Overview – Internal review

- Presentation title: Introduction to Pharmacokinetics for Toxicology Assessment
- Track title: Contamination
- Speaker: Andrew Bartholomaeus
- Date / Time: Tuesday
- Time allotted: 0900 - 1030
- Dot point overview (no more than 10 points):
  - A basic introduction to the essentials of pharmacokinetics relevant to the establishment of a PDE
  - Covers the principle concepts and processes related to Absorption, distribution, metabolism and excretion of drugs and other xenobiotics
  - Highlights pharmacokinetic issues that may present affect the PDE applicable for specific population groups

© PharmOut 2015

RISK IDENTIFICATION FOR MANUFACTURE IN SHARED FACILITIES

2

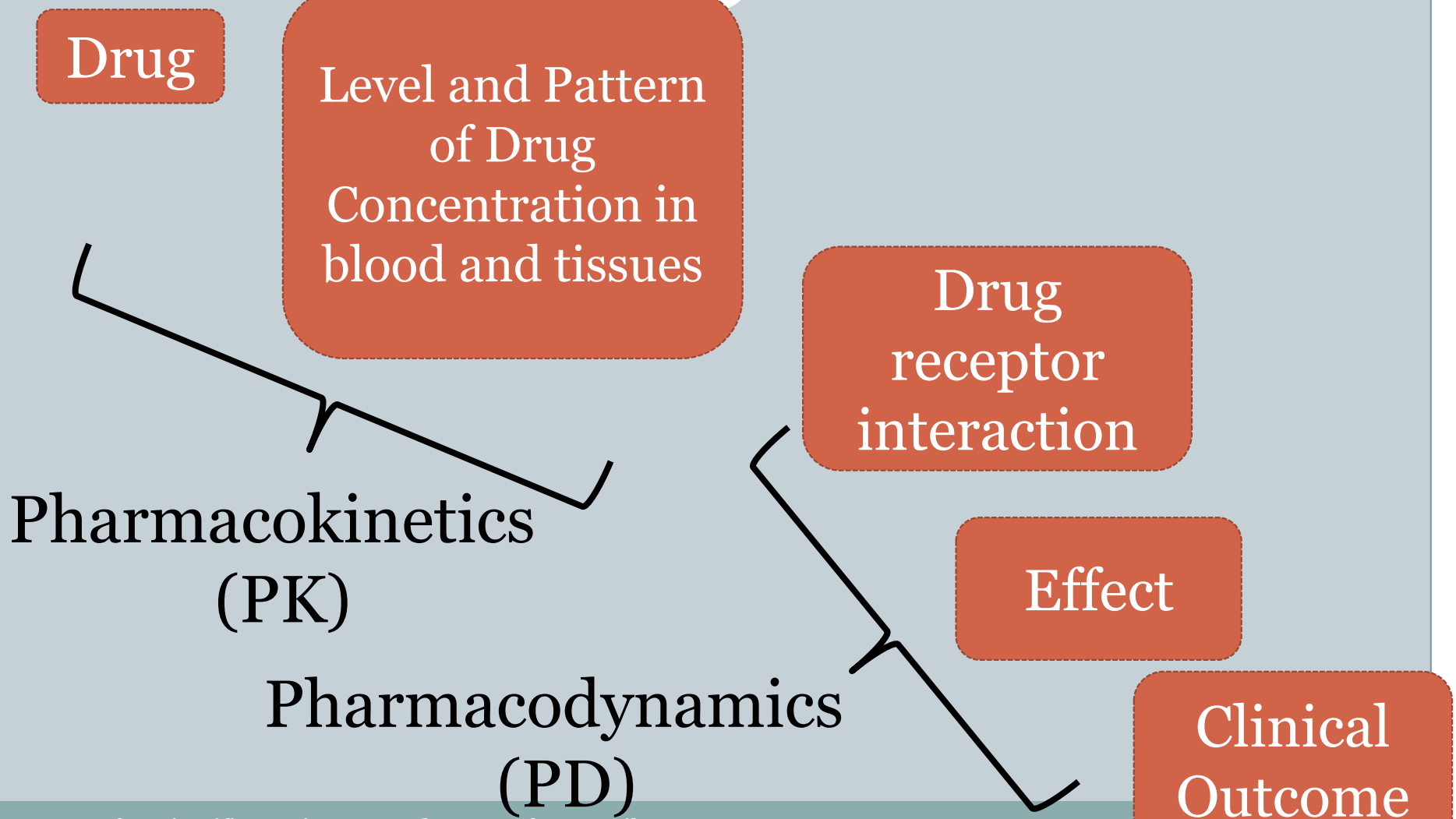
Introduction to Pharmacokinetics for Toxicology Assessment

PROF. ANDREW BARTHOLOMAEUS

SCHOOL OF PHARMACY, UNIVERSITY OF CANBERRA
THERAPEUTIC RESEARCH CENTRE,
SCHOOL OF MEDICINE, UNIVERSITY OF QUEENSLAND
CEO, BARTCROFTS SCIENTIFIC SERVICES PTY LTD

EMAIL; BARTCROFTS@GMAIL.COM

PharmacoKinetics (PK) Versus Pharmaco-Dynamics (PD)



Pharmacokinetics (PK)

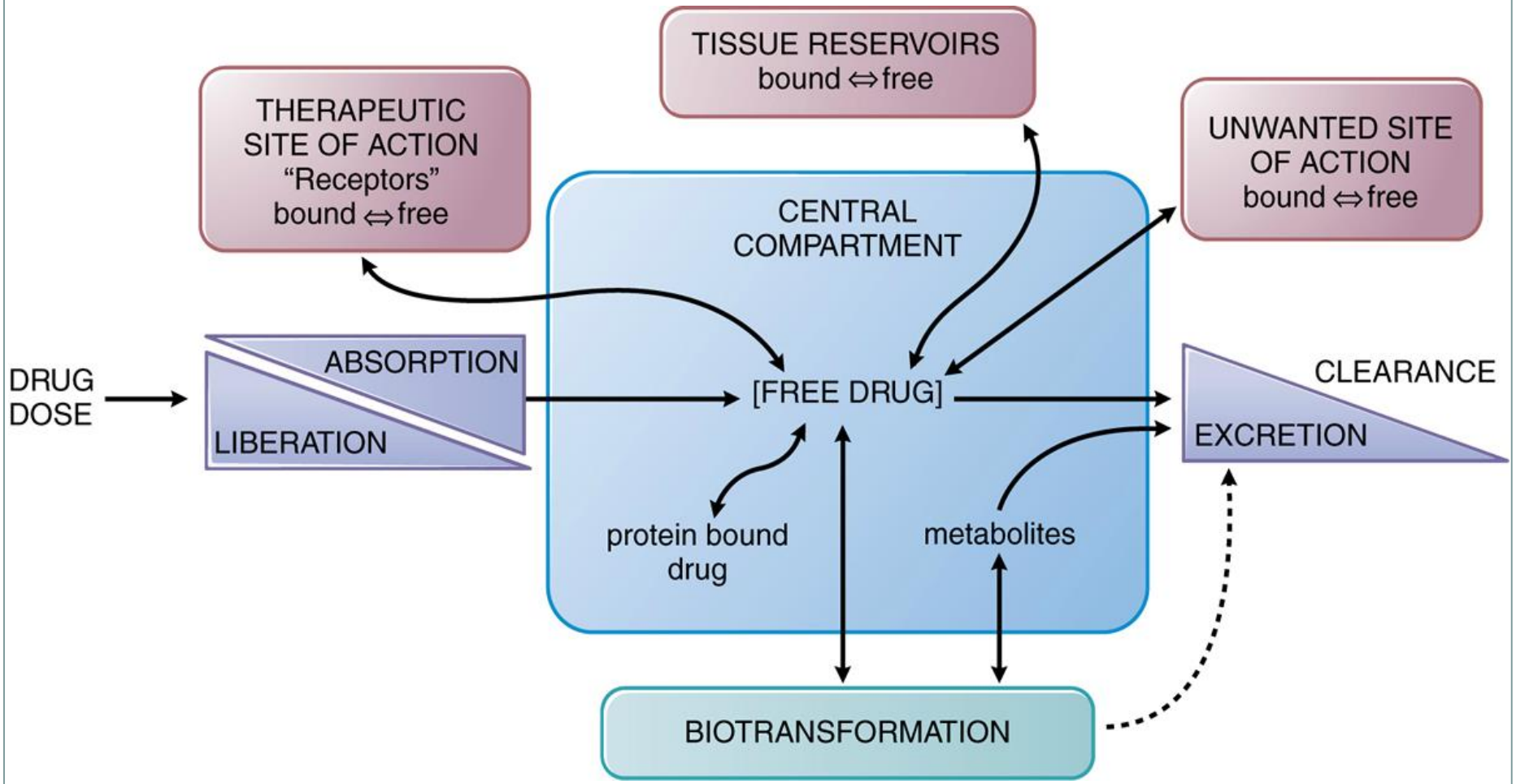
GENERAL PRINCIPLES

Why it Matters

- Dose comparisons between animals and man are based on comparative pharmacokinetics (PK) across species
  - Dose extrapolation across species is a critical aspect of PDE determination
- PK differences are a (the?) major determinant of interspecies differences in toxicity
- The amount of a chemical in the blood stream (Systemic exposure) is dependent on the PK of the route of administration (oral versus inhalation versus iv etc)
  - Sometimes the calculation of a PDE for one route of exposure will depend on data from another route of administration
- PK interactions between a contaminant and the API of a medicine may cause toxicity
  - Unlikely at very low exposures but there are always potential exceptions
- Disease states and physiological conditions (eg pregnancy) can alter PK and therefore toxicity
  - Contamination of an antibiotic used for meningitis may result in the contaminant crossing the blood brain barrier when it would not otherwise – unique toxicological effects

Key Components of Pharmacokinetics

- Absorption
  - Passage of a drug from the site of exposure into the systemic circulation
- Distribution
  - Movement of a drug into the different compartments of the body
- Metabolism
  - Alteration of the chemical structure of a drug to alter its solubility &/or activity
- Excretion
  - Removal of the drug from the systemic circulation
- Collectively referred to as ADME


TISSUE RESERVOIRS
bound ⇔ free
THERAPEUTIC SITE OF ACTION
"Receptors"
bound ⇔ free
UNWANTED SITE OF ACTION
bound ⇔ free
CENTRAL COMPARTMENT
DRUG DOSE
ABSORPTION
LIBERATION
[FREE DRUG]
CLEARANCE
EXCRETION
protein bound drug
metabolites
BIOTRANSFORMATION

Drug level in blood is the sum of multiple processes

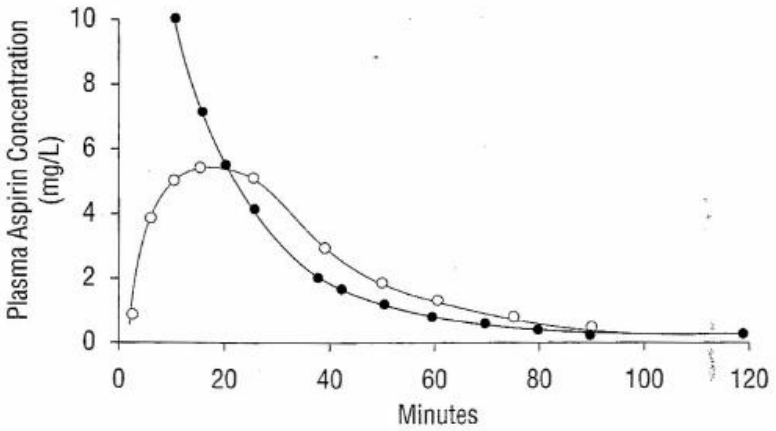
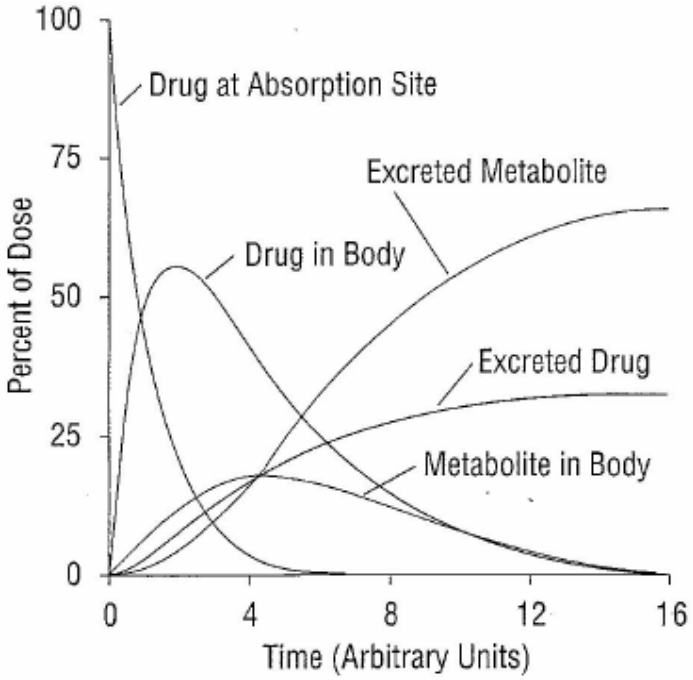


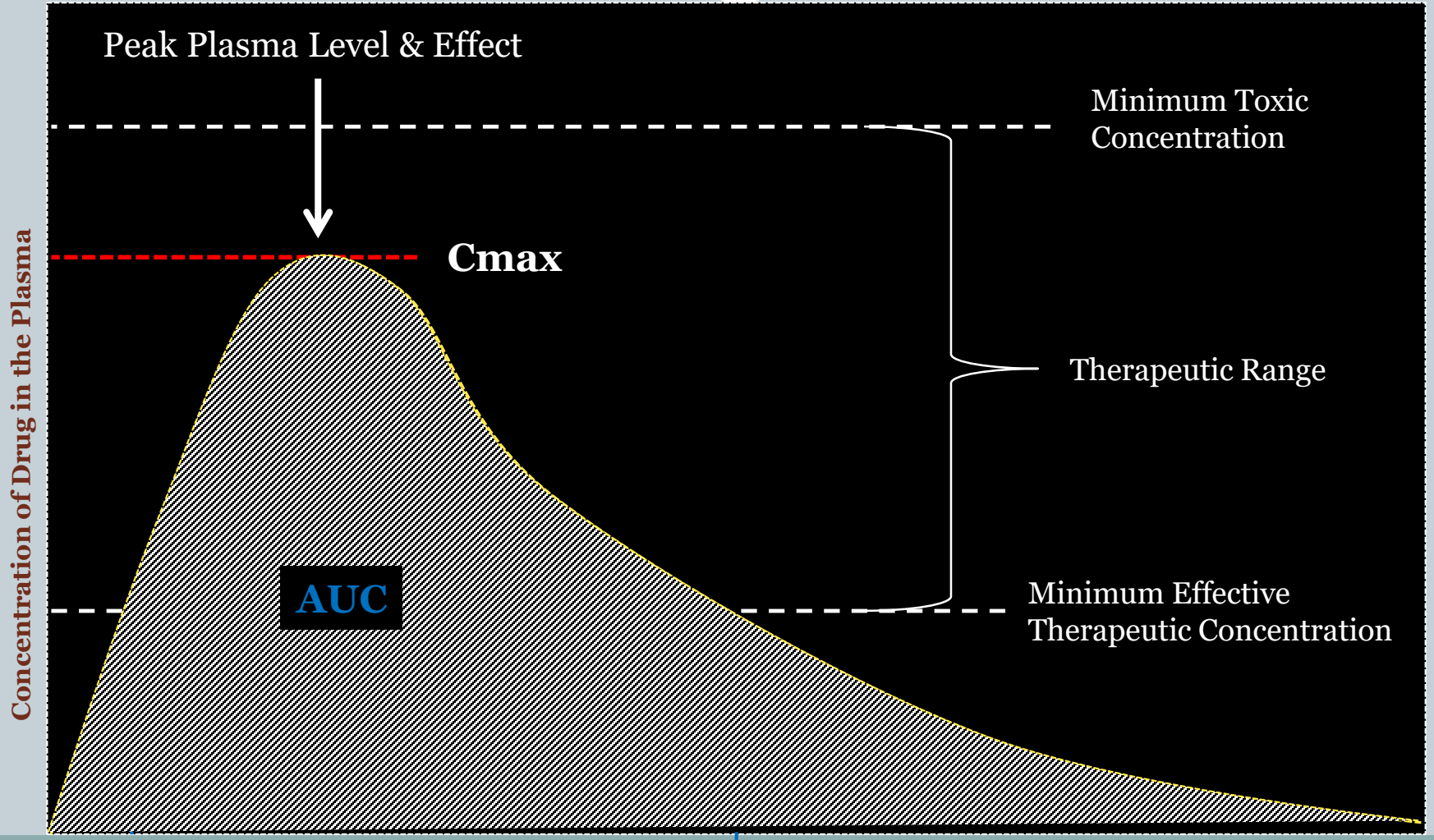
Fig. 4–2. Aspirin (650 mg) was administered as an intravenous bolus (●) and as an oral solution (○) on separate occasions to the same individual. Absorption causes a delay and a lowering of the peak concentration (1 mg/L = 5.5 μM). (Modified from the data of Rowland, M., Riegelman, S., Harris, P. A., and Sholkoff, S.D.: Absorption kinetics of aspirin in man following oral administration of an aqueous solution. J. Pharm. Sci., 67:379–385, 1972. Adapted with permission of the copyright owner.)



Key Pharmacokinetic Terms

- **AUC:** (area under blood concentration vs time curve)
  - proportional to the total amount of circulating drug – time weighted average blood concentration
  - characterises extent of absorption (bioavailability).
  - Chronic toxicity tends to be related to AUC
- **Cmax:**
  - maximum (or peak) drug concentration in the plasma.
  - a function of both the **rate** and **extent** of absorption
  - will increase with an increase in dose
  - will increase with an increase in absorption rate
  - Aaute toxicity tends to be related to Cmax
- **Tmax:**
  - time when Cmax occurs
  - reflects the rate of drug absorption
  - decreases as the absorption rate increases
  - The time of onset of acute toxicity often coincides with Tmax
- **T1/2:**
  - Time required for drug concentration to decrease by half
  - Reflects drug elimination (metabolism & excretion)
  - Calculation requires knowledge of the terminal elimination rate constant

The Plasma Concentration Time Curve



Therapeutic Range & Therapeutic Ratio

- Therapeutic Range
  - The range of plasma drug concentrations that are pharmacologically active without being toxic (excess pharmacological action &/or toxicological)
  - Doses above the maximum of therapeutic range (i.e. range of safe concentrations) is generally toxic due to excess pharmacological action
  - Dose below therapeutic range is sub-optimal and may not give therapeutic effect
- Therapeutic Ratio
  - Maximum non toxic dose ÷ minimum therapeutic dose
  - A measure of the safety margin of a drug
  - Narrow therapeutic ratio drugs must be dosed at near toxic levels
- All chemicals must get above a threshold level to have an effect (therapeutic or toxic)
- Many drugs have potential, non (primary) pharmacological, toxicity within the therapeutic range – eg teratogenesis, idiosyncratic reactions, "side effects"

Bioavailability

- Systemic effects require Systemic exposure
- **Bioavailability** is how much of the administered drug or chemical actually reaches the systemic circulation
  - And therefore how much of the chemical can potentially interact with drug or toxin targets.
  - IV bioavailability = 100%, oral can be anywhere between 0% and 100 % but as low as 1-3 % is pharmaceutically viable – eg bisphosphonates
- Why measure the chemical in the **systemic** circulation?
  - It is drug or chemical inside the body that can act on the targets (some drugs of course act outside the systemic circulation – eg topicals, antacids, inhalers, eye drops)
- Why not measure the level at the site of action ?
  - Technically difficult to measure the drug concentration at the site of action
    - blood is an accessible surrogate for concentration at active site
    - Blood level is usually, but **NOT** always, proportional to drug/chemical at the active site (eg digoxin)
  - Blood is easier to access so plasma concentration is used as a surrogate

Pharmacokinetics

13

A CLOSER LOOK AT

ABSORPTION

DISTRIBUTION

METABOLISM

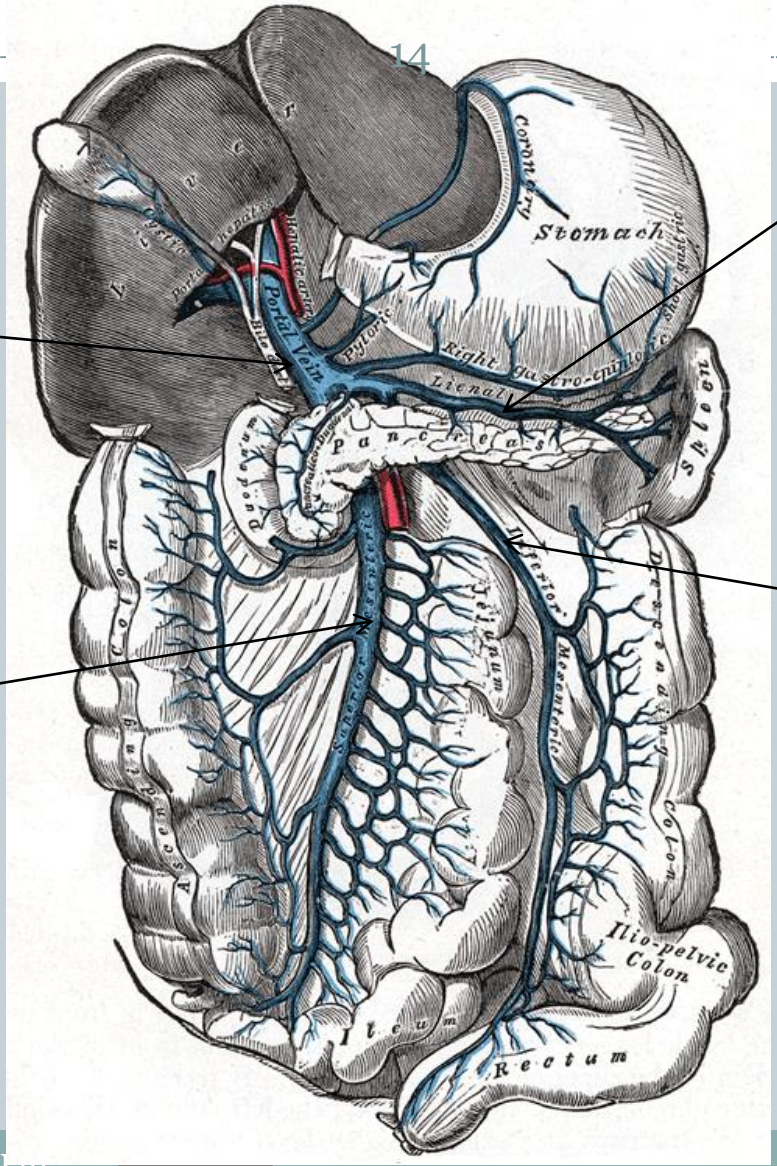
EXCRETION

BartCrofts Scientific Services Pty Ltd; Bartcrofts@gmail.com

Portal Circulation

Splenic vein

Hepatic portal vein



Superior mesenteric vein

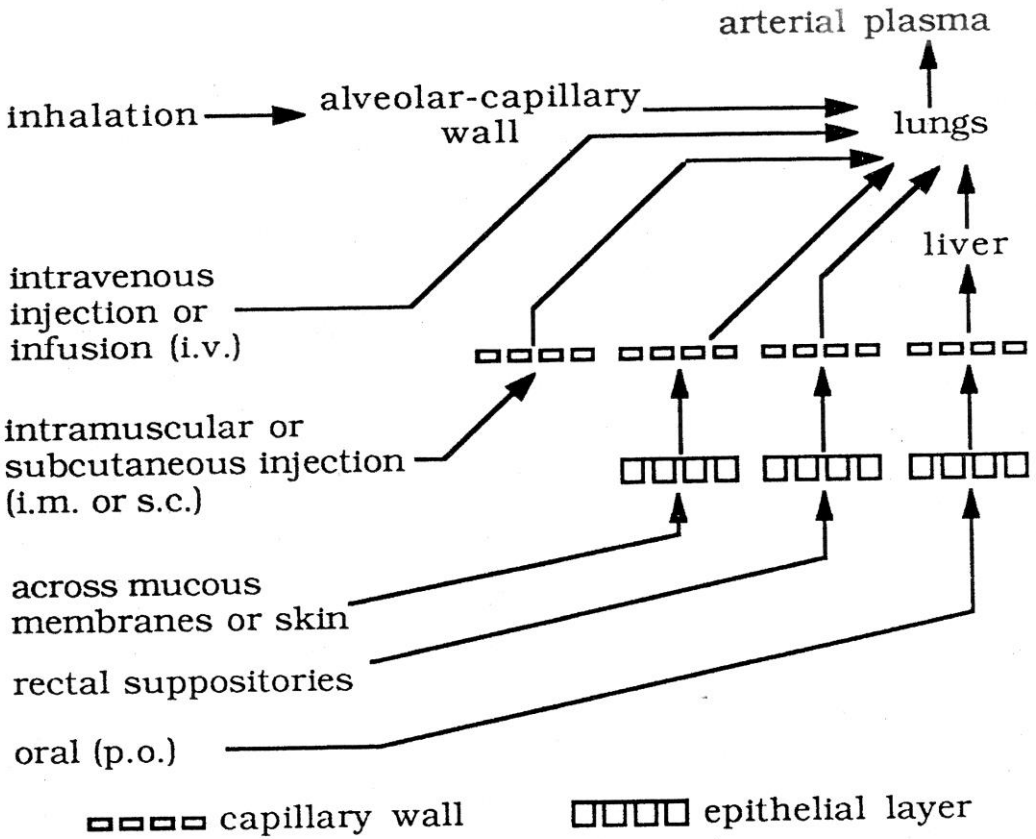
Inferior mesenteric vein

PD-EXP Gray's Anatomy Plate 591, Wikipedia

Absorption
Requires movement across multiple membranes

the movement of a drug from its site of application across biological membranes into the blood

Rate limiting step for bioavailability



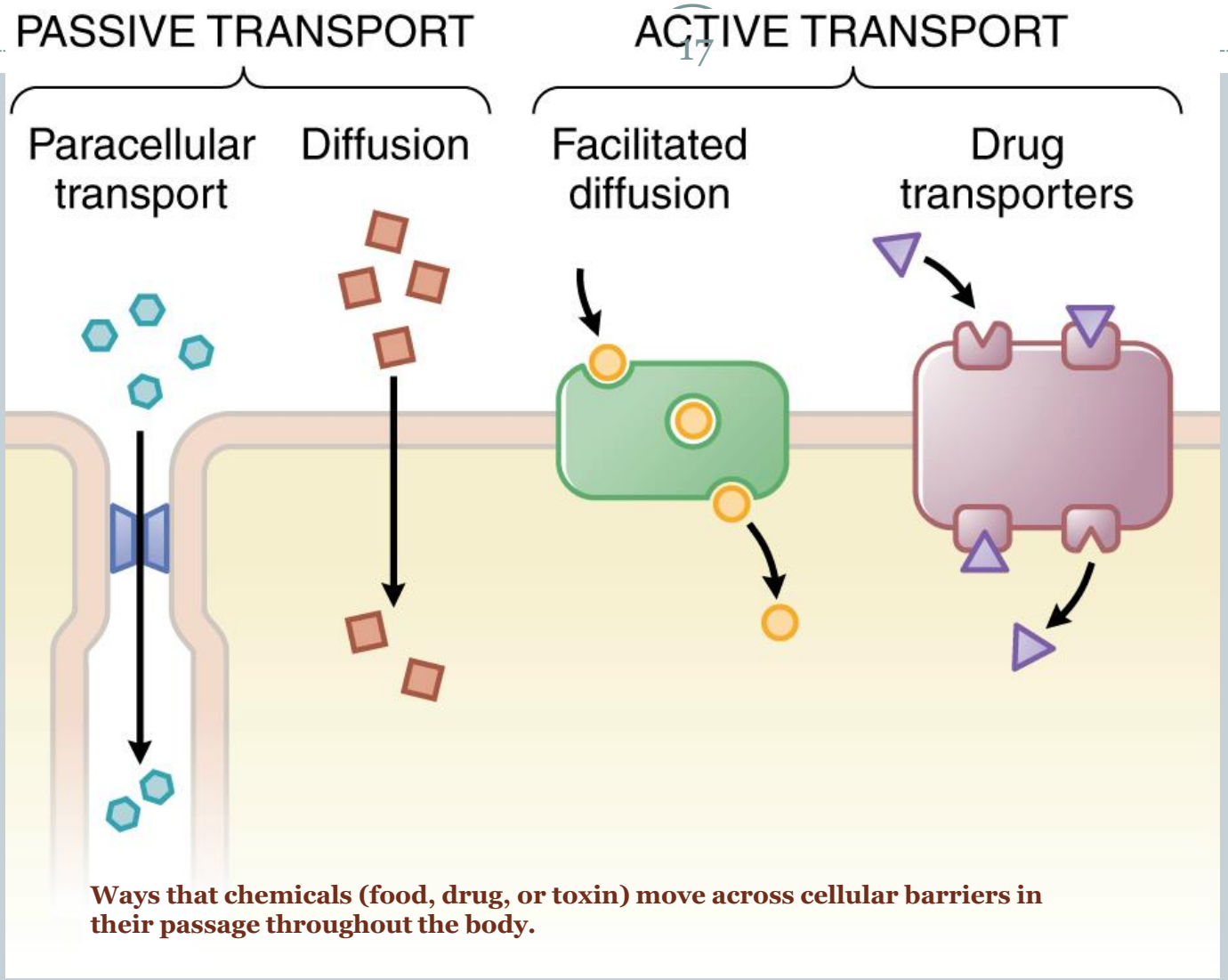
Routes of drug administration and the barriers a drug must pass to reach arterial blood plasma.

Hladky SB (1990) Pharmacokinetics Manchester University Press

Biological membranes

- The ability of a substance to pass through a membrane will depend on physico-chemical properties such as:
  - Size (molecular weight)
  - Lipid solubility
  - Similarity to endogenous molecules
  - Polarity / charge /ionisation
- These properties are also important for the interaction of the substance with its target
- **Paracellular transport** through the gaps between adjoining cells – minor in mammalian GIT
- **Diffusing directly** through the lipid probably the most important
  - Drug must be lipid soluble and non-ionised to pass the membrane
- **Aqueous pores** through the membrane allow diffusion or filtration but are too small for most compounds. Requires concentration gradient…
- **Carrier-mediated transfer**, facilitated diffusion, pinocytosis are active processes

Crossing Membranes



Ways that chemicals (food, drug, or toxin) move across cellular barriers in their passage throughout the body.

Solubility in blood

- Important to remember that the body is 70% water so compound needs to be able to dissolve in aqueous media (eg plasma) also!
  - pH is essential to solubility and stability
  - pH changes in the body
    - Stomach pH 1-3
    - intestine pH 5-8,
    - Blood pH 7.35-7.45
    - Breast milk pH 7.0 – 7.2 (7.45 colostrum)
    - CSF 7.28 – 7.32
    - Synovial fluid pH 7.7
  - This potentially changes ionisation in different fluid compartments/tissues – **ion trapping**

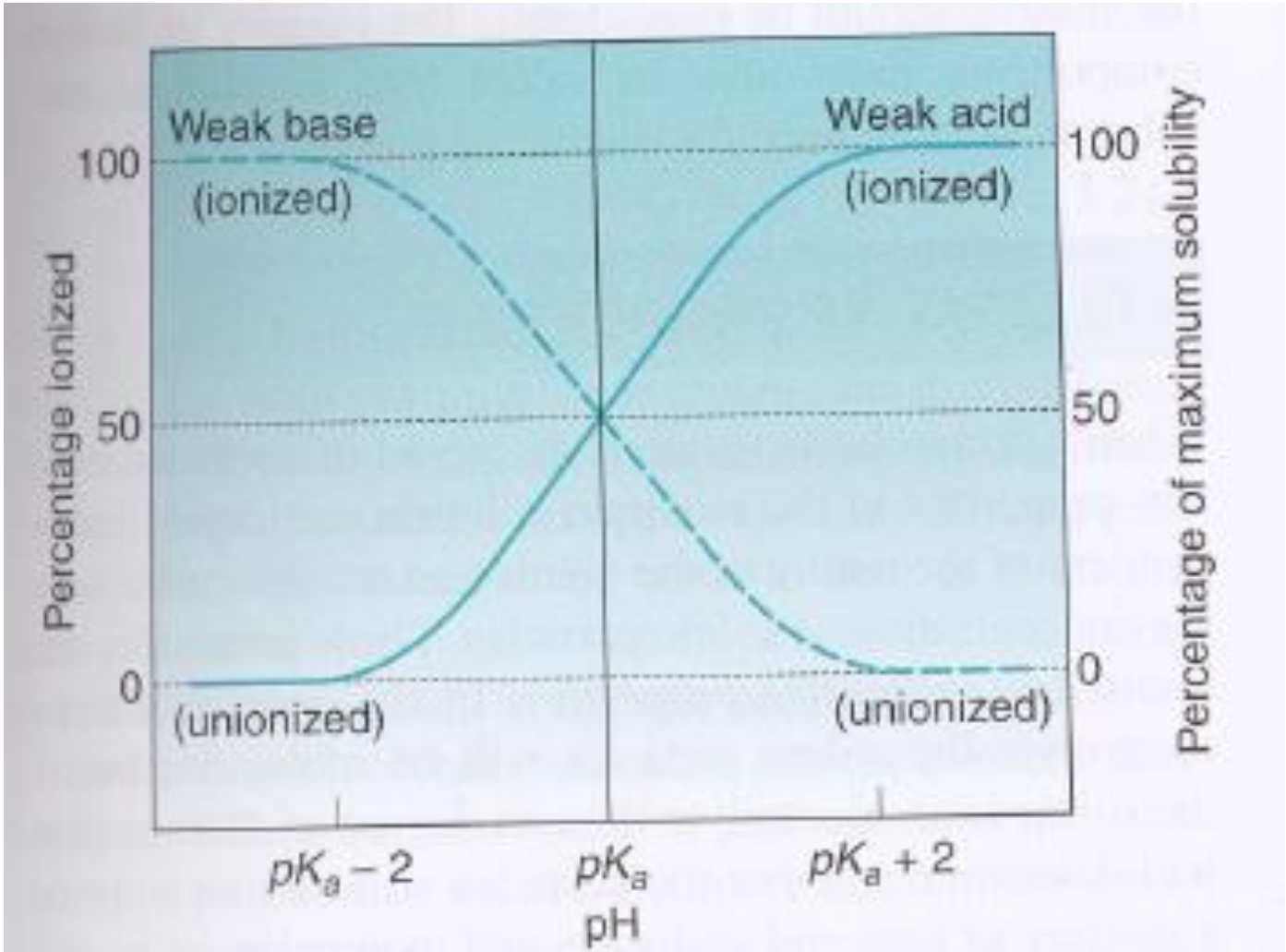


Fig. 3.1 Change in degree of ionization and relative solubility of weakly acidic and weakly basic drugs as a function of pH.

Aulton's Pharmaceutics

Drug trapping

- Changes in plasma pH will affect the degree of ionisation and hence the movement of drugs across membranes
- Normal plasma pH ≈ 7.4
  With acidosis, plasma pH may fall to 6.8
  With alkalosis, plasma pH may increase to 7.6
  - **disease states can alter distribution**
- Important in pregnancy with changes in pH on foetal side of placenta = foetal drug trapping and toxicity

Pharmacokinetics

21

A CLOSER LOOK AT

ABSORPTION

DISTRIBUTION

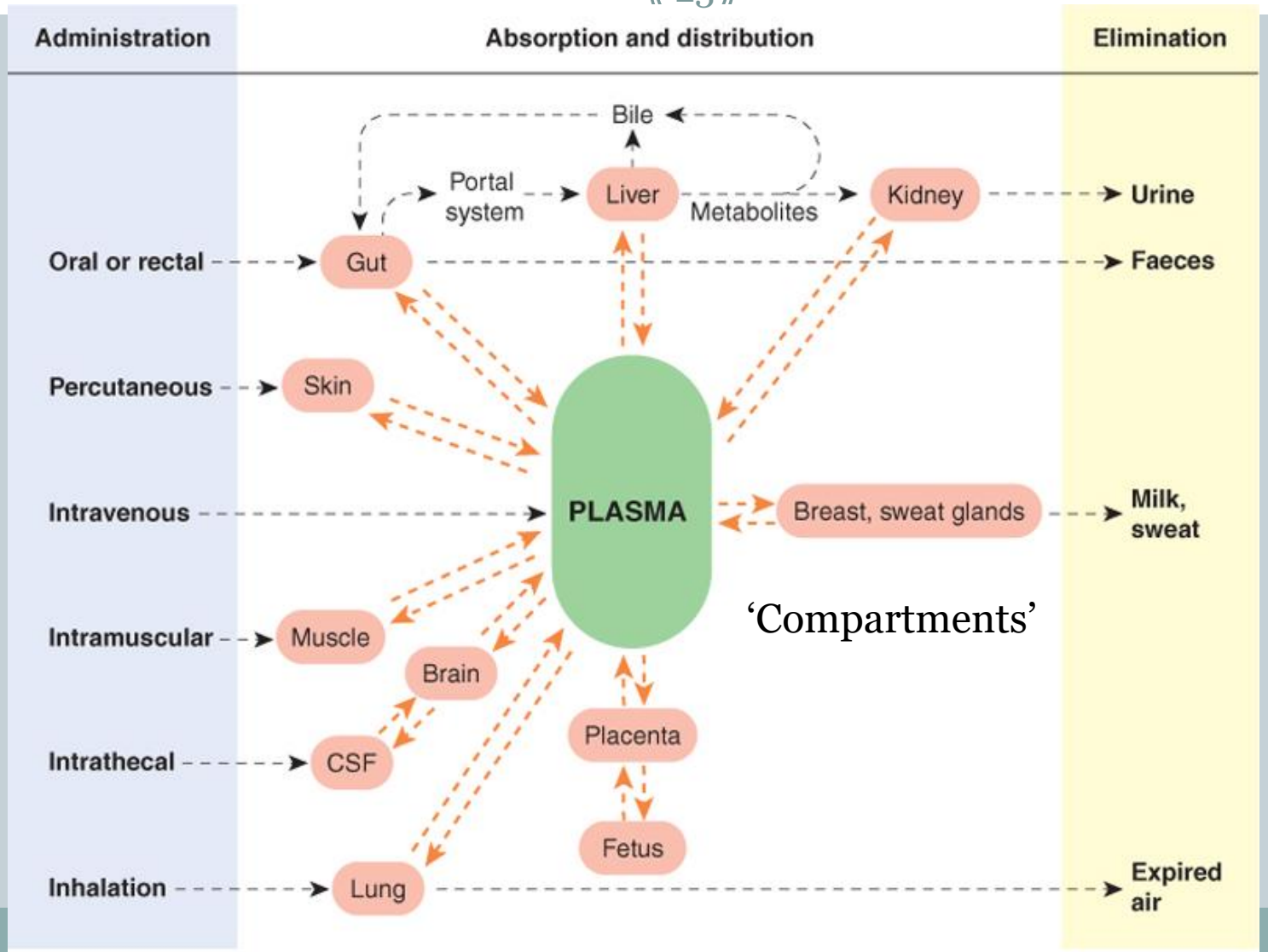
METABOLISM

EXCRETION

BartCrofts Scientific Services Pty Ltd; Bartcrofts@gmail.com

Why it matters

- Some disease states may alter the pattern of distribution and therefore the pattern of toxicity
- Distribution in pregnancy and lactation may determine foetal and neonatal toxicity
- Pathology dependent passage across the blood brain barrier may result in unexpected toxicity



© Elsevier. Rang et al: Pharmacology 6e - www.studentconsult.com

Ionisation and distribution

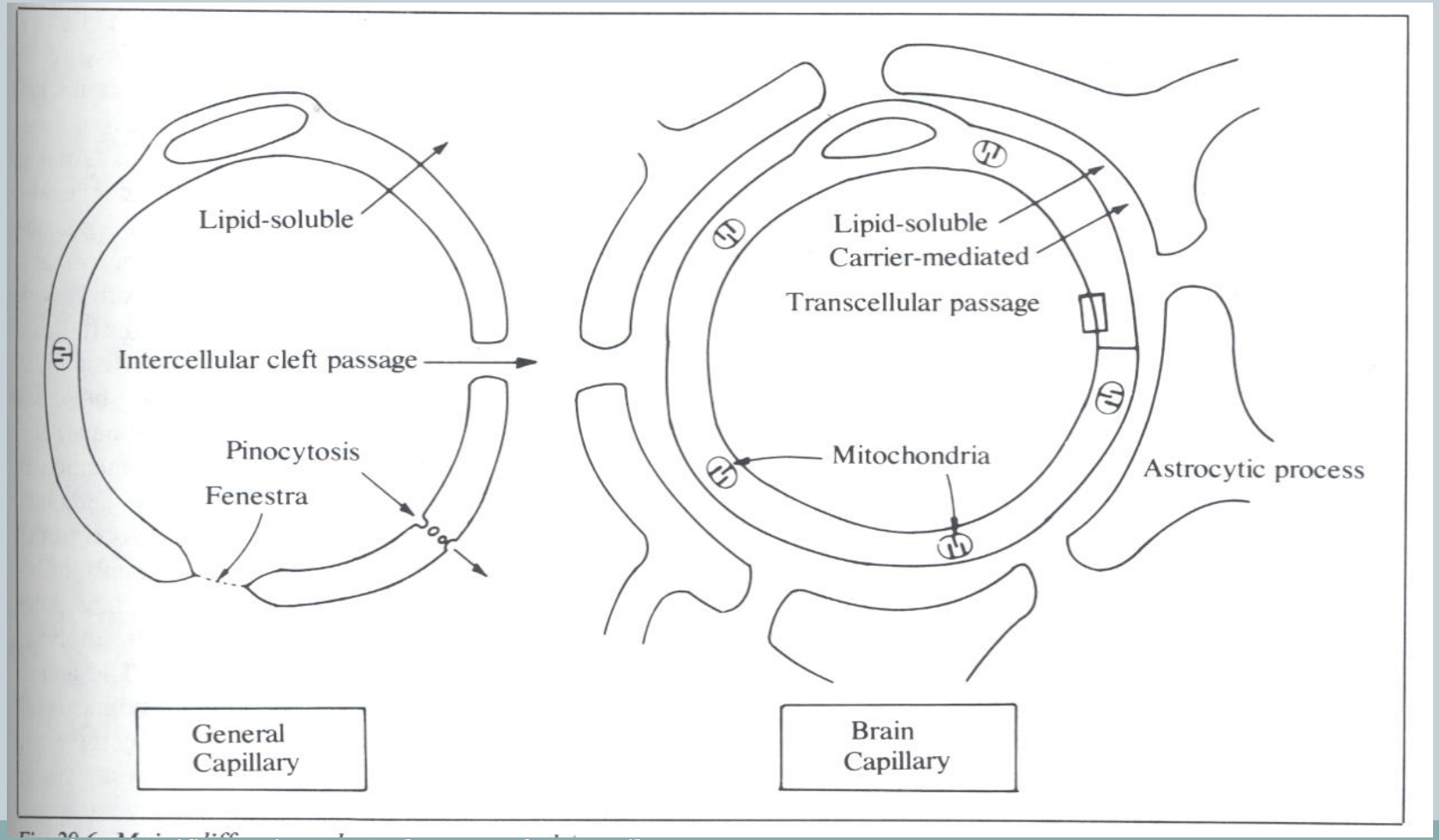
- These properties can lead to drug build-up in particular parts of the body which may effect the pattern of efficacy or toxicity
- A weakly acidic drug will concentrate in compartments with higher pH
  - ionised at higher pH so once in the compartment cannot diffuse out across lipid membrane
- Similarly a weakly basic drug will concentrate in the compartments with lower pH
- A highly lipophilic drug may find its way to the brain or adipose (fatty) tissue

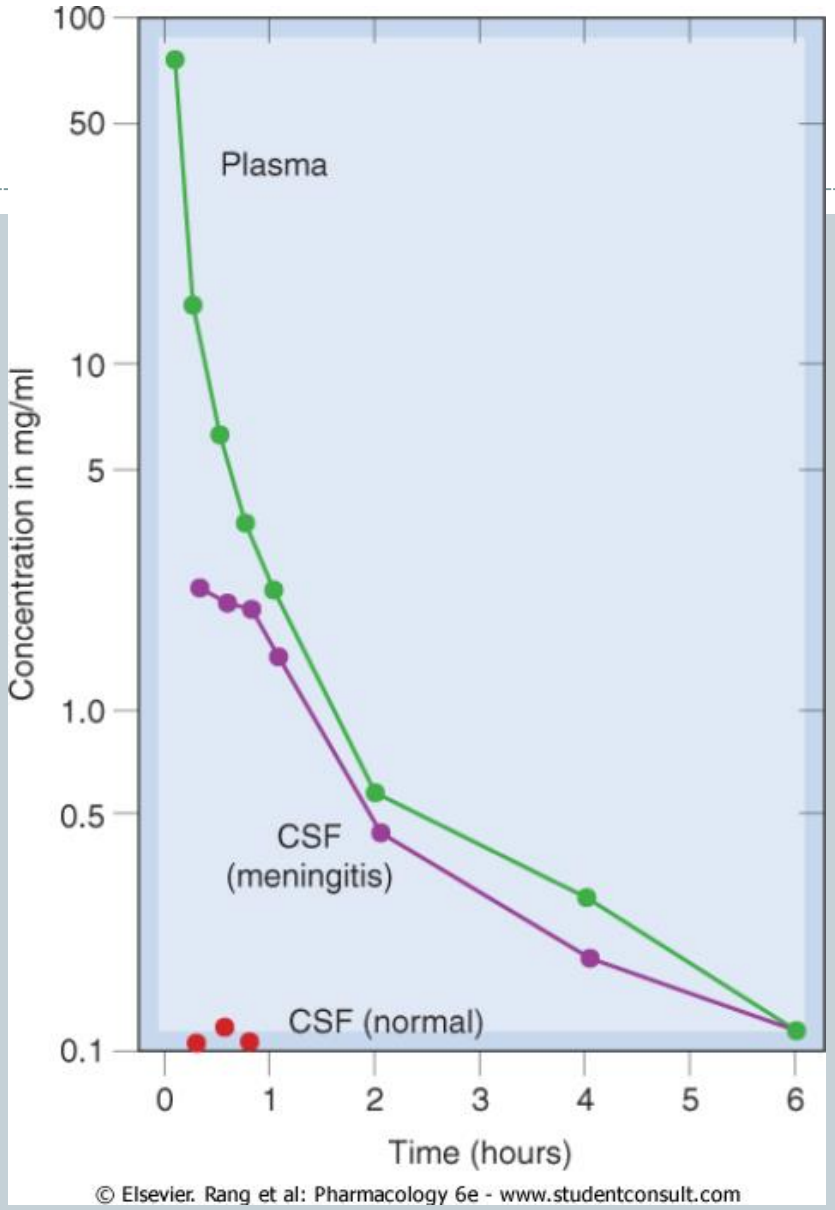
Factors opposing distribution

- Specialised barriers (eg brain, placenta)
- Binding to plasma proteins
- Distribution to storage sites (eg adipose)
- Association with intracellular proteins
- Export from cells (eg MDR transporter)

Blood-brain barrier

Craig (1986) Molecular Pharmacology. Little, Brown & Co.





Disease can alter drug distribution

Movement of the antibiotic thienamycin into cerebrospinal fluid

- CSF Level in healthy Individual
- CSF Level in patient with Meningitis

The toxicity of a contaminant may be dependent on the disease state of the patient

Pharmacokinetics

28

A CLOSER LOOK AT

ABSORPTION

DISTRIBUTION

METABOLISM

EXCRETION

Why it matters

- A contaminant might induce or inhibit an enzyme important for the activation or deactivation of another API
- Metabolism is a major determinant of toxicity
- Many individual factors affect the pattern and rate of metabolism in a patient (age, sex, disease etc)
- Some patient subpopulations may be more or less susceptible to toxicity as a consequence
- Variation mostly covered by Uncertainty/ Adjustment factors but a PDE assessor will need to be alert for potential exceptions.

A long and Winding Road from Dose to Destination

Metabolism occurs at multiple sites other than the liver

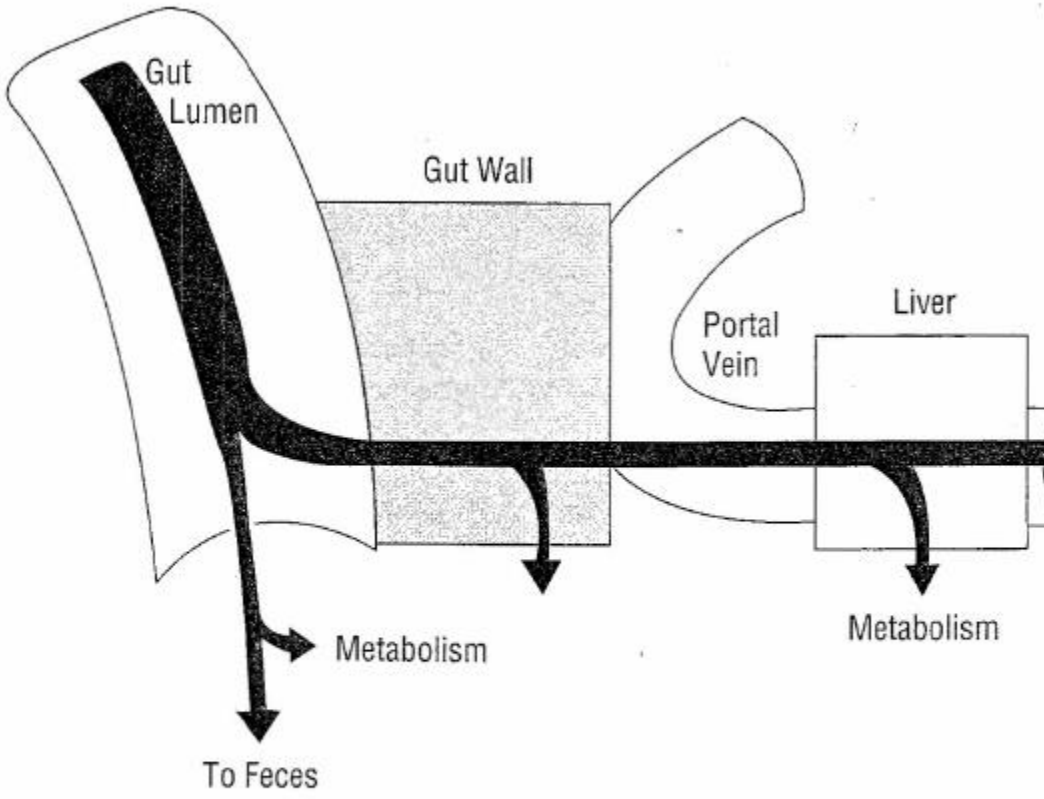


Fig. 2–2. A drug, given as a solid, encounters several barriers and sites of loss in its sequential movement during gastrointestinal absorption. Incomplete dissolution or metabolism in the gut lumen or by enzymes in the gut wall is a cause of poor absorption. Removal of drug as it first passes through the liver further reduces absorption.

Rowland and Tozer

Why metabolise Xenobiotics (drugs & natural and synthetic chemicals)

- The characteristics of drugs and chemicals that enable them to be readily absorbed also often make them hard to excrete.
- Drugs for example are usually lipid soluble, weak organic acids and bases that are not readily eliminated
  - this is good otherwise how would drugs get to their site of action!!
- In order to excrete these substances therefore their structure needs to be altered
- Biotransformation reactions generally result in metabolites that are more polar, more hydrophilic and therefore more soluble
- This allows them to be filtered at the glomerulus of the kidney without subsequent reabsorption resulting in renal elimination
- If drugs are unable to leave the body their build-up can lead to toxicity…eg barbital is only about 10% metabolised. Sedation can persist for up to 7 days after a single dose

Where the action is

- We generally think of metabolic enzymes as residing in the liver and this is certainly where the largest concentration and activity of these enzymes are found.
- BUT ALL tissues have some metabolic capability and some can be quite active including those of the gut, lungs kidneys and skin.
- Many metabolic enzymes have important endogenous functions such as the production of hormones

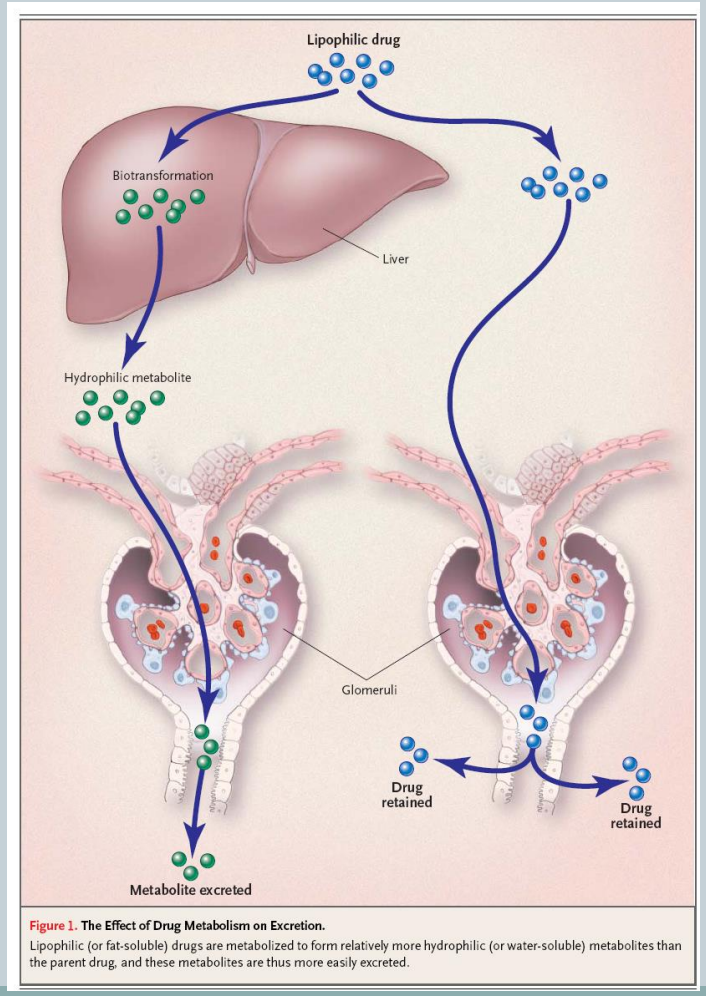
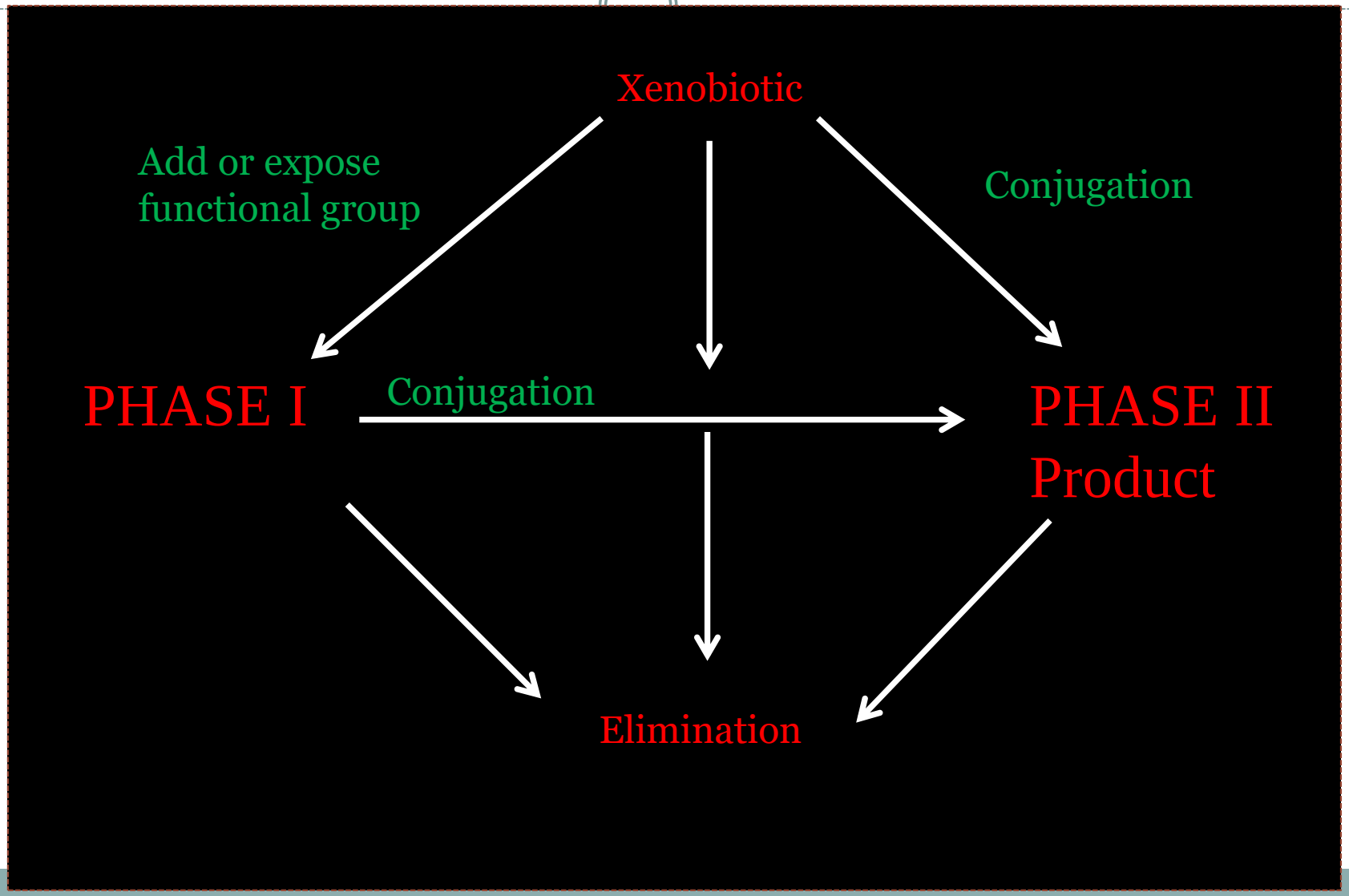


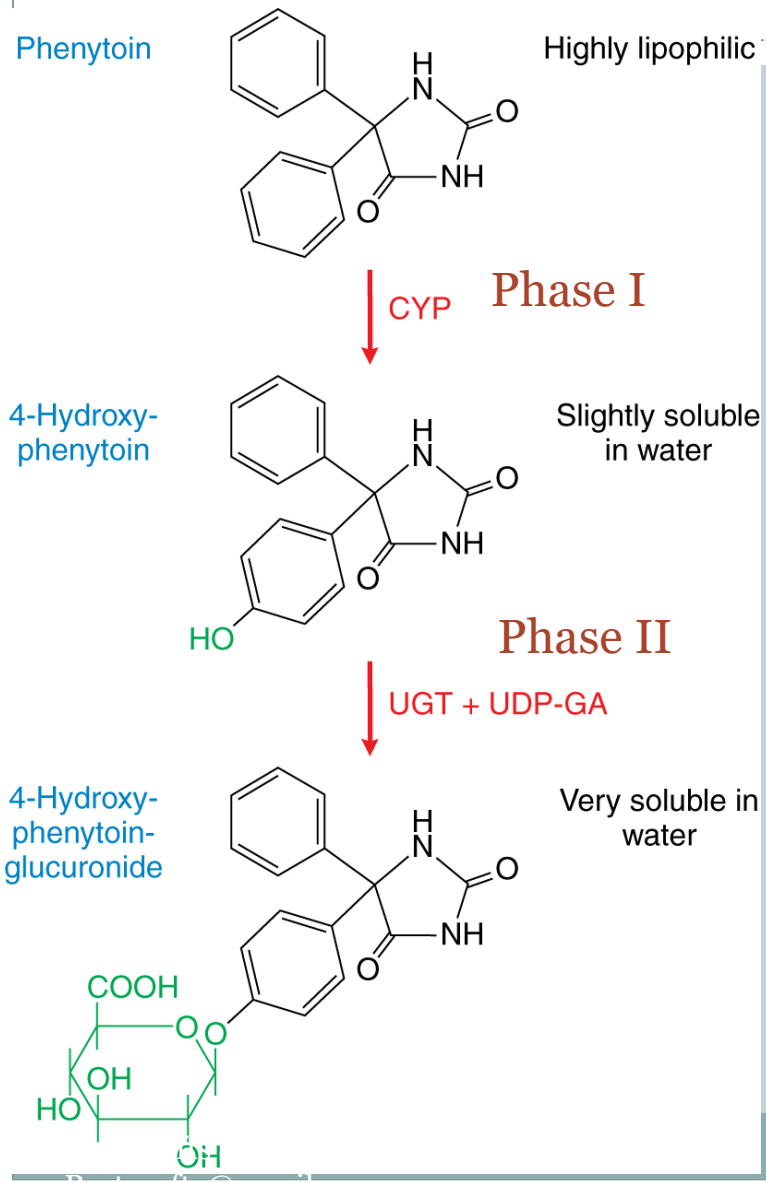
Figure 1. The Effect of Drug Metabolism on Excretion.
Lipophilic (or fat-soluble) drugs are metabolized to form relatively more hydrophilic (or water-soluble) metabolites than the parent drug, and these metabolites are thus more easily excreted.

Weinshilboum (2003) NEJM 348: 529

Metabolism Divided into Phase I & II



An Example



Benzene rings limit polarity and don't like to share electrons

Substitution with an hydroxyl group helps… it will share electrons and form hydrogen bonds improving water solubility

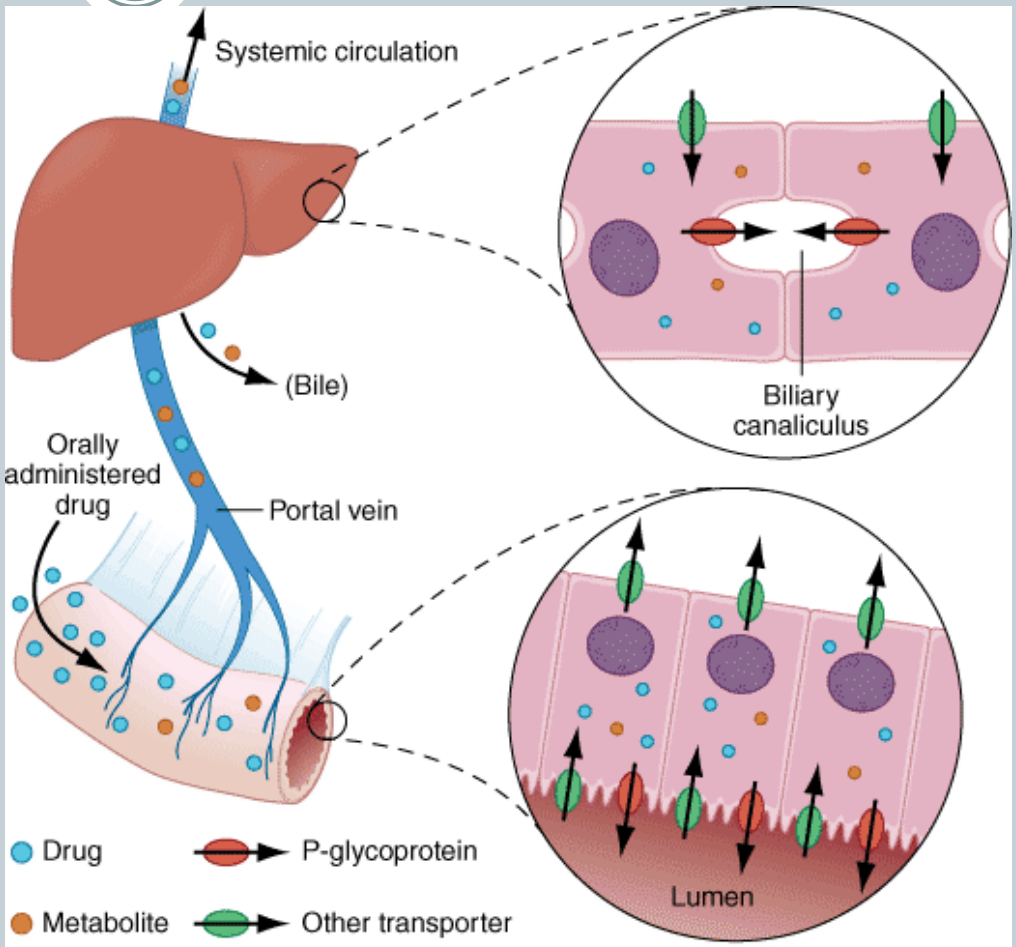
Conjugation adds Lots of hydroxyl groups willing to form hydrogen bonds greatly increasing solubility

UGT - Uridine 5'-diphospho-glucuronosyltransferase,
UDP-GA – UDP-glucuronic acid

First-pass effect (metabolism, extraction, clearance)

Extent to which a drug is removed (and inactivated) from the bloodstream on its first passage through the liver (normally coming in via portal vein)

Can be a major problem for oral administration



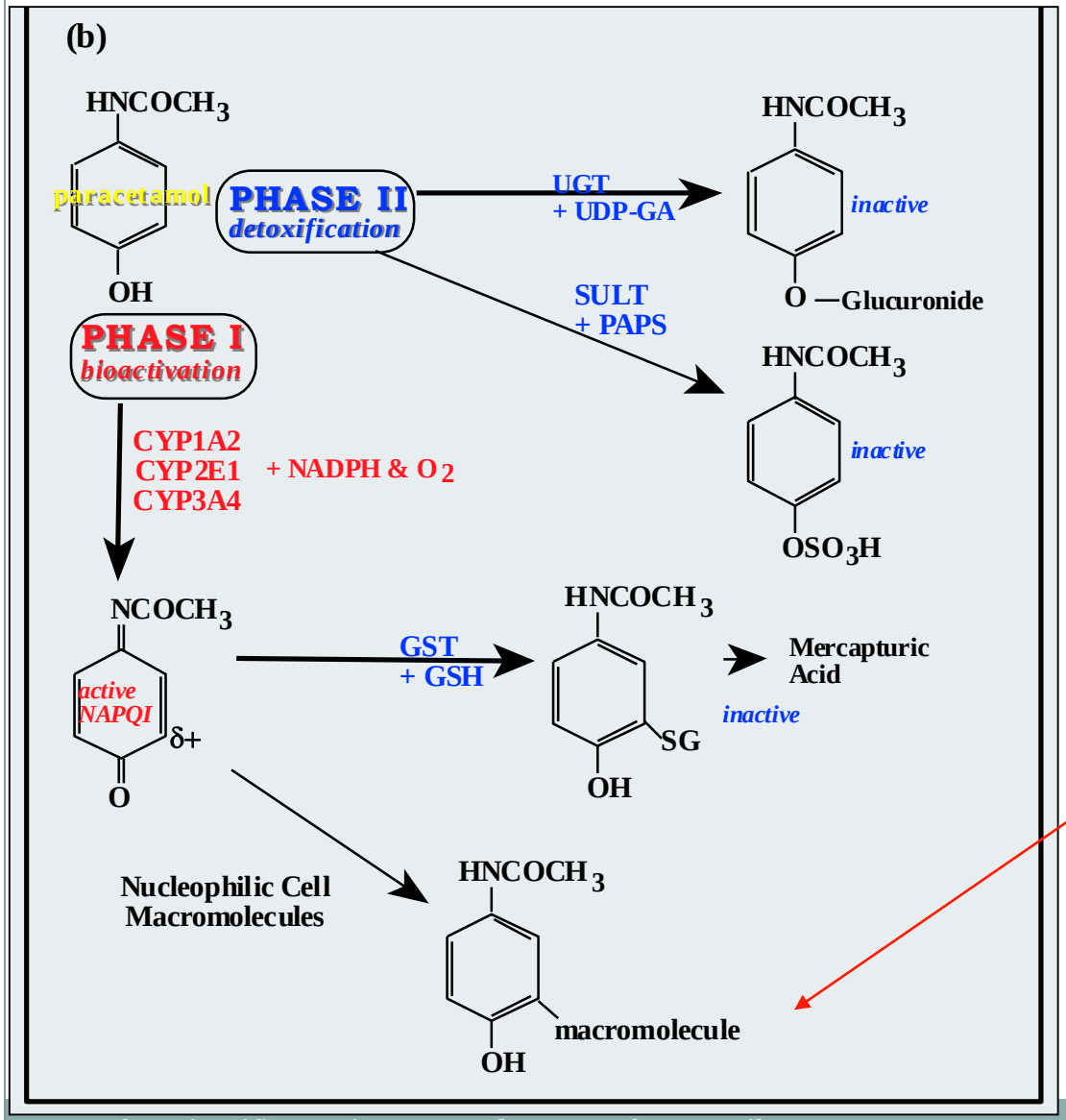
Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

Copyright © The McGraw-Hill Companies, Inc. All rights reserved.

Advantages and Disadvantages of metabolism

- Rapid first pass metabolism can
  - Break down a toxicant before it can reach a systemic target
    - This is protective,
  - limit the amount of drug getting to the required target
    - This would limit the therapeutic effectiveness of a drug
    - Sometimes we need to design drugs to be resistant to metabolism
- Some toxicants and some drugs require metabolism before they are active, this is called bio-activation
  - Drugs that require bio-activation are called prodrugs
    - Eg the active form of Minoxidil (Rogaine) is the sulfate metabolite

Paracetamol metabolism



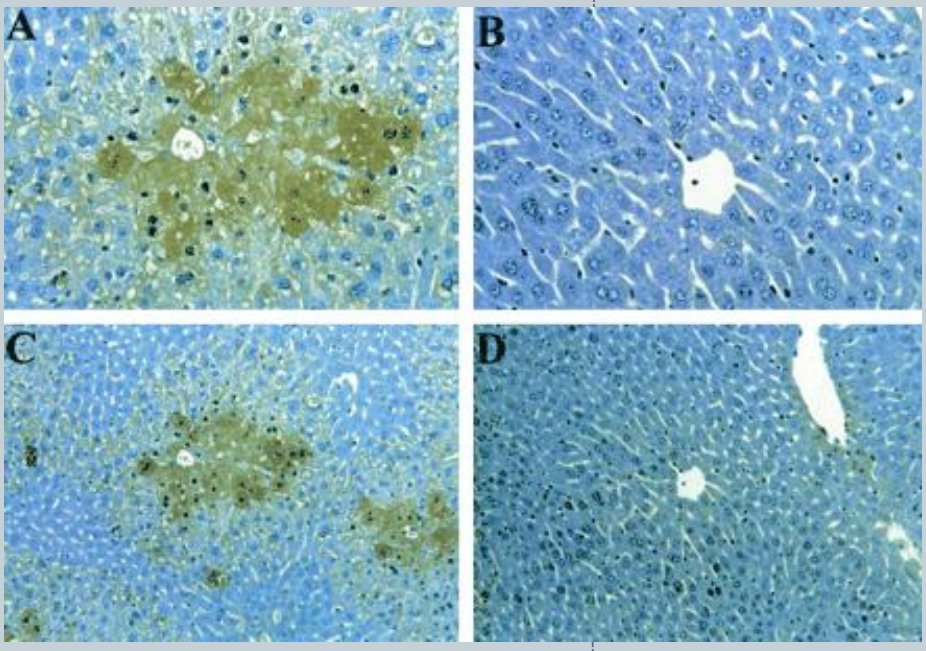
NAPQI - N-acetyl-p-benzoquinone imine

NAPQI is slightly electron deficient
Reacts with electron rich functional
Groups – eg thiols – forming an
Adduct – this damages structures &
Marks protein for destruction - disrupts
Intracellular homeostasis

TOXIC –
damages liver

Adapted from Park *et al.* (1995) Pharm Therap 68(3): 385-424 for Shield (2000) PhD thesis

WHAT HAPPENS WHEN IT GOES WRONG



A, mouse treated with 300 mg/kg paracetamol and sacrificed 4 h later (40×). The brown stain shows protein adducts. **B**, normal liver (40×). **C** paracetamol (20×). **D**, normal (20×). Injury pattern in the tissue reflects distribution & activity of specific CYP 450

James 2003 DRUG METABOLISM AND DISPOSITION. Vol. 31, No. 12

Factors influencing metabolism

- Age
- Gender
- Genetics
- Nutritional status
- Disease
- Inhibition
- Induction
- All these parameters may be different for a receiving (follow on) product compared to the intended population for the donating product

TABLE 1

Approximate incidence of the major pathways of drug elimination

Pathway	Incidence
	%
Renal unchanged	25
P450 metabolism	
CYP3A4	30
CYP2D6	20
CYP2C9/19	10
Glucuronidation	10
Other[a]	5

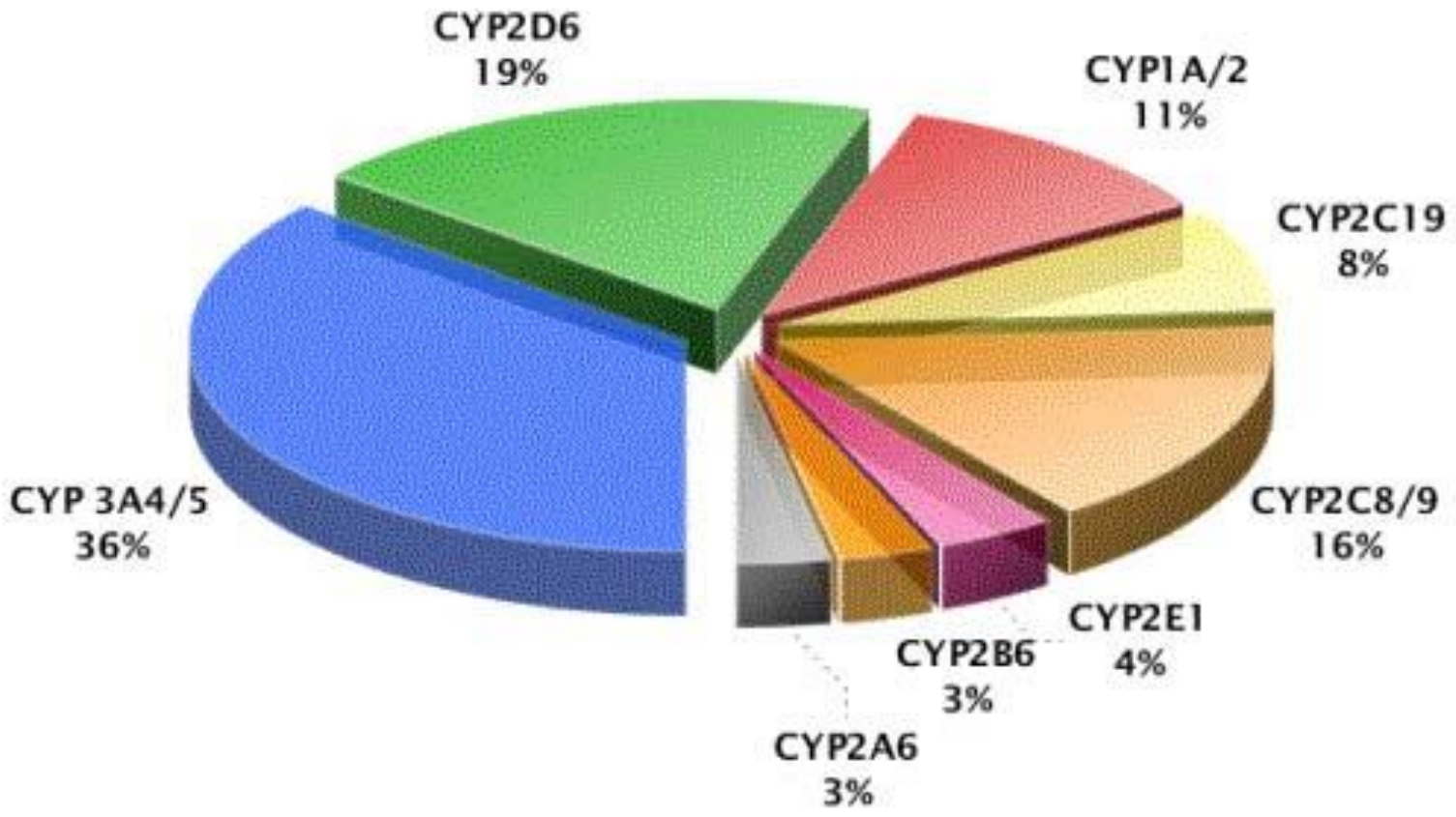
[a] Other includes acetylation, thiopurine methyltransferase, and dihydropyrimidine dehydrogenase.

Gardiner and Begg (2006) , Pharmacol Rev 58:521-590

Phase I – Addition or exposure of a functional group

- Addition or exposure of a functional group by oxidation, reduction or hydrolysis
  - -OH, -COOH, -NH_2, -O-, -SH
- Oxidation is the most common mechanism
- Cytochrome P450 family is dominant
  - 60+ functional genes in the human
    - 18 subfamilies
    - 60 pseudogenes
  - Found in most species from bacteria to plants and mammals
- Others include - dehydrogenases, monoamine oxidase, esterase, flavin-containing mono-oxygenase and epoxide hydrolase…

Proportion of Drugs Metabolized by P450 Enzymes



Adapted from: Wrighton SA et al. Crit Review Toxicology 1992;22:1-22.
Kashuba and Bertino. Mechanisms of drug interaction. In Drug Interactions in Infections Diseases. Humana Press. 2001.

Chemicals/Drugs that become **MORE** toxic through Metabolism

Table 6-3
Examples of Xenobiotics Activated by Human P450 Enzymes

CYP1A1
Benzo[a]pyrene and other polycyclic aromatic hydrocarbons

CYP1A2
Acetaminophen (Paracetamol)
2-Acetylaminofluorene
4-Aminobiphenyl
2-Aminofluorene
2-Naphthylamine
NNK*
Amino acid pyrrolysis products (DiMeQx, MeIQ, MeIQx, Glu P-1, Glu P-2, IQ, PhIP, Trp P-1, Trp P-2)
Tacrine

CYP2A6
N-Nitrosodiethylamine
NNK*

CYP2B6
6-Aminochrysene
Cyclophosphamide (Anticancer)
Ifosphamide

CYP2C8, 9, 18, 19
Tienilic acid
Valproic acid (Anticonvulsant)

CYP2D6
NNK*

CYP2E1
Acetaminophen
Acrylonitrile
Benzene
Carbon tetrachloride
Chloroform
Dichloromethane
1,2-Dichloropropane
Ethylene dibromide
Ethylene dichloride
Ethyl carbamate
Halothane
N-Nitrosodimethylamine
Styrene
Trichloroethylene
Vinyl chloride

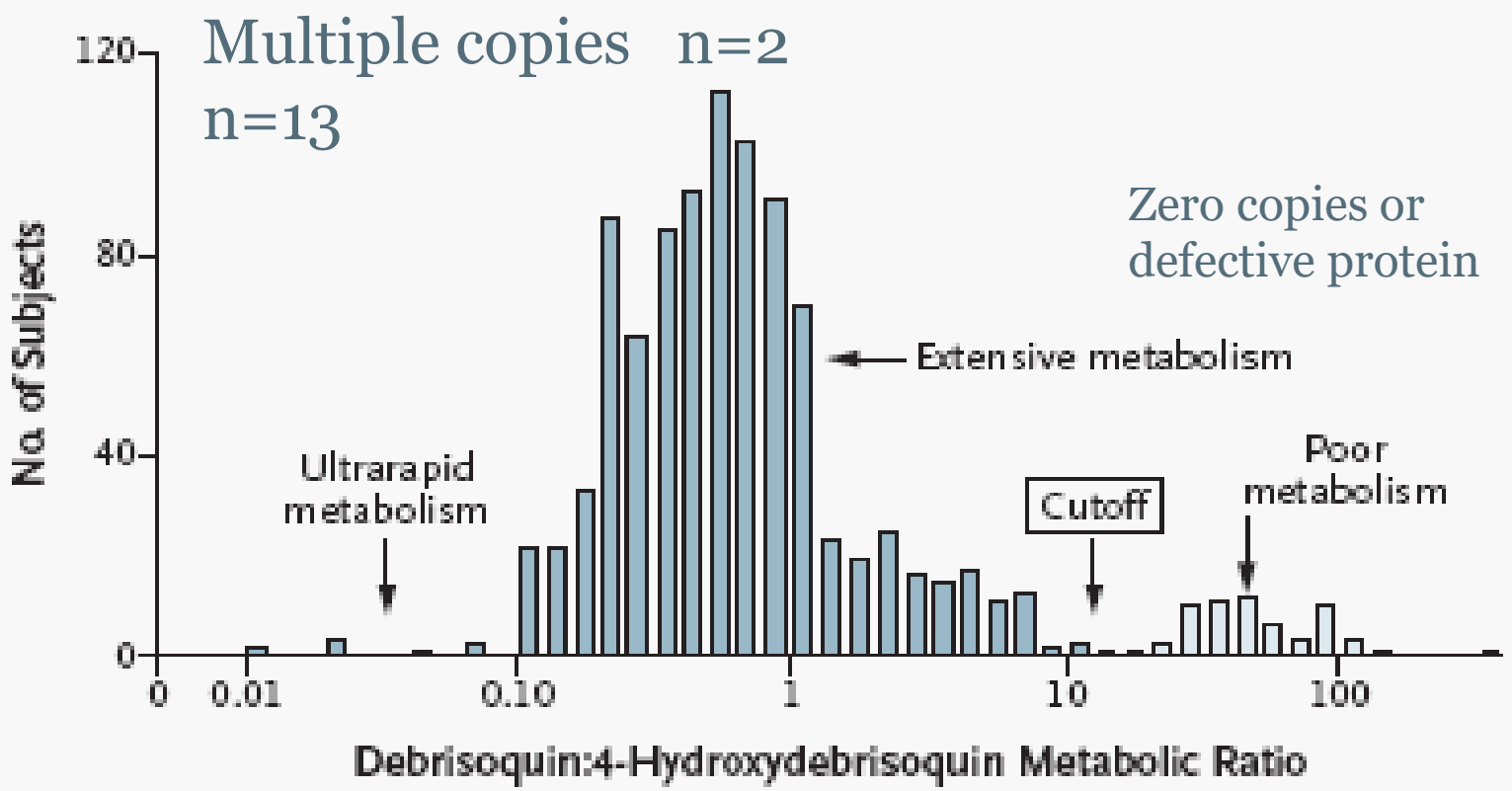
CYP3A4
Acetaminophen
Aflatoxin B_1 and G_1
6-Aminochrysene
Benzo[a]pyrene 7,8-dihydrodiol
Cyclophosphamide
Ifosphamide
1-Nitropyrene
Sterigmatocystin
Senecionine
Tris(2,3-dibromopropyl) phosphate

CYP4A9/11
None known

*NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, a tobacco-specific nitrosamine.

Cassaret & Doull's Toxicology

Substantial Genetic Polymorphism Creates Variation Across Populations



Data from 1970's

Figure 3. Pharmacogenetics of CYP2D6.

Urinary metabolic ratios of debrisoquin to its metabolite, 4-hydroxydebrisoquin, are shown for 1011 Swedish subjects. The Cutoff box indicates the cutoff point between subjects with poor metabolism as a result of decreased or absent CYP2D6 activity and subjects with extensive metabolism. Modified

Weinshilboum (2003)NEJM 348:529

Phase II Conjugation

- Common/Important Conjugation systems
  - UDP-glucuronosyl transferase (UGT)
  - Glutathione S-transferase (GST)
  - Sulfotransferase (SULT)
  - Methyltransferase
  - N-acetyltransferase
- Involves conjugation with a large polar molecule to increase solubility eg glutathione, sulfonate, methyl, glucuronyl group

UDP glucuronyl-transferases (UGT)

- Most prominent Phase II enzyme based on urinary metabolite profiles
- Cofactor is uridine diphosphoglucuronic acid (UDP)
- At least 17 functional UGT enzymes: 4 families
- Isoform distribution (& activity) varies across tissues

Sulfotransferases (SULT)

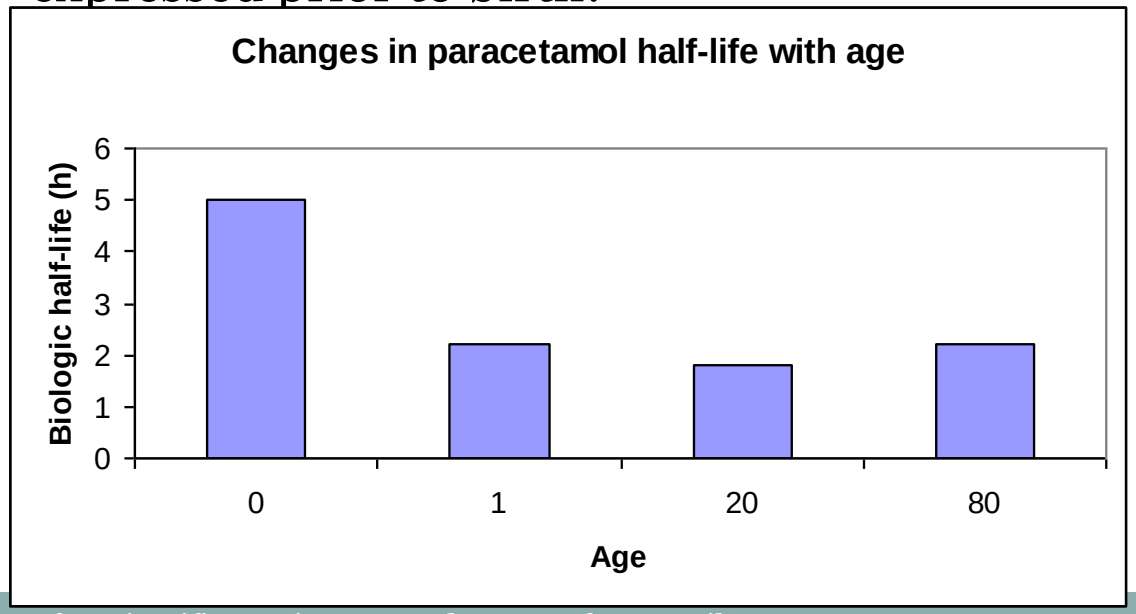
- Family of 10 known cytosolic enzymes in 3 families (SULT1, SULT2, SULT4)
- SULT1 isoforms metabolise phenolic compounds including paracetamol, estrogens, dopamine
- SULT2 isoforms metabolise steroids
- Found in the cellular cytosol

GSH & Glutathione Transferase (GST)

- 8 families of cytosolic enzymes in humans:
  - A, K, M, P, S, T, Z, O
- GSH is found in high concentrations in cell cytoplasm (10-25 mM)
- GSH can react directly (non enzymatically) with electrophiles and oxidants
- GST and GSH Prefer electrophilic substrates such as epoxides (e.g. styrene oxide) and activated polyaromatic hydrocarbons
- They have an important endogenous role – protecting against products of oxidative stress
- Reactive electrophiles can bind to the thiol groups of proteins that are critical to the structural integrity and functionality of cellular proteins.
- Glutathione provides a large pool of free thiol that can react directly with these electrophiles and therefore protect the proteins.

Effects of AGE

- Many drugs metabolised by UGTs eg morphine, paracetamol, diazepam
- Also important for metabolism of bilirubin
- Inability to do this causes yellowish skin tone which can be an indication of liver damage
- Why jaundice in newborns?
- UGT levels don't reach 'adult' levels for 2-4 years and are not expressed prior to birth!



In adults paracetamol is metabolised 60% by UGT's and 35% by SULT's – this relationship is reversed in children!

Effects of INHIBITION

- One chemical blocks the ability to metabolise another – the result
  - Less first pass metabolism
  - Decreased clearance
  - Higher plasma concentration
  - Longer $t_{1/2}$
  - Altered activity/toxicity

http://www.powernetdesign.com/grapefruit/

- Eg Grapefruit inhibits CYP3A4
- Lovastatin blood levels are increased by up to 12 X

Effect of ENZYME INDUCTION

- Occurs when increased synthesis of metabolic enzymes is triggered
- The effect of induction is
  - Increased first pass metabolism
    - Could be increased bio-activation to a toxic metabolite
  - Increased clearance
  - Decreased plasma concentration
  - Decreased $t_{1/2}$
  - Altered activity/toxicity
- Could be life threatening eg. for an anticoagulant

Pharmacokinetics

A CLOSER LOOK AT

ABSORPTION

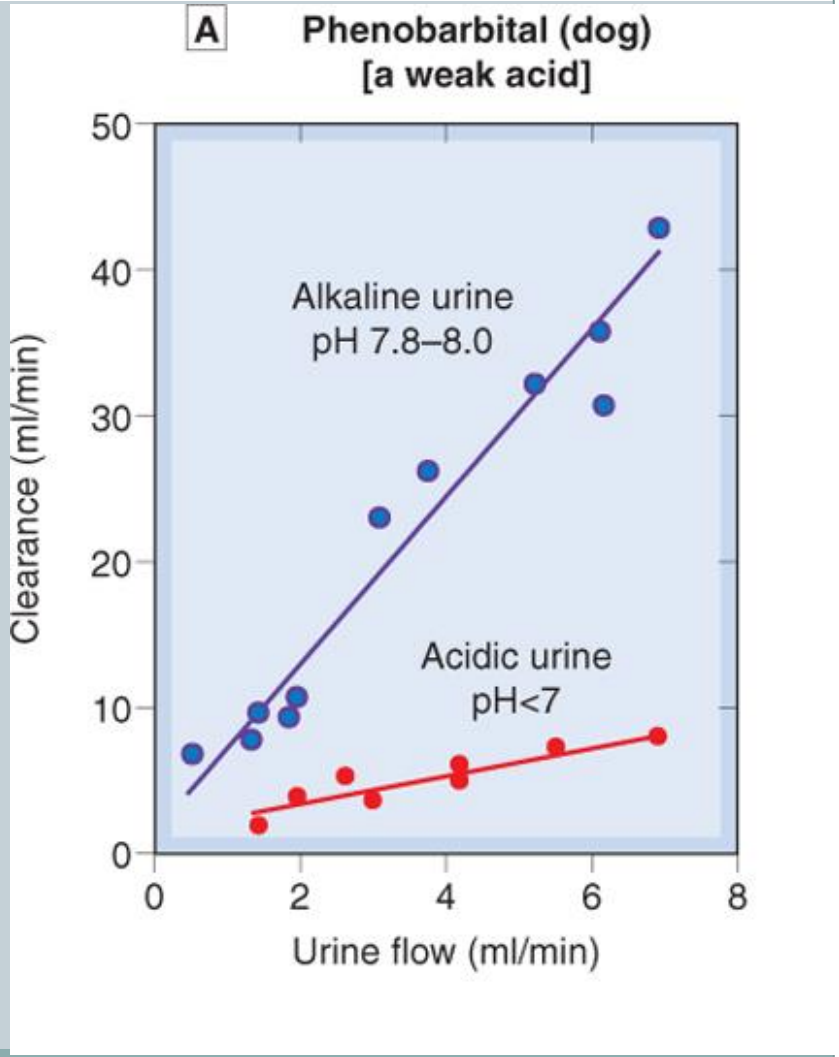
DISTRIBUTION

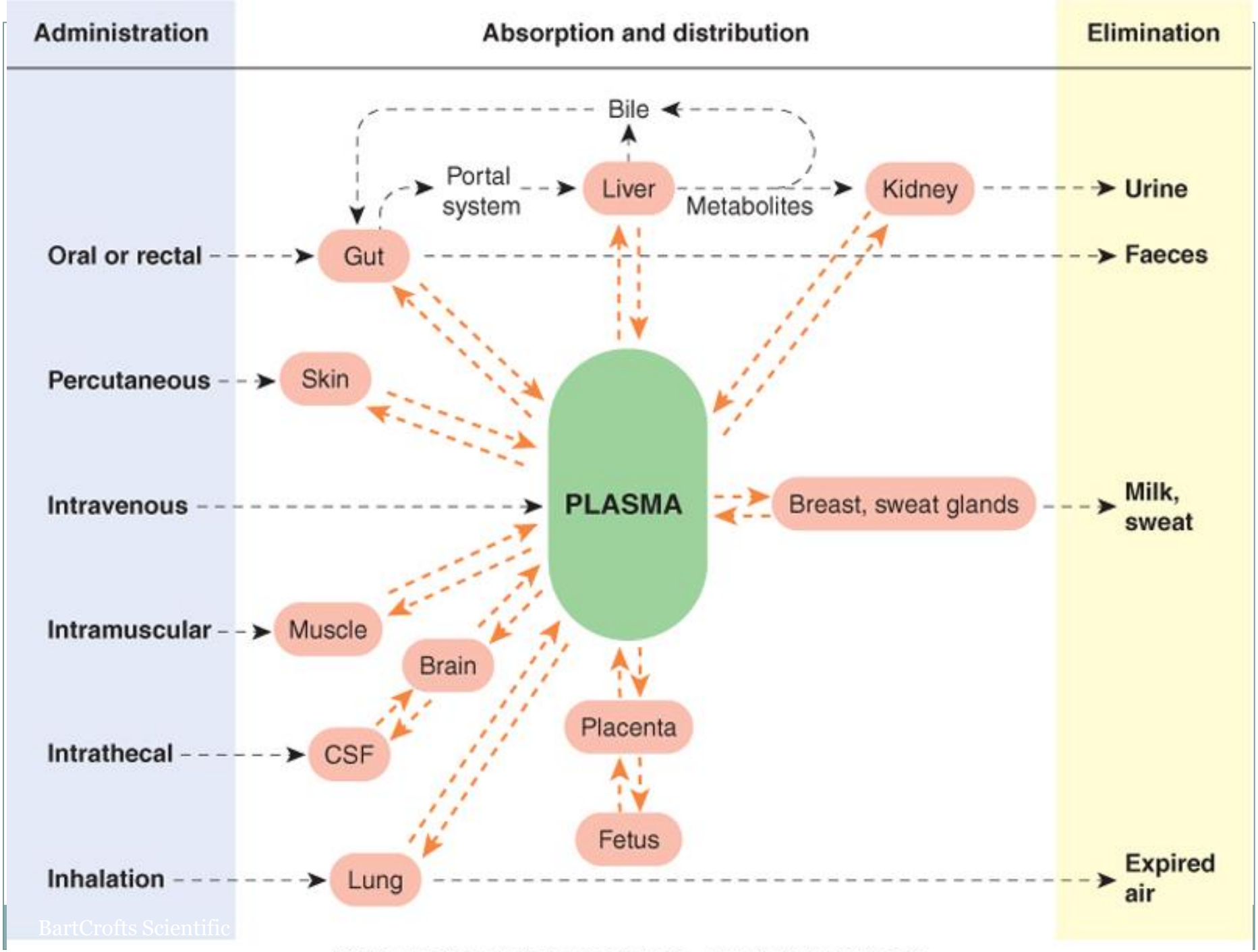
METABOLISM

EXCRETION

Why it matters

- Renal elimination is sensitive to urinary pH, & effects on active tubular secretion processes
- An API may alter the renal elimination of a contaminant – prolonging exposure for eg – and *vice versa*
- Similar issues apply to biliary excretion

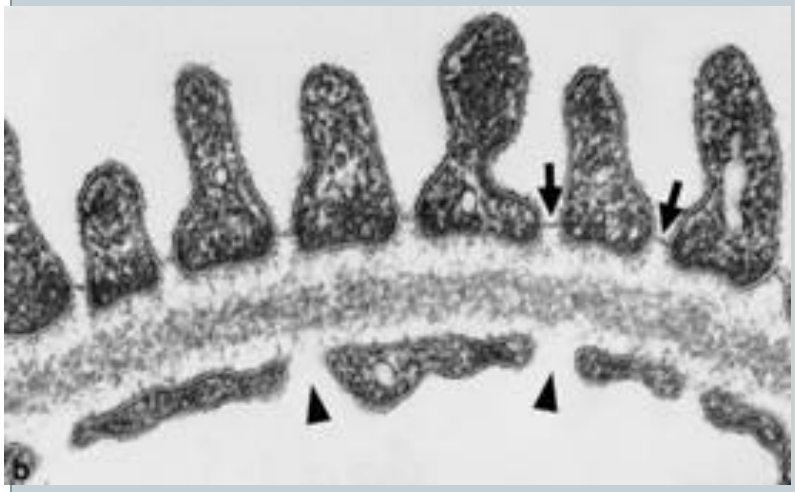


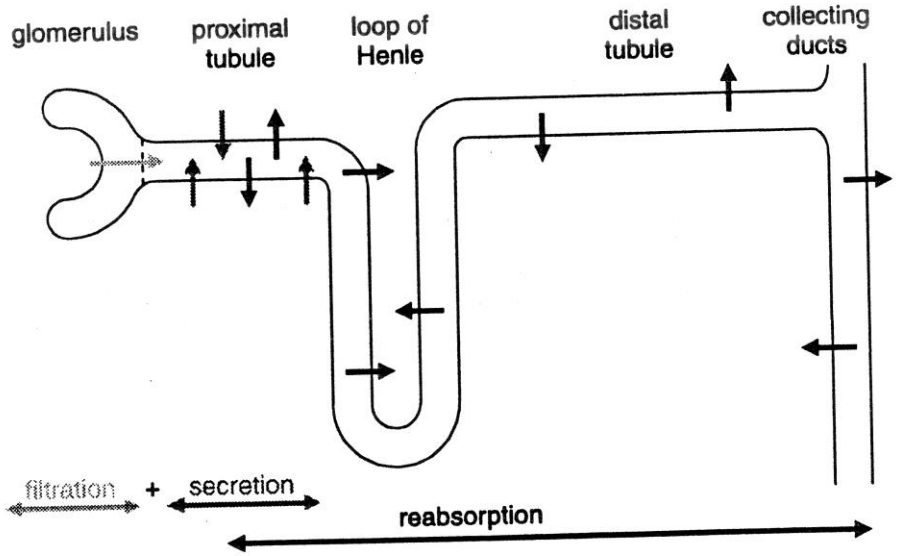


BartCrofts Scientific

© Elsevier. Rang et al: Pharmacology 6e - www.studentconsult.com

Renal Drug Elimination





Renal drug elimination

Renal drug clearance is the net result of filtration clearance (at the glomerulus) plus clearance by active secretion (in the proximal tubule) minus reabsorption which occurs all along the renal tubule.

Birkett, Pharmacokinetics Made Easy

Renal excretion

- Again polarity and ionisation states are critically important for most chemicals - There are three main processes:
  - glomerular filtration (+)
  - active tubular secretion (+)
  - passive tubular reabsorption (-)
  - Active secretion may be blocked by other chemicals leading to prolonged half lives – eg probenecid and ampicillin
- These processes rely on:
  - concentration gradients to drive compounds into urine
  - renal blood flow
  - active transport pumps
  - urine pH and flow rate
- Lipid soluble drugs may be reabsorbed back into the blood stream

Biliary excretion

- This route is important for large polar substances
- Alternative route out of the liver
- Active transporter important here
- Ultimately excreted in faeces
- Also susceptible to reabsorption (entero-hepatic [re]circulation)
- Can increase ‘half life’ (an elimination parameter) of compounds…

Elimination Versus Excretion

- Elimination
  - Removal of active drug or chemical from the systemic circulation - May be through
    - Metabolism causing inactivation
    - Sequestration in unresponsive tissues (eg distribution to fat)
    - Or passage out of the body in urine or bile
  - Excretion
    - Removal of the substance completely from the body
- Key measures
  - Half Life or t½
    - Time taken for the amount of (active) drug in the plasma to decrease by half
  - **Clearance**
    - The amount of blood completely cleared of drug over a given time
    - Can be divided into renal, hepatic other clearance

Why Pharmacokinetics Matters

- Dose comparisons between animals and man are based on comparative pharmacokinetics (PK) across species
  - Dose extrapolation across species is a critical aspect of PDE determination
- PK differences are a (the?) major determinant of interspecies differences in toxicity
- The amount of a chemical in the blood stream (Systemic exposure) is dependent on the PK of the route of administration (oral versus inhalation versus iv etc)
  - Sometimes the calculation of a PDE for one route of exposure will depend on data from another route of administration
- PK interactions between a contaminant and the API of a medicine may cause toxicity
  - Unlikely at very low exposures but there are always potential exceptions
- Disease states and physiological conditions (eg pregnancy) can alter PK and therefore toxicity
  - Contamination of an antibiotic used for meningitis may result in the contaminant crossing the blood brain barrier when it would not otherwise – unique toxicological effects

Key Considerations for API Risk Assessment

- Is there a specific patient group related to potentially contaminated product(s) that could be at higher risk from the API as a contaminant because of PK issues
  - Specific P450 inhibition
  - Toxicity related to Blood Brain or other Barrier impairment
  - Liver or kidney function compromised
  - Age or genetics related