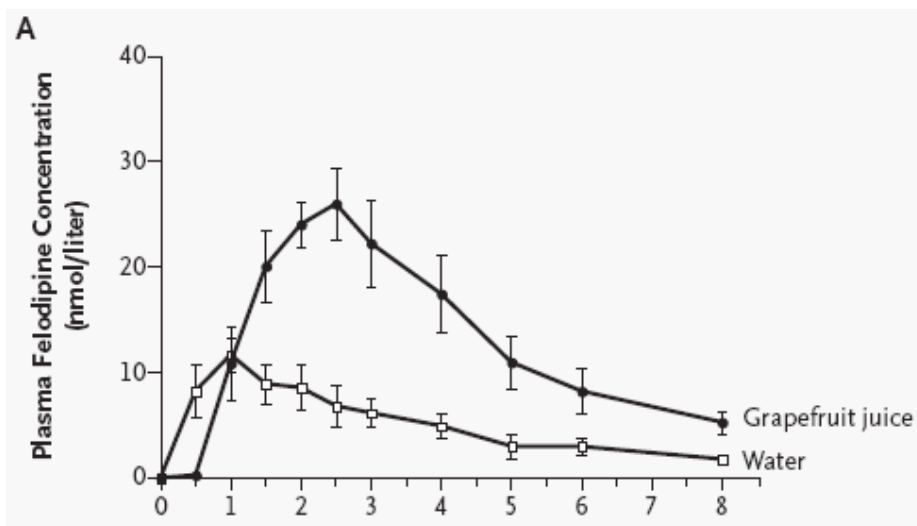
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Consequence of the inhibition of first-pass effect Example : Felodipine/Grapefruit juice interaction



Wilkinson GR. Drug metabolism and variability among patients in drug response. N Engl J Med 2005 May 26;352(21):2211-21.

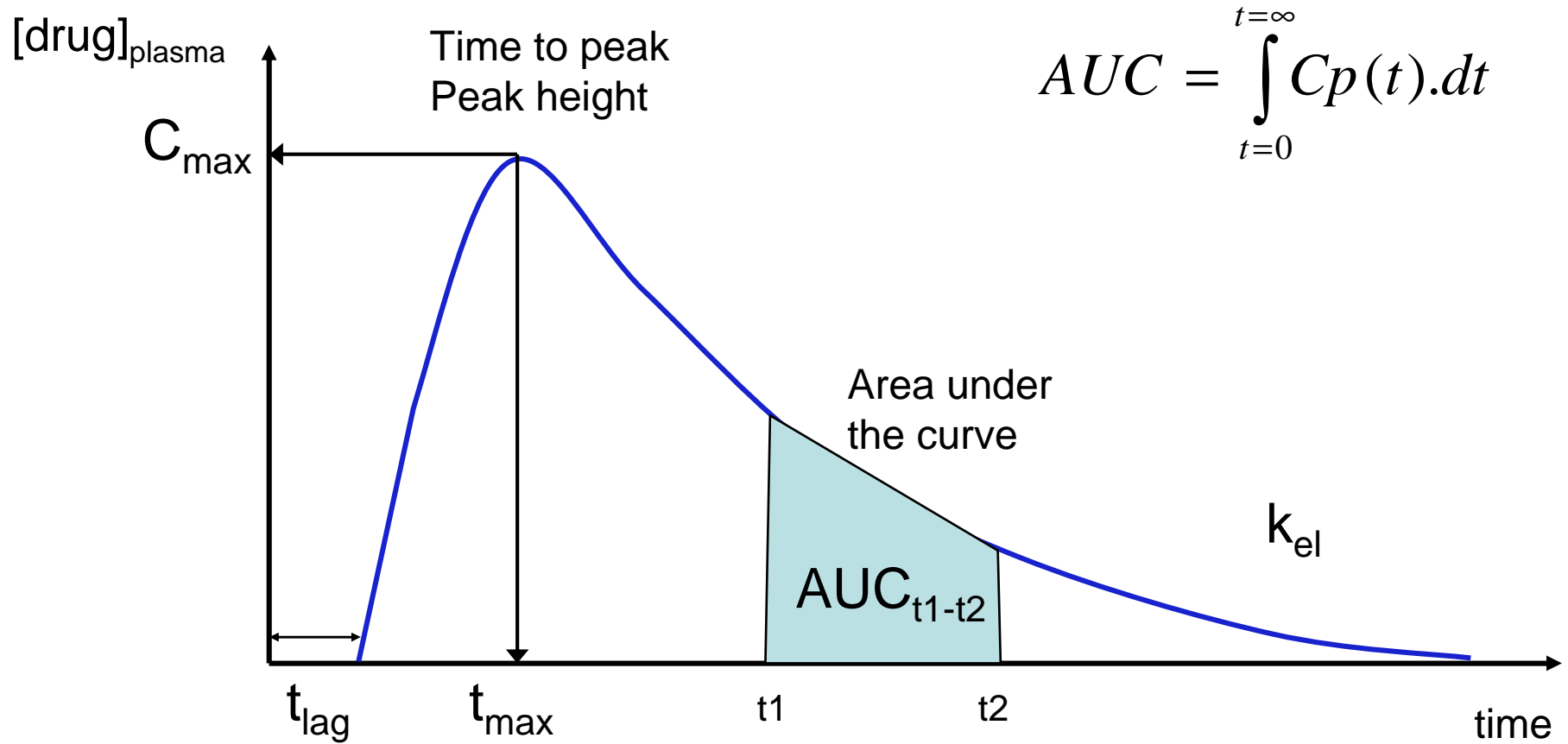
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PK parameters - oral administration





Bioavailability

Definition : the rate and extent of the active ingredient that reaches the systemic circulation

Can be calculated following:

$$F (\%) = \frac{AUC_{oral}}{AUC_{iv}} \times \frac{Dose_{iv}}{Dose_{oral}} \times 100$$





Distribution



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Distribution

The reversible transfer of a drug between the systemic circulation and the tissues

- ***Refers to movement of drug into tissues (usually a passive process)***

Major relevant parameters are:

- ***Plasma protein binding (albumin, alpha-1 acid glycoprotein)***
- ***Physicochemical properties (logD, clogP, pKa)***
- ***Specific/non-specific affinity for tissues***





Protein Binding

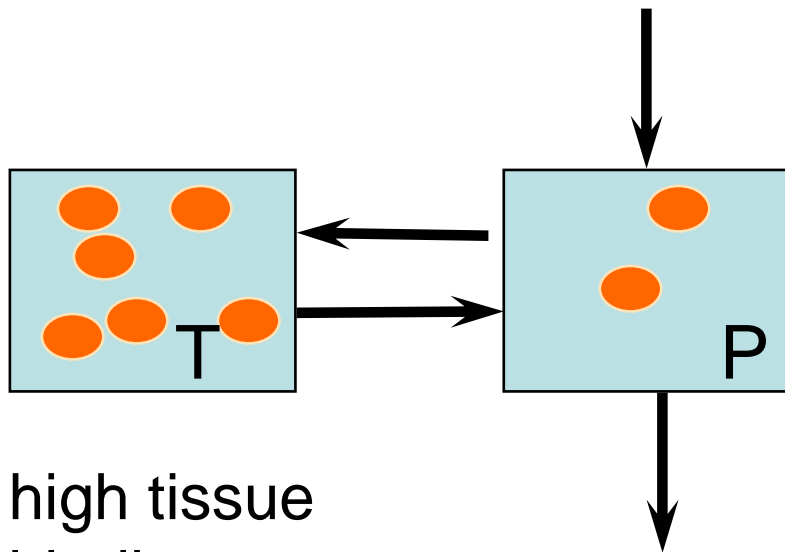
- Human Serum Albumin and other plasma proteins bind drugs***
- Only unbound fraction can interact with enzymes or receptors***
- Only unbound fraction is excreted by kidney***
- Compounds can compete for binding sites on HSA and tightly bound compounds can have suddenly high free fraction when displaced by other compounds***





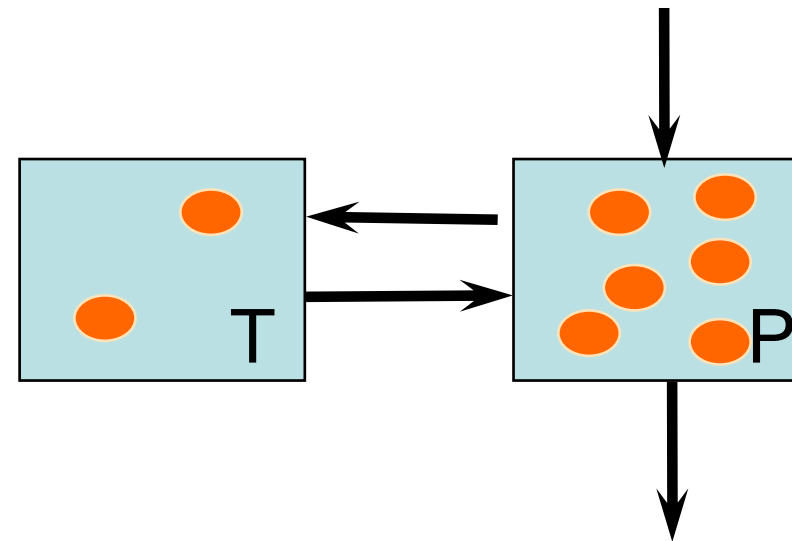
Volume of distribution

Drug with high V_d



high tissue
binding

Drug with low V_d





Volume of distribution

Definition : The apparent volume into which all the dose would have to be distributed to give the observed concentration in the plasma

$$V_d = \frac{\text{Total drug in body (mg)}}{\text{Plasma conc at } t = 0 \text{ (mg / mL)}}$$

If you dissolve 40 mg of X in an unknown volume and end up with a concentration of 2 µg/mL, the volume will be :

$$\text{Volume} = \frac{\text{amount}}{\text{concentration}} = \frac{40\text{mg}}{2\mu\text{g / mL}} = 20\text{L}$$





Volume of distribution

The apparent volume of distribution is a calculated space and does not always conform to any actual anatomic space

But if V_d is high, distribution into tissues is high

Rate of distribution dependent upon

- blood flow***
- physicochemical properties of the drug (affinity for the tissue, and the partition coefficient)***
- Protein Binding***

Units are L/kg (per kg enables cross species comparison)





Regional blood flow distribution (% cardiac output)

Tissue	Mouse ^a	Rat ^b	Dog ^c	Human ^d
Adipose		7.0		5.2
Adrenals		0.3	0.2	
Bone		12.2		4.2
Brain	3.3	2.0	2.0	11.4
Heart	6.6	5.1	4.6	4.0
Kidneys	9.1	14.1	17.3	17.5
Liver (Total)	16.1	18.3	29.7	22.7
Hepatic Artery	2.0	2.1	4.6	
Portal Vein	14.1	15.3	25.1	18.1
Lung	0.5	2.1	8.8	
Muscle	15.9	27.8	21.7	19.1
Skin	5.8	5.8	6.0	5.8
Thyroid				1.6

Brown RP, Delp MD, Lindstedt SL, Rhomberg LR, Beliles RP. Physiological parameter values for physiologically based pharmacokinetic models. Toxicol Ind Health 1997 Jul;13(4):407-84.

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Relationship between Vd and the extent of distribution

<i>Vd (L/kg)</i>	<i>Distribution in</i>	<i>Extent</i>
0.05 – 0.25	<i>plasma and/or extracellular fluids</i>	<i>Very low</i>
0.25 – 0.65	<i>intracellular water up to body water</i>	<i>Low</i>
0.65 - 5	<i>in total body fluids</i>	<i>Moderate</i>
> 5	<i>in deep tissues; bound to peripheral tissues</i>	<i>High</i>

Examples (L/kg) :

Chloroquine : 785

Hydroxychloroquine : 528

Digoxin : 8.6

Theophylline : 0.43

Gentamicin : 0.3

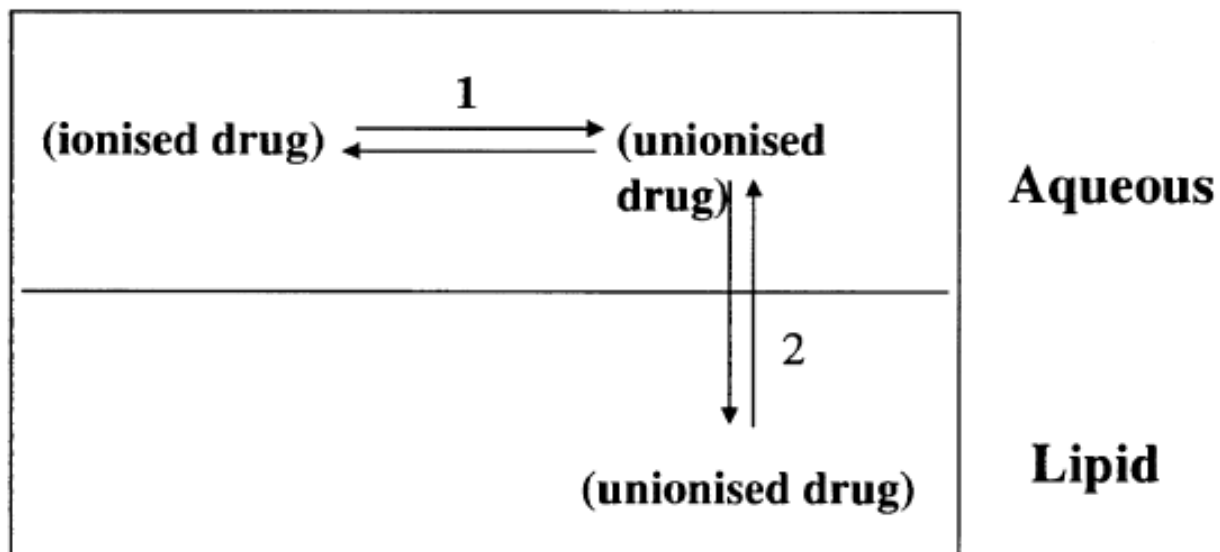
Warfarin : 0.13

Avastin : 0.1





Physico-chemistry and PK

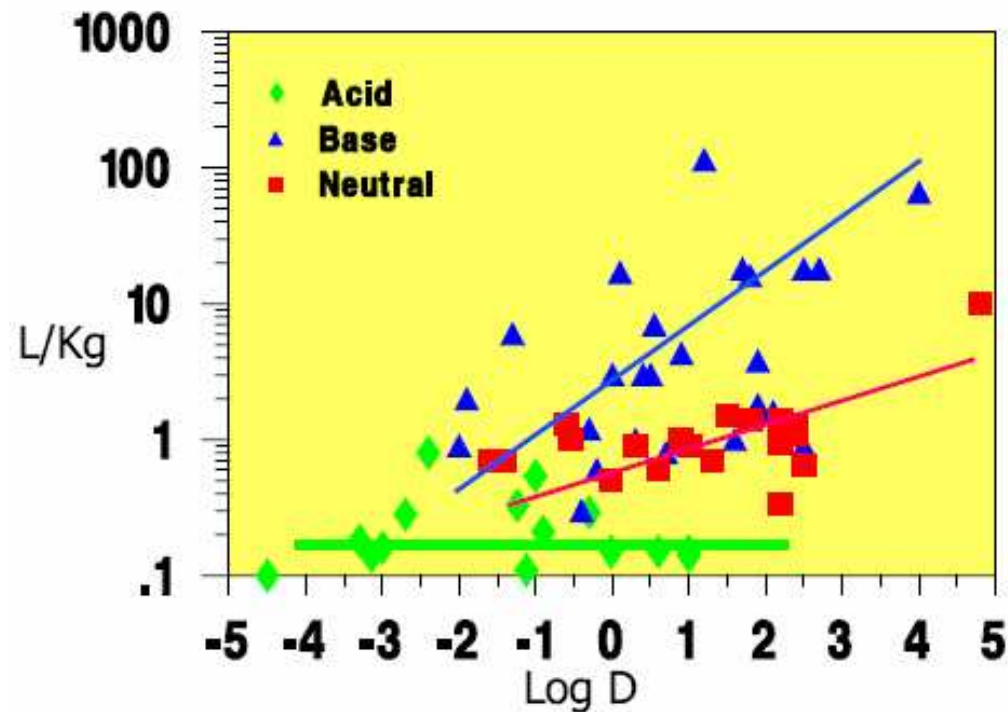


1. Is a function of acid/base strength pK_a
2. Is a function of P ($\log P$)





Volume of distribution for various drug classes



Neutral / bases : increase in lipophilicity = increase in Vd

Bases : increase in pKa = increase in Vd

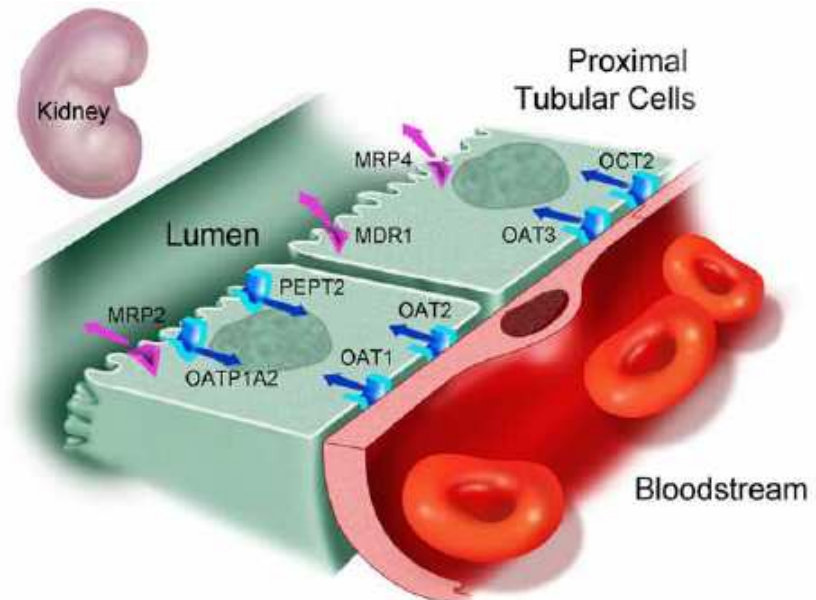
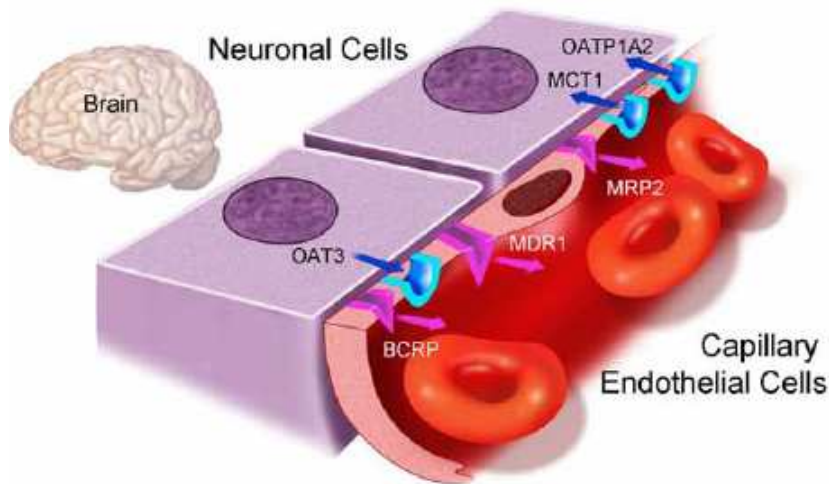
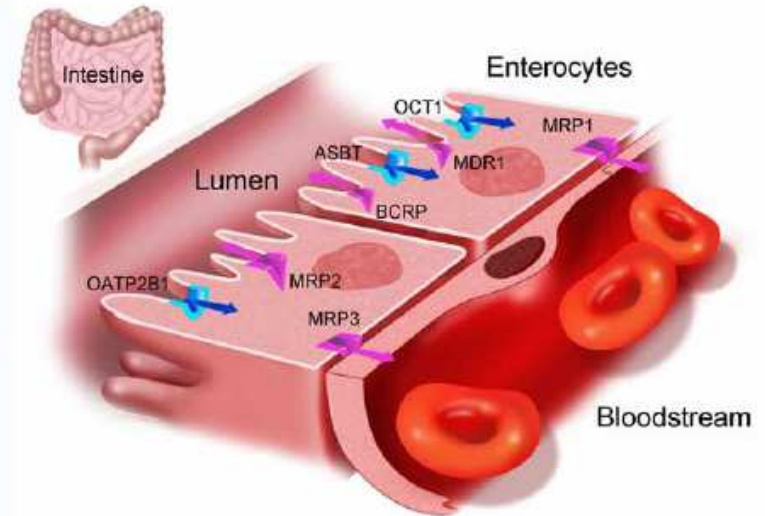
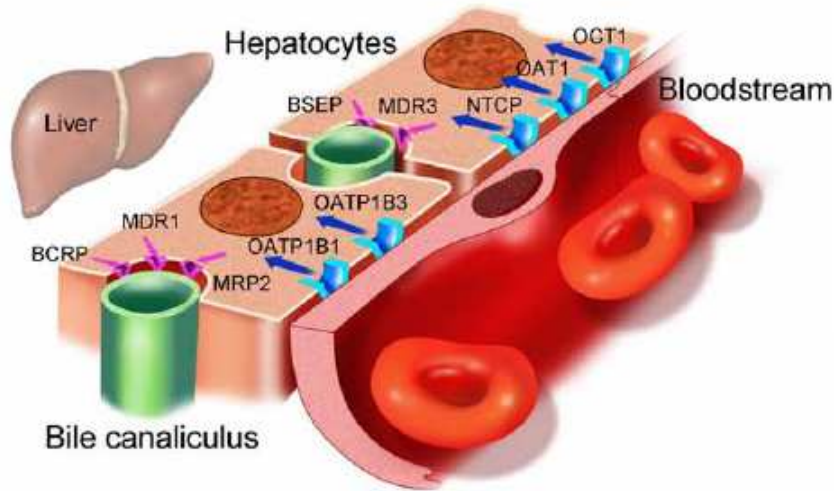
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Transporters and tissue drug distribution



Ho RH, Kim RB. Transporters and drug therapy: implications for drug disposition and disease. Clin Pharmacol Ther 2005 Sep;78(3):260-77.

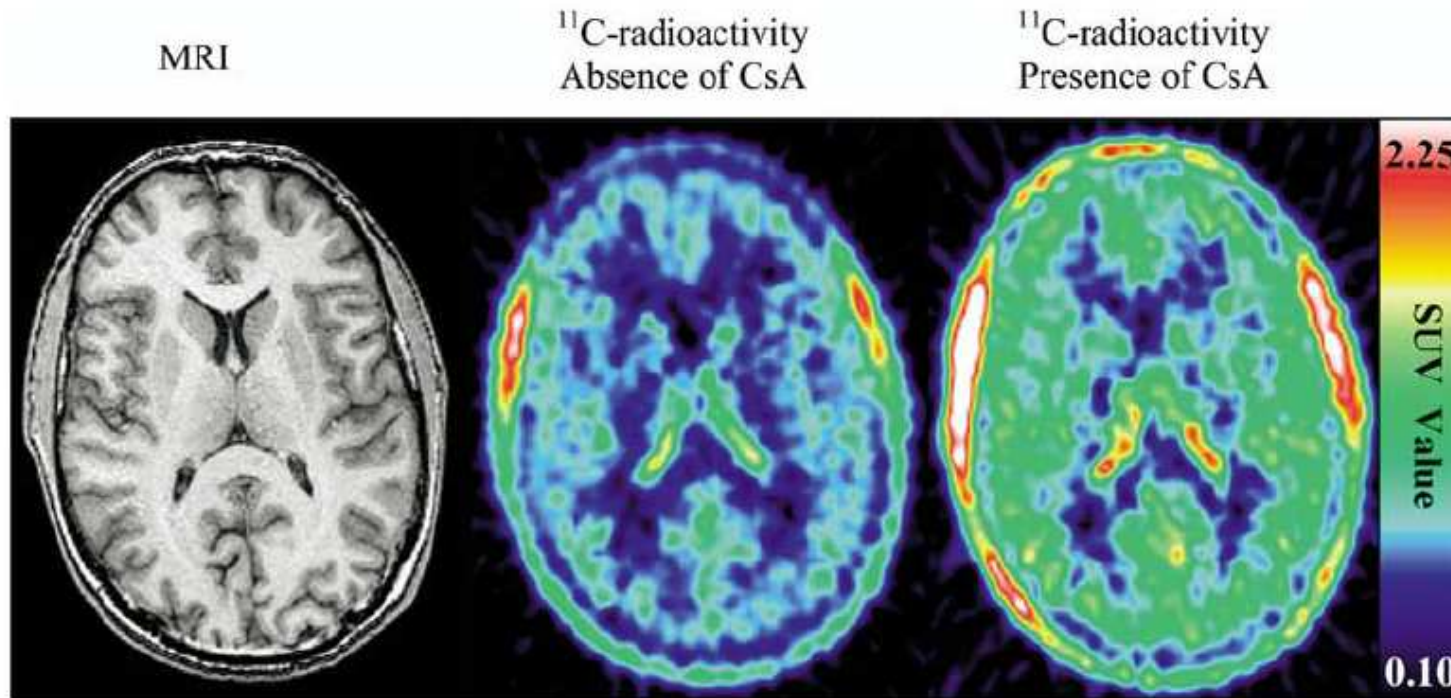
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L'essence de la science.





Role of P-gp in drug distribution into the human brain



^{11}C - verapamil : P-gp substrate

cyclosporine : P-gp inhibitor

Sasongko L, Link JM, Muzi M, Mankoff DA, Yang X, Collier AC, et al. Imaging P-glycoprotein transport activity at the human blood-brain barrier with positron emission tomography. Clin Pharmacol Ther 2005

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Metabolism



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Metabolism

Xenobiotic metabolism is a defense mechanism which living organisms need to survive

Bioprotective process concerned with removal of unwanted compounds from the body

Purpose: to enhance water solubility and excretability

Two classes of metabolism :

- Phase 1 (creation of a new chemical group on molecule)*
- Phase 2 (addition of endogenous molecule)*





What is Drug Metabolism or Biotransformation ?

Enzymatic, chemical, or stereochemical change to an administered drug; i.e. conversion of substance from :

- ***Active to Less Active or Inactive (Most cases):***

- ▶ Hydroxylation of Pentobarbital

- ***Active to Equivalent Activity:***

- ▶ Codeine to Morphine

- ***Inactive to Active:***

- ▶ Cyclophosphamide - anticancer drug

- ▶ Carbon Tetrachloride - carcinogen

LIVER is the primary site of metabolism although other organs are also capable of metabolic activity, i.e. lung, kidney, intestinal wall, gut microflora, blood

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Objectives of metabolism work

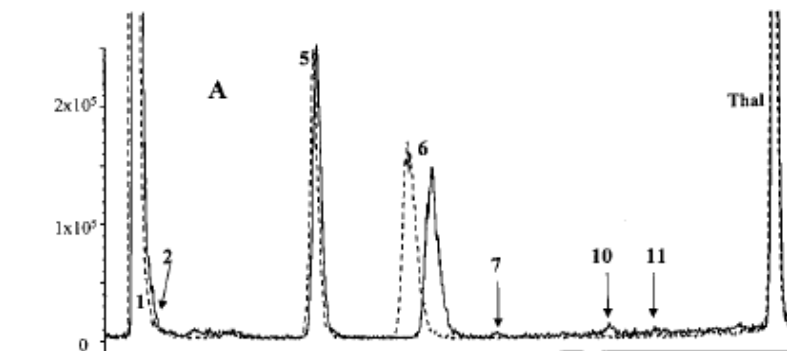
- Are all relevant metabolites present in humans covered by the toxicological species?***
- Are there specific metabolites formed in tox species which might explain an unexpected toxicity found in that species?***
- Are there pharmacologically active metabolites?***
- Are there metabolites with a drug-drug-interaction potential?***



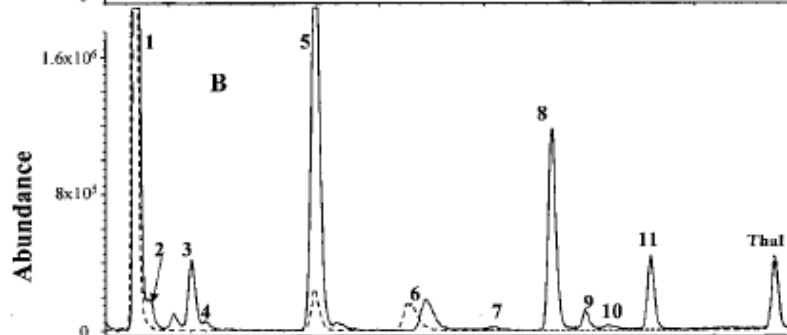


Example: Interspecies comparison of thalidomide metabolites formation in liver microsomes

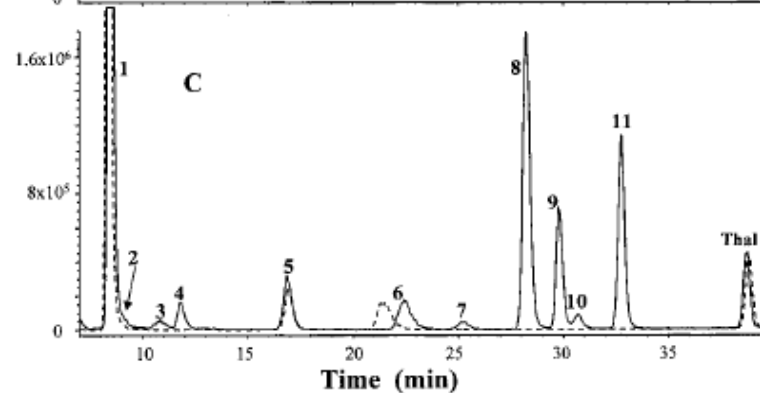
Human



Rabbit



Mice



Lu J, Helsby N, Palmer BD, Tingle M, Baguley BC, Kestell P, et al. Metabolism of thalidomide in liver microsomes of mice, rabbits, and humans. *J Pharmacol Exp Ther* 2004 Aug;310(2):571-7.

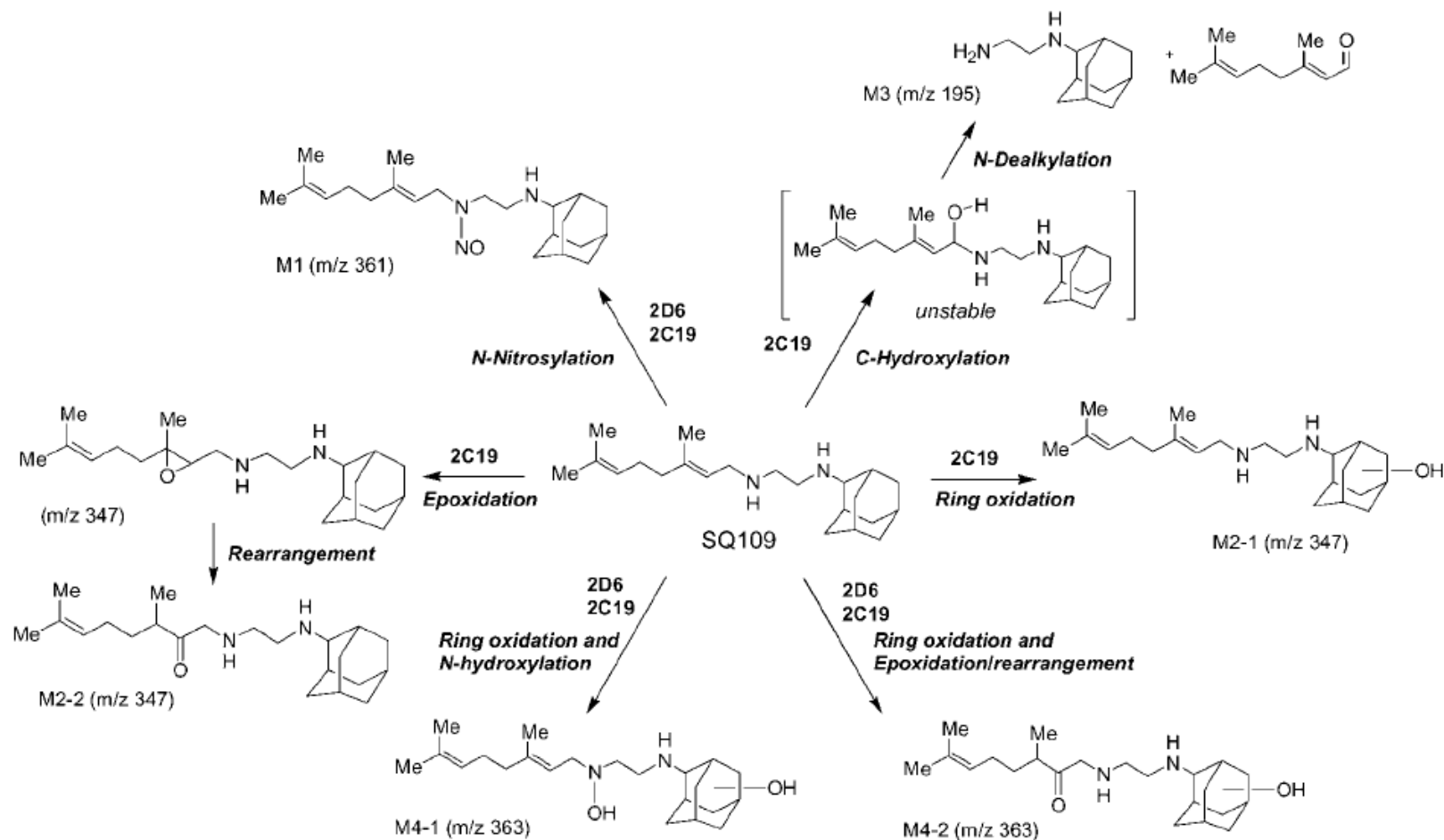
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Example: in vitro metabolic pathways of SQ109 and metabolites formed in liver microsomes.



Jia L, Noker PE, Coward L, Gorman GS, Protopopova M, Tomaszewski JE. Interspecies pharmacokinetics and in vitro metabolism of SQ109. Br J Pharmacol 2006 Mar;147(5):476-85.

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Drug Metabolizing Enzymes

Phase I

- *Cytochromes P-450 (CYP)*
- *Flavin Monooxygenase (FMO)*
- *Monoamine Oxidase (MAO)*
- *Aldehyde dehydrogenase*
- *Alcohol dehydrogenase*
- *Various amidases/esterases*

Phase II

- *UDP-glucuronosyl transferase (UGT)*
- *sulfotransferases*
- *glutathione transferases*
- *N-acetyltransferases*
- *methyl transferases*



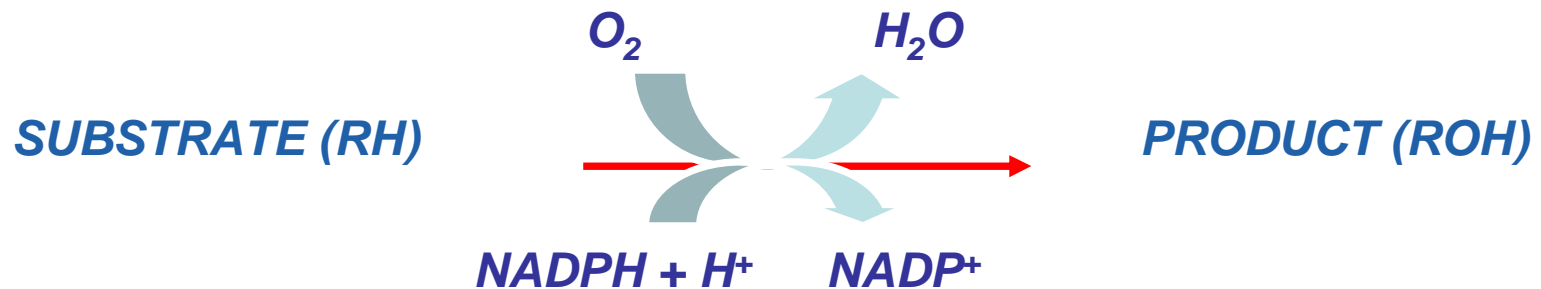


What are Cytochrome P450 (CYP) enzymes ?

CYP450 is a Heme (Iron-containing porphyrin, protoporphyrin IX) - protein (Apoprotein)

The basic reaction catalyzed by CYP450 is monooxygenation

- one atom of O₂ is incorporated into a substrate RH
- the other is reduced to water with reducing equivalents derived from NADPH, as follows:



- P450 = Peak at 450nm = absorbance peak of reduced P450 complexed with carbon monoxide

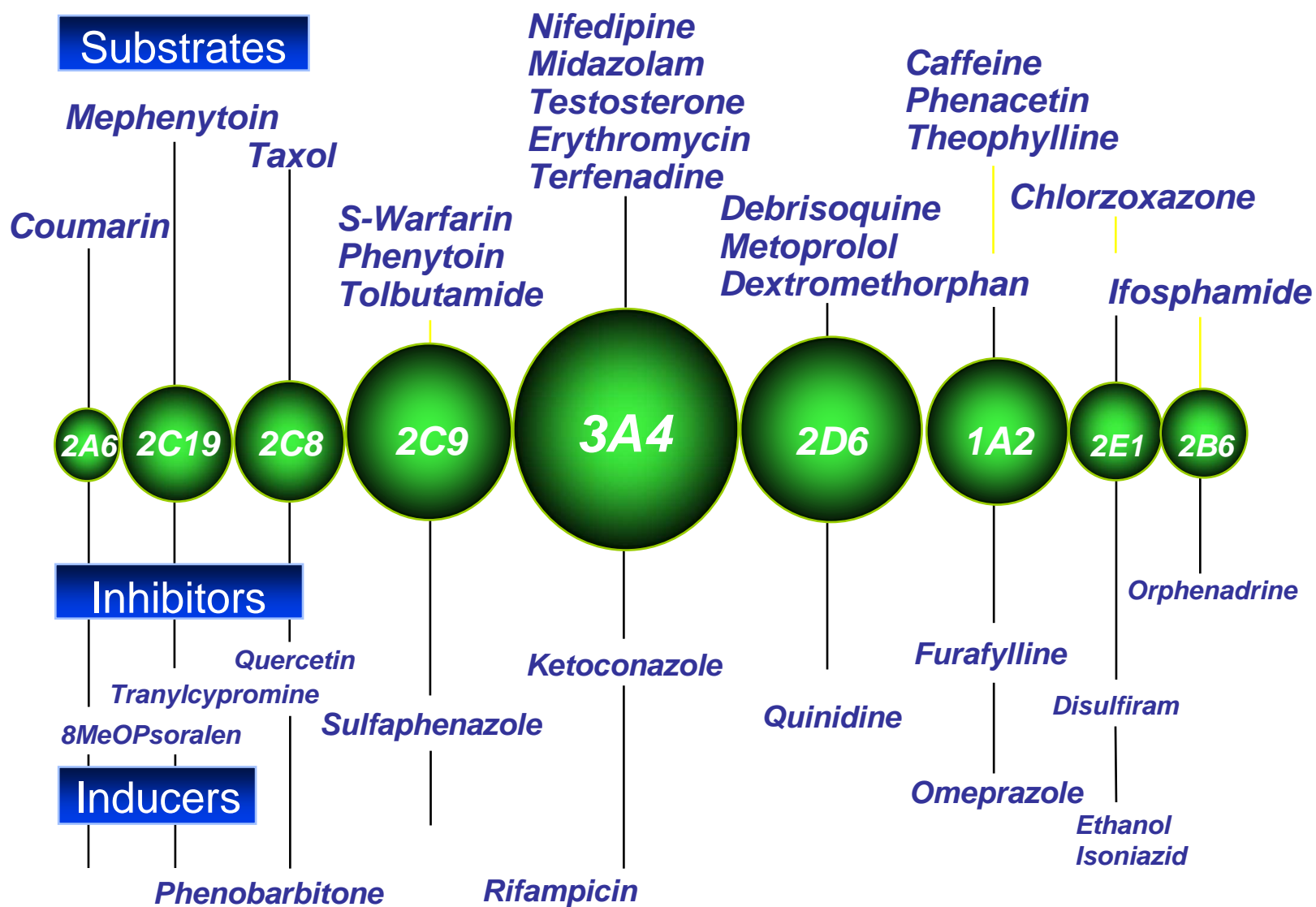
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Cytochrome P450 System



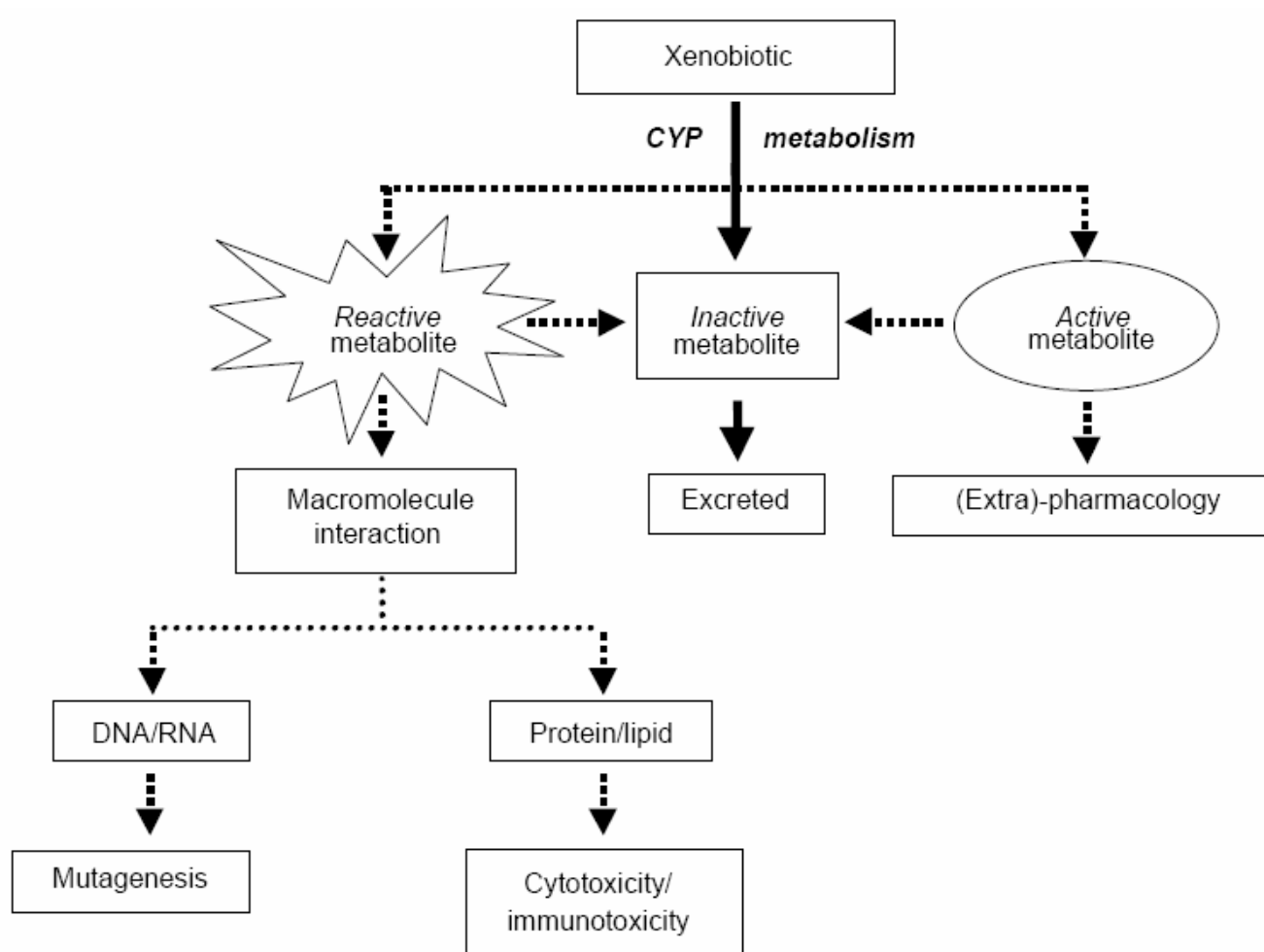
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Roles of CYP enzymes in drug efficacy and toxicity.





Phase II Metabolism

Also described as CONJUGATION reactions

Glucuronidation - most common, multiple forms, species differences

Drug conjugates excreted in the bile can be hydrolysed in the gut and the drug reabsorbed - Enterohepatic Recirculation

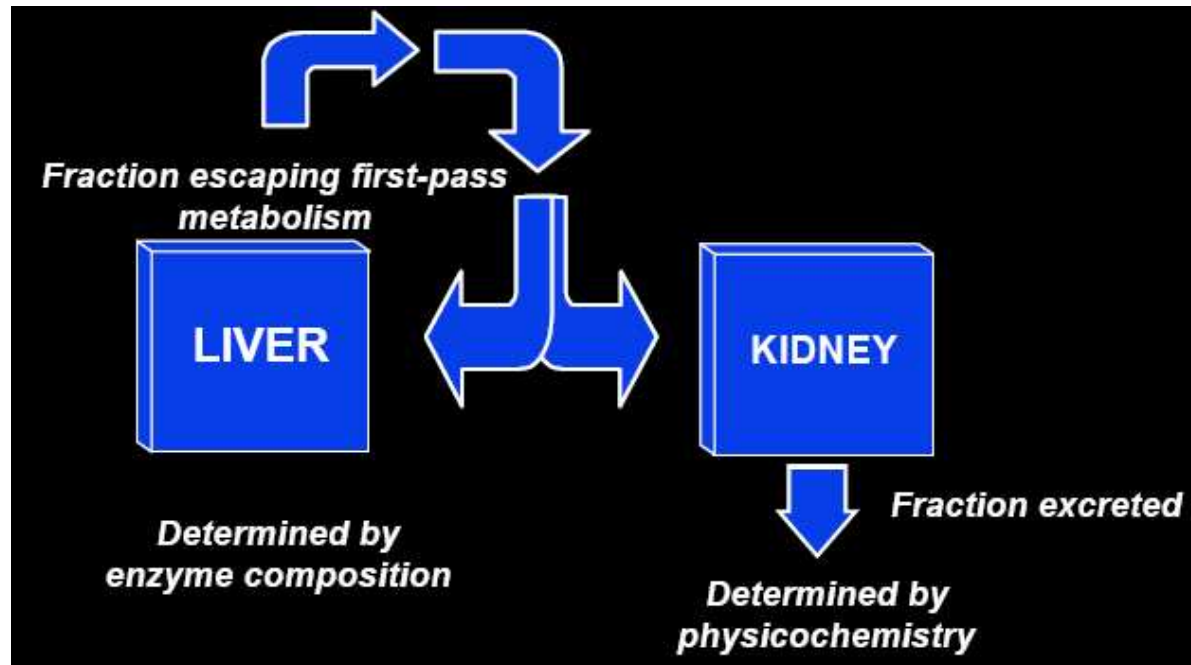




Drug clearance

Definition : the volume of blood cleared of drug per unit time

Clearance is the irreversible removal of drug



$$CL_{tot} = CL_R + CL_{met} + CL_{other}$$

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Calculation of clearance

Describes the efficiency of elimination

Volume of blood cleared of a drug per unit time

Units : volume/time : L/h

$$CL = \frac{Dose}{AUC}$$





Liver blood flow varies across the species

Liver blood flow (mL/min/kg)

● <i>Mouse</i>	<i>90</i>
● <i>Rat</i>	<i>80-100</i>
● <i>Dog</i>	<i>50</i>
● <i>Cynomolgus</i>	<i>45</i>
● <i>Human</i>	<i>25</i>

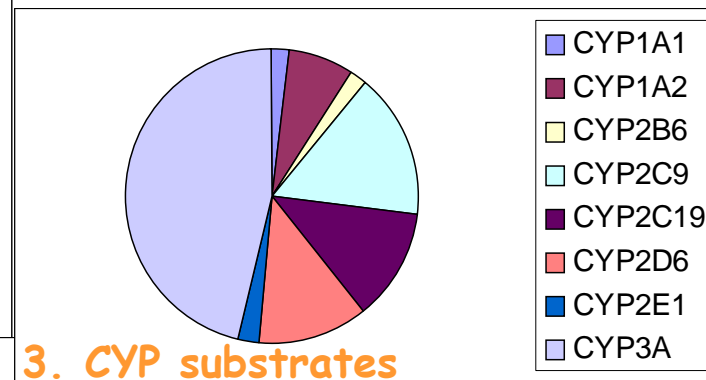
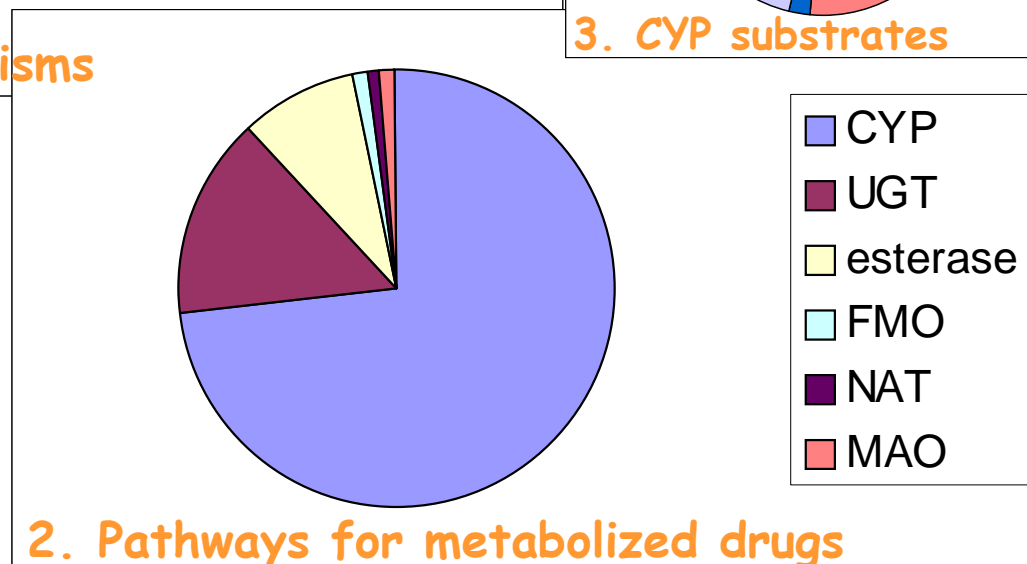
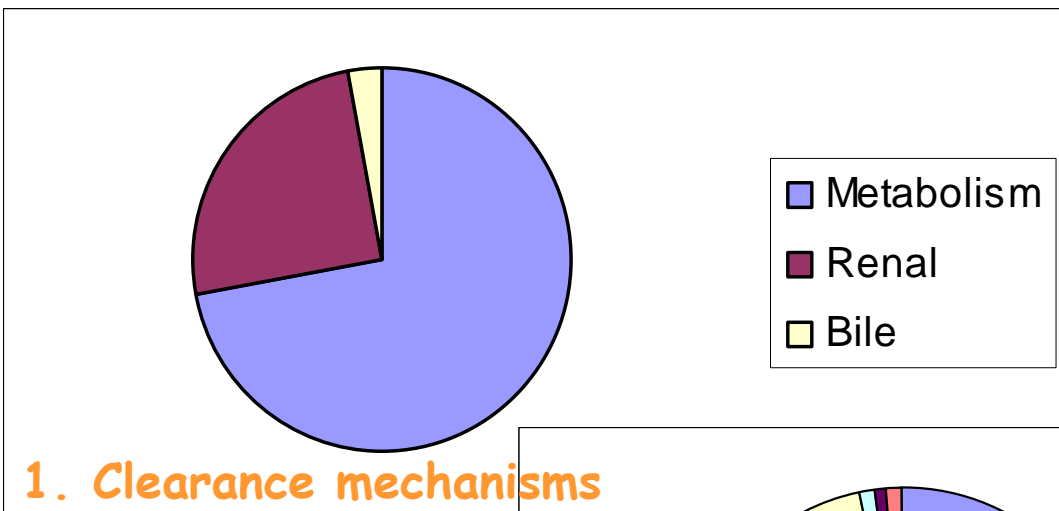
If clearance approaches liver blood flow, then a large proportion of the drug will be removed by the liver on each blood circulation

Implications for bioavailability (first-pass) and half-life





Clearance Mechanisms for the Top 200 Drugs Prescribed in 2002





Excretion



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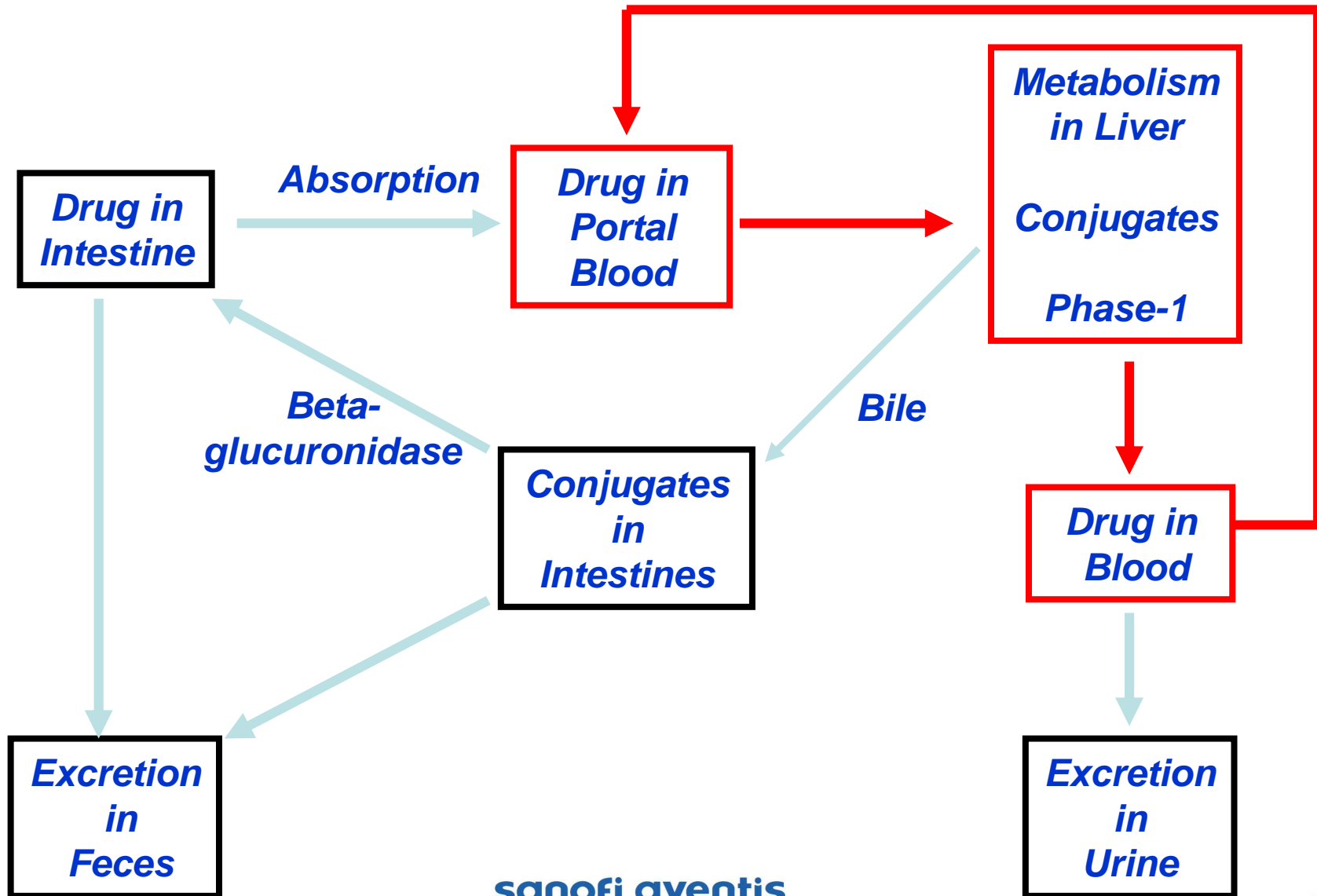
Excretion

- The final elimination of drug-related materials from the body*
- A drug may be excreted unchanged or as metabolites*
- A drug may be excreted via the urine, faeces, sweat, milk,*





Routes of Excretion



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Elimination

The irreversible removal of parent drug from the body

Elimination can be by

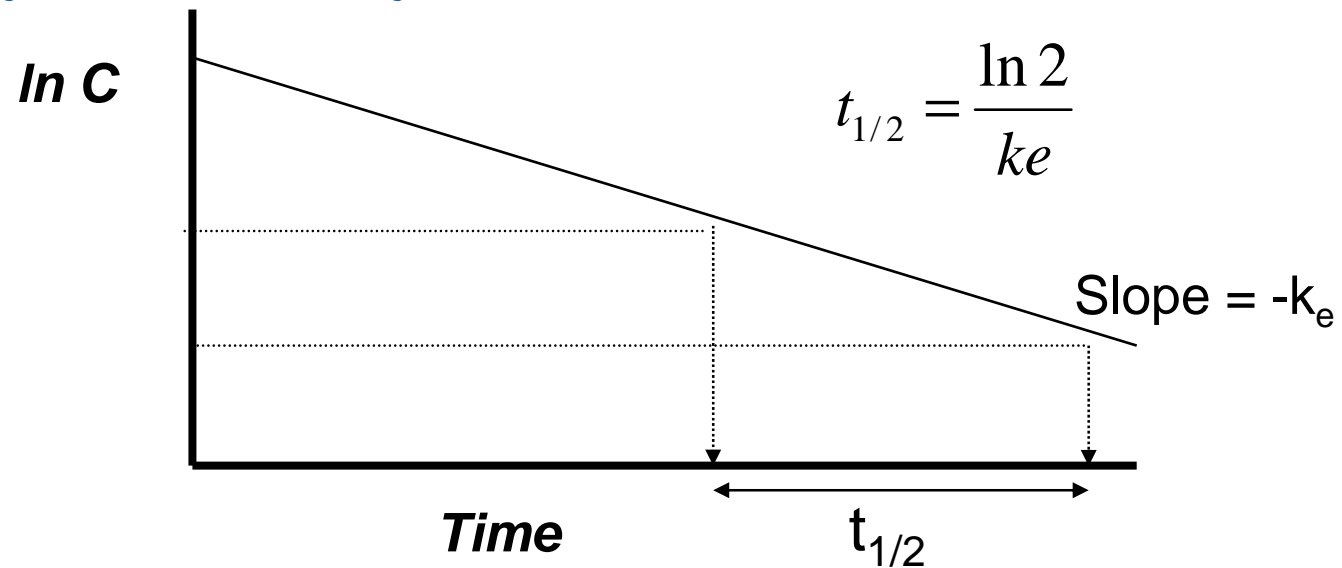
- *metabolism*
- *renal filtration (passive)*
- *renal secretion (active)*
- *biliary secretion (active)*
- *exhalation*





Half-Life

Time taken for concentration or the amount of drug in the body to decrease by 50%



Elimination of a drug is normally a first-order process





Elimination Half-Life

Half-life describes the exponential relationship between concentration and time and represents the interplay between clearance and volume of distribution

Elimination half-life is not a 'fundamental' property but depends upon 2 variables :

$$t_{1/2} = \frac{0.693 \times V}{CL}$$

Unit : hours (days)





Drug-drug interactions

Alterations in absorption

- ***Complexation/Chelation***
- ***Altered GI Transit***
- ***Altered Gastric pH***

Alterations in plasma protein binding

Alterations in hepatic metabolism

- ***Induction of Metabolism***
- ***Inhibition of Metabolism***

Alterations in renal clearance

- ***Increase in Renal Blood Flow***
- ***Inhibition of Active Tubular Secretion***
- ***Alterations in Tubular Reabsorption***





Prediction of human dose



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Interspecies Scaling

The relationship of some physiologic / pharmacokinetic parameters across species can be correlated with body weight.

$$Y = a \cdot W^b$$

- *Y = PK parameter*
- *W = Body weight*
- *a = allometric coefficient*
- *b = scaling exponent*

Parameters

- *Heart rate, Circulation time, Respiratory rate, Basal metabolic rate, Blood flow, Clearance (flow limited)*





Guidance for Industry

Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
July 2005
Pharmacology and Toxicology

Step 1

Determine NOAELs (mg/kg) in toxicity studies

Is there justification for extrapolating animal NOAELs to human equivalent dose (HED) based on mg/kg (or other appropriate normalization)?

Yes

HED (mg/kg) = NOAEL (mg/kg) (or other appropriate normalization)

No

Convert each animal NOAEL to HED (based on body surface area; see Table 1)

Step 2

Select HED from most appropriate species

Step 3

Step 4

Choose safety factor and divide HED by that factor

Maximum Recommended Starting Dose (MRSD)

Step 5

Consider lowering dose based on a variety of factors, e.g., PAD





Table 1: Conversion of Animal Doses to Human Equivalent Doses Based on Body Surface Area			
Species	To Convert Animal Dose in mg/kg to Dose in mg/m ² , Multiply by k _m	To Convert Animal Dose in mg/kg to HED ^a in mg/kg, Either:	
		Divide Animal Dose By	Multiply Animal Dose By
Human	37	---	---
Child (20 kg) ^b	25	---	---
Mouse	3	12.3	0.08
Hamster	5	7.4	0.13
Rat	6	6.2	0.16
Ferret	7	5.3	0.19
Guinea pig	8	4.6	0.22
Rabbit	12	3.1	0.32
Dog	20	1.8	0.54
Primates:			
Monkeys ^c	12	3.1	0.32
Marmoset	6	6.2	0.16
Squirrel monkey	7	5.3	0.19
Baboon	20	1.8	0.54
Micro-pig	27	1.4	0.73
Mini-pig	35	1.1	0.95

^a Assumes 60 kg human. For species not listed or for weights outside the standard ranges, HED can be calculated from the following formula:

$$\text{HED} = \text{animal dose in mg/kg} \times (\text{animal weight in kg/human weight in kg})^{0.33}$$

^b This k_m value is provided for reference only since healthy children will rarely be volunteers for phase 1 trials.

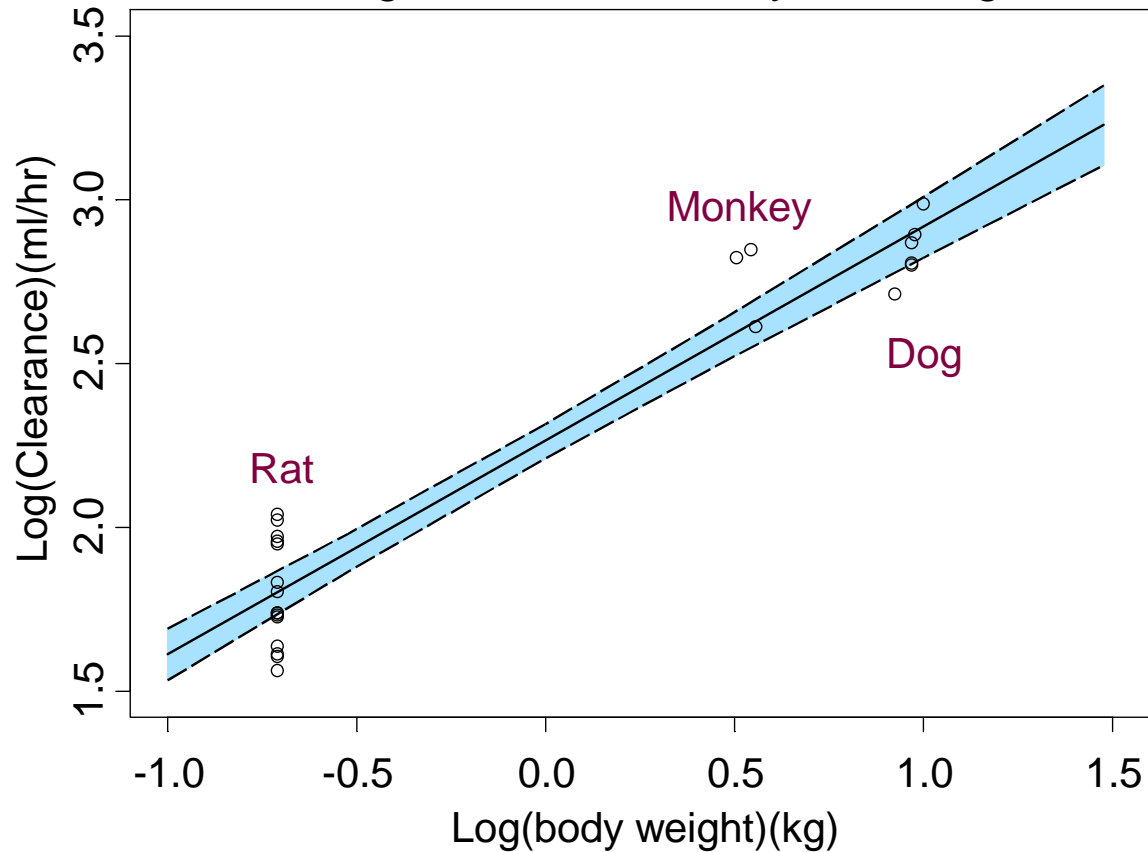
^c For example, cynomolgus, rhesus, and stump-tail.





Estimation of Human Clearance Using Allometric Scaling

Log Intravenous Clearance versus Log Body Weight for Rat, Monkey, and Dog



- Basic allometric equation:
 $Y=A \cdot B^X$
- Fit of above equation to rat, monkey, and dog data:
 $A=185 \pm 13.4$
 $X=0.653 \pm 0.0412$
- Estimated human intravenous clearance (median (90%CI)):
2940 (2100 - 4100)(ml/hr)





Proposed First-in-Human Dose : example

$$Dose = \frac{CL_{hum} \times AUC_{NOAEL} \times PBcf}{F \times S}$$

————— $CL_{hum} = 10 \text{ L/h}$

————— $AUC_{NOAEL} = 8490 \text{ ng.h/mL}$
(at 25 mg/kg, NOAEL dose, male rat)

————— $PBcf = \text{protein binding correction factor} = 0.25$
(estimated free fraction of 0.02% in rat and 0.08% in human)

————— $F = 1$

————— $\text{Safety Factor} = 10$

Proposed dose based on Allometry is 2 mg

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Conclusion



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ADME properties determined by:

Drug Characteristics

- ***Physicochemical properties***
- ***Structure***
- ***Suitability as substrates for receptors and enzymes***

Potentially modifiable by Drug Design

'Non-drug' characteristics

- ***Species***
- ***enzymology (Vmax/Km)***
- ***gender***
- ***age***
- ***genetics***
- ***food status***
- ***other drugs....***

Fixed and out of our control





Why PK could be critical?

- Low bioavailability
 - ➔ high variability, cost of goods
- Non-linearity
 - ➔ unpredictable PK, dose-effects
- Rapid metabolism
 - ➔ short duration of action, variability
- Extensive metabolism
 - ➔ unpredictable pharmacological / toxicological action
 - interspecies/-individual variability
- Poor distribution
 - ➔ lack of efficacy
- Enzyme inhib/induction
 - ➔ drug-drug interactions, nonlinear or time-dependent PK
- *High variability*
 - ➔ *unpredictable behaviour*
 - ➔ *difficult to develop (Nr of subjects↑)*
 - ➔ *reduced safety margin*





Toxicokinetics

The main objectives of toxicokinetics are to :

- *define the drug bioavailability,*
- *dose proportionality,*
- *gender differences*
- *species differences*

it also helps to

- *establish the correlation of systemic exposure with toxicological findings.*
- *aids in the determination of the margin of safety between nonclinical safety studies and human plasma concentrations achieved in clinical trials.*
- *predict the drug exposure and possible bioaccumulation under multiple dose regimes in humans*





Practical applications



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