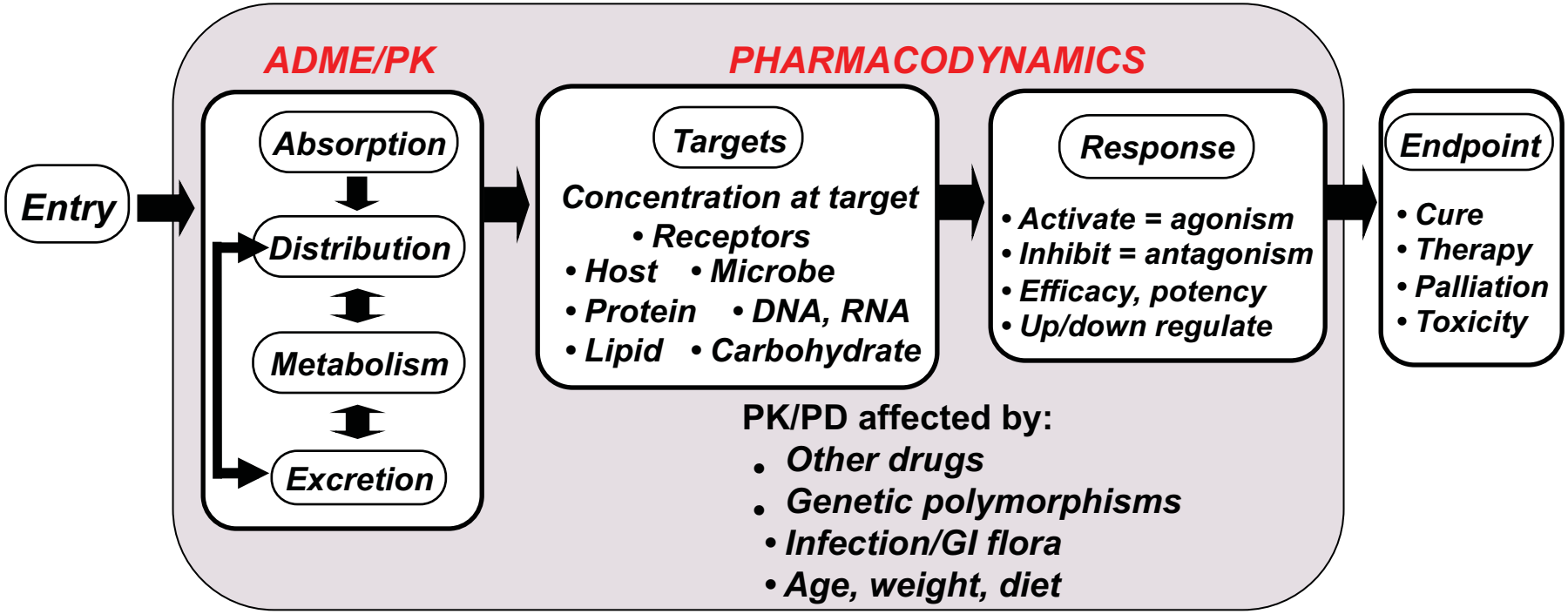
20.201 Mechanisms of Drug Action

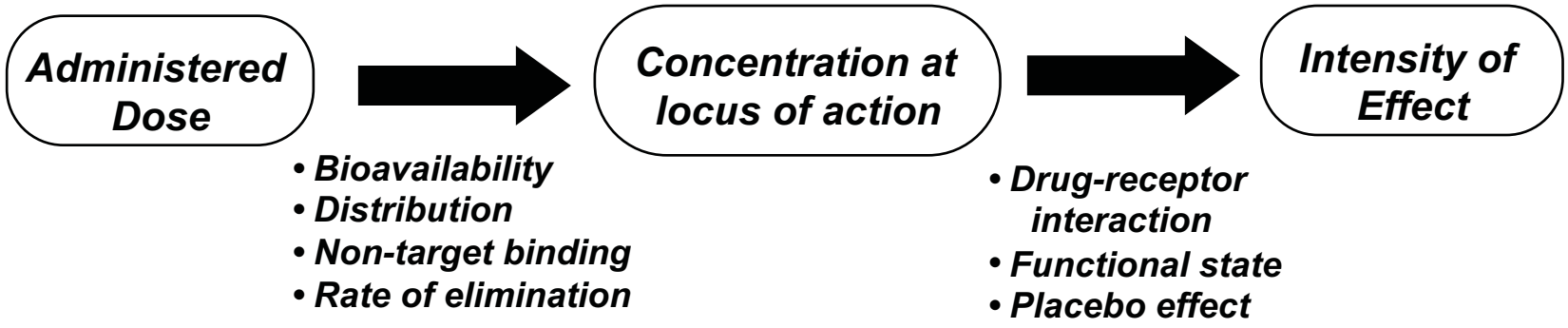
Lecture #4: Principles and Absorption

September 13 and 18, 2013

Paradigm for pharmacokinetics and pharmacodynamics



Paradigm relating drug dose and drug effect



Defining terms in drug development

- **Target discovery** – finding targets specific to a disease
- **"Drugability"** – suitability of a target for drug development and the suitability of a compound to be administered as a drug (Can the compound reach the target?)

drug → drugable → drugability

- **Drug discovery** – finding candidate drugs that are active against a defined target (*e.g.*, kinase inhibitors).
- **Hits** – compounds that give a minimal positive response during screening (*e.g.,* bind to a receptor)
- **Lead** – the most promising compound arising in a screen
- **Food and Drug Administration (FDA)** – the agency of the US government responsible for monitoring drug development

Defining terms in drug development

- **New Chemical Entity (NCE), New Molecular Entity (NME)** – Application to FDA for permission to develop a drug candidate and proceed to pre-clinical research
- **Pre-clinical research** – testing the drug candidates in cells and animals for efficacy, toxicity, pharmacokinetics
- **Good Laboratory Practice (GLP)** – FDA and international rules and regulations under which preclinical drug development studies are planned, performed, monitored, recorded, reported and archived
- **Investigational New Drug (IND)** – application to the FDA for permission to proceed with clinical trials

Defining terms in drug development

- **Clinical research/trials** – human testing for safety and efficacy; challenge: defining the dose; phases I-IV
- **Good Clinical Practice (GCP)** – FDA rules and regulations governing studies in people; data from pre-clinical studies guides human use.
- **Good Manufacturing Practice (GMP)** – FDA rules and regulations ensuring consistent formulation and manufacture of a drug; pill, solution, shelf-life, etc.
- **New Drug Application** – the request to the FDA for permission to market a new drug if a drug candidate is proved to be safe and effective in humans

Defining terms in drug development

- **Pharmacokinetics** - What the body does to the drug; determines how much reaches the target
- **Pharmacodynamics** – What the drug does to the body; therapeutic or toxic outcome; mechanism of action
- **ADMET** – an acronym for the main determinants of pharmacokinetics:

A ~ Uptake = absorption
D ~ Distribution
M ~ Metabolism
E ~ Elimination
T ~ Toxicology (politically correct: “Drug Safety”)

Steps in drug development

(1) **Target identification**

~ Disease mechanism – biochemical hypothesis

~ Target type and 'drugability'

~ Functional genomics

Gastric H^+ ➔ ulcers ➔ inhibit H^+ production

(2) **Target validation**

~ Knock-out/-in, transgenics, antisense, RNAi, chem. genetics

~ Pathways and pathophysiology

~ Clinical data – 2nd/3rd generation drugs

Na^+/H^+ pump well defined – discovered 1970's

(3) **Assay development** – for screening drugs

~ *In vitro* (cell-based), *in vivo* (animal-based)

~ High throughput screening (HTS)

Timoprazole discovered to inhibit Na^+/H^+ ATPase

(4) **Screening** – identifying "hits" & narrowing to "leads"

~ Compound/combinatorial libraries; *in silico* (CADD, SBDD)

~ Primary screen – rapid screen for hits; single dose

~ Potency and dose-response – refine analysis of hits

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Steps in drug development

(5) **Lead optimization** – improving the lead compounds

~ Medicinal chemistry
~ Animal PK/PD/ADME/Toxicity
~ Formulation and delivery
~ Apply to FDA for a New Chemical Entity (NCE)

Synthesized derivatives of timoprazole: Omeprazole

(6) **Development** – test safety and efficacy in animals

~ Pre-clinical data – safety and efficacy in animal models
~ Process development – drug properties and formulation
~ Investigational New Drug (IND) application for clinical trials

(7) **Clinical trials**

~ Phase I – PKs, toxicity, dosing
~ Phase II – small scale efficacy
~ Phase III – large scale efficacy

Omeprazole found to be safe and effective in increasing gastric pH

(8) Apply to FDA for approval as a drug

(9) Perform Phase IV follow-up studies

Lansoprazole, Dexlansoprazole, Pantoprazole, Rabeprazole, Ilaprazole…

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Concepts covered in the next two lectures

- ***Pharmacodynamics*** - quantitative relationship between drug binding to a target and the ultimate pharmacological effect; concentration at the target drives ligand-receptor binding.
- ***Receptor*** – broad term for a protein molecule that binds a ligand and participates in a signaling pathway.
- ***Drug-based definition of a "receptor"*** – macromolecule altered by drug binding. The "receptive substance" noted by Langley (1905) and the "chemoreceptor" coined by Ehrlich (1900).
- ***Drug target*** – broader definition of drug receptor: any macromolecule that specifically recognizes a drug and carries out a function in response to drug binding. ***Binding TD, competition, agonists, antagonists***
- ***Dose-response relationship*** - correlative relationship between the degree of a response in a biological system and the amount of drug or toxicant administered. ***Efficacy, potency, therapeutic index***
- ***Barriers to absorption*** - epithelial tissues
- ***Mechanisms of absorption*** - diffusion, facilitated transport, active transport; predicting bioavailability; prelude to Keith Hoffmaster lectures

Target identification: Types of drug targets

- ***Receptors*** - direct ligand-gated ion channel receptors, G-protein-coupled receptors, cytokine receptors, TNFα receptors, integrin receptors, receptors associated with a tyrosine kinase, nuclear receptors
- ***Enzymes*** – oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases (*e.g.*, HMG CoA reductase for statins)
- ***Ion channels*** - voltage-gated Ca^{+2} channels, K^{+} channels, Na^{+} channels, ryanodine-inositol 1,4,5-triphosphate receptor Ca^{+2} channel (RIR-CaC) family, transient receptor potential Ca^{+2} channel (TRP-CC) family, Cl^{-} channels
- ***Transporters*** - cation-chloride co-transporter (CCC) family, Na^{+}/H^{+} antiporters, proton pumps, Na^{+}/K^{+} ATPase, eukaryotic sterol transporter (EST) family, neurotransmitter/Na^{+}, symporter (NSS) family
- ***Macromolecules*** – DNA, RNA, spindle proteins/tubulin; ribosomes
- ***Atypical targets*** – metabolites (urate, asparagine), lipid membranes, hydronium ion (antacids), photons (sunscreen)

*P. Imming, C. Sinning and A. Meyer (2006) Nat Rev Drug Disc **5**: 821-834*

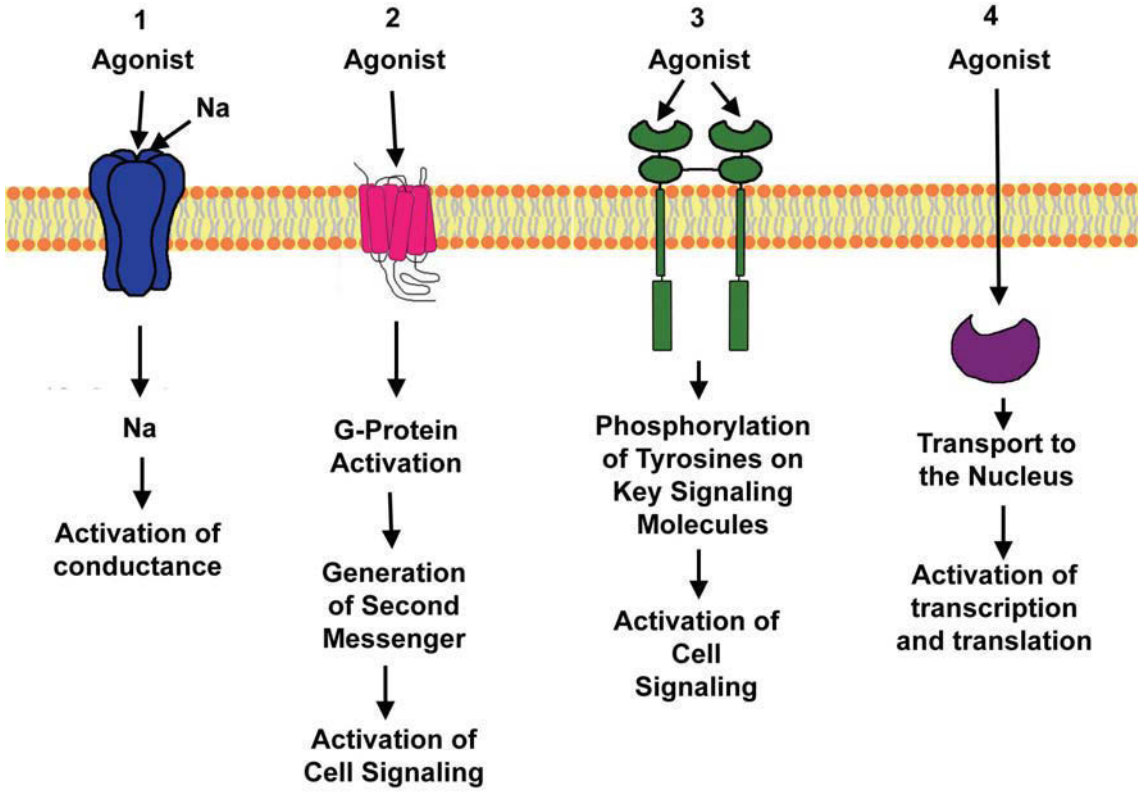
Types of classical receptors

- ***Transmembrane ion channels***

  ~ Conduct ions across membrane in response to ligand binding, voltage gradient or second messenger

  ~ Examples: H^+/K^+-ATP'ase, Na^+/K^+-ATP'ase

- ***Transmembrane linked to intracellular G protein*** - *e.g.*, adrenergic receptors
- ***Transmembrane with cytosolic domain*** - *e.g.*, receptor tyrosine kinases
- ***Intracellular*** - cytoplasm or nucleus; *e.g.,* DNA, estrogen receptor



Atypical drug targets and mechanisms

Table 8 | **Various physicochemical mechanisms**

Mechanism	Agent
Ion exchange	Fluoride
Acid binding	Magnesium hydroxide, aluminium hydroxide
Adsorptive	Charcoal, colestyramine
Adstringent	Bismuth compounds
Surface-active	Simeticone, chlorhexidine, chloroxylene
Surface-active on cell membranes	Coal tar
Surface-active from fungi	Nystatin, amphotericin B
Mucosal irritation	Anthrones, anthraquinones
Osmotically active	Lactulose, dextran 70, polygeline, glucose, electrolyte solutions, mannitol
Water binding	Urea, ethanol
UV absorbant	4-Aminobenzoic acid derivatives
Reflective	Zinc oxide, titanium dioxide
Oxidative	Tannines, polyphenoles, dithranol, polyvidon iodide, silver nitrate, hypochlorite, permanganate, benzoylperoxide, nitroimidazoles, nitrofuranes, temoporfin (mainly via singlet oxygen, cytostatic drug), verteporfin (mainly via singlet oxygen, ophthalmic drug)
Reduce disulphide bridges	D-Penicillamine, *N*-acetyl-cysteine
Complexing agents	Al^{3+}, arsenic compounds
Salt formation	Sevelamer
Modification of tertiary structure	Enfuvirtide (from HIV glycoprotein 41)

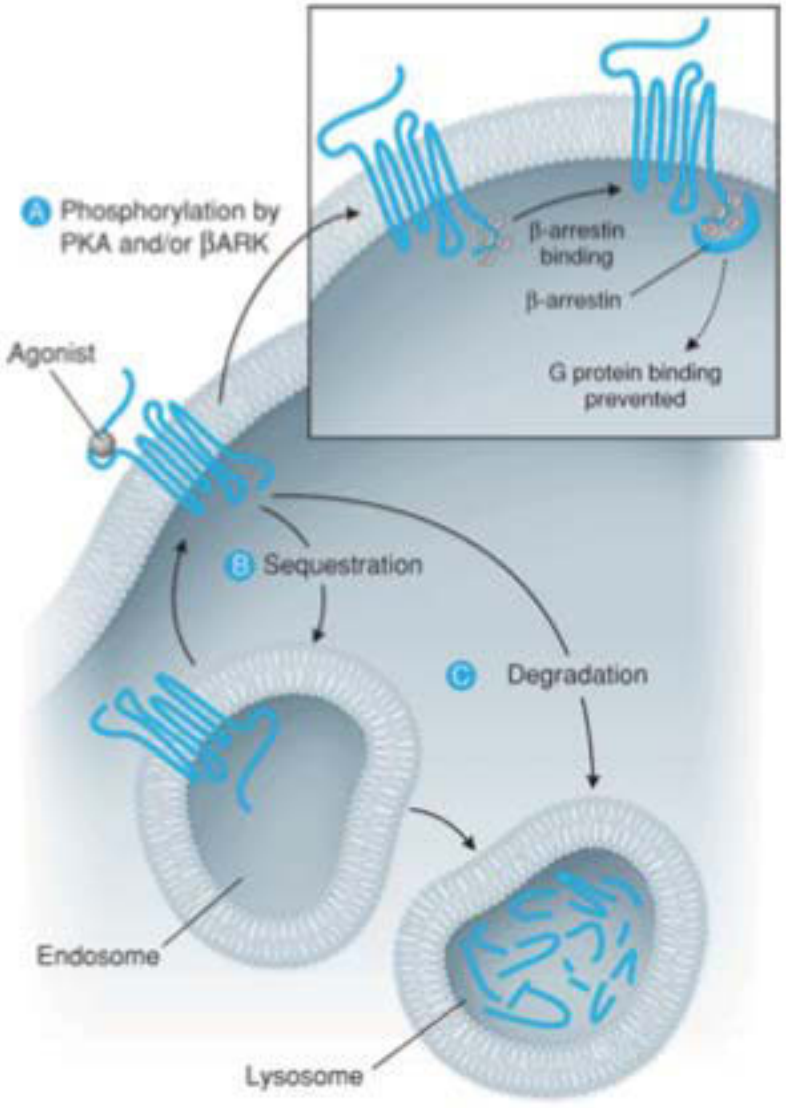
Source: Imming, Peter, Christian Sinning, et al. "Drugs, Their Targets and the Nature and Number of Drug Targets." *Nature Reviews Drug Discovery* 5, no. 10 (2006): 821-34.

P. Imming, C. Sinning and A. Meyer (2006) Nat Rev Drug Disc ***5***: 821-834

Drug-receptor interactions: Regulating receptor activity

TABLE 1-6 Mechanisms of Receptor Regulation

MECHANISM	DEFINITION
Tachyphylaxis	Repeated administration of the same dose of a drug results in a reduced effect of the drug over time
Desensitization	Decreased ability of a receptor to respond to stimulation by a drug or ligand
Homologous	Decreased response at a single type of receptor
Heterologous	Decreased response at two or more types of receptor
Inactivation	Loss of ability of a receptor to respond to stimulation by a drug or ligand
Refractory	After a receptor is stimulated, a period of time is required before the next drug-receptor interaction can produce an effect
Down-regulation	Repeated or persistent drug-receptor interaction results in removal of the receptor from sites where subsequent drug-receptor interactions could take place



Source: Golan, David E., Armen H. Tashjian, et al. Armstrong, eds. *Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy*. Lippincott Williams & Wilkins, 2011.

Drug-receptor interactions: Quantitative principles

- Quantitative principles govern drug-receptor interactions:
  - ~ Specificity
  - ~ Affinity
  - ~ Intrinsic activity
  - ~ Saturability

- Simple **thermodynamics and kinetics** govern interaction of drug molecule with its receptor
  - ~ Association/dissociation constants
  - ~ Kinetic parameters of on-rate and off-rate

  define the binding constant

Characteristics of a receptor

- ***Specificity***
  - Receptor interacts with one type of ligand or structurally related ligands
  - Competition between related ligands
  - Example: glucose transporter and D-glucose

- ***Affinity***
  - The energetics of ligand receptor interactions
  - Energetics of binding contribute to specificity

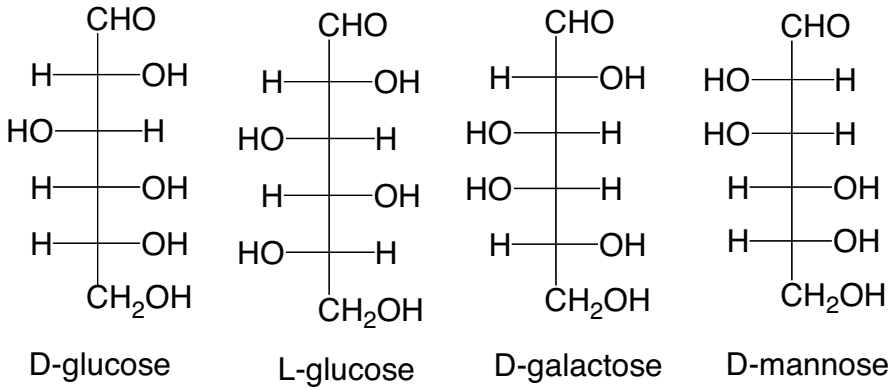
- ***Intrinsic activity***
  - A measure of the ability of a bound drug to activate the receptor
  - Distinguishes agonist from antagonist

- ***Saturability***
  - Finite number of binding sites on a receptor and their specificity for a ligand imply that binding sites can become fully occupied with ligand molecules
  - Additional ligand leads to non-specific binding

Substrate	K_m ***(M)***
L-Glucose	>3000
Galactose	30
Mannose	20
D-Glucose	1.5

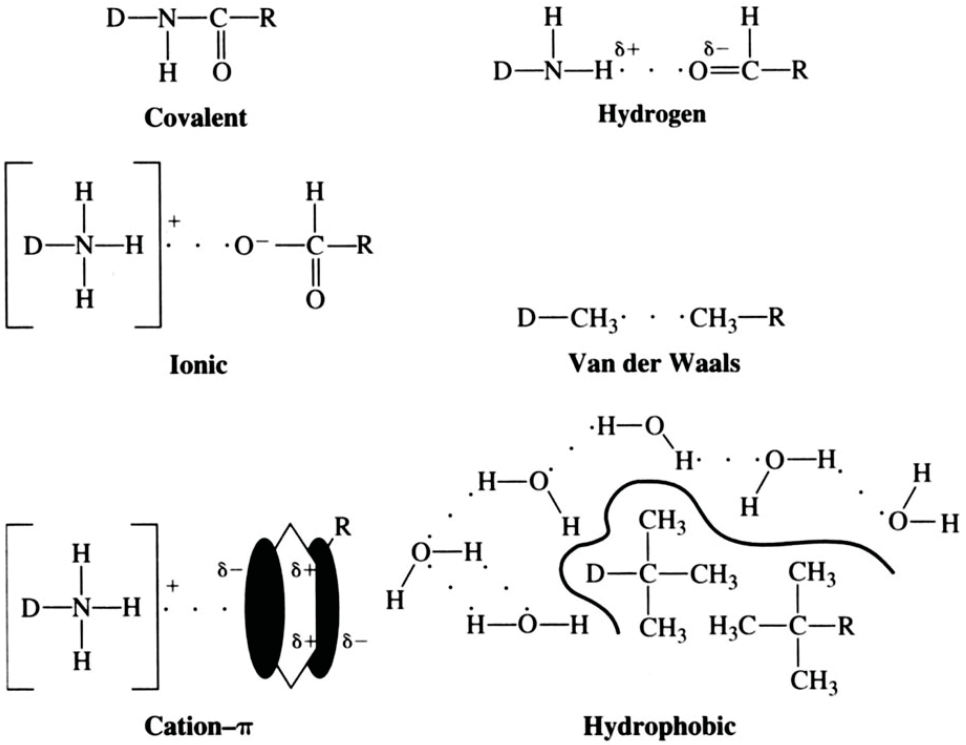
Unnatural substrate, low affinity binding

Native substrate, high affinity binding

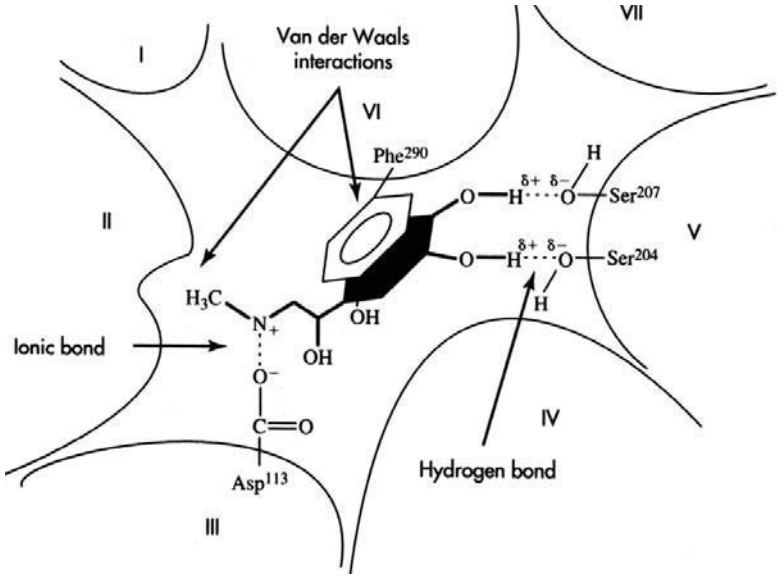


Types of chemical bonds that govern ligand-receptor interactions

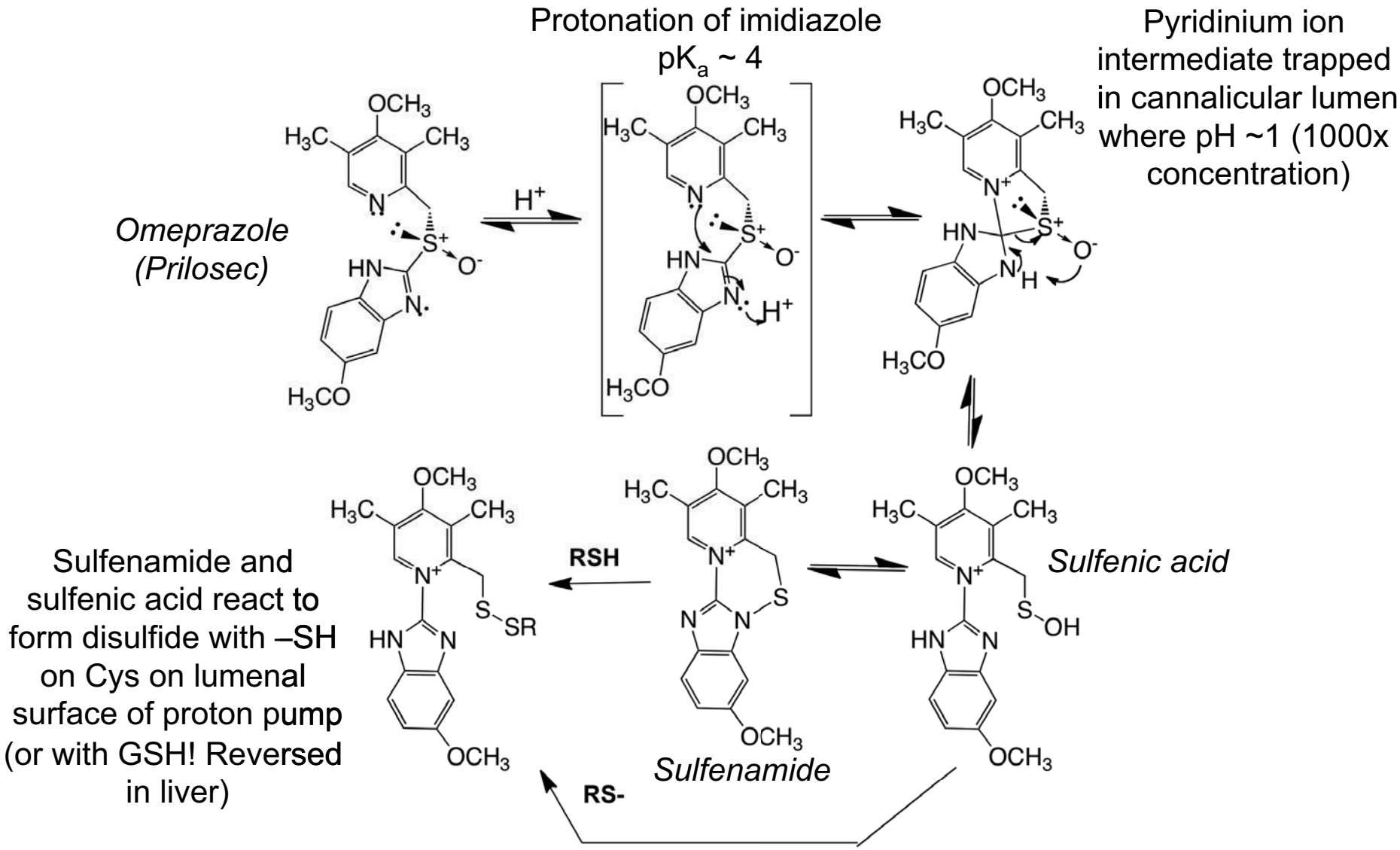
- Affinity and specificity - based on chemical bonds
- Covalent binding of omeprazole occurs only after non-covalent, specific interaction with H^+/K^+-ATPase
- Ionic bonds ➙ initial attraction
- Cation-π interactions, H-bonds ➙ improved binding, some specificity
- Van der Waals forces, hydrophobic interactions ➙ most specificity



Epinephrine and the β-adrenergic receptor



Covalent bonds in ligand-receptor interactions: Example with omeprazole



Quantitation of ligand-receptor interactions: Kinetics and thermodynamics

• Consider interaction of drug (X) with receptor (R) containing a single binding site

$$R + X \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} RX$$

$$K_a = \frac{1}{K_d} = \frac{[RX]}{[R][X]}$$

- Association constant; not acidity constant!
- [R] = unoccupied receptor
- [X] = free (unbound) drug concentration

$$\Delta G^{\circ}_f = -RT\ln(K_a)$$

- R = gas constant; T = temperature
- -ΔG = tight binding

• Define **"saturation fraction" = r** (a.k.a. percent occupancy)

• **r** = average number of ligands bound per receptor molecule (Langmuir isotherm)

$$r = \frac{[X]_{bound}}{[R]_{total}} = \frac{[RX]}{[R]_{free} + [RX]}$$

$$K_a = \frac{[RX]}{[R]_{free}[X]_{free}} \Rightarrow r = \frac{K_a[R]_{free}[X]_{free}}{[R]_{free} + (K_a[R]_{free}[X]_{free})} = \frac{K_a[X]_{free}}{1 + K_a[X]_{free}}$$

• For receptor with "n" binding sites: $$r = \frac{nK_a[X]_{free}}{1 + K_a[X]_{free}}$$

Parallel "drug-receptor" relationships in pharmacology: Michaelis-Menten kinetics and binding thermodynamics

- Michaelis-Menten equation is relevant to simple enzyme kinetics according to the reaction:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$v_o = \frac{v_{max} \bullet [S]}{K_m + [S]}$$

- When product release is not rate-limiting (i.e., k_2 is small), the Michaelis constant $\mathbf{K_m}$ is a dissociation constant (unit: M) governing substrate binding to the enzyme

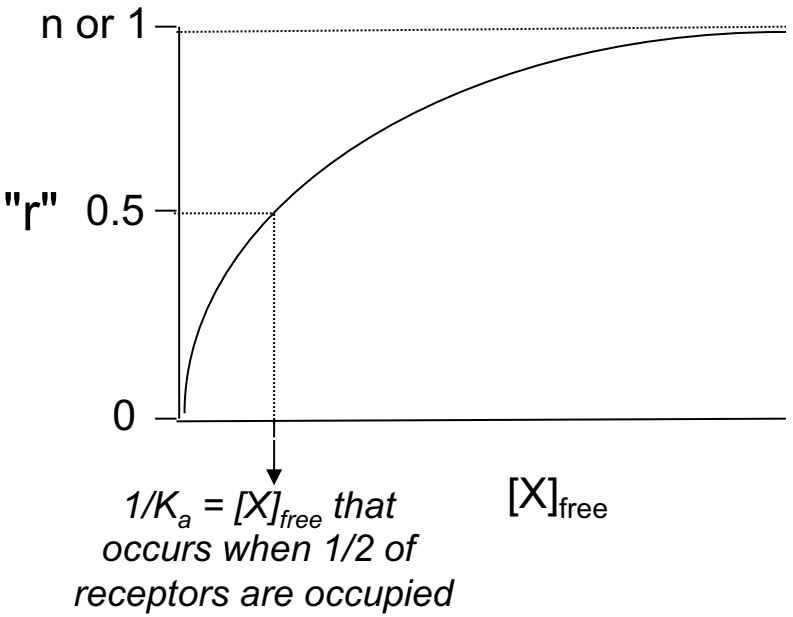
$$K_m = \frac{k_{-1} + k_2}{k_1}$$

When product release is not rate-limiting:

$$K_m \approx \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]} = K_d$$

Quantitation of ligand-receptor interactions

- *Binding isotherm*: increase [ligand] and measure bound and free (constant temp)
- Nonlinear regression to fit the data and determine K_a



$$r = \frac{nK_a[X]_{free}}{1 + K_a[X]_{free}}$$

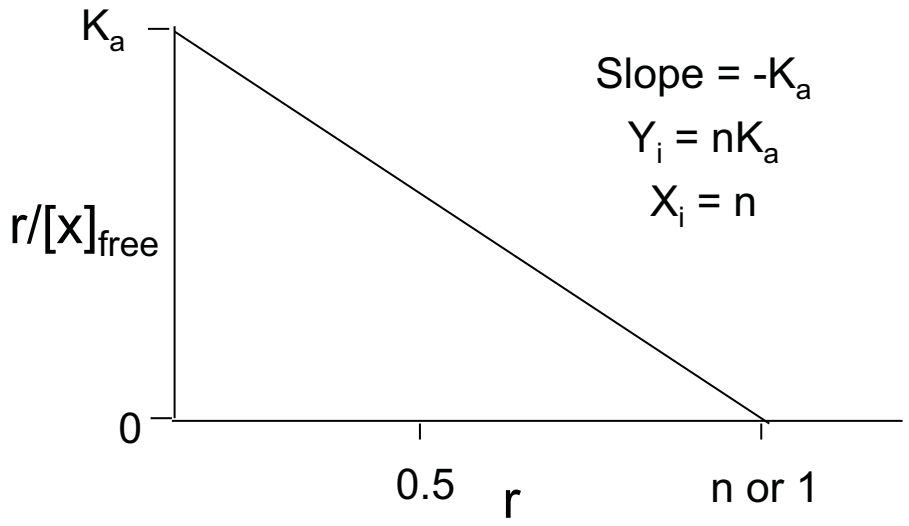
$$r = 0.5 = \frac{K_a[X]_{free}}{1 + K_a[X]_{free}}$$

$$r = 0.5 \Rightarrow [R]_{free} = [RX] \Rightarrow K_a = \frac{1}{[X]_{free}} \text{ and } \frac{1}{K_a} = [X]_{free\,1/2}$$

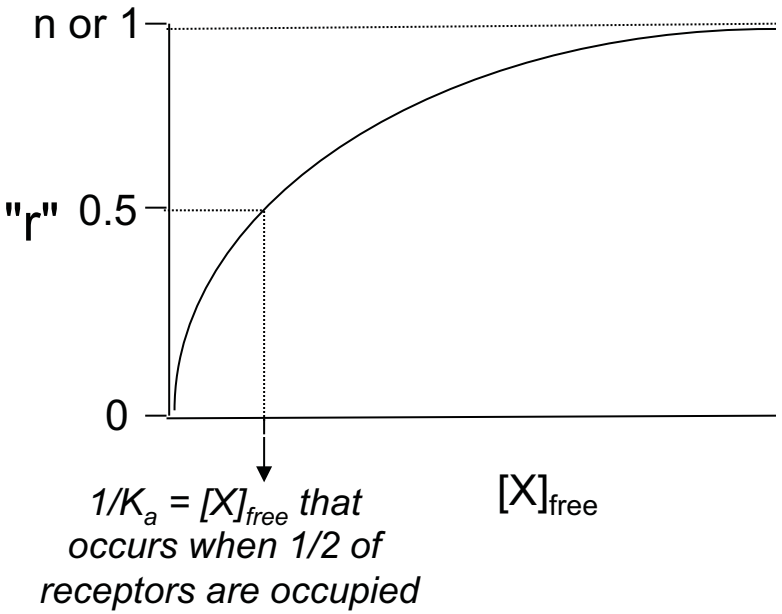
Scatchard plot

- Rearrange equation: linear plot
- $r/[X]_{free}$ versus r

$$r = \frac{nK_a[X]_{free}}{1 + K_a[X]_{free}} \Rightarrow \frac{r}{[X]_{free}} = nK_a - K_a r$$



Quantitation of ligand-receptor interactions

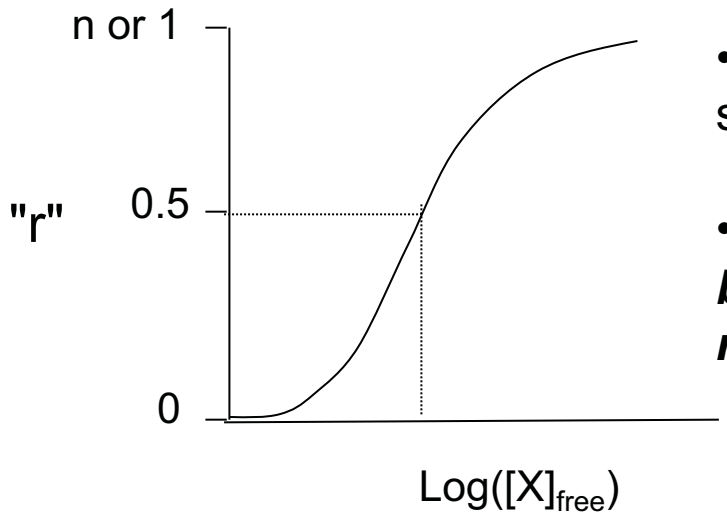


$1/K_a = [X]_{free}$ that occurs when 1/2 of receptors are occupied

$$r = \frac{nK_a[X]_{free}}{1 + K_a[X]_{free}}$$

$$r = 0.5 = \frac{K_a[X]_{free}}{1 + K_a[X]_{free}}$$

$$r = 0.5 \Rightarrow [R]_{free} = [RX] \Rightarrow K_a = \frac{1}{[X]_{free}} \text{ and } \frac{1}{K_a} = [X]_{free1/2}$$



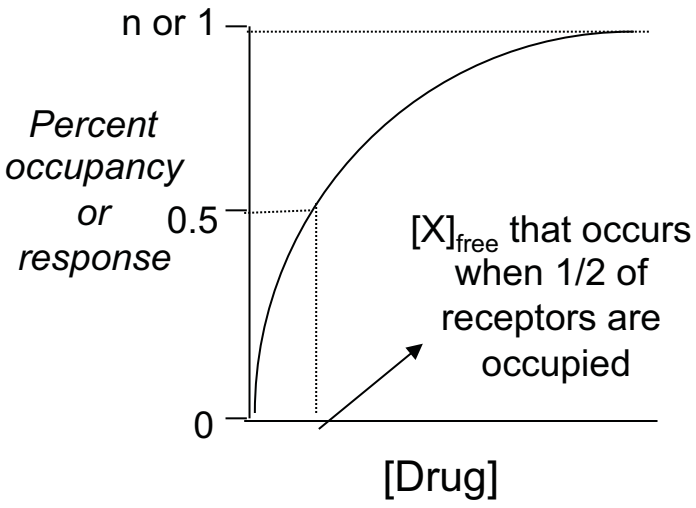
- Replot binding isotherm as semi-log plot: log[ligand]
- ***Sigmoidal shape typical for binding isotherms and dose-response relationships!***

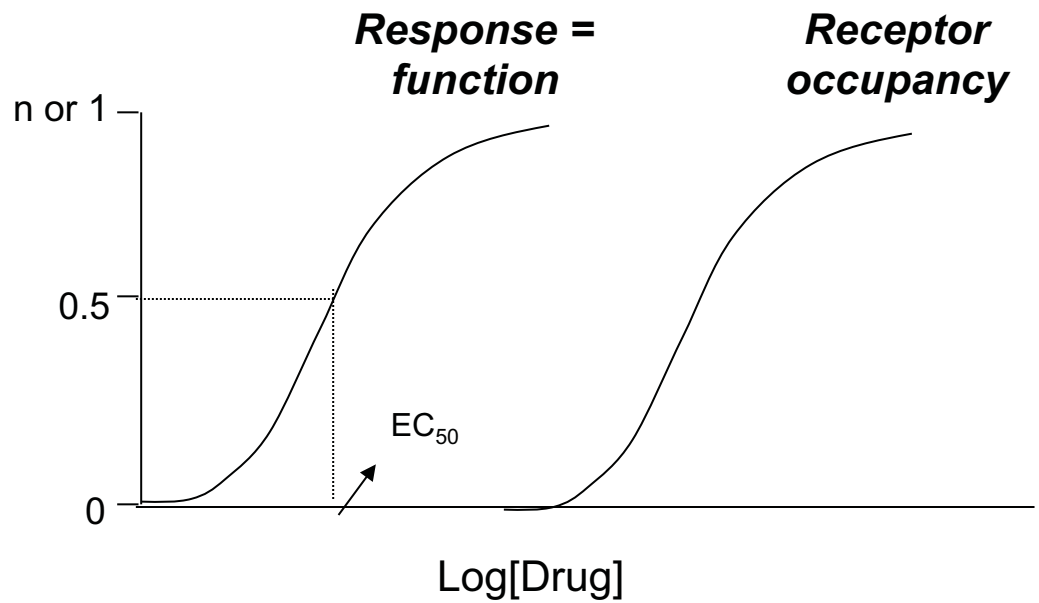
Linking receptors to ultimate effects: Drug-receptor interactions parallel the pharmacological effect

- **Classic receptor theory**: response emanates from receptor occupied by drug
- Drug can do two things to receptors:
  - ~ bind to them
  - ~ alter their activity

$$D + R \underset{}{\overset{K_a}{\rightleftharpoons}} DR \longrightarrow \text{Response}$$

- Binding is due to affinity of drug for receptor: chemical bonds
- Degree of change in the activity of the receptor referred to as "**efficacy**" (ε)
- Shape of binding curve (isotherm) and dose-response curve are the same
  - ~ Flattening of binding curve due to saturation
  - ~ plot occupancy or response vs. log[Drug] yields sigmoidal curve (similar to pH titration, etc.)





The dose-response relationship

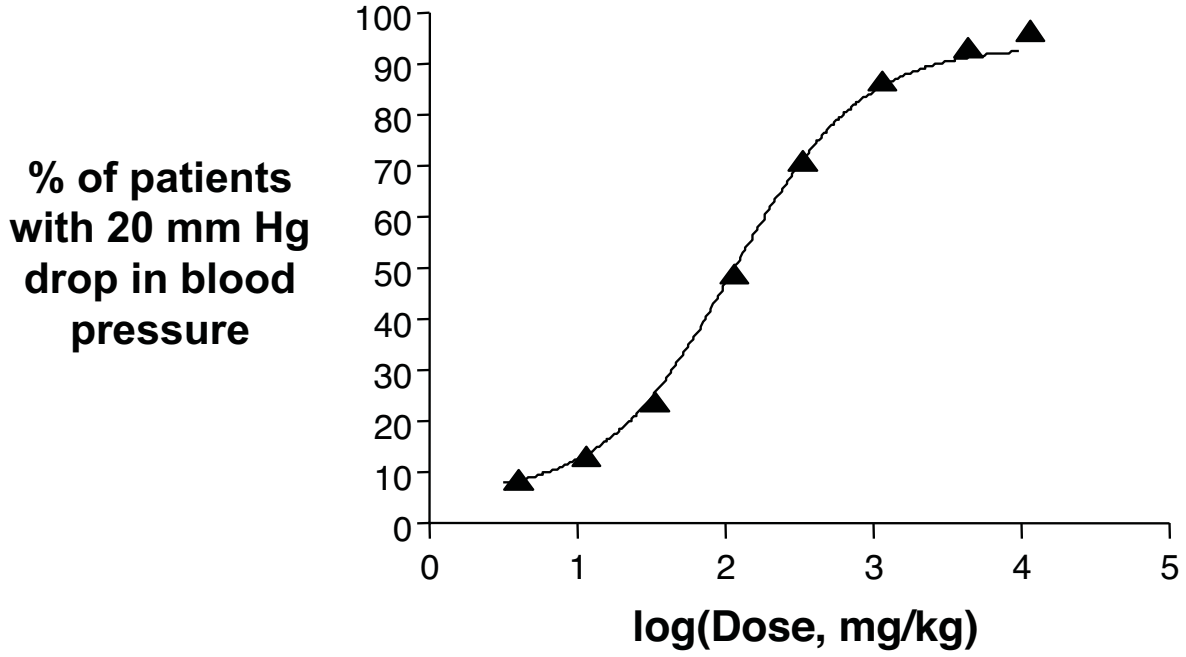
- The relationship between the quantity of response and the dose of drug or toxicant
- Foundation for receptor theory of pharmacology, drug action
- Prerequisites and assumptions for defining a dose-response relationship:
  - ~ *Response is due to the drug*
  - ~ *Degree of response is due to the drug concentration*
  - ~ *Have a quantifiable (measurable) response parameter*
- Types of dose-response relationships:
  - ~ **Individual, graded or continuous**: dose-related change in intensity of response in an individual. Example: enzyme inhibition, blood pressure change
  - ~ **Quantal**: effect of various drug doses on a population; single end-point ("quanta") study in which an organism either responds or it does not. Example: death

The dose-response relationship

- Example: drug for control of blood pressure
  - ~ groups of 10 patients
  - ~ one dose of drug per group
  - ~ administer 8 doses of drug and then measure blood pressure
  - ~ “response” = ≥ 20 mm Hg drop in blood pressure
  - ~ plot % of patients responding versus log of dose

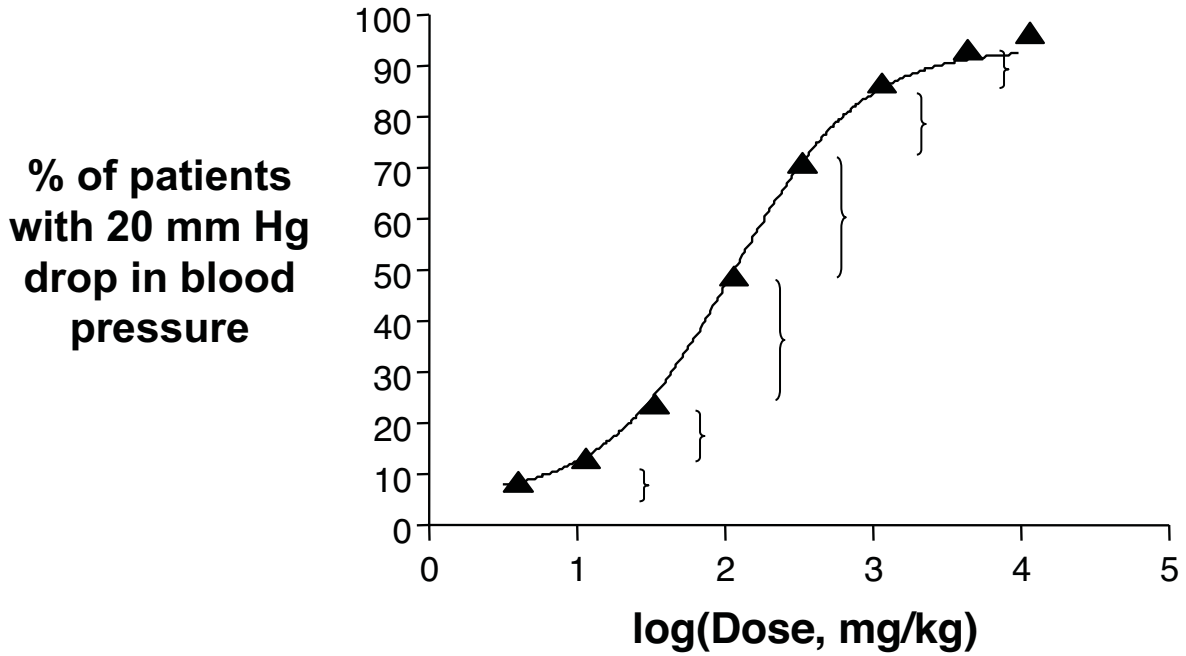
- Sigmoidal dose-response curve is typical

- Similar to ligand-receptor binding curve, cell response curve, etc.



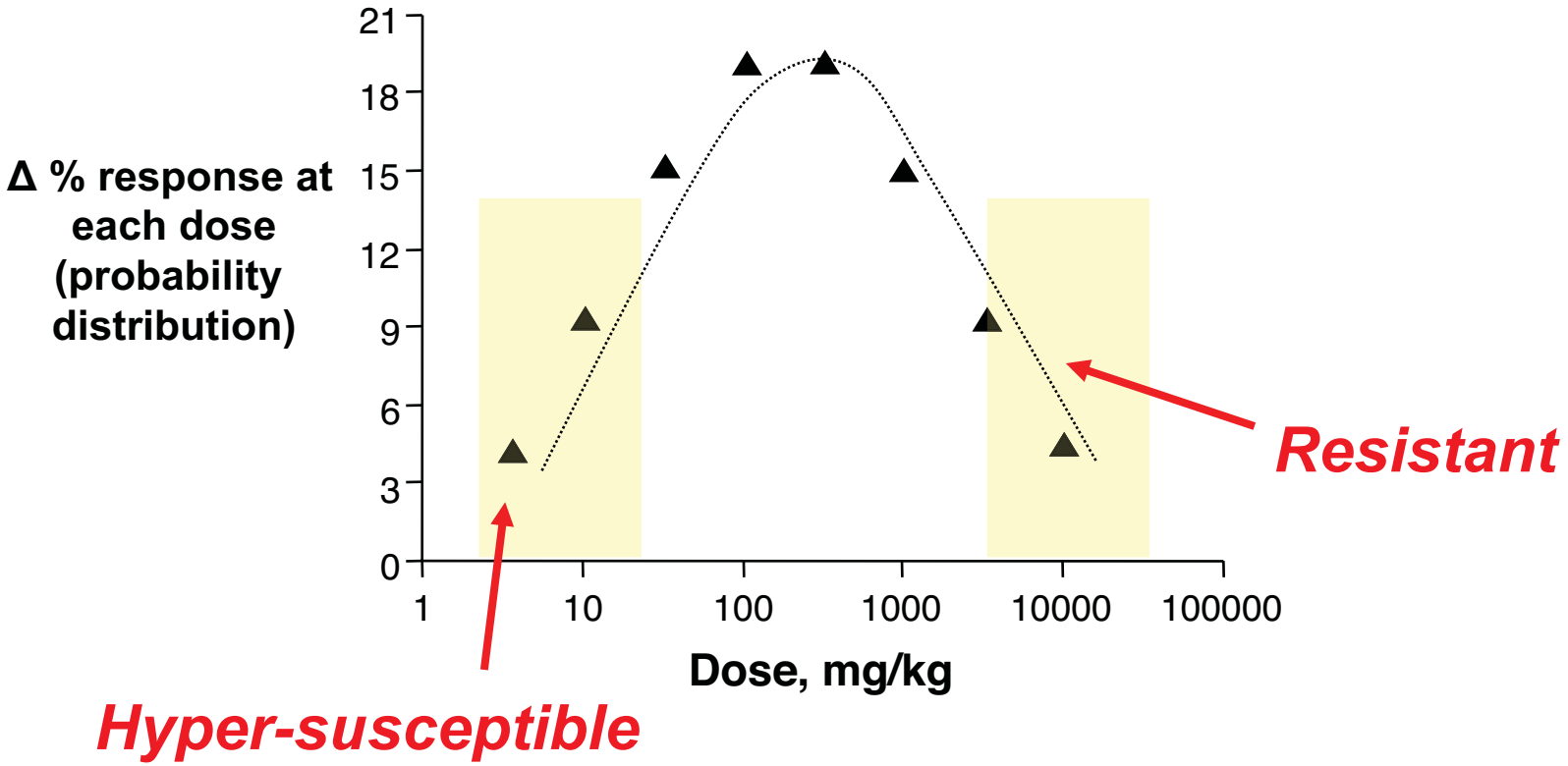
The dose-response relationship

- Calculate net increase in % response with each higher dose
- Replot the change in % response or frequency



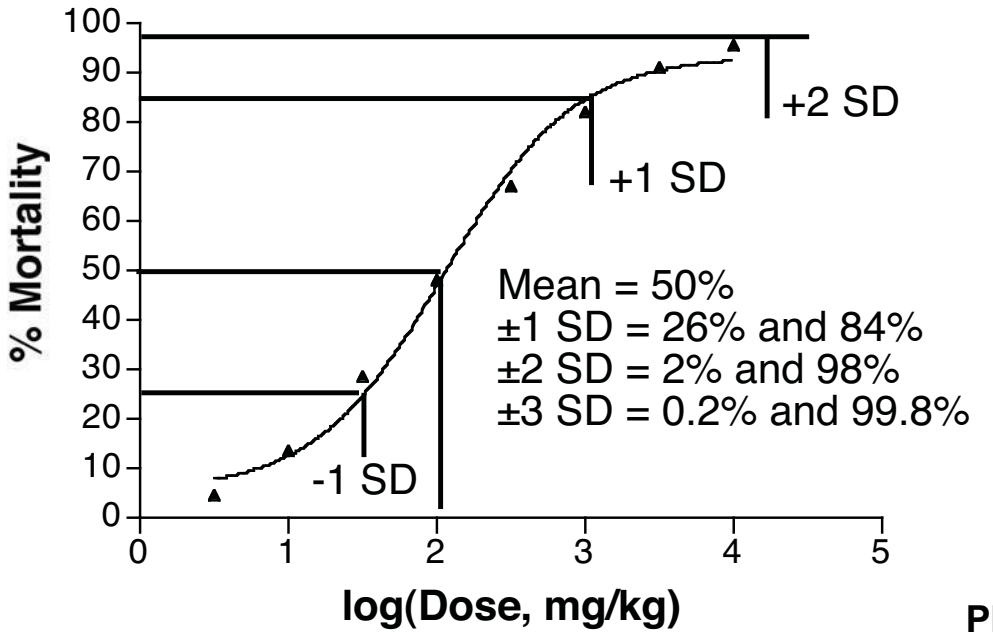
The dose-response relationship

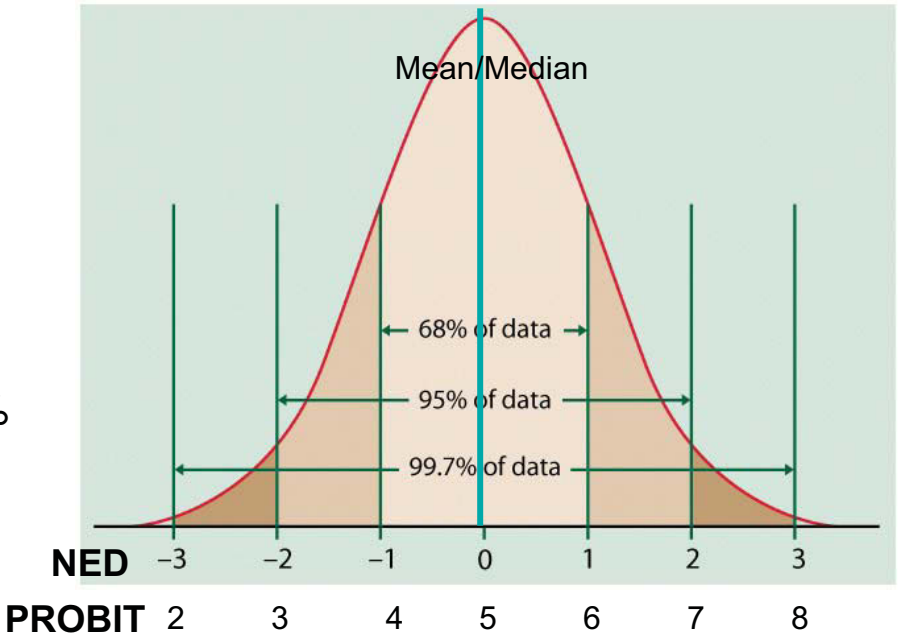
- Replot response as % change in response per dose (% increase in response with each higher dose)
- Bell-shaped probability distribution: dose-response data *generally* follows a Gaussian distribution ("normally distributed")



The dose-response relationship

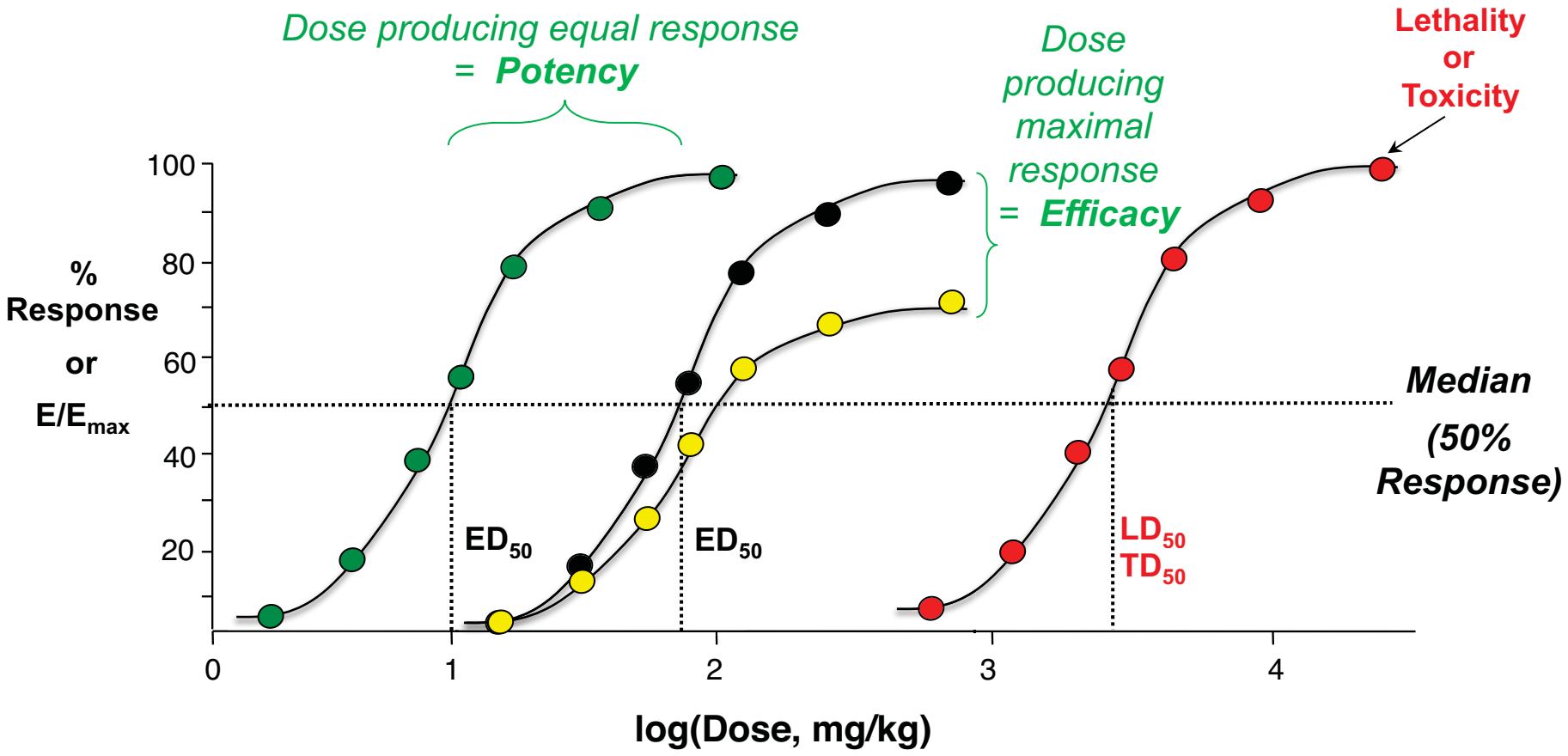
- In population with normal distribution (mean = median):
  - ~ mean ± 1 SD…..68.3% of the population
  - ~ mean ± 2 SD…..95.5% of the population
  - ~ mean ± 3 SD…..99.7% of the population
- Convert % response to units of deviation from the mean = **NED** (normal equivalent deviations) +5 = **PROBIT**:
  - ~ 16% response (-1 SD)….NED = -1….PROBIT = 4
  - ~ 50% response (0 SD)…..NED = 0…..PROBIT = 5
  - ~ 84% response (+1 SD)…NED = +1…PROBIT = 6





The dose-response relationship

- **Definitions -** Effective dose = **ED**; Toxic dose = **TD**; Lethal dose = **LD**
- **Potency -** Range of doses over which a drug produces increasing responses
- **Efficacy -** Maximal response; plateau of the dose-response curve



Drug-receptor interactions: Agonists and Antagonists (distinguishing binding from response)

- ***Agonist***
  - Ligand that binds to receptor and stabilizes an "active state" of the receptor
  - "Active state" is defined as the functionally activated form (e.g., open ion channel, activated tyrosine kinase)
  - Endogenous ligands are generally agonists: neurotransmitters

- ***Antagonist***
  - A ligand that binds to the receptor with affinity/specificity but does not have intrinsic activity
  - Inhibits the action of an agonist but has no activity in the absence of agonist
  - ***Receptor antagonist*****:** binds to the active site or an allosteric site *reversibly* or *irreversibly*
  - ***Non-receptor antagonist*****:** binds to molecule downstream in activation pathway, or acts in a pathway that opposes the agonist pathway
    - ~ *Chemical antagonist*: protamine binds to and inhibits heparin, an anticoagulant
    - ~ *Physiological antagonist*: β-adrenergic receptor agonists block the tachycardia caused by hyperthyroidism (though thyroid hormone acts by a different receptor)

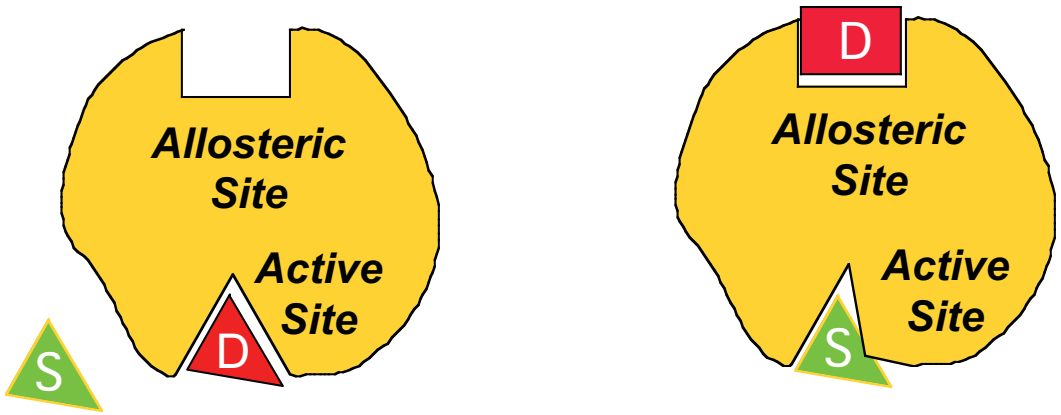
Agonists

- Ligand that binds to receptor and stabilizes an "active state" of the receptor
  - "Active state" represents conformational change caused by agonist binding
  - Binding can occur at the active site or at another region of the receptor (exerts allosteric effects)
  - The kinetics of drug binding and receptor activation are distinct

$$D + R \underset{k_{off}}{\overset{k_{on}}{\leftrightarrows}} DR \underset{k_{\beta}}{\overset{k_{\alpha}}{\leftrightarrows}} DR^*$$

Potency ***Efficacy***

- ***Potency*** related to drug binding affinity *(i.e.*, association constant)
- ***Efficacy*** related to the rate and extent of receptor activation AFTER drug binding



$$D + R \underset{k_{off}}{\overset{k_{on}}{\leftrightarrows}} DR \underset{k_{\beta}}{\overset{k_{\alpha}}{\leftrightarrows}} DR^*$$

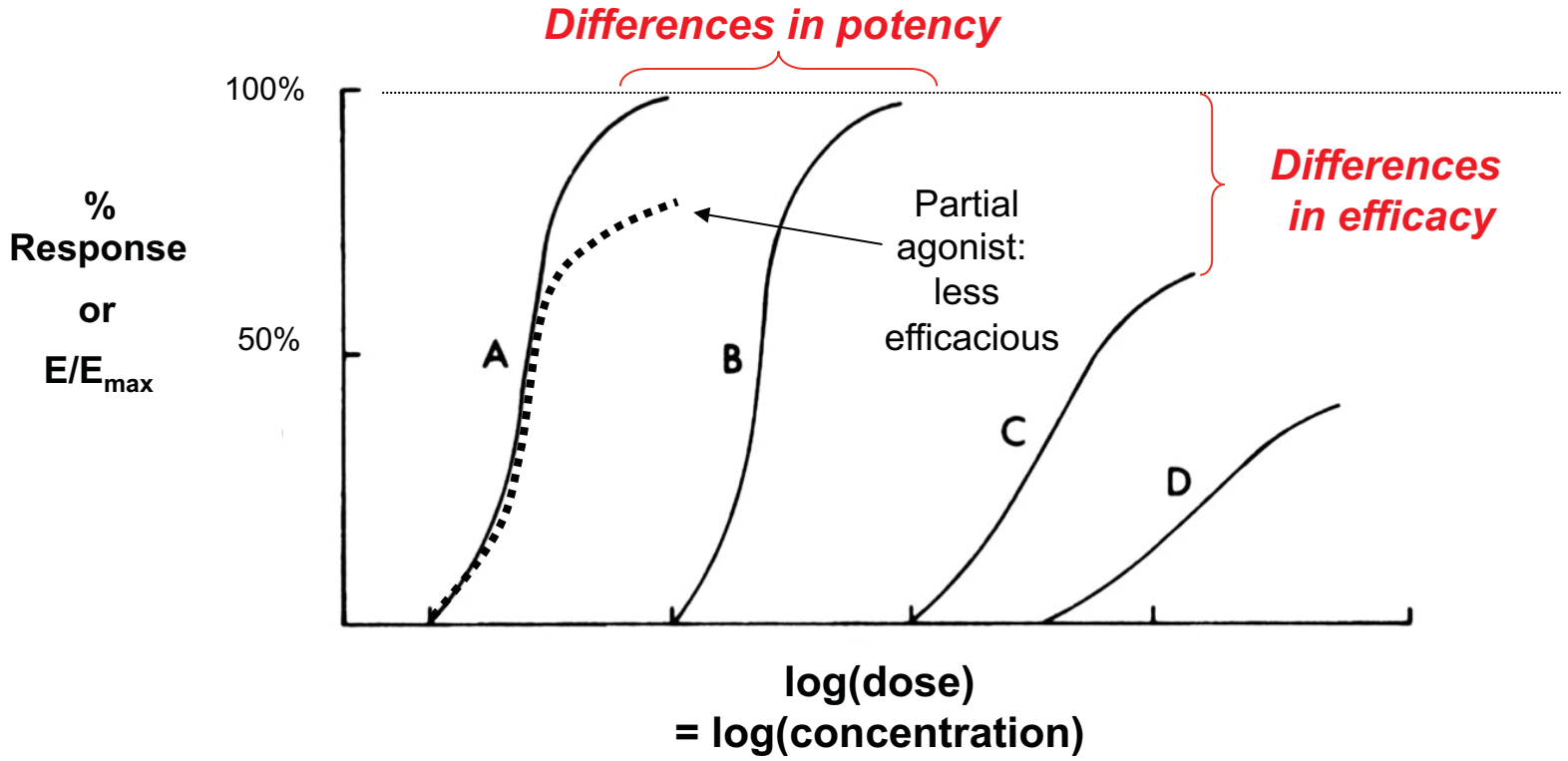
Potency ***Efficacy***

Agonists

- ***Potency*** related to drug binding affinity (i.e., association constant)

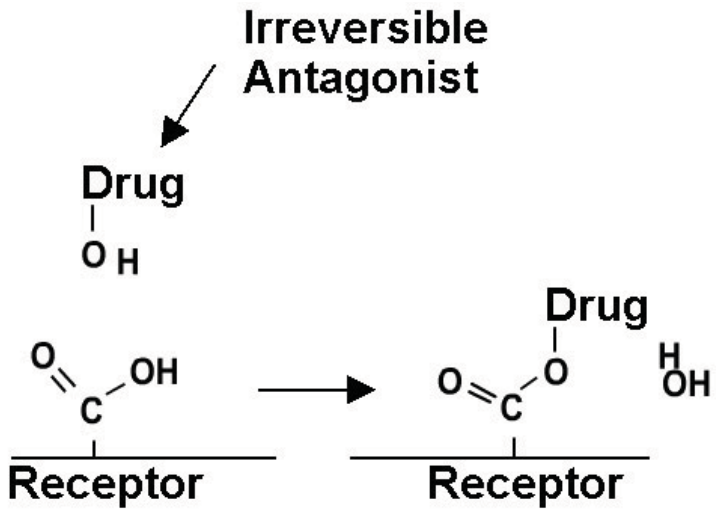
- ***Efficacy*** related to rate and extent of receptor activation AFTER drug binding

- ***Partial agonist***: sub-maximal response when drug binds to receptor; judged relative to the most efficacious drug in class



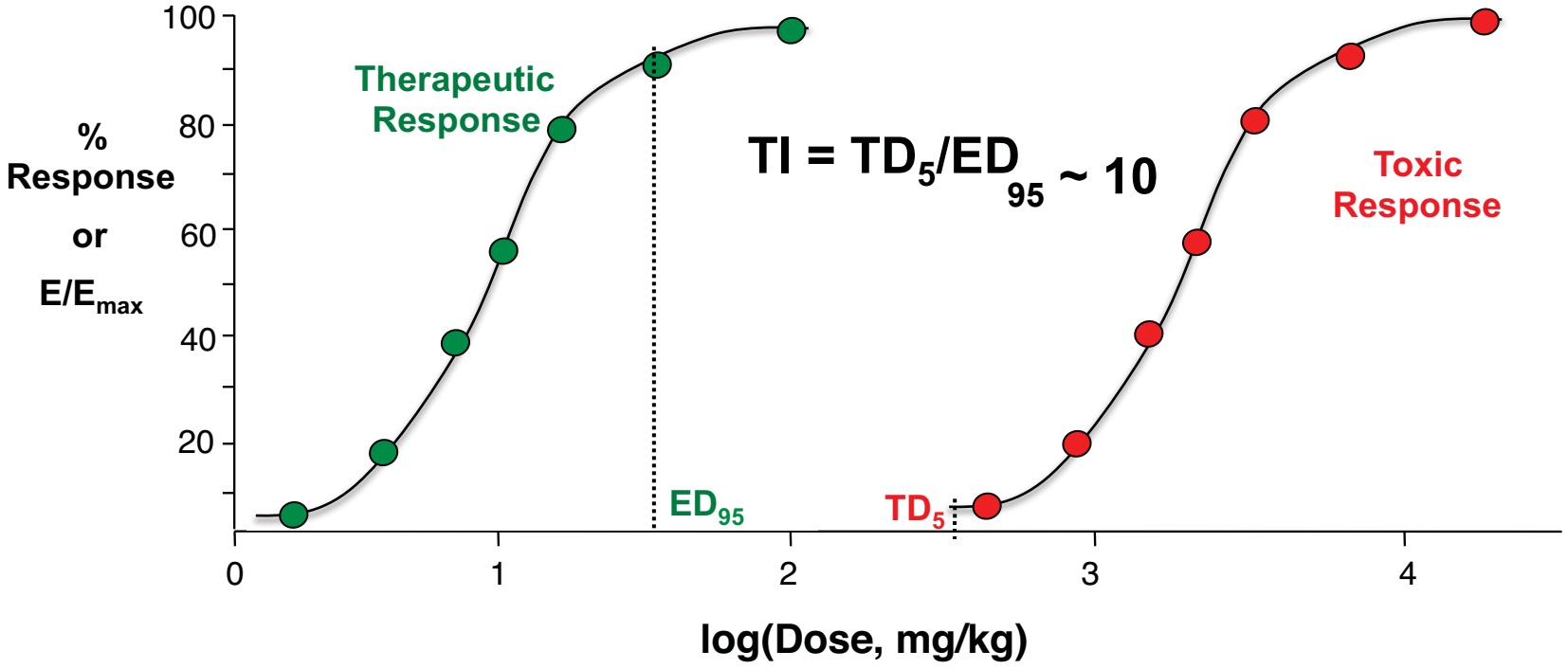
Irreversible Antagonists

- ***Irreversible Antagonist = Noncompetitive Antagonist***
- Drug binds to receptor at active or allosteric site with extremely high affinity or by covalent bonds
- Example: omeprazole
- Antagonist action terminates when receptor degraded



Therapeutic Index

- **Goal**: wide difference between toxic and therapeutic doses
- Quantify this concept in the **Therapeutic Index**: **$TI = TD_x / ED_x$**
- Conservative approach: use $TD_{5\text{-}10}$ and $ED_{90\text{-}95}$
- More conservative: **Margin of Safety (MOS) = TD_{01}/ED_{99}**
- Safe drug: TI > 10 versus MOS > 1
- Usually based on animal studies; clinical experience guides human "TI" estimates



Drugs with narrow therapeutic indices

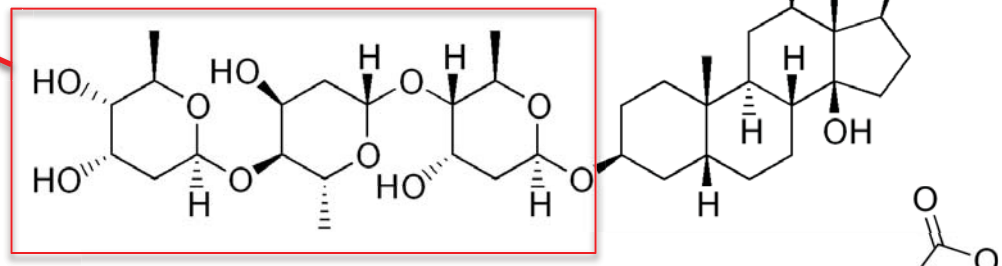
Drug	*Therapeutic Range*	*Toxicity*	*TI*
Phenytoin	10-12 mg/L	>25 mg/L	**2-2.5**
Phenobarbitone	10-30 mg/L	>35 mg/L	**3-3.5**
Carbamazepine	5-12 mg/L	>12 mg/L	**1-2.4**
Ethosuximide	40-100 mg/L	>100 mg/L	**1-2.5**
Valproic acid	50-100 mg/L	>100 mg/L	**1-2**
Digoxin	1-2.5 μg/L	>3 μg/L	**1-3**
Digitoxin	10-25 μg/L	>38 μg/L	**1.5-4**
Quinidine	3-6 mg/L	>6 mg/L	**1-2**
Procainamide	3-10 mg/L	>10 mg/L	**1-3**
Lithium	0.8-1.0 mmol/L	>1.5 mmol/L	**1.5-1.8**
Nortryptyline	50-160 μg/L	>210 μg/L	**1-4**
Theophylline	10-20 mg/L	>20 mg/L	**1-2**

From Avery's Drug Treatment (1987) edited by T.M. Speight, Williams and Wilkins, Baltimore. 3rd Edition.

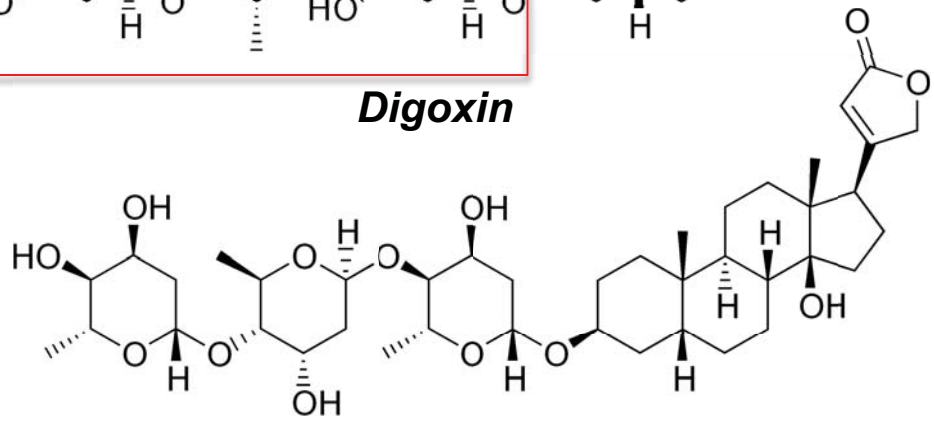
Pharmacodynamics and receptors:
Case study with cardiac glycosides

Digitalis purpurea
(Common Foxglove)
(Dead men's bells)

- **Digitalis**
  - ~ genus of flowering plants ("foxglove")
  - ~ 20 species: *Digitalis purpurea*
  - ~ Source of cardiac glycosides (Digtalins)
- **Caridac glycosides**
  - ~ Carbohydrate conjugate
  - ~ Aglycone = digoxigenin
- **Digoxin** - "Digitalis"
  - ~ Lanoxin (GlaxoSmithKline)
  - ~ excreted mainly in urine
- **Digitoxin** -
  - ~ Not available in US
  - ~ excreted mainly in bile (liver)
- **Main indications** for use:
  - ~ Chronic heart failure = inotropic agent
  - ~ Atrial fibrillation = anti-arrhythmic agent



Digoxin

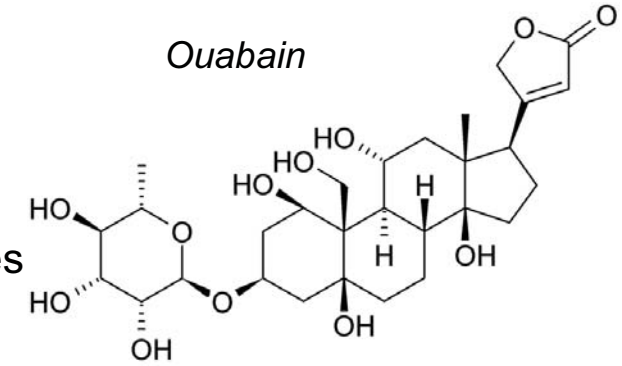


Digitoxin

Pharmacodynamics and receptors: Binding of digoxin to Na^+/K^+-ATPase

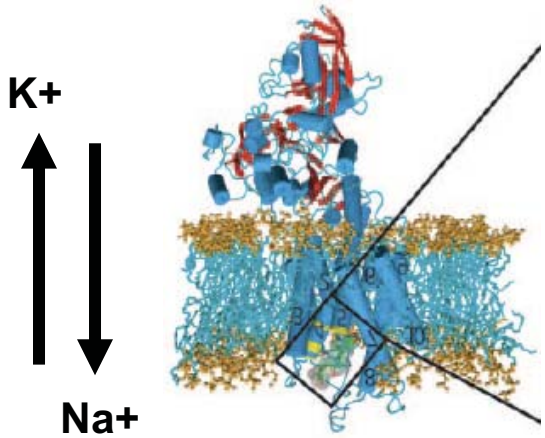
- **Mechanism of action:**
  - ~ Inhibits Na^+/K^+-ATPase – ***the "receptor"***
  - ~ *Direct effect*: ↑force/velocity cardiac contraction
    "positive inotropism"
  - ~ *Indirect effects*: ↓BP, ↓HR (autonomic nervous system)
- **Na^+/K^+-ATPase**
  - ~ trans-membrane
  - ~ ion transport protein: Na^+ out, K^+ in
  - ~ extracellular binding site
- Inhibition: ↑ intracellular Na^+ ⇒ ↑ intracellular Ca^{+2}
- Recent crystal structure with ouabain
- **Ouabain**
  - ~ Digoxin surrogate
  - ~ "g-strophanthin" (extract)
  - ~ cardiac glycoside
  - ~ endogenous, plant sources (Strophanthus gratus)

Ouabain

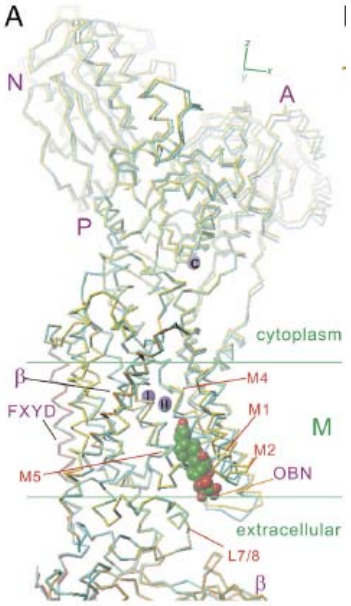


Ogawa *et al.* (2009) *PNAS* **106**: 13742

Qiu *et al.* (2005) *J. Biol. Chem.* **280**: 32349



Source: Qiu, Li Yan, Elmar Krieger, et al. "Reconstruction of the Complete Ouabain-binding Pocket of Na, K-ATPase in Gastric H, K-ATPase by Substitution of only Seven Amino Acid." *Journal of Biological Chemistry* 280, no. 37 (2005): 32349-55.

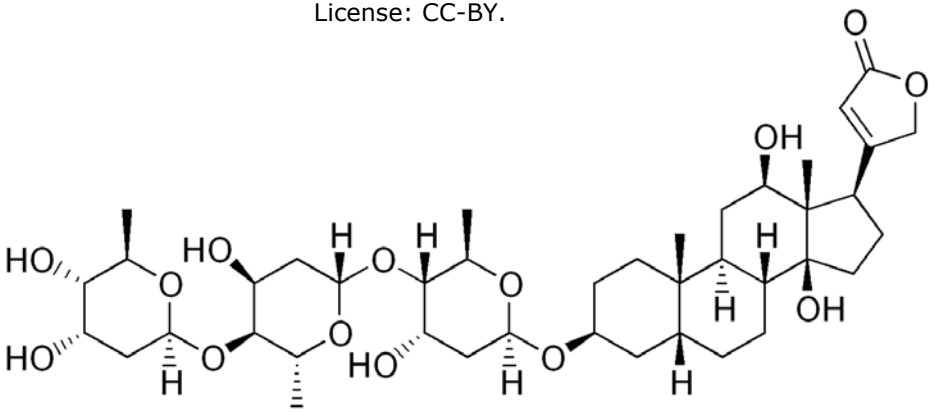


Courtesy of the authors. Used with permission.
Source: Ogawa, Haruo, Takehiro Shinoda, et al."Crystal Structure of the Sodium-Potassium Pump(Na+, K+-ATPase) with Bound Potassium andOuabain." *Proceedings of the National Academy of Sciences* 106, no. 33 (2009): 13742-7.

Pharmacodynamics and receptors:
Case study with cardiac glycosides

Digitalis purpurea
(Common Foxglove)
(Dead men's bells)

- **Toxicity**:
  - ~ ↑sympathetic nervous system activity
  - ~ atrial and ventricular arrythmias
  - ~ xanthopsia - yellow vision; Van Gogh "Yellow Period"
- **Narrow Therapeutic Index**
  - ~ Toxic level/therapeutic level = 1-3
  - ~ variations in blood level are bad
- **Digoxin ADME:**
  - ~ 70% oral bioavailability
  - ~ V_d 7.3 L/kg (511 L in 70 kg)
  - ~ $t_{1/2}$ 36-48 hr, t_{max} 1-3 hr
  - ~ 30% plasma protein binding

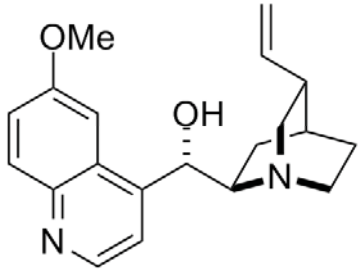


Digoxin

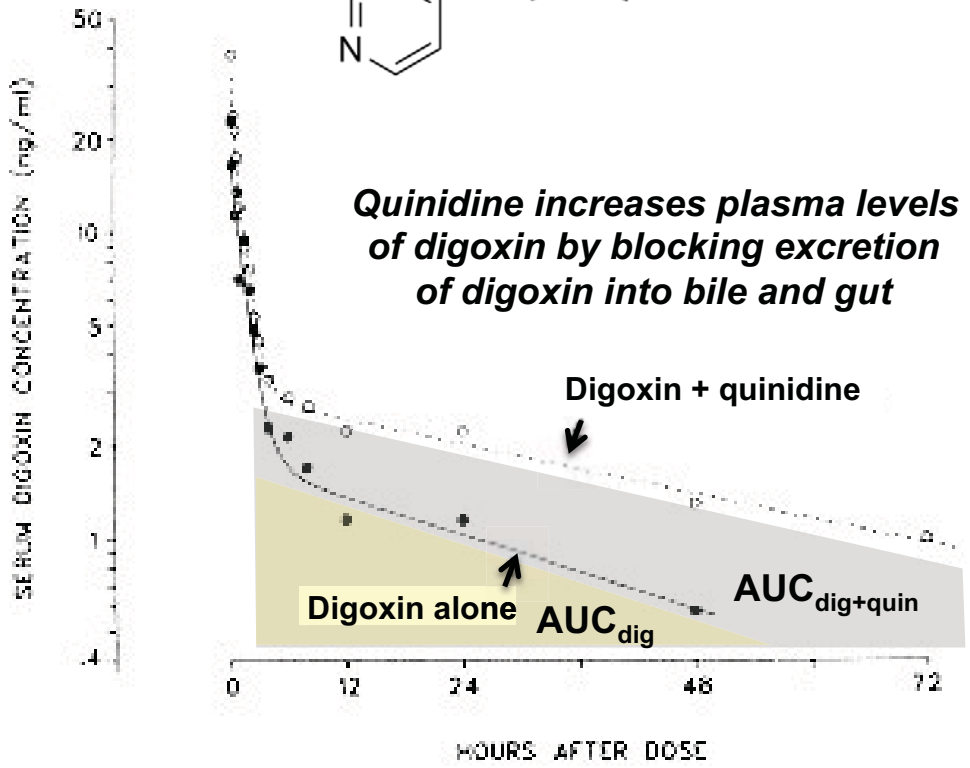
- **Metabolism**:
  - ~ Reductive metabolism by *E. lentum* in gut
  - ~ Liver: 3-keto-digoxigenin, 3-epidigoxigenin, conjugation
  - ~ Metabolic clearance ~0.8 mL/kg/min
- **Excretion:**
  - **~ Mediated by MDR1 transporter**
  - ~ 50-70% unchanged in urine
  - ~ 10-30% biliary excretion
  - ~ 10-20% direct transport into gut

MDR1/P-Glycoprotein: Digoxin Case Study

- Major role for MDR1 in excretion of digoxin
- MDR1 mediates digoxin excretion:
  ~10-30% of dose into bile
  ~20% of dose in gut lumen
- MDR1 mutation or competition for binding: major effects on digoxin PKs
- ***Competition for MDR1***:
  ~ binding thermodynamics!
  ~ Elevated plasma digoxin levels when other MDR1-transported drugs are administered (*e.g.*, quinidine)
- ***Over-expression of MDR1***:
  decreased serum digoxin levels when some drugs (*e.g.*, rifampin) cause increased MDR1 expression



Quinidine
Antiarrhythmic agent



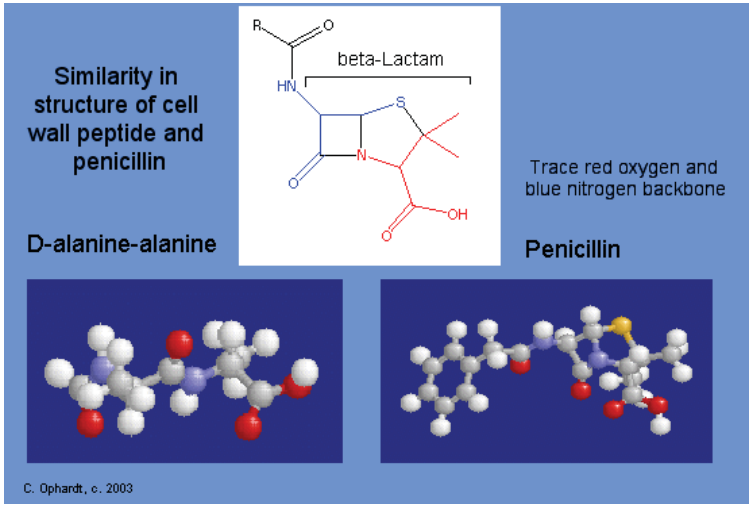
Quinidine increases plasma levels of digoxin by blocking excretion of digoxin into bile and gut

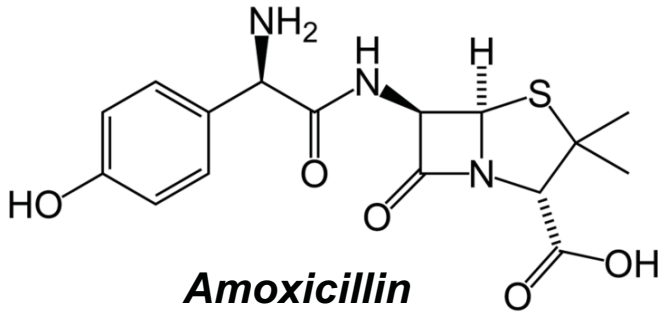
Fig. 1. Serum digoxin concentrations and pharmacokinetic functions for a representative subject (study 1) following intravenous administration of digoxin in the control state and during coadministration of quinidine.

Drescher et al. (2003)
Clin Pharm Ther **73**: 223-31.

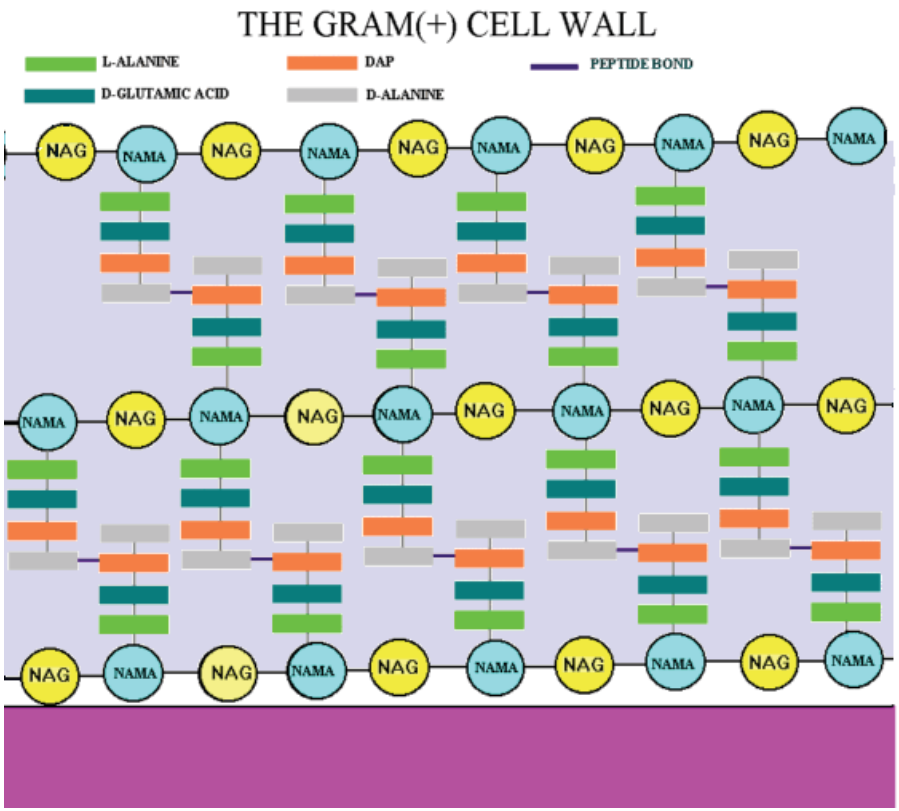
Source: Drescher, Siegfried, Hartmut Glaeser, et al. "P-glycoprotein-mediated Intestinal and Biliary Digoxin Transport in Humans." *Clinical Pharmacology & Therapeutics* 73, no. 3 (2003): 223-31.

Drugs with a high TI: Amoxicillin

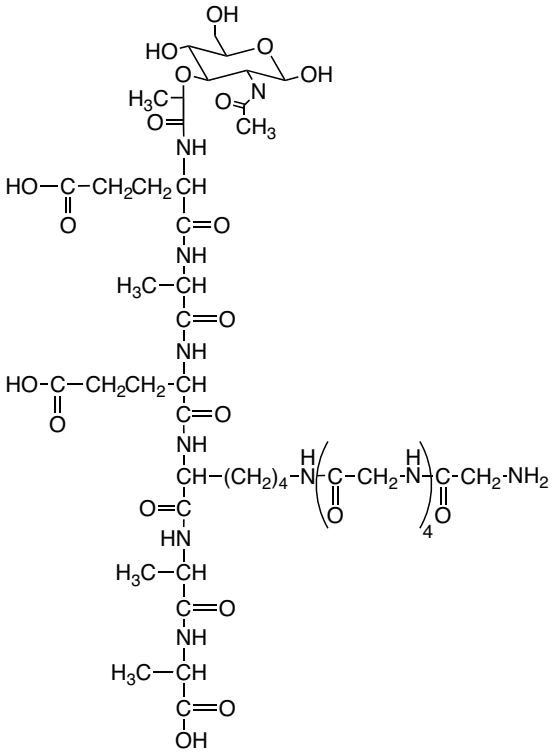


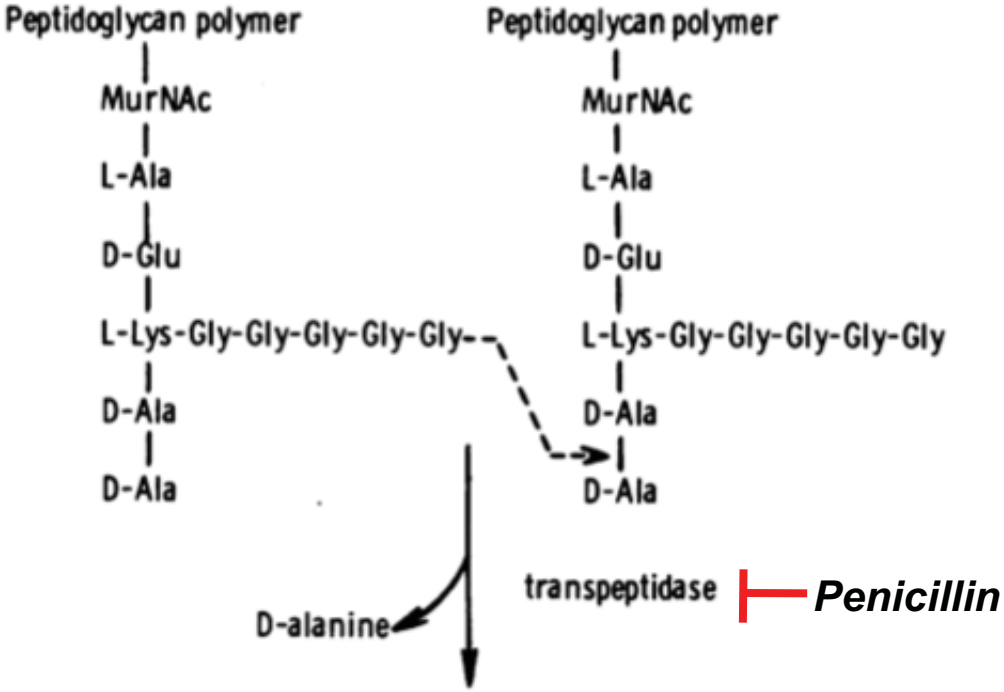


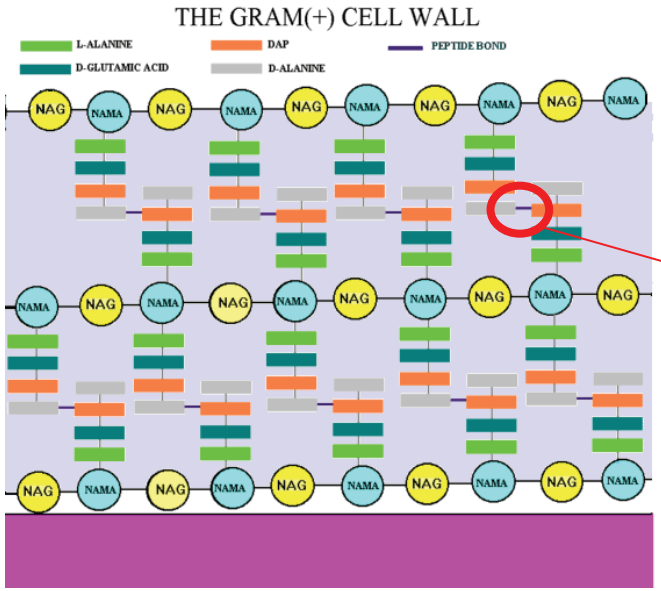
Amoxicillin

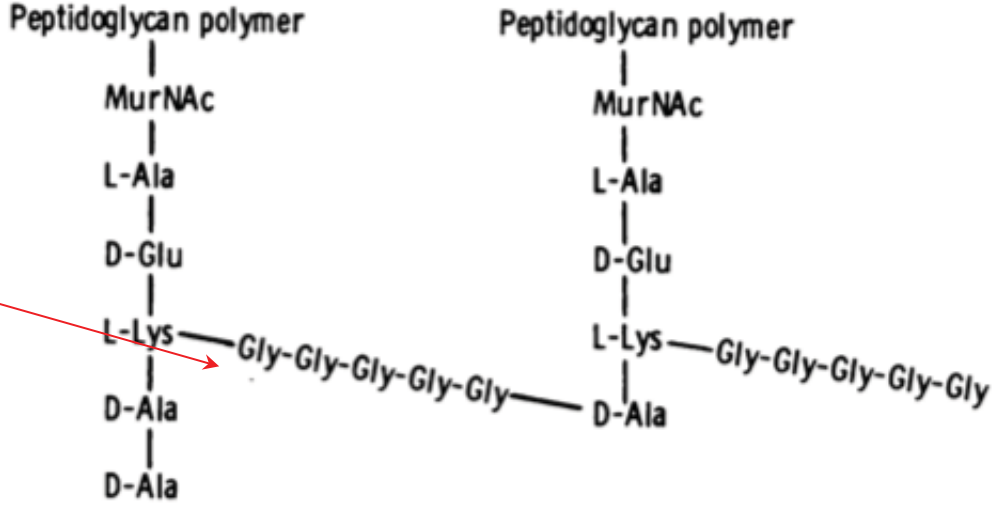


- **Amoxicillin**: penicillin family of antibiotics
- Penicillins – β-lactams
  - ~ bacteriocidal
  - ~ inhibit cell wall synthesis
  - ~ non-competitive transpeptidase inhibitor
- Analogs of D-ala-D-ala that is cross-linked by transpeptidase
- Weakened cell walls: osmotic rupture
- **LD_{50} ~ 12-25,000 mg/kg (mice, rats, rabbits)**
- **ED ~ 7 mg/kg ⇒ TI > 1000**









Outline

- Fundamental concepts in Tox and Pharm

A • Absorption, transporters, oral activity

D • Distribution, protein binding

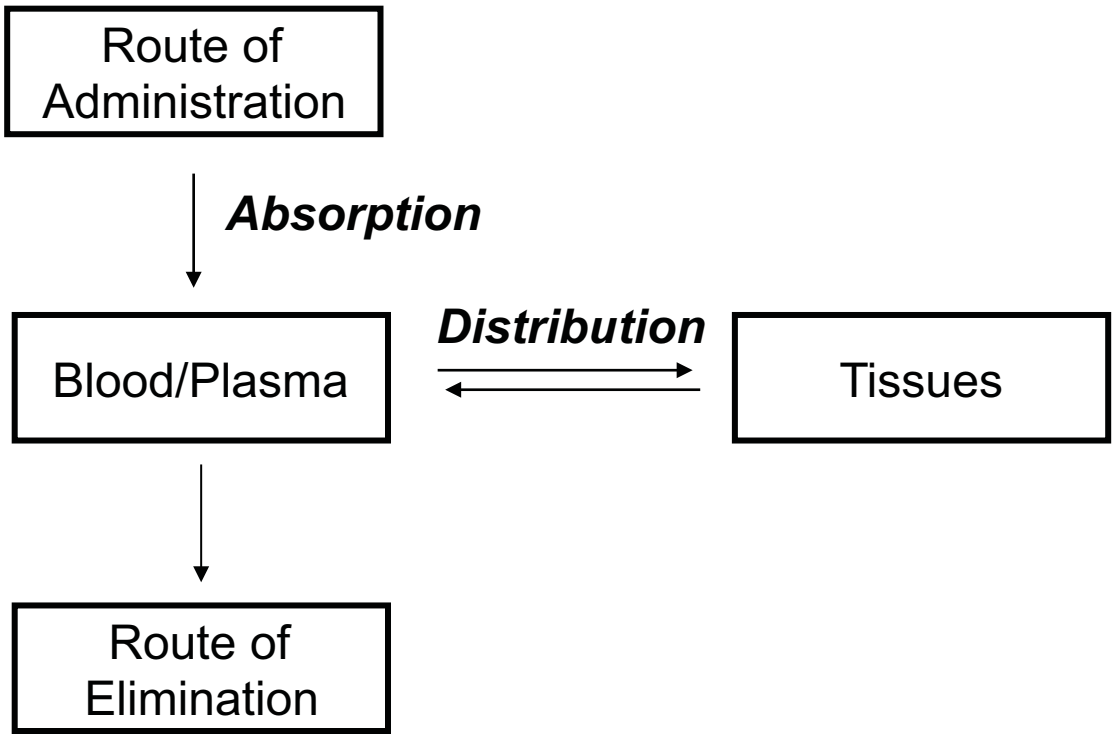
M • Metabolism, the liver and portal circulation

E • Excretion pathways

- Pharmacokinetics
- Genetic variation and ADME

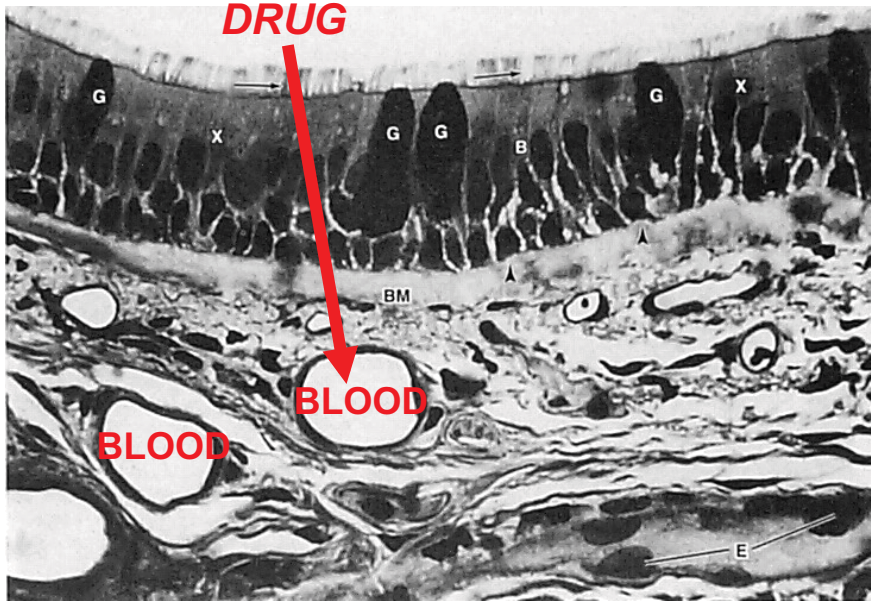
Absorption and Distribution

- Route of administration determines PKs
- ***Enteral***
  - ~ oral
  - ~ sublingual
  - ~ rectal
- ***Parenteral***
  - ~ intravenous (iv)
  - ~ intramuscular (im)
  - ~ subcutaneous (sc)
- ***Other routes***
  - ~ inhalation
  - ~ topical
  - ~ transdermal



Absorption

- Common feature of all routes of administration (except IV): epithelial barriers
- Uptake of drugs from GI tract, lungs or skin must cross epithelial cell barriers to gain access to systemic circulation
- Epithelial tissues as barriers to drug absorption
  - ~ Sheets of polyhedral cells lining all body surfaces and cavities
  - ~ Varying thickness: Single-cell (gut, lungs) to multilayer (skin)
  - ~ Cell-cell junctions prevent movement around cells
  - ~ Drugs must cross lipid bilayers
- Physicochemical factors that affect kinetics of absorption:
  - ~ pH ~ blood flow
  - ~ gastric emptying, bowel transit
  - ~ surface area: lungs - 140 m^2; GI tract - 300 m^2; skin - 1.5-2 m^2



Absorption: Epithelial tissue barriers

- *Four types of tissue*: collections of similar cells and intercellular material
  - ~ **Epithelial**: sheets of polyhedral cells lining body cavities and surfaces
  - ~ **Connective**: cells in intercellular matrix; form, adhesion, and support
  - ~ **Muscle**: contractile elements
  - ~ **Nervous**: impulse conducting
- Epithelial tissues as barriers to drug absorption
  - ~ Varying thickness: Single-cell (gut, lungs) to multilayer (skin)
  - ~ Cell-cell junctions prevent movement around cells
  - ~ Drugs must cross lipid bilayers
- Functions:
  - ~ Protection/hydration - skin
  - ~ Protection/absorption - GI tract
  - ~ Gas transfer - respiratory tract
  - ~ Hormone production - glandular elements
  - ~ Barrier to fluid movement - lining of blood vessels
- Epithelium and cancer:
  - ~ ***Carcinoma*** - malignant neoplasm of epithelial origin
  - ~ ***Sarcoma*** - malignant neoplasm of connective tissue origin
  - ~ epithelial cells account for >90% of tumors in adults >45 yo
  - ~ children <10 yo: hematologic>>neuronal>connective tissue (bone)>epithelial

Epithelial Structure/Function: Cell Structure Review

Images of cell structures from textbooks removed due to copyright restrictions.

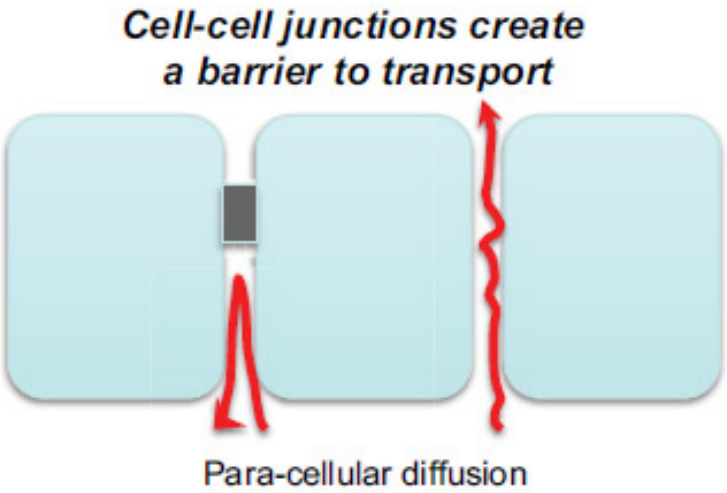
Epithelial Structure/Function: Tissue Architecture

Images of cell structures from textbooks removed due to copyright restrictions.

Epithelial Structure/Function: Cell Decorations and Attachments

Image of cell-cell junctions removed due to copyright restrictions.

Scanning electron micrographs of the surface of rat respiratory mucosa removed due to copyright restrictions. See Plate 8 of Andrews, Peter M. "A Scanning Electron Microscopic Study of the Extrapulmonary Respiratory Tract." *American Journal of Anatomy* 139, no. 3 (1974): 399-423.



Electron micrograph of microvilli removed due to copyright restrictions. See the image here.

Mechanisms of drug transport

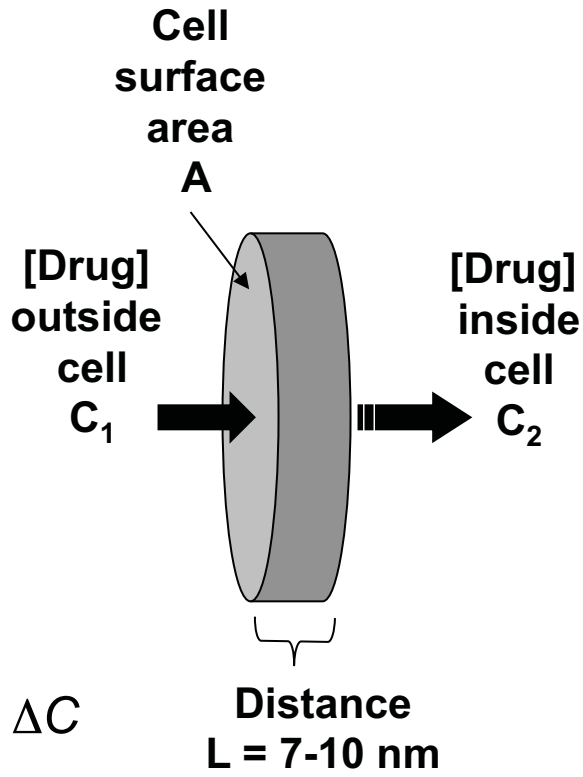
- ***Diffusion*** – *previously thought to be the major form of drug transport*
  ~ low MW, lipophilic drugs
  ~ requires a concentration gradient to provide the energy
- ***Carrier-mediated transport***
  ~ ligand binds to receptor on cell surface
  ~ complex internalized by endocytosis
  ~ Example: cholesterol/lipoproteins bind to LDL receptor
  ~ Example: steroid hormone/binding globulin complexes bind to megalin proteins
- ***Facilitated transport***
  ~ mediated by membrane transport proteins
  ~ energy provided by concentration gradient
  ~ glucose transporter
- ***Active transport –*** *now thought to be the major form of drug transport*
  ~ mediated by membrane transport proteins
  ~ transport against a concentration gradient
  ~ temperature-dependent and saturable

Mechanisms of transport: Diffusion

- Passive mechanism for small lipophilic drugs
- **Fick's law**: flux of diffusing material is proportional to the local density gradient
  - ~ J = flux along density gradient (mol•m^{-2}•s^{-1})
  - ~ D = diffusion coefficient or diffusivity (m^2/s)
  - ~ ø = density or concentration; mol/m^3
  - ~ x = position

$$J = -D\frac{\partial \phi}{\partial x} \propto \left(\frac{D}{L}\right) \bullet \Delta C$$

- Rate of diffusion affected by distance, surface area, and properties of drug and medium
  - ~ A = area available for exchange (m^2)
  - ~ L = distance (m); 7-10 nm lipid bilayer
  - ~ ΔC = concentration difference (mol/m^3)



- ***Occurs <u>down</u> a concentration gradient***:

$$C_2 < C_1 \Rightarrow -\Delta C \Rightarrow -\frac{\partial \phi}{\partial x}$$

- P = permeability coefficient (m/s) = D/l $\quad J = P \bullet \Delta C$
- For a specific area (A, m^2): $\quad \frac{mol}{s} = A \bullet J = P \bullet A \bullet \Delta C$

First-order processes in the body

- Definition of a first-order process: a reaction or activity the rate of which depends on the concentration of reactants or the chemical of interest

- Most of the processes of absorption, distribution, metabolism, elimination are first-order

- ***Absorption***: Rate of diffusion depends on the concentration gradient (i.e., the concentration of the "reactant")

$$-\frac{dQ}{dt} = P \bullet A \bullet \Delta C$$

- ***Metabolism and transport proteins*** - Enzyme kinetics generally first -order, except under conditions of substrate saturation:

$$\frac{dProduct}{dt} = V = \frac{V_{max} \bullet [S]}{K_m + [S]}$$

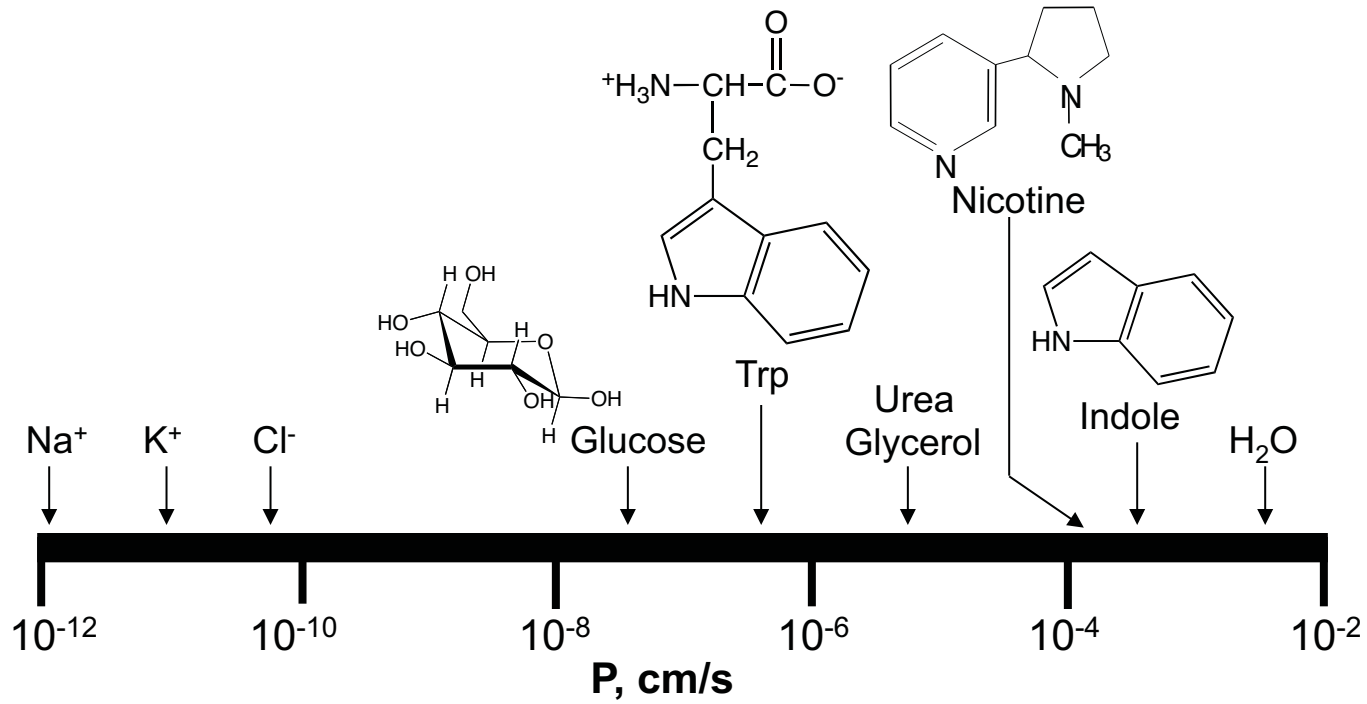
when Km>>[S], then $\frac{dProduct}{dt} = V = \frac{V_{max}}{K_m} \bullet [S] = k_{met} \bullet [S]$

Permeability of a drug determines the rate of diffusion

- Fisk's law states that movement through the membrane will be proportional to ΔC and P

$$\frac{mol}{s} = A \bullet J = -\frac{dQ}{dt} = P \bullet A \bullet \Delta C$$

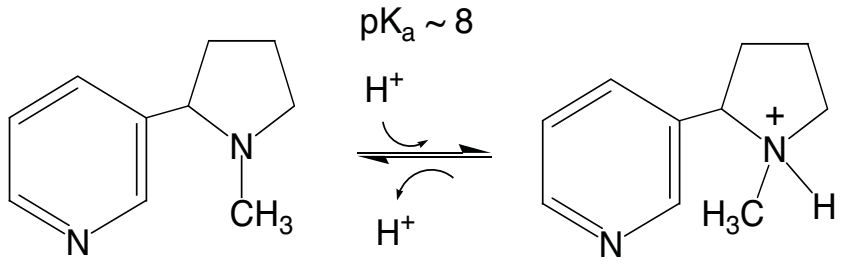
- ***Cell membrane is essentially impermeable to most charged and highly polar substances except H_2O***
- H_2O moves 10^9-times faster than Na^+ or K^+



Chemical transport and pH: The chemistry of nicotine delivery

- **Nicotine**
  - ~ addictive component of cigarette smoke
  - ~ tertiary amine - acid/base chemistry
- **Bioavailability** of nicotine is affected by pH
  - ~ Uncharged/base form: volatile; vapor phase (rapid uptake)
  - ~ Charged/acid form: non-volatile; particulate phase (slow uptake)
- Manufacturers control pH of cigarette smoke to control nicotine uptake
- Flue-cured tobacco: pH <6; air-cured dark tobacco: pH >7 - more nicotine?

Nicotine



Uncharged, base form *Charged, acid form*

$RNH_3^+ \leftrightarrow RNH_2 + H^+$

$K_a = \frac{[RNH_2][H^+]}{[RNH_3^+]}$

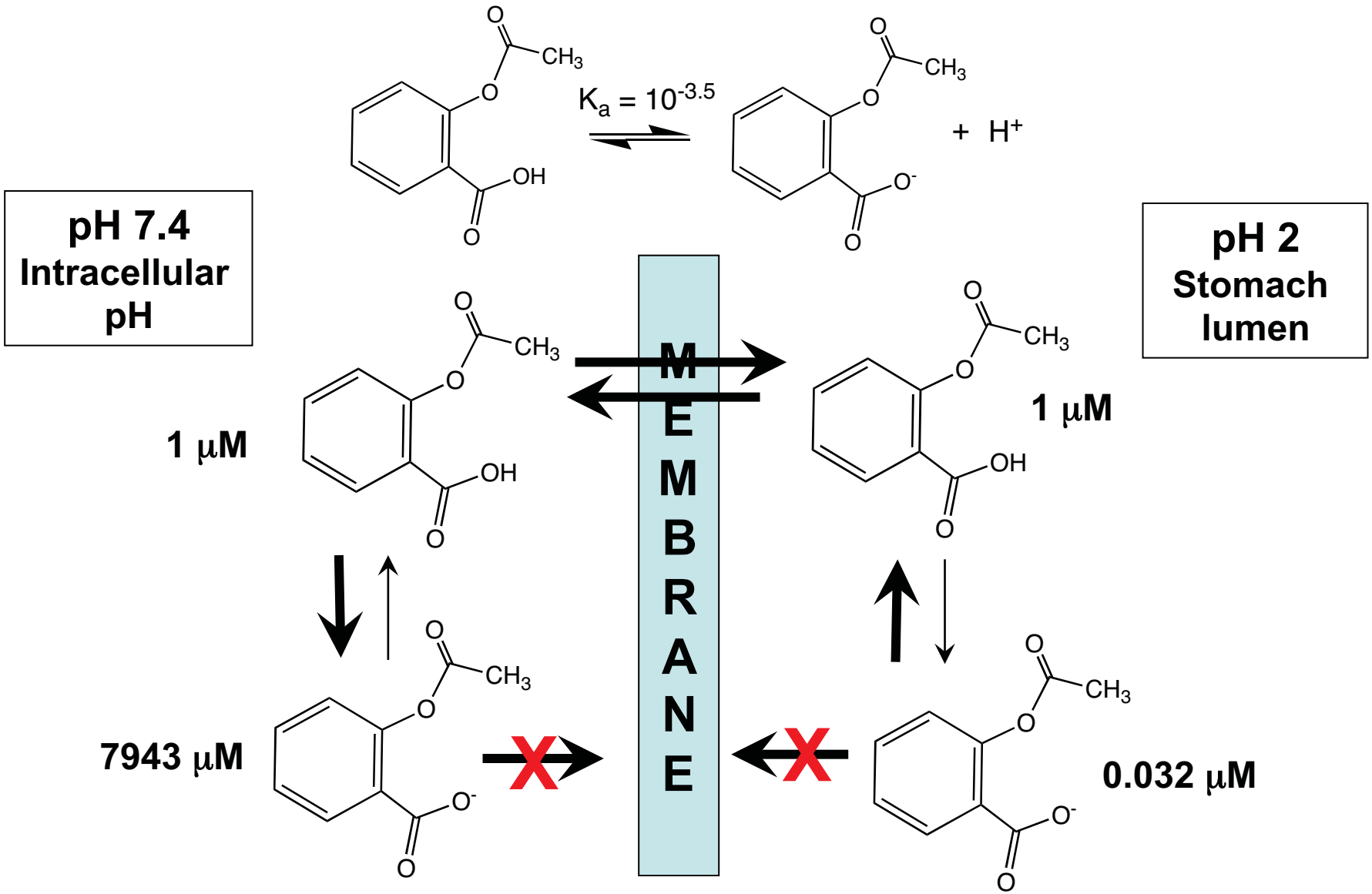
$pK_a = -\log(K_a)$

$pH = -\log[H^+]$

Henderson-Hasselbalch equation:

$pH = pK_a + \log([base]/[acid])$

pH-Dependent diffusion of aspirin can influence toxicity

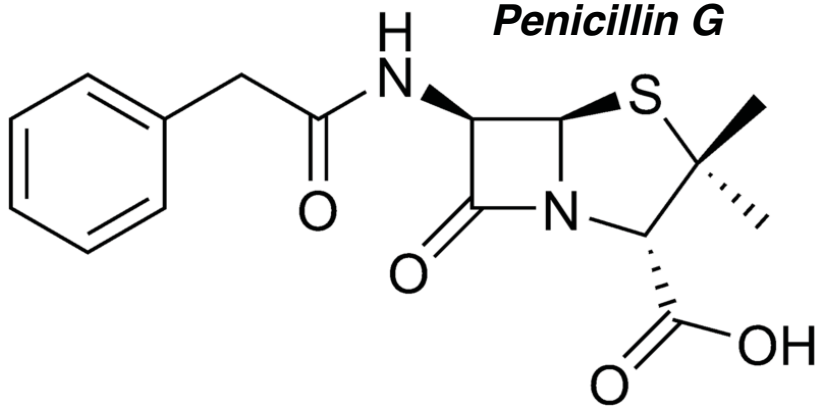


High concentration of aspirin inside gastric epithelial cells if mucous layer disturbed

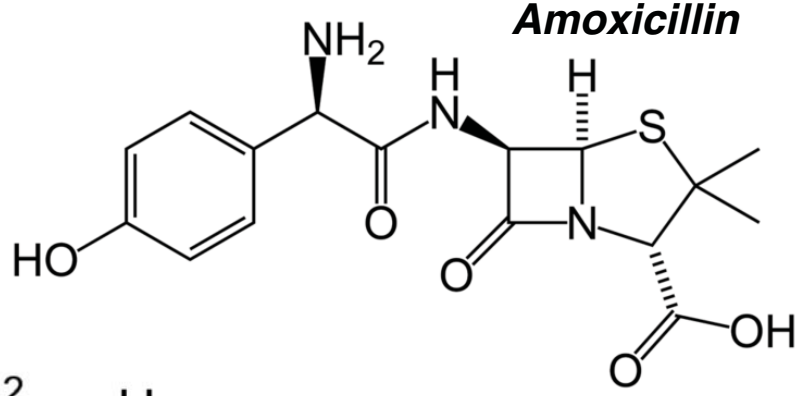
pH effects on drug stability

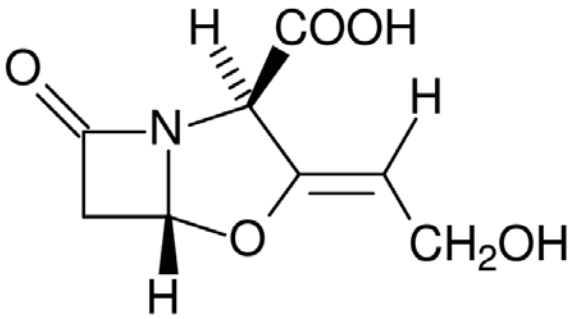
- **Penicillins**
  - ~ lactam instability at low pH (stomach)
  - ~ given by injection or IV
- **Amoxicillin**
  - ~ modify structure to confer acid stability
  - ~ first acid-stable, oral penicillins
- Most frequent form of resistance to penicillins: bacterial β-lactamase
- Solution to β-lactamase:
  - ~ Alter structure to confer β-lactamase resistance (cephalosporins, etc.)
  - ~ include β-lactamase inhibitor (e.g., clavulanic acid) in the drug formulation ("Augmentin")

Penicillin G

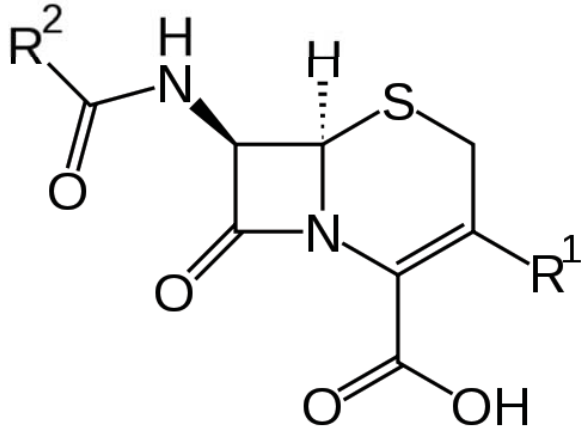


Amoxicillin





Clavulanic acid

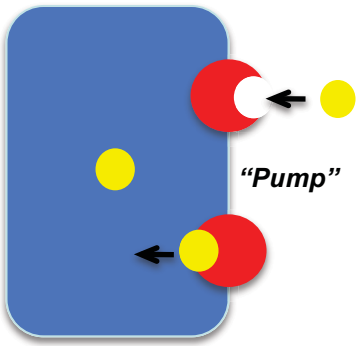


Cephalosporin core structure

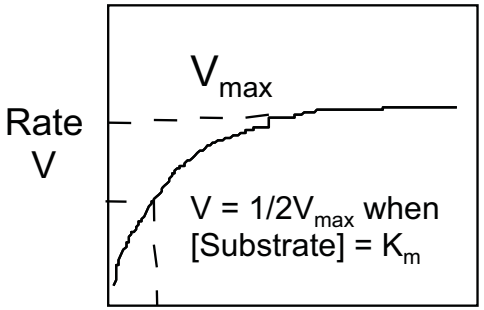
Facilitated or carrier-mediated diffusion

- Simple diffusion is too slow for most physiologic substances:
  Example - glucose diffusion very slow: P ~ 10^{-7}-10^{-8} cm/s
- Speed up diffusion by introducing a carrier protein in membrane: a "pore"
- Movement is still <u>down</u> the concentration gradient of the chemical!
- Characteristics of **facilitated diffusion**:
  - ~ Transport ***rate is greater than Fick's Law predicts***:
    simple diffusion is too slow!
  - ~ The transport protein is ***specific*** for a chemical structure
  - ~ Transport is ***saturable***: finite # binding sites on a protein and finite rate of transport
  - ~ Obeys ***Michaelis-Menten kinetics***

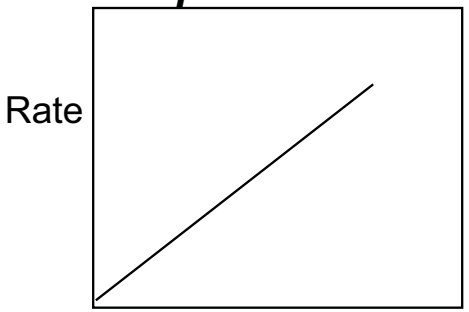
$$V = \frac{V_{max} \bullet [S]}{K_m + [S]}$$



Facilitated Diffusion



Simple Diffusion



<u>Km</u>	
L-glucose	>3000
galactose	30
mannose	20
D-glucose	1.5

Predicting “drug-like behavior”

- Problem: Poor bioavailability and pharmacokinetics were major causes of drug failure for orally active drugs

- Problem: evolution of drug development to high-throughput screening of large, DMSO-solvated drug libraries (combinatorial) led to drug structures with potentially undesirable properties: large size, poor water solubility, high lipophilicity

- Problem: how to screen early drug leads for good behavior as an “orally active drug”?

- Christopher Lipinski (1997) studied thousands of successful drugs to determine which physicochemical properties accounted for successful orally active drugs: what was the structure of a drug that was well absorbed and pharmacokinetically well behaved?

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* **46:** 3–26

Predicting "drug-like behavior"

- Lipinski compared the structures of drugs that had entered Phase II clinical trials in the US
- Rationale: Phase II trials serve as a filter (albeit an expensive one!) for rejecting drug candidates that were poorly soluble, poorly permeable or poorly absorbed in the gut. Any drug making it into Phase II trials was a well behaved drug candidate.
- Lipinski selected 2500 drugs from World Drug Index, a computerized database of about 50000 drugs that had reached Phase II trials
- Collected data for several criteria for each drug:
  ~ ***Molecular weight*** –high molecular weight ➔ poor permeability
  ~ ***Lipophilicity*** – Calculated LogP (P = octanol/H_2O partition coefficient) between -0.5 and 5 (not too lipophilic and not too polar)
  ~ ***# H-bond donors/acceptors*** – High # ➔ poor membrane permeability

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* **46:** 3–26

Predicting "drug-like behavior": Lipinski's "Rule of 5"

- A well behaved, orally active drug should have:
  - ~ less than **5** H-bond donors
  - ~ less than **10** H-bond acceptors
  - ~ molecular weight less than **500**
  - ~ MLogP less than **5**

  } ***"Rule of 5"***

- Drugs exceeding one or more of these values has a high probability of failing

- Average values derived from the 2500 Phase II candidates studied:
  - ~ MlogP = 1.80
  - ~ Molec. weight = 408
  - ~ H-bond donor = 2.53
  - ~ H-bond acceptor = 6.95

- Relatively good predictor: Alerts for possible poor absorption-12%

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* **46:** 3–26

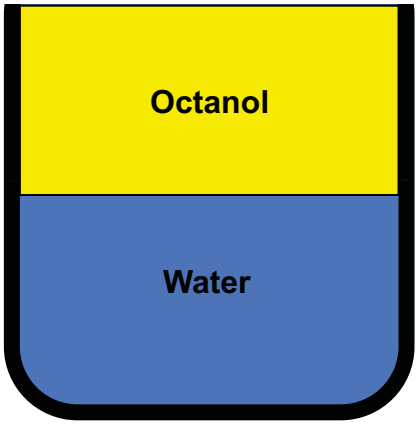
Strengths and weaknesses of LRO5

- Most drugs are intended for oral route of administration, but predicting oral absorption and behavior is difficult
- LRO5 provides a computational tool to screen out the least “drug-like” molecules from a large library
- LRO5 increases the probability that a drug lead will have acceptable oral activity (bioavailability)
- However, LRO5 is very crude in its predictive power
  ~ Many LRO5 compliant molecules turn out to be poor drugs
  ~ LRO5 rules out the worst cases, so many bad candidates are missed
- Exceptions to LRO5
  ~ Applies only to orally active drugs
  ~ Applies only to drugs absorbed by passive mechanisms (diffusion)
  ~ Does not apply to drugs with transporters involved in absorption
- Most drugs now appear to have transporters involved at some point in their ADMET, so parallel LRO5 calculation and transporter assessment is critical

TH Keller, A Pichota, Z Yin *Current Opinion in Chemical Biology* **10**: 357–361, 2006

LogP and the activity of a drug

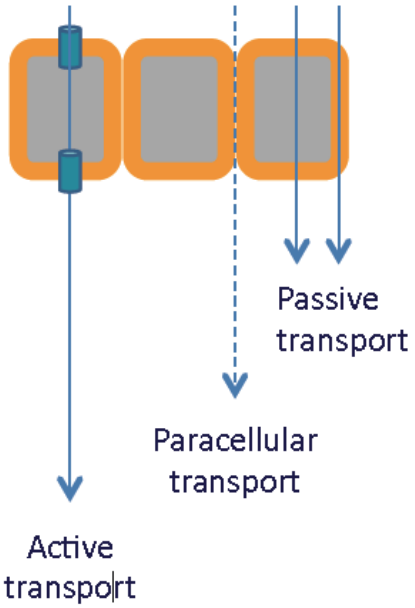
• IUPAC definition of lipophilicity:
"…represents the affinity of a molecule or moiety for a lipophilic environment. It is commonly measured by its distribution in a biphasic system (e.g., partition coefficient in octanol-water)."



$$P = \frac{\text{Concentration in organic solvent}}{\text{Concentration in water}}$$

• Lipophilicity is usually a major factor involved in determining the biological activity of a drug

• Rule of thumb: Optimize/minimize LogP to reduce toxicity, non-specific binding, increase bioavailability.



Adapted from John Comer, CSO, Sirius Analytical Ltd.

LogP and the activity of a drug

- Relationships between Log P and activity vary :

  Linear: Activity= m•log P + k
  Parabolic: Activity= m•log P - c(log P)2 – k
  Rectilinear: Activity= m•log P – c(blog P +1) – k

- Most useful relationships between activity and physico-chemical parameters (e.g., LogP, pK_a) come from multivariate statistical analysis.
- Correlation of LogP with activity is mostly empirical - organic solvents are simple models of lipid bilayers etc. and cannot explain everything.
- CNS, gastric absorption: parabolic LogP relationship holds (Log P ~2±1), from experiments relating radiolabelled compounds and behavior.
- Sophisticated analysis of molecular properties such as “partial charged surface area” and H-bonding properties better predict oral absorption.

LogP and the activity of a drug

- Literature survey reveals general guidelines for optimal Log P values for penetration or absorption:
  - ~ CNS: LogP = 2
  - ~ Oral: LogP = 1.8
  - ~ Intestinal: LogP =1.4
  - ~ Colonic: LogP = 1.3
  - ~ Sublingual: Log P = 5.5
- Formulation and dosing forms:
  - ~ Low Log P (below 0) Injectable
  - ~ Medium (0-3) Oral
  - ~ High (3-4) Transdermal
  - ~ Very High (4-7) Toxic build up in fatty tissues
- Drug Clearance and Toxicity:
  - ~ $LogD_{7.4}$ >0: ↓ renal clearance, ↑ metabolic clearance.
  - ~ High $LogD_{7.4}$: metabolised by hepatic P450 enzymes.
  - ~ High ionisation: keeps drugs out of cells, ↓ systemic toxicity.
  - ~ pKa 6~8 advantageous for membrane penetration

The battle between active and passive transport mechanisms

1. Paper suggesting that Active Transport is the main route across membranes

Dobson, P. D.; Kell, D. B.,
Carrier-mediated cellular uptake of pharmaceutical drugs: an exception or the rule?
Nat Rev Drug Discov 2008, 7 (3), 205-20.

2. Pharmaceutical scientists respond: "Passive & Active Transport Co-exist"

Sugano, K.; Kansy, M.; Artursson, P.; Avdeef, A.; Bendels, S.; Di, L.; Ecker, G. F.; Faller, B.; Fischer, H.; Gerebtzoff, G.; Lennernaes, H.; Senner, F.,
Coexistence of passive and carrier-mediated processes in drug transport.
Nat Rev Drug Discov 2010, 9 (8), 597-614.

3. Re-iteration of the Active Transport argument

Kell, D. B.; Dobson, P. D.; Oliver, S. G., Pharmaceutical drug transport: the issues and the implications that it is essentially carrier-mediated only.
Drug Discov Today 2011, 16 (15-16), 704-14.

4. Pharmaceutical scientists: "Let's look at the evidence"

Di, L.; Artursson, P.; Avdeef, A.; Ecker, G. F.; Faller, B.; Fischer, H.; Houston, J. B.; Kansy, M.; Kerns, E. H.; Kramer, S. D.; Lennernas, H.; Sugano, K., Evidence-based approach to assess passive diffusion and carrier-mediated drug transport.
Drug Discov Today 2012.

From John Comer, CSO, Sirius Analytical Ltd.

Active transport

- Emerging appreciation for major role of active transport in drug uptake and distribution
- Transport proteins that require chemical energy source
- Can occur ***up*** a concentration gradient
- ***Primary active transport***
  ~ ATP hydrolysis as energy source
  ~ E.g.: p-glycoprotein (P-GP), multi-drug resistance protein (MRP)
- ***Secondary active transport***
  ~ Ion gradients generated by 1° transport provide energy for transport of drugs up a concentration gradient
  ~ Co-transport and counter-transport
  ~ Examples: Na^+-taurocholate cotransporter; Na^+/H^+-antiporter
- ***Tertiary active transport***
  ~ ion gradients from 2° transport drive molecules against electrochemical gradient
  ~ Example: OH^-/HCO_3^- anion exchange

20.201 Mechanisms of Drug Actions

Fall 2013