20.201 Mechanisms of Drug Action

Uptake and Distribution

Pharmacokinetics

October 9, 2013

Review and Agenda

- Covered significant portions of ADMET
  - **A** ~ Uptake = absorption
  - **D** ~ Distribution
  - **M** ~ Metabolism - Tannenbaum
  - **E** ~ Elimination
  - **T** ~ Toxicology - Wright, Tannenbaum

  } Transporters - Hoffmaster

- Pharmacokinetics was defined as 1/2 of pharmacology:
  - ~ “Pharmacokinetics” - getting to the target
  - ~ “Pharmacodynamics” - action at the target

- Now look at pharmacokinetics in a more practical, quantitative sense

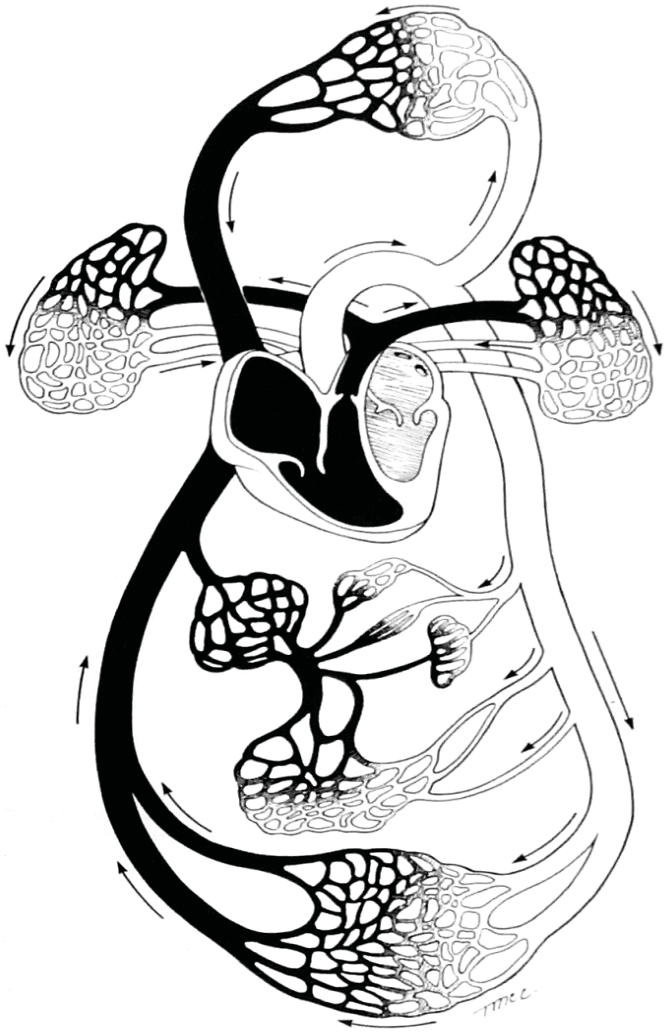
Things to learn today

- Volume of distribution
- Portal circulation/Hepatic extraction
- Fluid compartments
- Protein binding concepts and constants
- Drug-drug interactions due to protein binding
- Routes of administration
- Bioavailability/bioequivalence
- Area under the plasma concentration-time curve
- Zero-, first-, second-order kinetics
- Plasma half-life
- Clearance
- Pharmacokinetic models – one-, two-, multi-compartment
- Dosing calculations

Drug Distribution

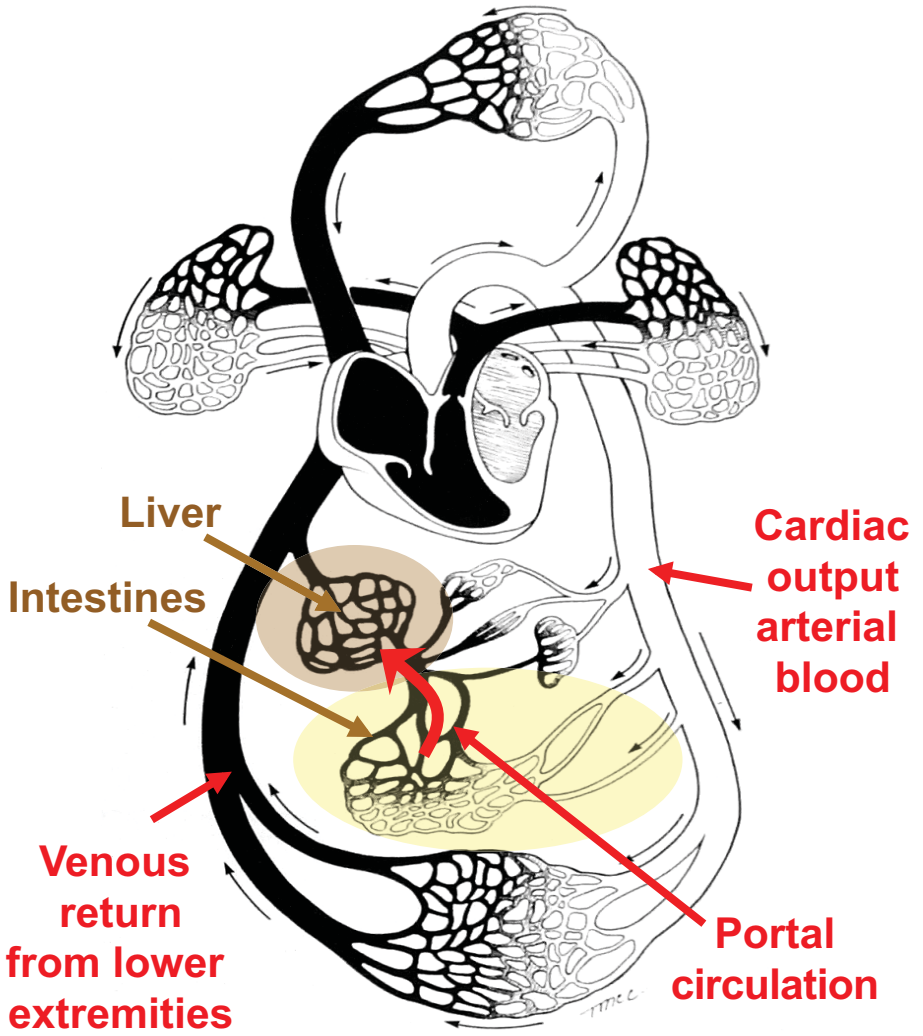
- Once absorbed, a drug molecule is subject to distribution throughout body by the circulatory system

- **Major concepts of drug distribution**
  - **~ portal circulation**
  - **~ plasma protein binding**
  - **~ fluid compartments**
  - **~ Volume of Distribution (V_d)**



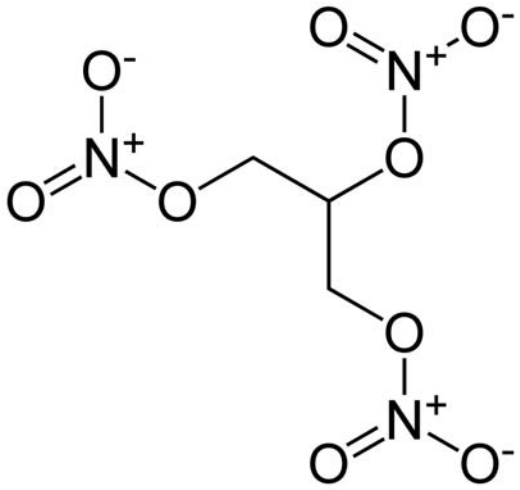
Drug Distribution

- Unique circulatory system for intestines and liver: **portal circulation**
- Venous outflow from GI tract (lower stomach, small intestine, upper colon) enters portal vein
- Portal vein enters liver and branches as capillaries to deliver blood to hepatocytes
- 80% of blood entering liver from portal vein; 20% from hepatic artery
- **Net result: orally administered drugs must pass through the liver before entering circulation**



Drug Distribution

- ***Hepatic extraction***: degree to which drug is removed from blood on each pass through the liver
- Example: 63% of *rosuvastatin* is "captured" by liver on each pass
- ***First-pass metabolism***: degree to which a drug is metabolized on first pass through liver in portal circulation
- Example: *nitroglycerin* for angina
- >90% first-pass metabolism demands alternate route for administration
- Sublingual and rectal routes: venous absorption leads to systemic circulation and bypasses liver



<u>Nitroglycerin ADME</u>

- V_d ~200 L
- $t_{1/2}$ ~1-4 min
- Metabolism: 1,3- & 1,2-dinitroglycerol (active, $t_{1/2}$ 3-4 hr); 2 inactive mets.
- 60% protein bound
- Renal excretion of parent, metabolites

Apparent Volume of Distribution (V_d)

- *Hypothetical volume into which the drug is dissolved or distributed*

V_d = (total amount of drug)/(plasma concentration) = Dose/C_{p0}

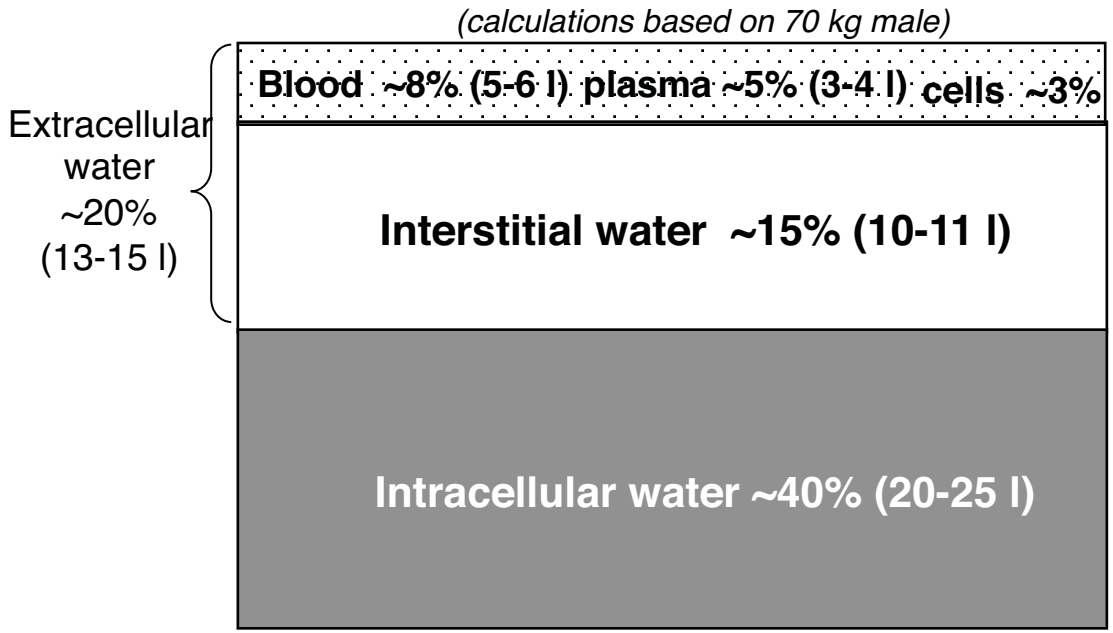
- Limited physical interpretation but useful concept to understand water compartments and gross physicochemical properties of drug

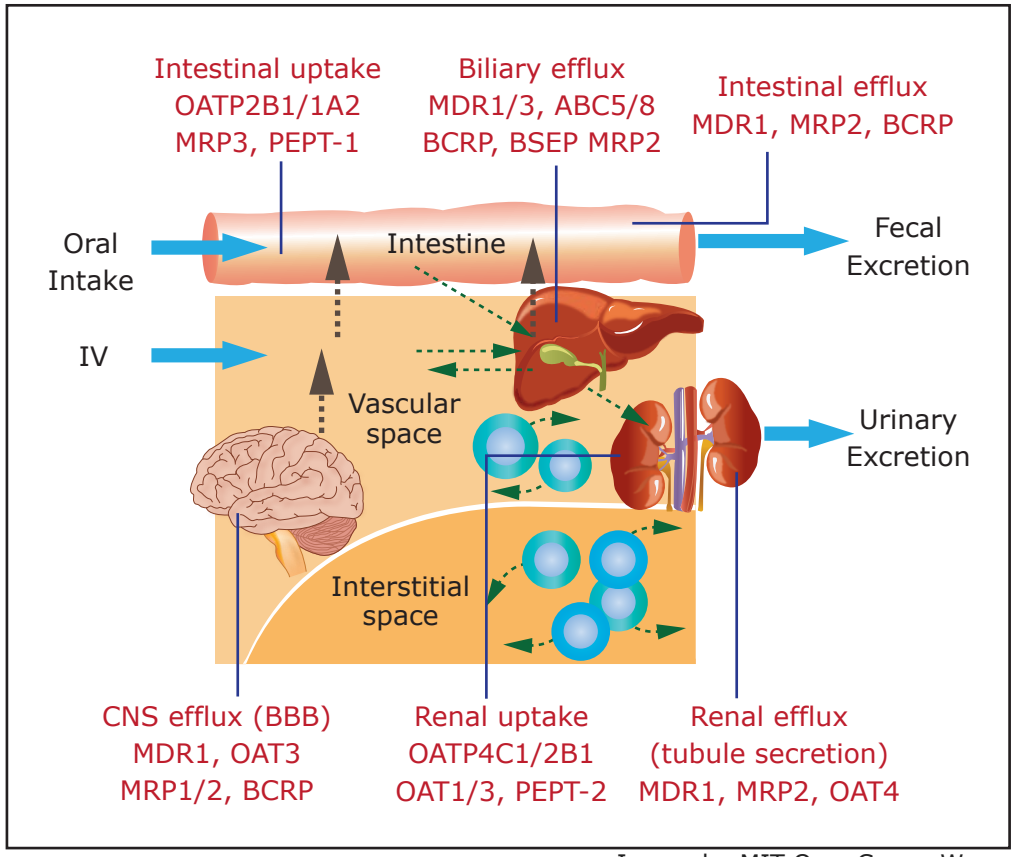
- Affected by: plasma protein binding, binding in tissues, lipid solubility, etc.

- *Lipid soluble drugs have a high apparent volume of distribution*

- Concept of V_d reflects fluid compartments
  - ~ Total body water is ~ 60% of mass
  - ~ Three fluid compartments:
    - blood
    - interstitial
    - intracellular
  - ~ Epithelial barriers

- V_d's often reflect real fluid compartments





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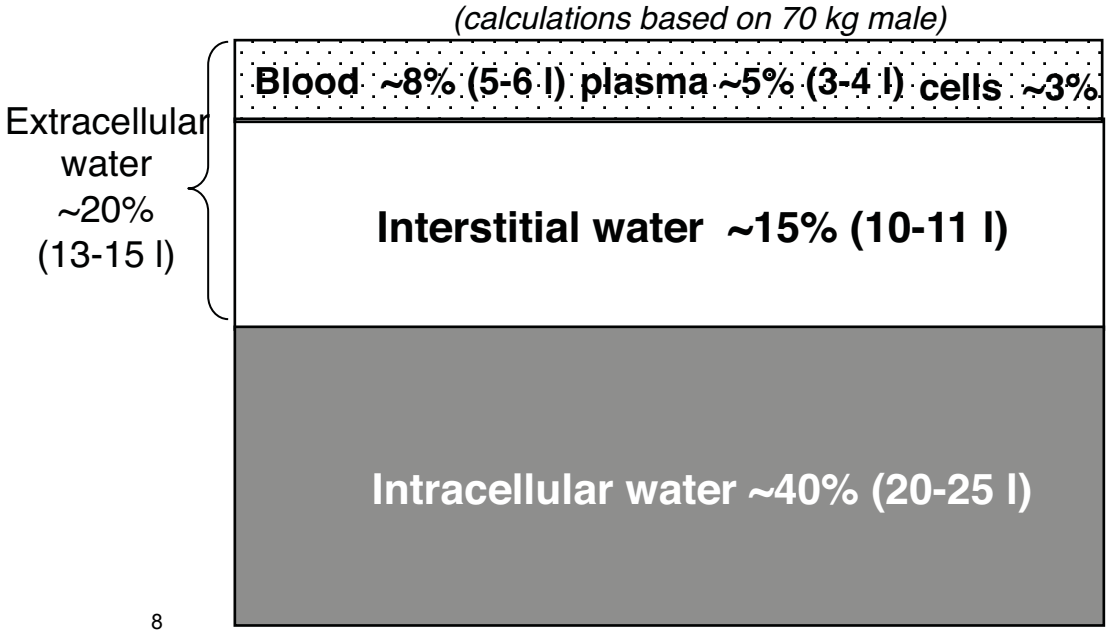
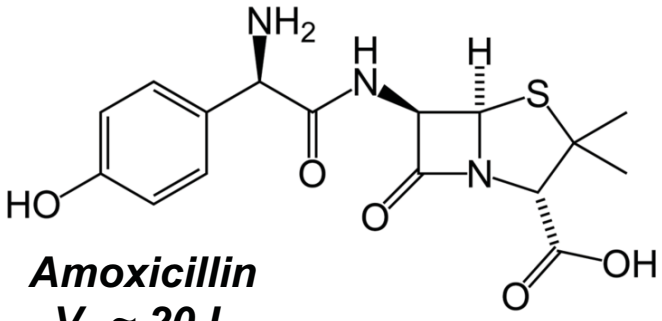
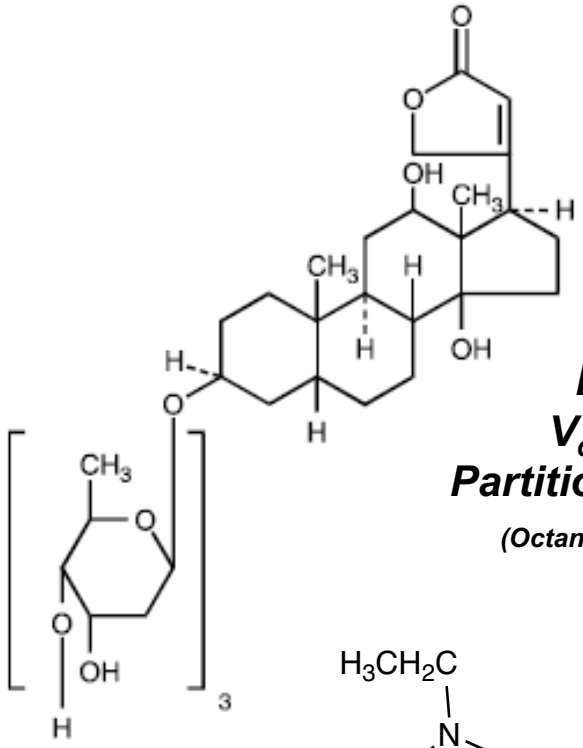




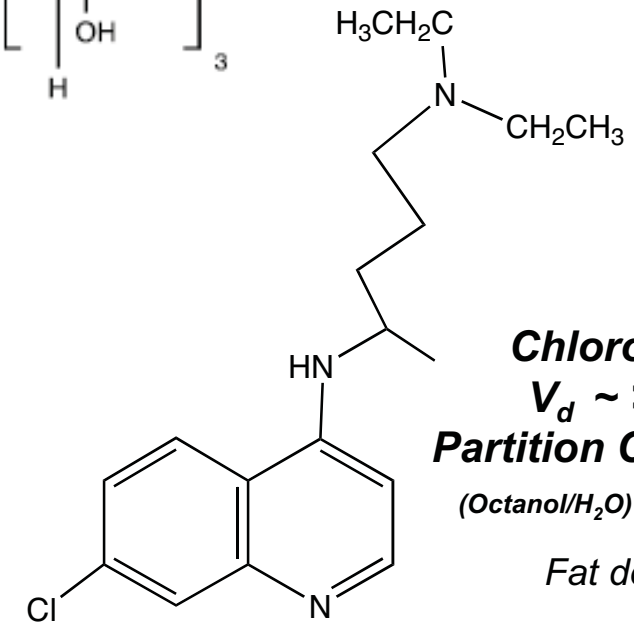
Amoxicillin
V_d ~ 20 L
Partition Coefficient
(Octanol/H_2O) = 0.03

Blood and interstitial fluids

Drug	V (L/Kg)	V (L, 70 Kg)
Sulfisoxazole	0.16	11
Amoxicillin	0.3	20
Phenobarbital	0.55	38
Phenytoin	0.63	44
Diazepam	2.4	168
Digoxin	7	490
Chloroquine	>100	$>10^4$



Digoxin
V_d ~ 490 L
Partition Coefficient
(Octanol/H_2O) = 18.4



Chloroquine
V_d ~ $>10^4$L
Partition Coefficient
(Octanol/H_2O) = 52,000

Fat depot!

Concepts of distribution: Protein binding

• Binding of drugs to proteins in blood is a major determinant of PKs and a source of toxic drug-drug interaction

• Binding generally depends on charge and water solubility: hydrophobic drugs bind to hydrophobic pockets in serum proteins

• Importance of protein binding:

~ "active" drug = unbound drug = can bind to target

~ binding affects concentration of "active" drug at the site of action

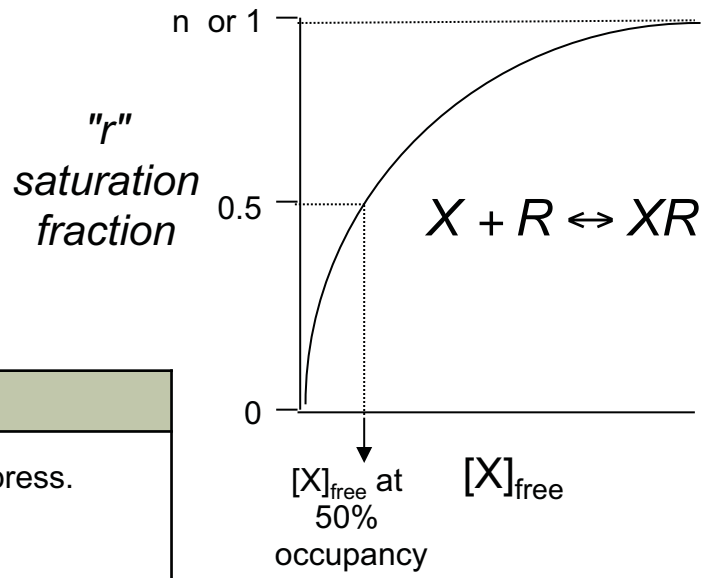
~ wide variation in serum protein concentrations in different diseases

~ drug-drug interactions can involve competition for protein binding

~ "bumping" a drug off of protein increases its unbound concentration

Concepts of distribution: Protein binding

- Focus on two critical serum proteins:
  - ***~ albumin***
  - ***~ α1-acid glycoprotein***
- Fundamental binding isotherm quantifies binding affinity



Proteins in serum

Molecule	*KDa*	*G/dL*	*μM*	*Function*
Albumin	66.5	4.5	670	Chemic. trans., oncotic press.
Globulins				
Immunoglobulins (IgG)	150	1.5-2	130	Humoral immunity
Lipoprotein				Lipid and chemical transport
Transferrin	79	0.2	17	Iron transport
Ceruloplasmin	150	0.3	20	Copper transport
Haptoglobin				Binds to hemoglobin
Steroid-binding globul.	53	0.05	0.8	Transport of steroid hormones
Thyroid-binding globul.				Transport of thyroxin
Macroglobulins				
α1-Acid glycoprotein	42	0.4-1	9	Acute phase reactant, chem. trans.
Fibrinogen	400	0.5	12	Clot formation
Complement proteins				Immune function

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

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

$$r = 0.5 \Rightarrow [R]_{free} = [RX]$$

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

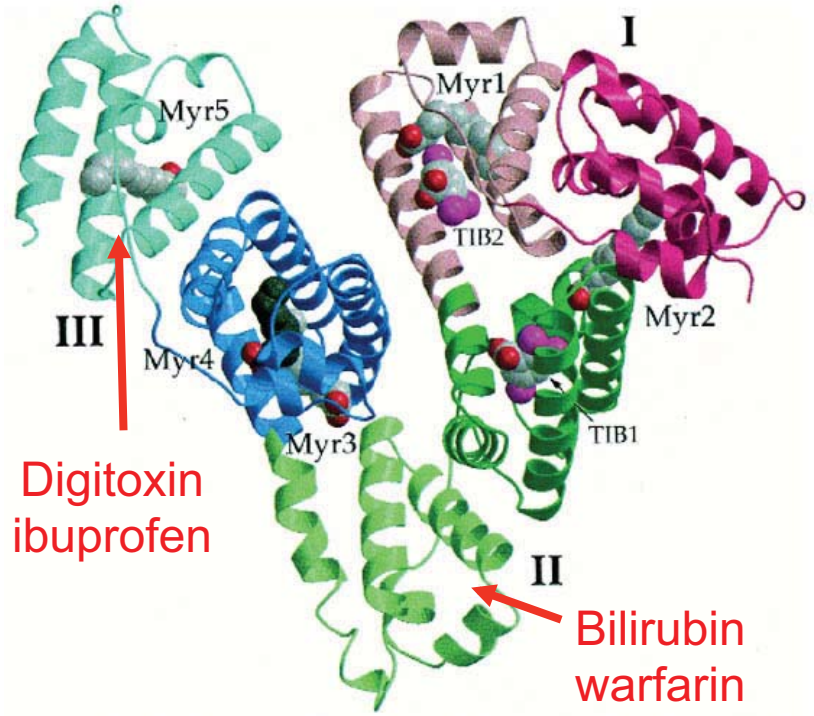
Serum albumin as a drug transport protein

- Most abundant protein in plasma, most important protein for drug

- Member of a protein family
  - ~ α-fetoprotein, vit. D binding protein
  - ~ 3 heart-shaped domains
  - ~ ***most drugs bind subdomains IIA, IIIA***
  - ~ IIA and IIIA have hydrophobic pocket
  - ~ I lacks hydrophobic pocket

- Endogenous ligands: fatty acids, bilirubin, steroids, NO, metals

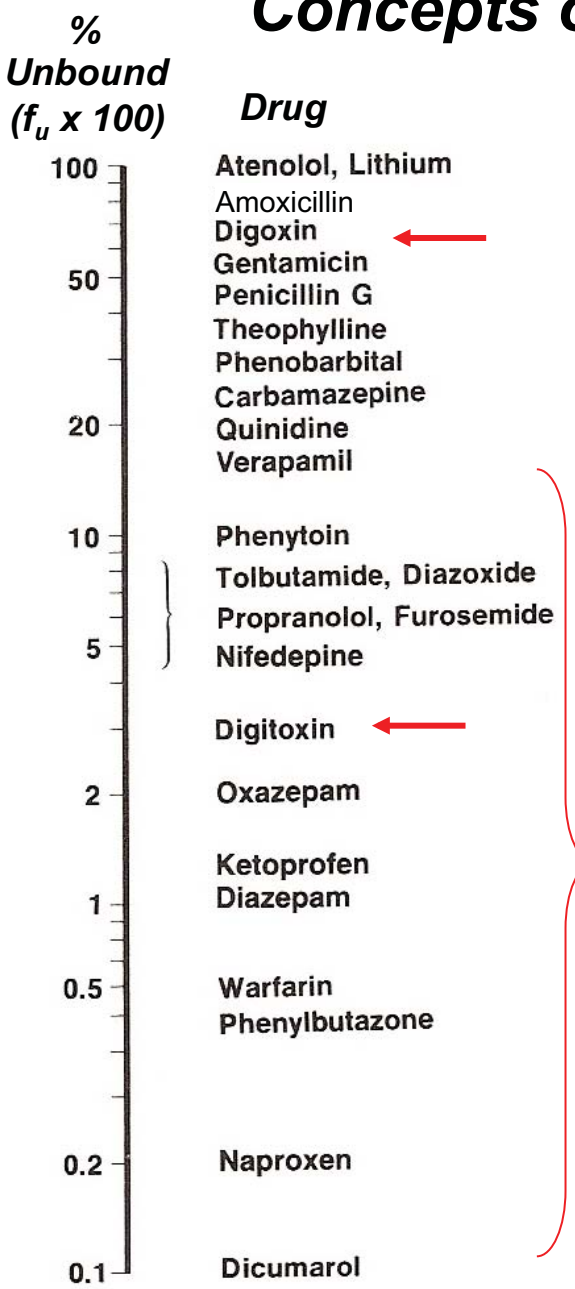
- Drug binding
  - ~ Most drugs bound less tightly than endogenous chemicals:
  - ~ 1-4 primary/high-affinity binding sites; many weaker/nonspecific binding sites



Chemical	K_a, M^{-1}
bilirubin	10^8
oleate	10^8
Ca^{+2}	10^2
drugs	10^4-10^6

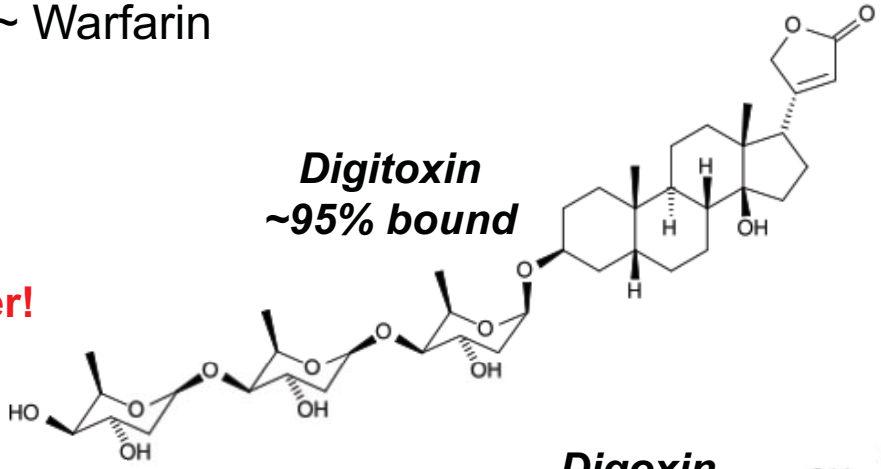
He and Carter (1992) Atomic structure and chemistry of human serum albumin. *Nature* **358**: 209-15

Concepts of distribution: Protein binding

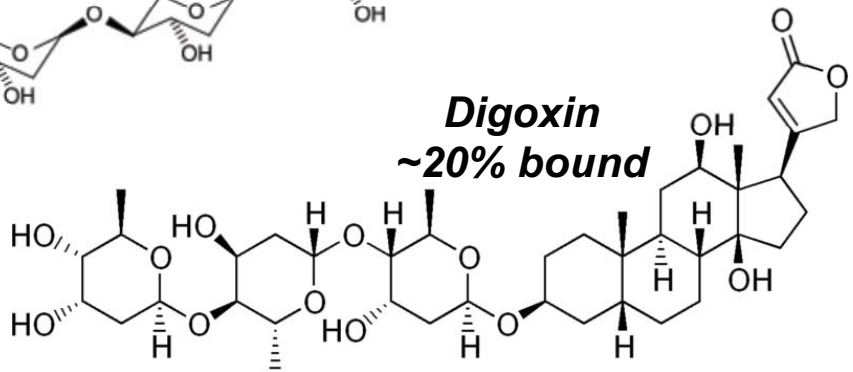


- *Bound drugs can be displaced by competition*
- Competition by endogenous ligands or other drugs
- Net result: increase in the unbound/free concentration of a drug
- Danger for drugs with narrow TI!
  - ~ Digitoxin (compare to digoxin)
  - ~ Warfarin

Digitoxin
~95% bound

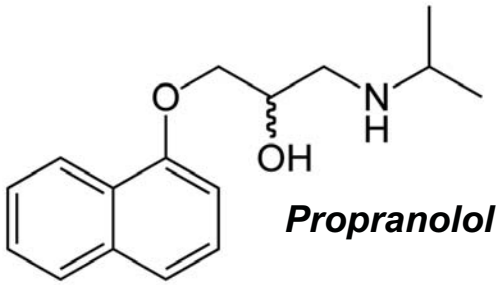


Digoxin
~20% bound



Consequences of altered protein binding in disease

- **Propranolol**: β-adrenergic receptor antagonist used to treat hypertension, tachyarrythmias, migraine
- Bound extensively to **α-acid glycoprotein**: cationic charge
- *What happens to the level of drug binding when the protein level is altered by disease?*



Propranolol

$$K_a = \frac{[X]_b}{[X]_f \bullet [P]_f}$$

Binding of drug X to protein P
X_f = free or unbound drug
X_b = bound drug
P_f = free or unoccupied protein

$$f_u = \frac{[X]_f}{[X]_f + [X]_b} = \frac{[X]_f}{[X]_t}$$

$$f_u \bullet [X]_t = [X]_f$$

$$(1 - f_u) \bullet [X]_t = [X]_b$$

f_u = Fraction of drug unbound

$$f_{P_f} = \frac{[P]_f}{[P]_t}$$

f_{Pf} = Fraction of protein unoccupied

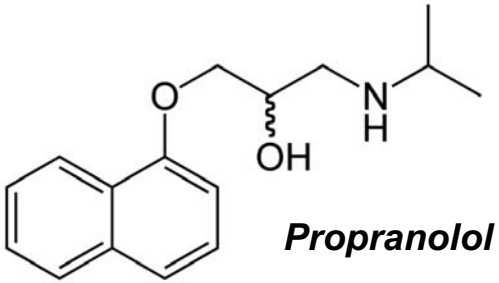
Substitute $$K_a = \frac{(1 - f_u) \bullet [X]_t}{f_u \bullet [X]_t \bullet [P]_f}$$

Solve for f_u $$f_u = \frac{1}{1 + K_a \bullet f_{P_f} \bullet [P]_t}$$

- Free concentration of drug depends on binding constant, concentration of unoccupied binding sites on protein, and protein concentration
- In general, f_{pf} ~ 1: most sites are unoccupied
- ***Thus, concentration of free drug depends on protein concentration and is relatively constant at different drug concentrations (steep part of binding isotherm)***

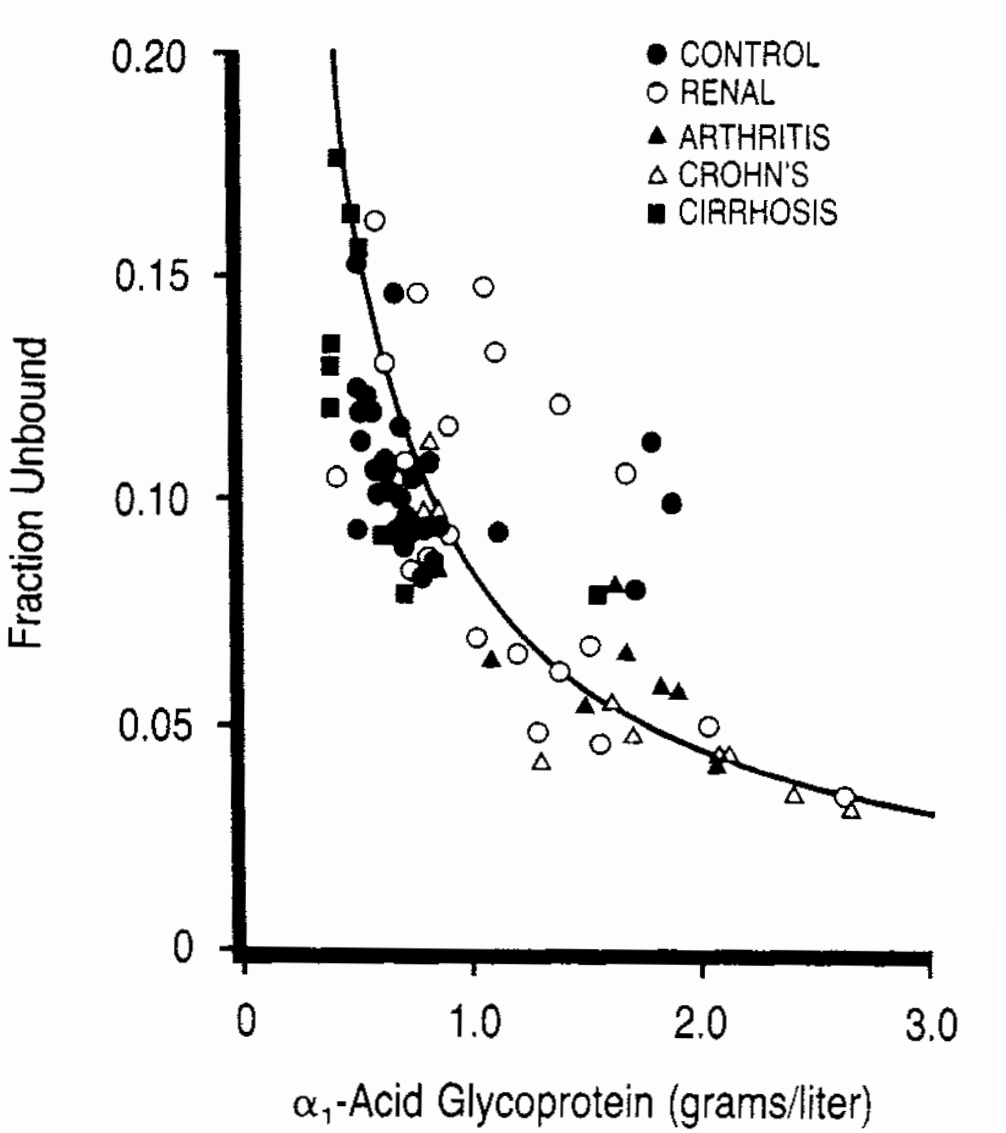
From: *Clinical Pharmacokinetics: Concepts and Applications* (1989) ed. Rowland and Tozer, Lea and Febiger, Philadelphila.

Consequences of altered protein binding in disease



Propranolol

- **Propranolol**: β-adrenergic receptor antagonist used to treat hypertension, tachyarrythmias, migraine
- Bound extensively to **α-acid glycoprotein**: cationic charge
- The level of α-acid glycoprotein changes as a function of inflammation and disease (*acute phase reactant*)
- *A reduction in the level of the protein leads to an increase in the proportion of unbound drug*



From: *Clinical Pharmacokinetics: Concepts and Applications* (1989) ed. Rowland and Tozer, Lea and Febiger, Philadelphila.

BREAK

• Two drugs bind to albumin with the following dissociation constants:

Drug A	***Drug B***
K_d ~ 1 pM	K_d ~ 1 μM

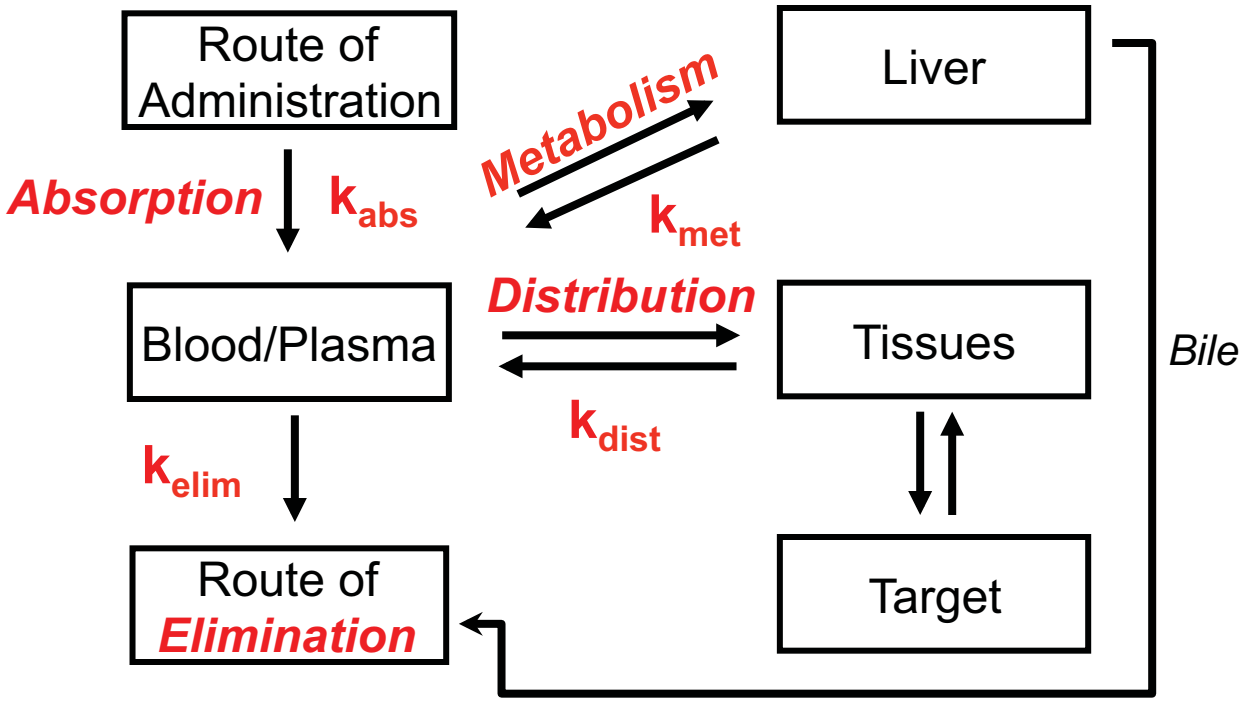
• Which drug has a higher affinity for albumin?

• Which drug would be displaced by bilirubin, which has a K_d ~ 10 nM

Pharmacokinetics and the Fate of Drugs in the Body

- **Definition of Pharmacokinetics/Toxicokinetics**: quantitative temporal analysis of the processes of ADME; how much of and how fast the drug reaches its target
- **Compare to pharmacodynamics**: mechanism by which a chemical or agent exerts its effects (*e.g.*, binding to receptor, interfering with cell wall formation)
- ***Applications in pharmacology:*** determine how often to administer a drug to maintain therapeutic concentration
- ***Applications in toxicology***: define the association between exposure and the progression of disease
- ***Approaches to pharmacokinetic analysis***:
  ~ Simple compartment models
  ~ Physiologically-based pharmacokinetic models (PBPK)

Paradigm for Pharmacokinetics Concepts



Routes of administration and absorption

- Already looked at ***mechanisms*** of absorption
- Now look at quantifying the ***kinetics*** of absorption
- Rates of absorption dictated by route of administration:
  - ~ Enteral *vs* parenteral
  - ~ Vascular *vs* extravascular

- ***Enteral routes***
  - ~ Oral - portal!
  - ~ Sublingual - bypass portal
  - ~ Rectal - bypass portal

- ***Parenteral routes***
  - ~ Intravenous (iv)
  - ~ Intramuscular (im)
  - ~ Subcutaneous (sc)
  - ~ Topical/transdermal
  - ~ Inhalation/nasal
  - ~ Ocular

Factors affecting absorption from site of administration

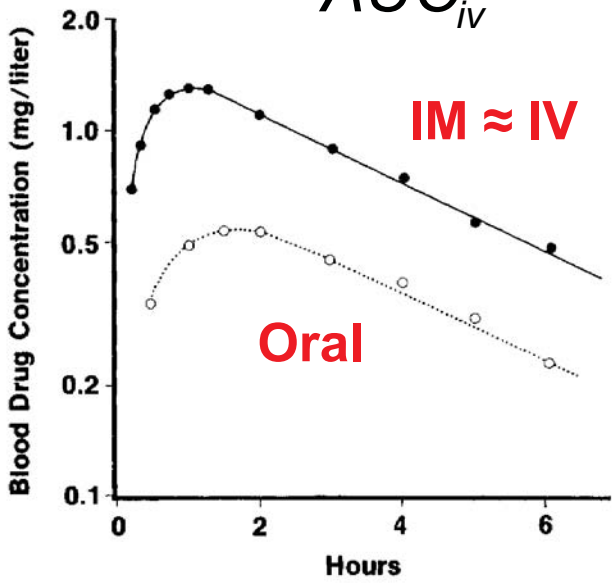
- Quantitative aspects of absorption are important for GI, lung and topical routes
- Transport
  - ~ diffusion - not saturable
  - ~ active, facilitated; saturable
- pH effects
  - ~ charge affects transport/diffusion
  - ~ pH stomach ~ 2; tissue pH ~6.5-8
- Physical factors at the site of absorption
  - ~ blood flow
  - ~ surface area
    - lungs 140 m^2 - skin 1.5-2 m^2
    - GI tract 300 m^2 (small intestine)
  - ~ contact time

Quantifying absorption: Bioavailability

- Concept of ***AUC***:
  - ~ area under plasma concentration vs time curve
  - ~ measure of the total quantity of drug entering the general circulation
- ***Bioavailability***
  - ~ defined as fraction (**F**) of administered drug entering general circulation
  - ~ calculate as plasma AUC_{oral} /AUC_{IV}
- Determinants of bioavailability
  - ~ Formulation (salt form, particle size, excipients) affects rate of dissolution
  - ~ Chemical stability - E.g. penicillin unstable at acid pH of stomach
  - ~ Hepatic extraction - E.g. nitroglycerin has >90% 1st pass metabolism
- ***Bioequivalence*** - relative bioavailability of two drugs

From: *Clinical Pharmacokinetics: Concepts and Applications* (1989) ed. Rowland and Tozer, Lea and Febiger, Philadelphila.

$$F = \frac{AUC_{ev}}{AUC_{iv}}$$



- *500 mg of a drug administered IM and orally to same subject*
- *Quantify [drug] in plasma vs time*

	Plasma Data		***Urine Data***
Route	**AUC (mg•hr/L)**	**$t_{1/2}$ decay phase (min)**	**Cumul. Excret. (mg)**
IV	7.6	190	152
IM	7.4	185	147
Oral	3.5	193	70

EXERCISE

Basic Kinetics

- Use elements of chemical kinetics to develop pharmacokinetic concepts
- Basic rate law for a reaction in which molecule A is converted to molecule B:

$$A \rightarrow B \qquad -\frac{dA}{dt} = \frac{dB}{dt} = k \bullet [A]^n$$

- **Zero-order kinetics: n = 0**

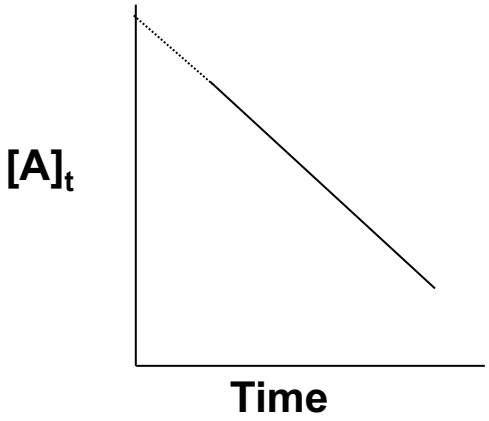
~ $-dA/dt = k \bullet [A]^n$ becomes $-dA/dt = k \bullet 1$

~ Rearrange and integrate rate equation:

$$\int -dA = k \bullet dt$$

$$[A]_t = -k \bullet t + C \qquad t = 0 \Rightarrow C = [A]_0$$

$$[A]_t = -k \bullet t + [A]_0$$



~ Rate of the reaction is **independent of substrate concentration**

~ Rate constant k has units of concentration per unit time

~ Concentration versus time **plot is linear**

Basic Kinetics

- **First-order kinetics: n = 1**

~ -dA/dt = k•[A]^n becomes -dA/dt = k•[A]

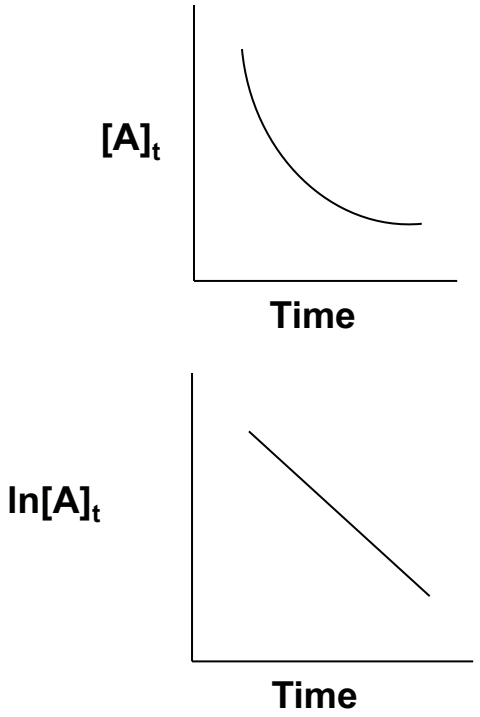
~ Rearrange and integrate rate equation:

$$\int -\frac{dA}{[A]_t} = k \bullet dt$$

$$\ln([A]_t) = -k \bullet t + C \qquad t = 0 \Rightarrow C = \ln([A]_0)$$

$$\ln([A]_t) = -k \bullet t + \ln([A]_0)$$

$$\ln\left(\frac{[A]_t}{[A]_0}\right) = -k \bullet t \qquad [A]_t = [A]_0 e^{-kt}$$



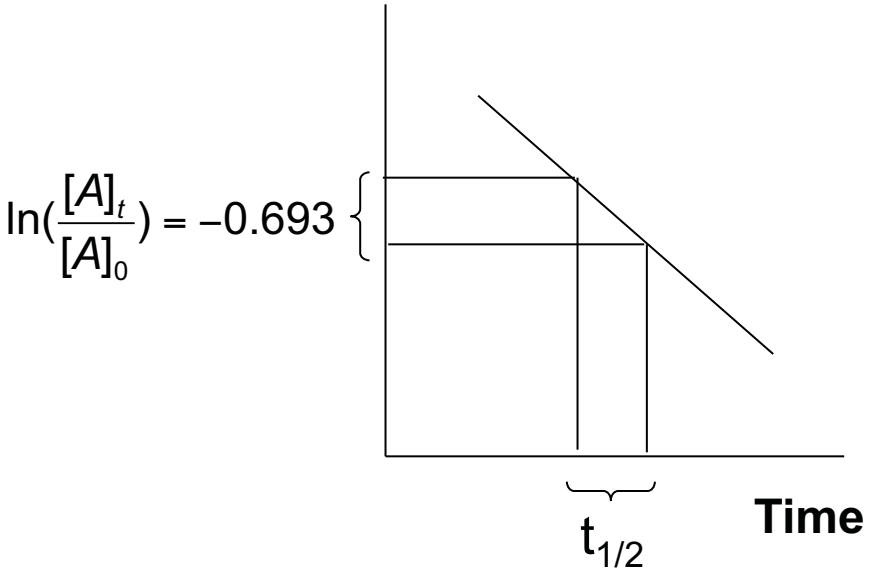
- Rate of the reaction is **dependent on substrate concentration**
- Rate constant k has **units of reciprocal time**
- ln[A] *vs.* time plot is **linear**

Basic Kinetics

- **Half-life** - fundamental pharmacokinetic concept and parameter
- Definition: time to decrease concentration by one-half
- Define mathematically by setting $[A]_t = [A]_0/2$

$$\ln\left(\frac{[A]_t}{[A]_0}\right) = \ln(0.5) = -0.693 = -k \bullet t$$

$$t_{1/2} = \frac{0.693}{k}$$



Basic Kinetics: Processes subject to zero-order kinetics

- "Saturable" processes: ligand molecules completely occupy available binding sites
- Metabolic enzymes
  - ~ ***Aspirin*** - glycine conjugation and phenolic glucuronidation
  - ~ ***Ethanol*** - alcohol/aldehyde dehydrogenase
  - ~ ***Phenytoin*** - CYP2C9; K_m~ 5 mg/L; therapeutic range 10-20 mg/L
- Transporters: ***glucose transporter*** in renal tubule (filtered [glucose] > 320 ng/min)
- Mathematical basis for zero-order kinetics
  - ~ Michaelis-Menten rate equation considerations:

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

  - ~ When [S] >> K_m, all substrate binding sites occupied and enzyme operates at V_{max}

Basic Kinetics: Processes subject to first-order kinetics

- 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 processes of absorption, distribution, metabolism, elimination are first-order***
- ***Diffusion***: 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:

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

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

Concept of clearance

- **Clearance (Cl)**: rate of removal of a chemical from any compartment (blood, tissue, entire body) by any process (metabolism, excretion, distribution to another tissue, etc.)
- Whole body or systemic Cl is sum of other Cl's: $Cl_s = Cl_{hepatic} + Cl_{renal} + Cl_{other}$
- Physical interpretation: volume of blood/tissue "cleared" of chemical per min
  Example: Cl = 100 ml/min ⇒ chemical removed from 100 ml of blood/min
- Mathematical definitions:

$$CL = k_{el} \bullet V_d$$

where k_{el} is the first-order rate constant for elimination of a chemical from the blood or tissue; V_d is the apparent volume of distribution of the chemical

$$CL = \frac{Dose}{AUC_0^{\infty}}$$

where AUC over the time period t = 0 to t = ∞

$$CL_{organ} = Q\left(\frac{C_A - C_V}{C_A}\right) = Q \bullet E$$

where Q is blood flow to the organ, C_A is the arterial blood concentration, C_V is the venous blood conc. and E is the extraction ratio

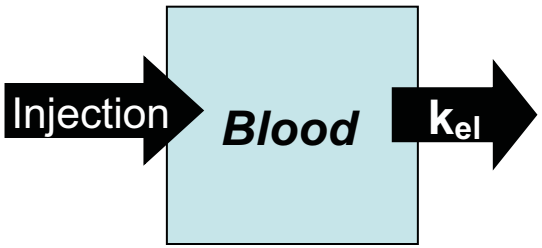
- **Intrinsic clearance (Cl_{int})**: the contribution of metabolism to the overall clearance associated with an organ; CL_{int} is independent of blood flow

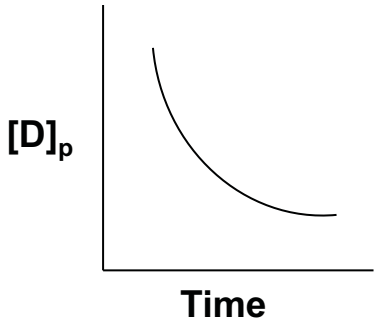
Pharmacokinetic Models

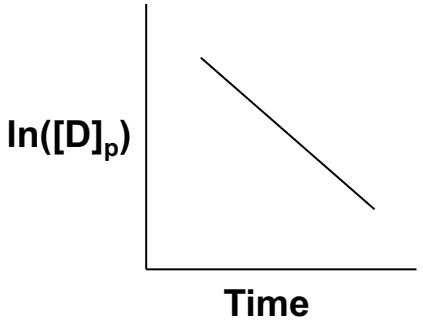
- Build an understanding of PK'S with simple models
- More complicated physiologically-based models combine many simple models
- **Single compartment with I.V. injection and first-order elimination**
  - ~ Consider the body as a "box" with blood as the **sampling compartment**
  - ~ Rapid injection and presumed rapid ("instantaneous") distribution
  - ~ Obtain blood sample and quantify drug as a function of time
  - ~ ***First-order*** - linear plot of ln(plasma concentration) vs time
  - ~ The rate constant, k, is now the **elimination rate constant, k_{el}**
  - ~ Plasma half-life = $0.693/k_{el}$
- Loss of drug from plasma due to metabolism, excretion, distribution to tissue…

$$\ln([D]_p) = -k_{el} \bullet t + \ln([D]_{p_{t=0}})$$

Already wrote and solved the mass balance differential equation!







Pharmacokinetic Models

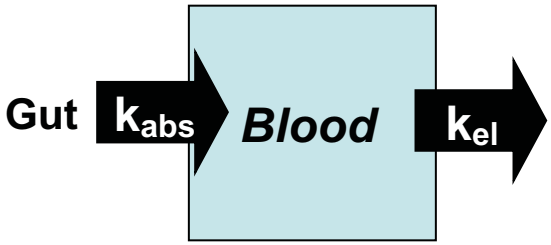
- **Single compartment with absorption from gut and first-order elimination**

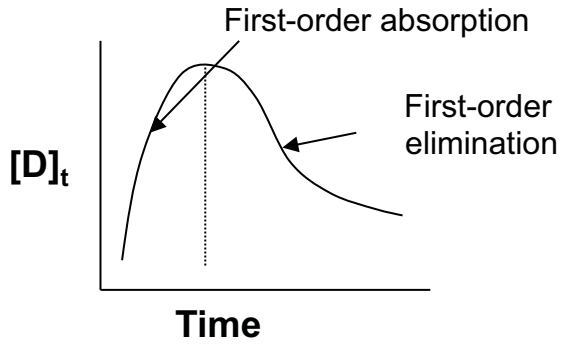
  ~ Factor in kinetics of absorption with kinetics of elimination from blood
  ~ Distribution is no longer instantaneous
  ~ Assume first-order absorption from gut (why?)
  ~ Write rate equation that accounts for 1° absorption and 1° elimination

$$d\frac{[D]_p}{dt} = k_{abs}[D]_{gut} - k_{el}[D]_p$$

$$d\frac{[D]_p}{dt} = k_{abs}[D]_{gut} - k_{el}[D]_p = k_{abs}\left([D]_{gut0}e^{-k_{abs}t}\right) - k_{el}[D]_p$$

$$Integrate \Rightarrow [D]_{p_t} = [D]_{gut_0}\left(\frac{k_{abs}}{k_{abs}-k_{el}}\right)\left(e^{-k_{el}t} - e^{-k_{abs}t}\right)$$



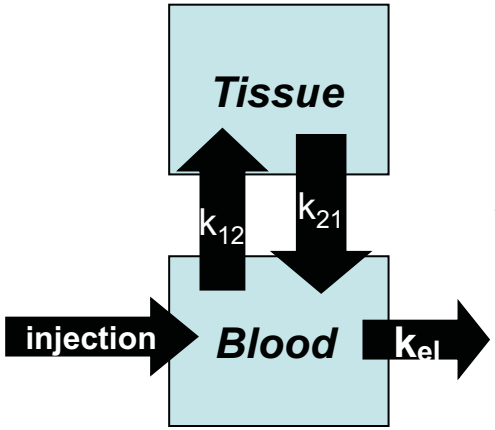


~ As drug absorbed from gut, $e^{-k_{abs}t}$ goes to zero and $[D]_p$ dominated by k_{el}

Pharmacokinetic Models

- **Two compartments with I.V. injection and first-order elimination**

  ~ Rate equation now has 3 terms
  ~ Injected drug distributes in blood compartment “instantaneously”
  ~ Observe two "phases"
  ~ Rapid movement of drug out of blood into tissue compartment
  ~ Slower phase: as plasma concentration falls below tissue concentration, drug moves into blood

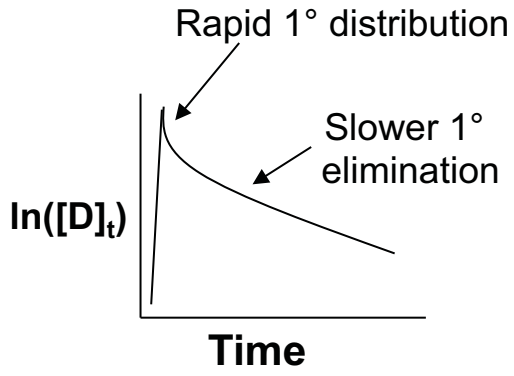


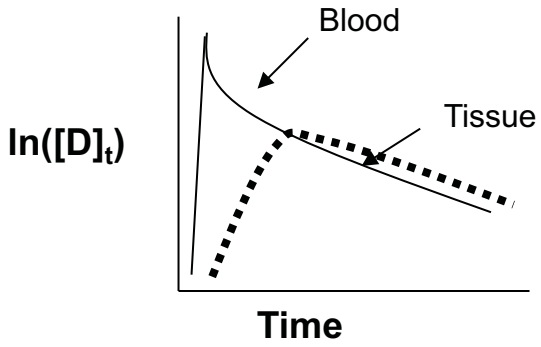
$$d\frac{[D]_p}{dt} = k_{21}[D]_{tis} - k_{12}[D]_p - k_{el}[D]_p$$

$$Integrate \Rightarrow [D]_{p_t} = Ae^{-\alpha t} + Be^{-\beta t}$$

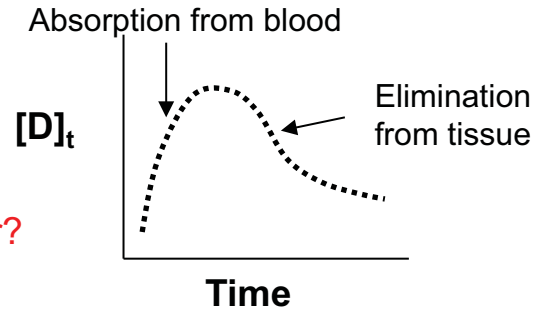
$$\alpha + \beta = k_{12} + k_{21} + k_{el} \qquad A = [D]_{p_0}\left(\frac{\alpha - k_{21}}{\alpha - \beta}\right)$$

$$\alpha \bullet \beta = k_{21} \bullet k_{el} \qquad B = [D]_{p_0}\left(\frac{k_{21} - \beta}{\alpha - \beta}\right)$$





Linear plot
looks familiar?



Pharmacokinetic Models

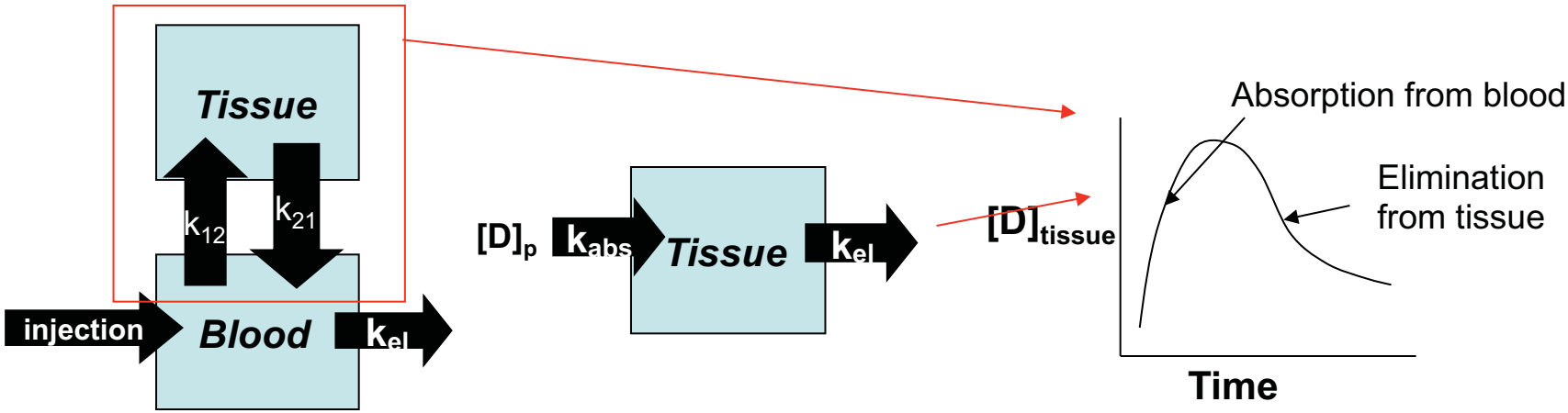
- **Correlate single- and multi-compartment models**

~ Graph of $[D]_t$ *vs*. time for the tissue compartment of a 2-compartment model is identical to graph of 1 compartment model with 1° absorption and 1° elimination

~ $k_{abs} = k_{12}$ and $k_{el} = k_{21}$

~ Easy: **string together single compartment models for each entry and exit component, solve ordinary differential equations (Physiologically-based PK models; PBPK)**

~ Don't hassle with the complexity of ≥ 2 compartment models



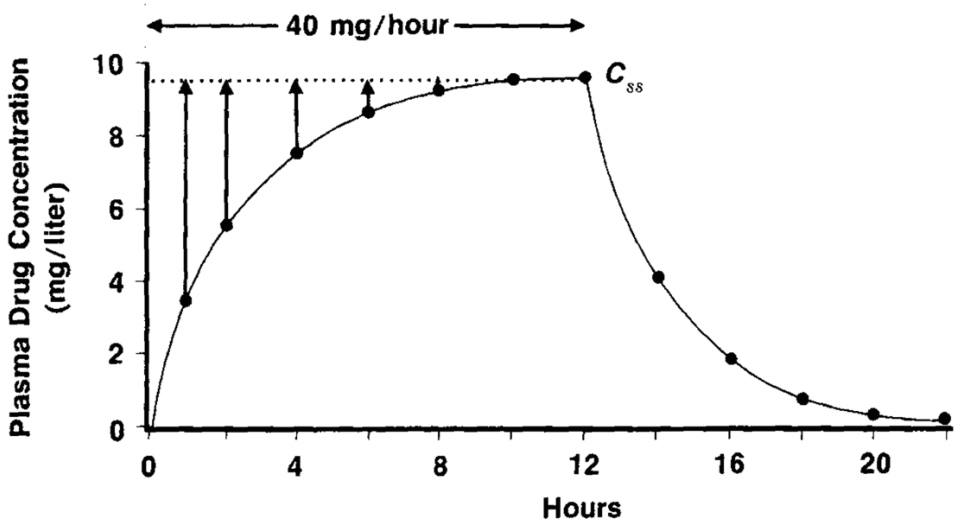
Pharmacokinetics of Multiple Doses

- Need to determine how frequently to give a drug so that we maintain blood concentration in the therapeutic range and below the toxic range
- Define the concept of steady-state concentration of drug in blood (C_{ss}):
  - ~ balance of rates: dosing, absorption, elimination
  - ~ reach a state in which drug concentration fluctuates within a narrow window
- Achieve C_{ss} after ~4 half-lives
- First example with **constant infusion**:

$$C_t = \left(\frac{k_{inf}}{Cl}\right)\left(1 - e^{-k_{el} \cdot t}\right)$$

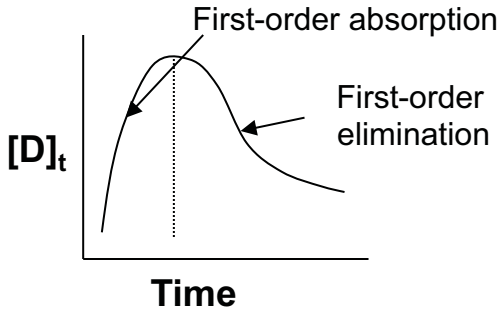
$$C_{ss} = \frac{C_t}{[1-(0.5)^{(t/t_{1/2})}]} = \frac{k_{inf}}{k_{el} \bullet V_d}$$

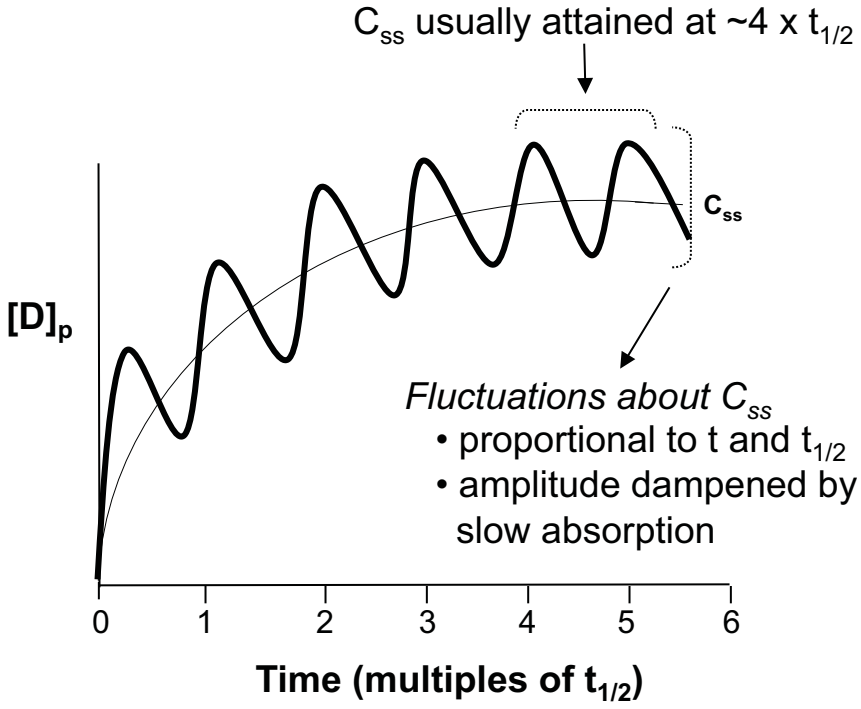
k_{inf} = rate of infusion
k_{el} = elimination rate constant
C_{ss} = steady-state concentration (mg/mL)
C_t = concentration at time = t
t = time
$t_{1/2}$ = half-life



Pharmacokinetics of Multiple Doses

- Consider the case of multiple daily doses
- Now see saw-tooth drug concentration profile due to peak and trough fluctuation
- Simply string together 1° abs/1° elim graphs
- Achieve C_{ss} after ~4 half-lives: quantify average $[D]_p$ at $t > 4 \times t_{1/2}$





$$C_{ss} = \frac{F \bullet dose}{CL \bullet T} = \frac{F \bullet dose}{k_{el} \bullet V_d \bullet T}$$

C_{ss} = steady-state concentration (mg/mL)
F = fractional bioavailability
CL = blood clearance (mL/min)
T = dosage interval (min)
Dose in mg

Pharmacokinetics Web Sites

- Excellent web site for pharmacokinetics:
http://www.boomer.org/c/p1/index.html

- JAVA calculator for plotting blood concentrations approaching steady-state:
http://www.boomer.org/c/p1/Ch15/Fig57/Fig57.html

20.201 Mechanisms of Drug Actions

Fall 2013