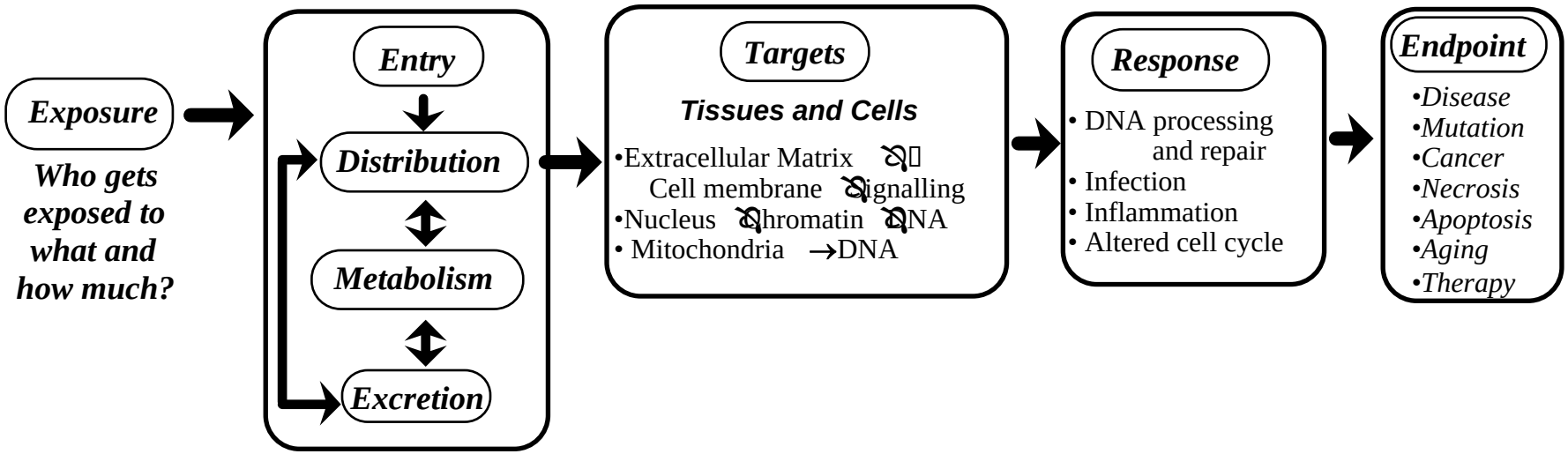
20.201 Mechanisms of Drug Action

Session #3: Principles, Uptake and Distribution

Toxicology/Pharmacology Paradigm



- **Definitio**n of a dose-response relationship: correlative relationship between he degree of a response a biological system and the amount of drug or toxicant administered.

- Why do we need to understand this relationship?
  ~ Society and government needs to know if a chemical is safe
  ~ Physicians need to know how much drug to give, how the patient will respond, if a drug is safe

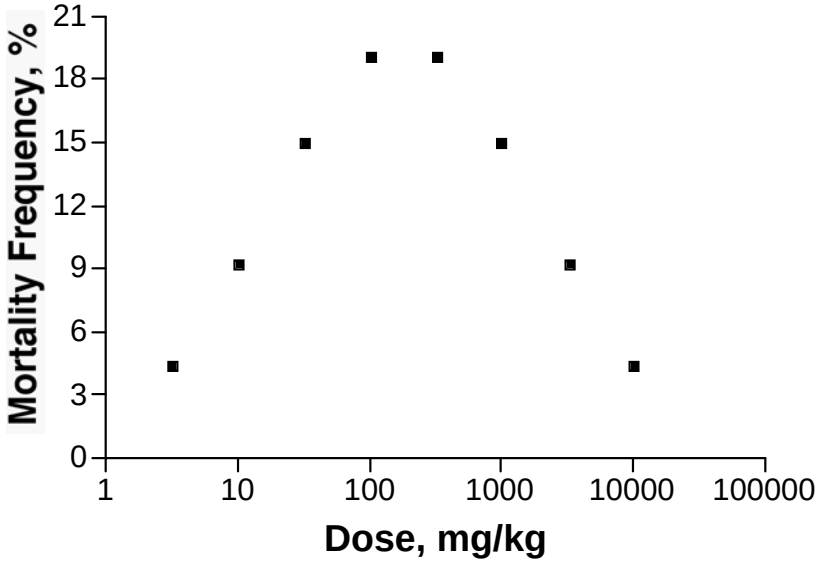
- Prerequisites for defining a dose-response relationship:
  ~ Certainty that the response is due to the chemical (otherwise it is an epidemiologic study and not a dose response)
  ~ Certainty that the level of response is due to the level of chemical
  ~ A quantifiable parameter for the response and a means to measure it

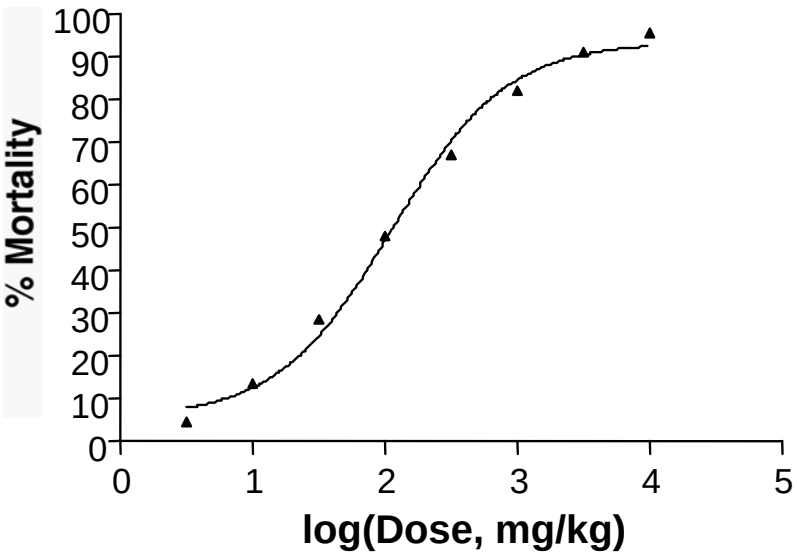
- Types of dose-response relationships:
  ~ **Individual or graded or continuous**: study that demonstrates dose-related increase in the intensity of a response; *e.g.*, inhibition of an enzyme as function of dose
  ~ **Quantal**: single end-point ("quanta") dose-response study in which an organism either "responds" or doesn't. Focus on this type

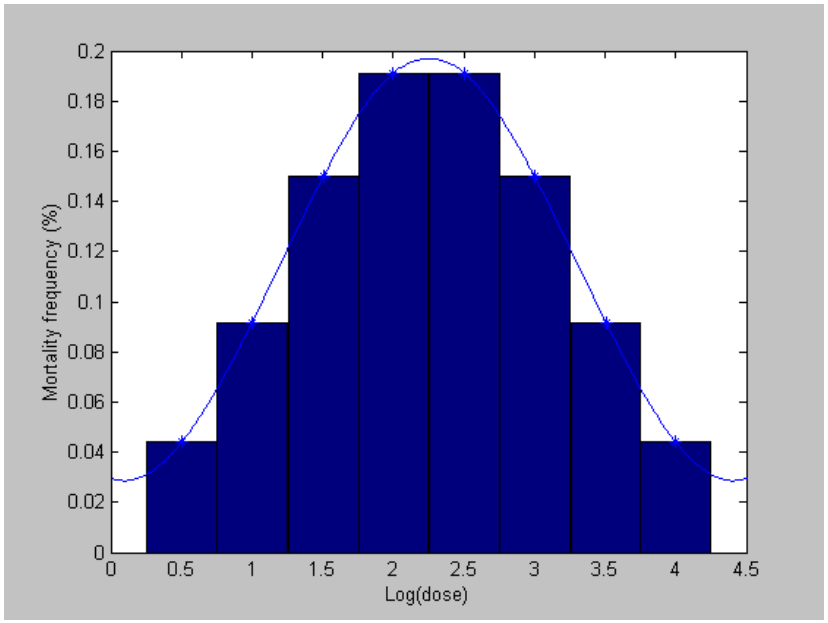
• Degree of response to different doses of chemical often often follows a normal distribution (Gaussian distribution)

• Bell-shaped probability distribution:
  ~ Left end - hypersusceptible
  ~ Right end - resistant

• Recalculate response as cumulative response and replot versus logarithm of dose
  ~ Sigmoidal curve
  ~ More useful for comparisons







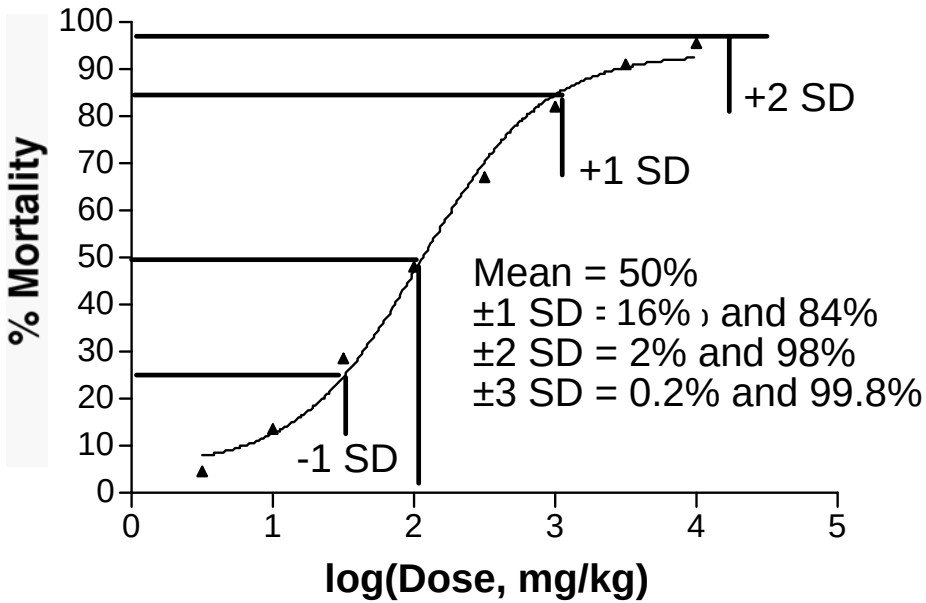
• Normally-distributed sigmoid cumulative distribution

• In population with normal distribution:

mean ± 1 SD…68.3% of population
mean ± 2 SD…95.5% of population
mean ± 3 SD…99.7% of population

• Convert % response to units of deviation from the mean = **NED** (normal equivalent deviations):

~ 16% response (-1 SD) NED = -1
~ 50% response (0 SD) NED = 0
~ 84% response (+1 SD) NED = +1

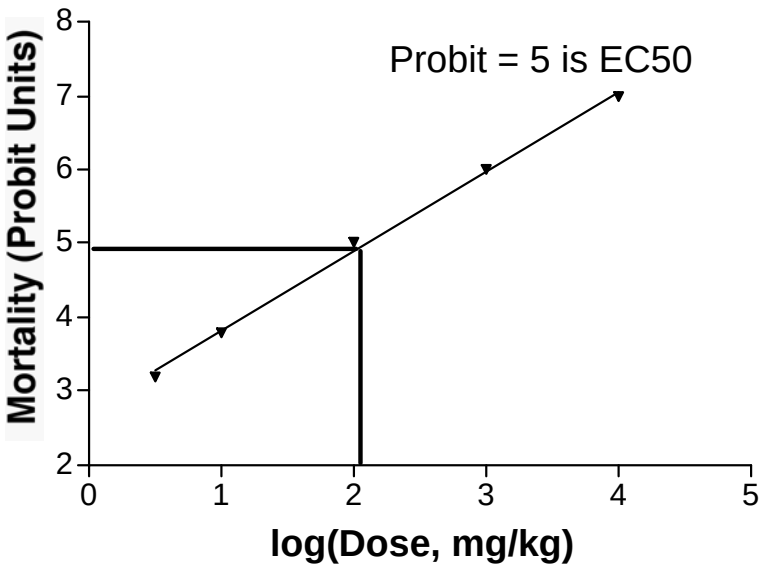


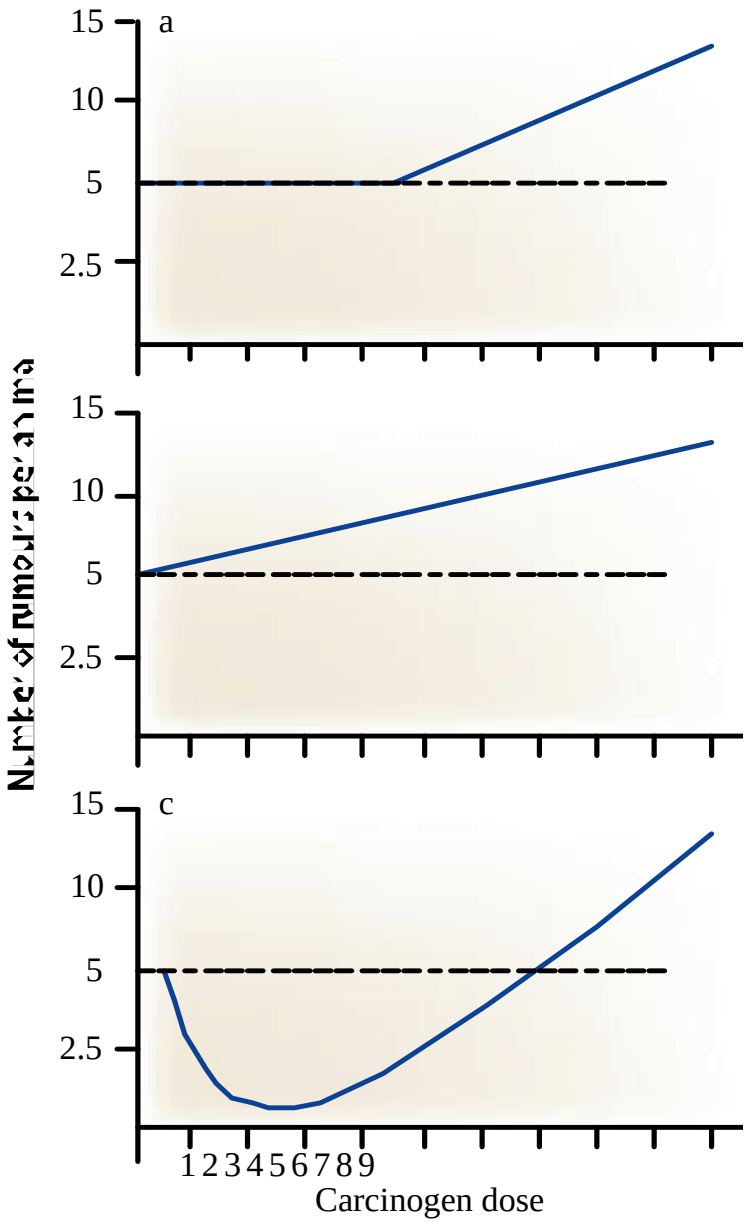
• Avoid negative numbers: convert to Probit scale

• **Probit** (***prob***ability un***it***) = NED + 5

16% response (-1SD) NED = -1 probit = 4
50% response (0 SD) NED = 0 probit = 5
84% response (+1 SD) NED = 1 probit = 6

• Probits plot as a straight line
LD_{50} obtained interpolation of linear regression line at probit = 5





Hormesis

• Engineering definition: non-linear response in a device due to failure to return to the original ground state with each iteration

• Medical/toxicological: phenomenon in which small doses of otherwise toxic chemicals or agents cause enhanced cell growth or a reduction in an endpoint that, at higher doses, represents a toxic response.

• Examples:
  ~ Radiation
  ~ Alcohol

Effective dose (ED)
Toxic dose (TD)
Lethal dose (LD)

Therapeutic Index (TI)
Relative safety of the drug
Comparison of the effective dose and the toxic or lethal dose
Ratio of lethal or toxic dose to (therapeutically) effective dose
The larger the ratio, the greater the relative safety
Most commonly used index is the median dose,
ie the dose required to elicit response in 50% of a population
note: the median does not give information about
the slope of the dose-response curve

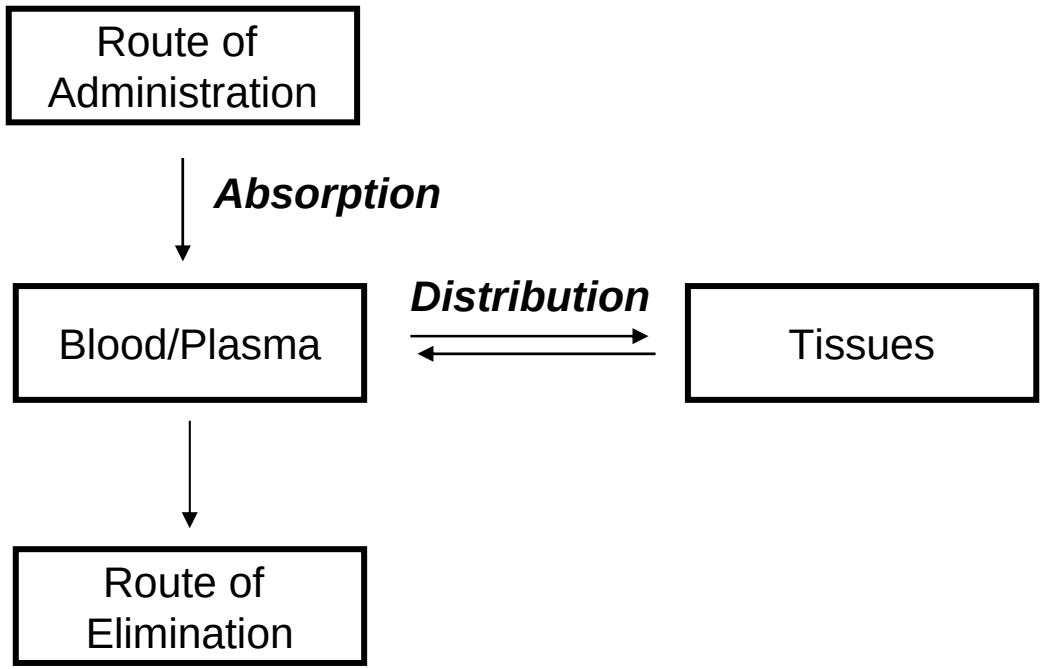
$TI = LD_{50} / ED_{50}$

Comparison of toxic effects
Potency and efficacy

Potency
Range of doses over which a chemical produces increasing responses

Efficacy
Maximum response
Limit / plateau of dose-response curve on the response axis

Uptake and Distribution



Routes of administration

(1) Enteral -
- oral
- sublingual
- rectal

(2) Parenteral -
- intravenous (iv)
- intramuscular (im)
- subcutaneous (sc)

(3) Other -
- inhalation
- topical
- transdermal

Absorption

Quantitative aspects of absorption are important for GI, pulmonary and topical administration. Chemicals must cross epithelial cell barrier to gain access to blood stream and thus be distributed around body

(1) Epithelia

(2) Transport
- ***passive***
  - ~ simple diffusion
  - ~ facilitated diffusion
- ***active*** *COVERED BY KEITH HOFFMASTER*
  - ~ anion/cation transporters
  - ~ p-glycoprotein and multi-drug transporters
  - ~ glucuronide transporters
  - ~ glutathione conjugate transporters

(3) pH effects

(4) Physical factors
- blood flow through organ
- surface area: lungs, 140 m^2; GI tract, 300 m^2 (small intestine); skin, 1.5-2 m^2
- contact time (e.g., gastric emptying time, bowel transit time)

Epithelial Tissue Barriers

- ***Concept of tissue*** organ structure = tissues

  1) *Definition*: collection of similar cells and the intercellular substances surrounding them.
  2) *Four types of tissue*:
     - **epithelial**: sheets of polyhedral cells lining body cavities and surfaces
     - **connective**: less organized collection of cells embedded in intercellular matrix; provides form, adhesion, and support.
     - **muscle**: contractile elements
     - **nervous**: impulse conducting

- To enter body, *all chemicals must pass through epithelial tissue*

- 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

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:

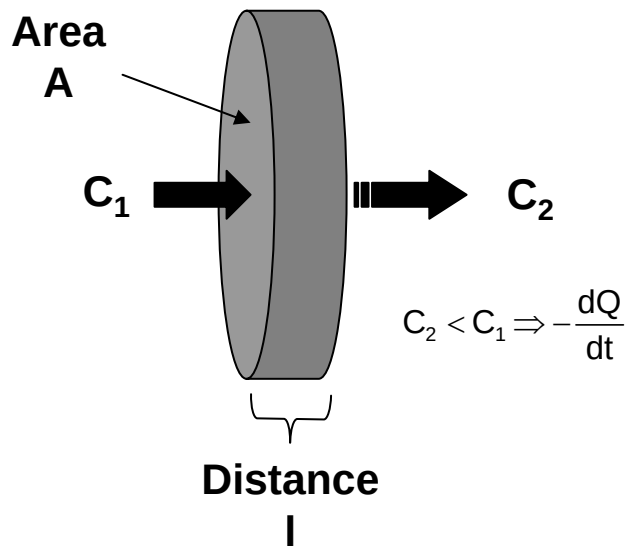
$$\frac{dProduct}{dt} = V = \frac{V_{max} \bullet [S]}{K_m + [S]}$$

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

Diffusion

- Important process for many chemicals and toxins
- Occurs only when there is a concentration gradient
- Rates of diffusion: air>>water>>tissue
- Fick's first law of diffusion: rate of movement of a chemical through a medium is affected by
  1) distance traversed
  2) surface area through which diffusion occurs
  3) properties of chemical and medium.

$$J = -\frac{dQ}{dt} = \left(\frac{D \bullet A}{l}\right) \bullet \Delta C$$



~ dQ/dt = quantity moving per unit time (mol/s)
~ D = diffusion coefficient (m^2/s); a proportionality constant; for lipid bilayer contains terms for:
- gas constant (R) (J/(mol•°K))
- temperature (°K)
- volume occupied by the molecule
- viscosity of the membrane ( )
- solubility of the chemical in lipid (oil-water partition coefficient)

~ A = area available for exchange (m^2)
~ l = distance over which diffusion takes place (m)
~ ΔC = concentration difference across membrane (mol/m^3)

- Membrane thickness nearly constant at 7-10 nm

$\Rightarrow$ replace D/l by P = permeability coefficient (m/s)

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

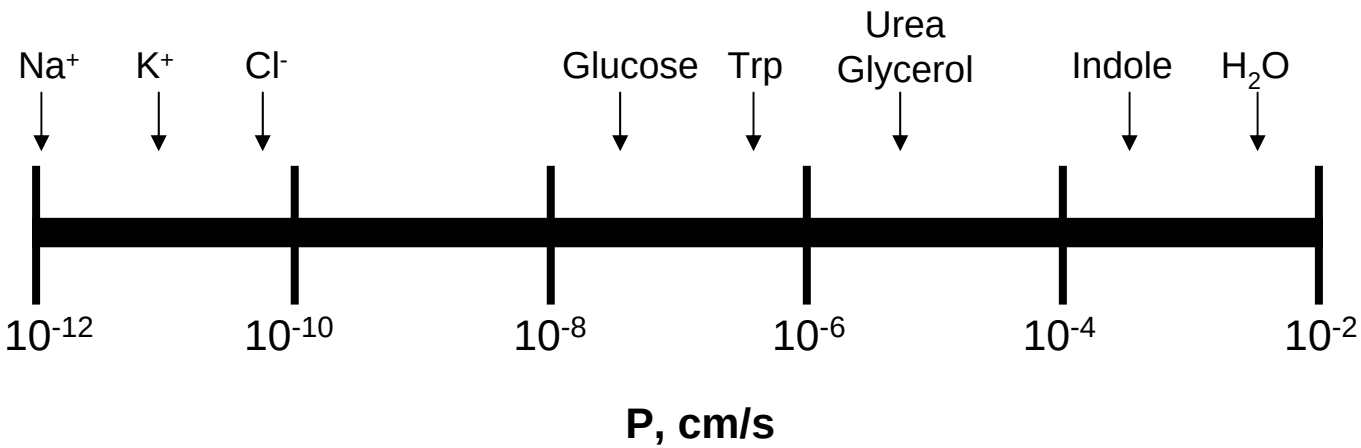
Examples of differential permeability in cell membranes

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

• 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^+

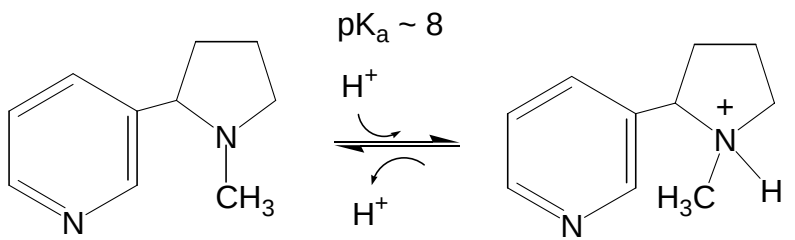
$$J = -\frac{dQ}{dt} = P \bullet A \bullet \Delta C$$



Chemical transport and pH: The Chemistry of Nicotine Delivery

- Nicotine: addictive chemical in cigarette smoke; the driving force for cigarette sales
- Nicotine is a base: undergoes acid/base chemistry
- Acid/base chemistry determines the bioavailability of nicotine and thus the qualities of the biological effect
- Uncharged, base form: volatile; present in vapor phase (rapid uptake into body)
- Charged, acid form: non-volatile; present in the particulate phase (slow uptake into body)
- Cigarette manufacturers control the pH of cigarette smoke to control nicotine uptake
- Flue-cured tobacco: pH <6; air-cured dark tobacco: pH >7. Which delivers more nicotine?

Nicotine



Uncharged, base form *Charged, acid form*

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

$$K_a = \frac{(RNH_2)(H^+)}{(RNH_3^+)}$$

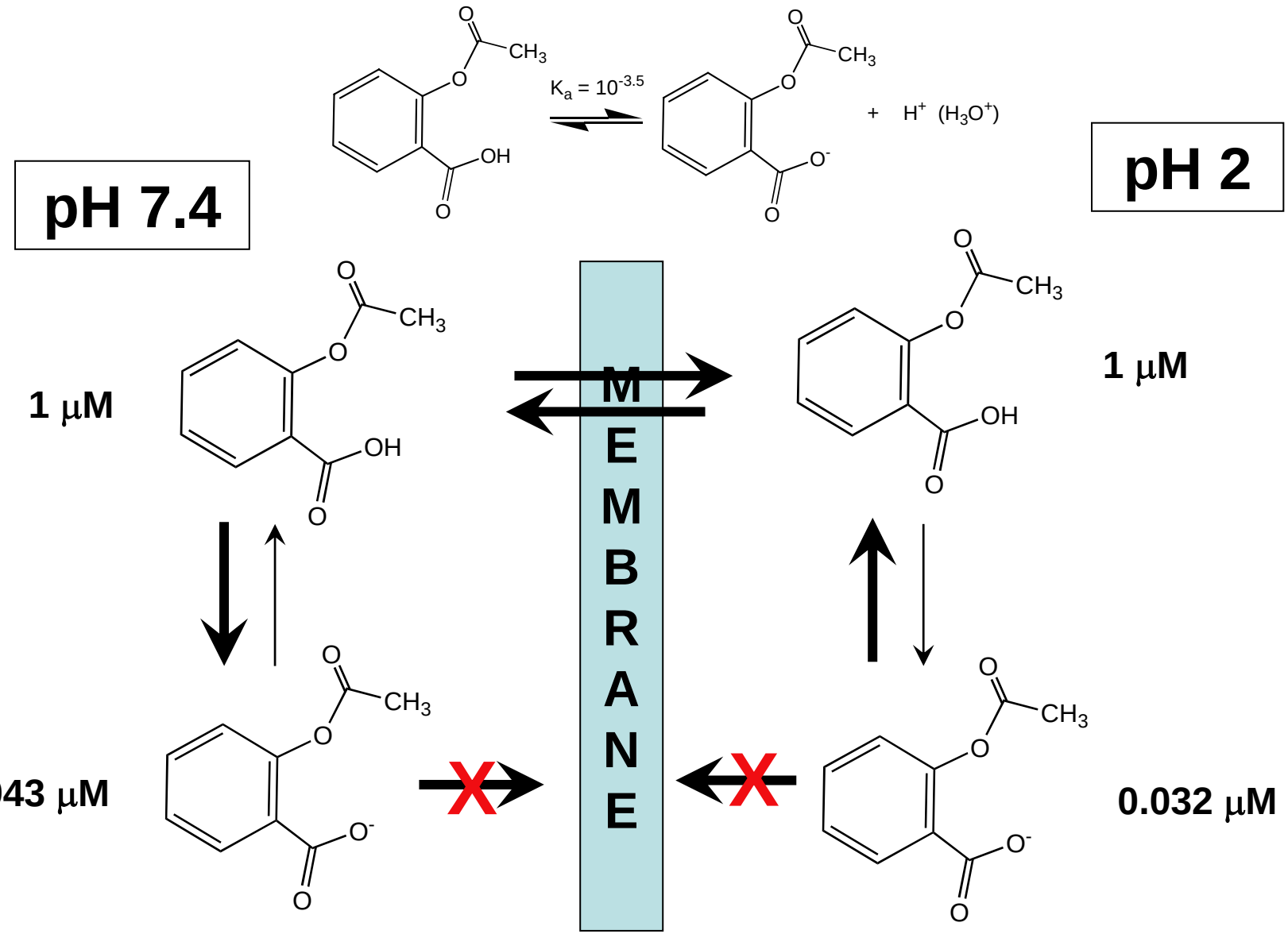
$$pK_a = -\log(K_a)$$

$$pH = -\log(H^+)$$

Henderson-Hasselbalch equation:

$$pH = pK_a + \log(\text{base/acid})$$

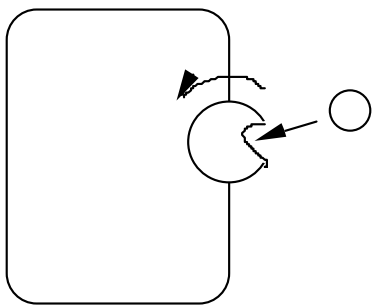
Chemical transport and pH

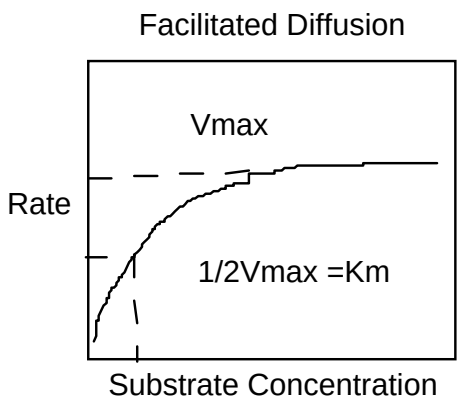


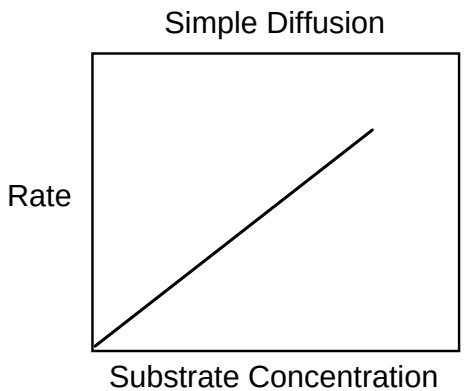
Facilitated Diffusion

- Simple diffusion too slow for most physiologic substances:
  glucose diffusion very slow…..P = 10^{-7}-10^{-8}.
- Speed up diffusion by introducing a carrier protein in membrane: a "pore"
- Movement is still down 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-Menton kinetic model

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







Km	
l-glucose	>3000
galactose	30
mannose	20
D-glucose	1.5

Distribution of Drugs

Process by which drug leaves site of absorption and enters tissues

(1) Blood flow: rate varies widely as function of tissue structure/function:

Organ/Tissue	Resting Blood Flow (ml/min)	Ml/min/100 g
Liver	1350 (27%)	95
Muscle	750 (15%)	4
Kidney	1100 (22%)	360
Heart	200 (4%)	70
Skin	300 (6%)	3
Brain	700 (14%)	50
Bronchi	100 (2%)	25
Other	500 (10%)	

(2) Capillary structure:

- Most capillaries are “leaky” and do not impede diffusion of drugs
- blood-brain barrier formed by high level of tight junctions between cells
- BBB is impermeable to most water-soluble drugs

(3) Plasma protein binding: Albumin!

Blood Cells

Classes		*Cell Type*	*Products*	*Function*	*Size*	*Number*	*Lifetime*
Erythrocytes		Erythrocytes	Hemoglobin	O_2 transport	7.5x2.5 um	$5x10^6$/ul (M) $4x10^6$/ul (F)	120 days
Leukocytes	Granulocytes	Neutrophils	Oxidative chemicals	Bacterial phagocytosis	15 um	5000/ul	6-7 hr blood 1-4 d tissues
		Eosinophils	Inflamm. modulating chemicals	Parasite defense, mod. of inflamm.	15 um	300/ul	
		Basophils	Histamine, heparin	Allergic reactions	15 um	100/ul	
	Mononuclear Leukocytes	Monocytes	Lysosomal enzymes	Phagocytosis of viruses, protozoa, dead cells	20 um	500/ul	12-100 hr blood
		B-Lymphocyte T-Lymphocyte Killer Cells	Antibodies (IgG, IgM, IgE) Cytokines Cytokines, etc.		6-18 um 6-18 um 6-18 um	2-3000/ul	
Platelets		Platelets	Blood clotting elements	Clot formation	2-4 um	$3x10^5$/ul	10 days

Proteins in blood

Molecule	***Mol. Wt.***	***G/dL***	***μM***	***Function***
Albumin	66,500	4.5	670	Chemical transport, plasma oncotic pressure
Globulins (α, β, γ)				
immunoglobulins (IgG, etc.)	150,000	1.5-2	130	Humoral Immunity
lipoproteins				Lipid and chemical transport
transferrin	79,000	0.2	17	Iron transport
ceruloplasmin	150,000	0.3	20	Copper transport
gluco/mucoprotein:haptoglobin				Binds to hemoglobin
coagulation factors (~10)				Clot formation
steroid-bindfing globulin	53,000	0.05	0.8	Transport of steroid hormones
thyroid hormone-binding globulin				Transport of thyroxine
macroglobulins				
α1-acid glycoprotein	42,000	0.4-1	9	Acute phase reactant, chemical transport
Fibrinogen	400,000	0.5	12	Clot formation
Complement proteins(~12)				Antibody-dependent responses and bacterial immunity

Serum albumin as a drug transport and depot protein

1) Most abundant protein in plasma and probably most important protein for transport of chemicals
2) Trx numerous endogenous chemicals: FA, bilirubin, steroids, NO, metals (Ca^{+2}, Cu, Zn), amino acids.
3) Structural Characteristics:
   a) 585 a.a., ~66,500 MW, 17 disulfides and one free SH
   b) Part of larger family of proteins that includes α-fetoprotein, and vit. D binding protein
   c) AA sequence reveals 3 domains that share strong sequence similarity and 2° structure; 10 helical segments in each domain, 5-6 S-S bonds
   e) May have arisen by duplication of gene encoding for single domain
4) Drug binding sites:
   a) Extensive literature on drug binding; most drugs bound less tightly than endogenous chemicals:
   b) Number of 1° sites range: 1-4; weaker binding sites (nonspecific) 1-40
   c) Biochemical and spectroscopic studies = 5 major binding sites (chemical x-linking, competition, binding isotherms)
   d) Binding assignments are very gross estimates: digitoxin site unknown; warfarin/bilirubin bind to domain #2; indole and FA bind to domain #3
5) Recent structural studies: X-ray crystallography (He and Carter, Nature 7/92)
   a) Heart-shaped overall structure with 3 triangular domains
   b) each domain has two subdomains
   c) low resolution studies to define binding sites for several chemicals
      - most chemicals bound to subdomains IIA and IIIA
      - IIA and IIIA have hydrophobic pocket formed by leu, ala, ile, phe
      - IIA and IIIA differ slightly in structure, accounts for different chemicals
      - no binding in I: no hydrophobic pocket present due to influence of neighboring II and III domains

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