Enzymatic Reactions

\[ S + E \rightleftharpoons ES \rightleftharpoons E + P \]
Enzymatic Reactions

\[ S + E \rightleftharpoons ES \rightleftharpoons E + P \]
Michaelis-Menten Kinetics

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

$V =$ Reaction velocity
Rate of P formation

$V_{\text{max}}$
Michaelis-Menten Kinetics

• Adding S ➔ More P formation ➔ Faster V
• Eventually, reach Vmax
Michaelis-Menten Kinetics

• At $V_{\text{max}}$, enzymes saturated (doing all they can)
• Only way to increase $V_{\text{max}}$ is to add enzyme
Enzyme Kinetics

\[ V_{\text{max}} \]

\[ V_{\text{max}} \]

More Enzyme

[S]
Michaelis-Menten Kinetics

\[ V = V_{\text{max}} \cdot \frac{[S]}{K_m + [S]} \]

- \( V \) = Reaction velocity
- \( V_{\text{max}} \) = Rate of P formation
- \( K_m \) = Michaelis constant
- \([S]\) = Concentration of substrate
Michaelis Constant (Km)

\[ V = \frac{V_m \times [S]}{K_m + [S]} \]

Key Points:
1. Km has same units as [S]
2. At some point on graph, Km must equal [S]
Michaelis Constant (Km)

\[
V = \frac{V_m \times [S]}{[S] + [S]} = \frac{V_m \times [S]}{2 \times [S]} = \frac{V_m}{2}
\]

When \( V = \frac{V_m}{2} \)

\[ [S] = K_m \]
Michaelis Constant (Km)

\[ V = \frac{V_m \times [S]}{K_m + [S]} \]
Michaelis Constant (Km)

- Small Km $\rightarrow$ Vm reached at low concentration [S]
- Large Km $\rightarrow$ Vm reached at high concentration [S]

$$V = V_m \frac{[S]}{K_m + [S]}$$
Michaelis Constant (Km)

- Small Km $\rightarrow$ Substrate binds easily at low $[S]$
  - High affinity substrate for enzyme
- Large Km $\rightarrow$ Low affinity substrate for enzyme

\[
V = \frac{V_{\text{max}}}{K_m + [S]} \quad [\text{V = V}_m^* [S] \quad \frac{1}{K_m + [S]}]
\]
Key Points

• Km is characteristic of each substrate/enzyme
• Vm depends on amount of enzyme present
• Can determine Vm/Km from
  • Michaelis Menten plot V vs. [S]
  • Lineweaver Burk plot 1/V vs. 1/[S]
Lineweaver Burk Plot

\[ V = \frac{V_m \times [S]}{K_m + [S]} \]

\[ \frac{1}{V} = \frac{K_m + [S]}{V_m [S]} = \frac{K_m}{V_m [S]} + \frac{[S]}{V_m [S]} \]

\[ \frac{1}{V} = C \times \frac{1}{[S]} + \frac{1}{V_m} \]
Lineweaver Burk Plot

\[ \frac{1}{V} \]

\[ \frac{1}{S} \]

\[ \frac{1}{V_m} \]

\[ -\frac{1}{K_m} \]

\[ \frac{K_m}{V_m} \]
Enzyme Inhibitors

Jason Ryan, MD, MPH
Enzyme Inhibitors

- Many drugs work through enzyme inhibition
- Two types of inhibitors:
  - Competitive
  - Non-competitive
Enzymatic Reactions

\[ S + E \rightleftharpoons ES \rightleftharpoons E + P \]
Enzyme Inhibitors

**Competitive**
- Competes for same site as $S$
- Lots of $S$ will overcome this

**Non-competitive**
- Binds different site $S$
- Changes $S$ binding site
- $S$ cannot overcome this
- Effect similar to no enzyme
Competitive Inhibitor

\[ V_{\text{max}} \]

\[ \frac{V_{\text{max}}}{2} \]

\[ [S] \]

Normal

Inhibitor

Same V\text{m}
Higher K\text{m}
Non-competitive Inhibitor

\[ [S] \]

\( V_{max} \)

\( V_{max}/2 \)

\( V_{max}/2 \)

\( K_m \)
Competitive Inhibitor

\[
\frac{1}{V} = \frac{1}{V_m} - \frac{1}{K_m}
\]

\[
\frac{1}{S}
\]
Competitive Inhibitor

\[
\frac{1}{V} = \frac{-1}{K_m} + \frac{1}{V_m}
\]

- Normal
- Inhibitor
Non-competitive Inhibitor
Inhibitors

**Competitive**
- Similar to S
- Bind active site
- Overcome by more S
- Vm unchanged
- Km higher

**Non-competitive**
- Different from S
- Bind different site
- Cannot be overcome
- Vm decreased
- Km unchanged
Dose-Response

Jason Ryan, MD, MPH
Efficacy

- Maximal effect a drug can produce
  - Morphine is more efficacious than aspirin for pain control
Potency

• Amount of drug needed for given effect
  • Drug A produces effect with 5mg
  • Drug B produces same effect with 50mg
  • Drug A is 10x more potent than drug B

• More potent not necessarily superior
• Low potency only bad if dose is so high it’s hard to administer
Pain Control

![Graph showing the relationship between dose (mg) and analgesia for Morphine and Aspirin.](image)
Dose-Response

• For many drugs we can measure response as we increase the dose
• Can plot dose (x-axis) versus response (y-axis)
Dose-Response

- Graded or quantal responses
- Graded response
  - Example: Blood pressure
  - Can measure “graded” effect with different dosages
- Quantal response
  - Drug produces therapeutic effect: Yes/No
  - Example: Number of patients achieving SBP<140mmHg
  - Can measure “quantal” effect by % patients responding to dose
Graded Dose Response Curve

The graph illustrates a graded dose response curve, which shows the relationship between dose and effect. The curve is asymptotic, indicating that as the dose increases, the effect also increases, but at a decreasing rate. Key points include:

- **$E_{max}$**: The maximum effect achievable.
- **$E_{50}$**: The dose at which the effect is half of $E_{max}$.

The x-axis represents the dose, while the y-axis represents the effect. The curve reaches $E_{max}$ as the dose increases, approaching an asymptote.
Graded Dose Response Curve

\[ \text{Log [Dose]} \]

\[ E_{\text{max}} \]

\[ E_{50} \]

\[ \downarrow \text{EC50} = \uparrow \text{Potency} \]
Graded Dose Response Curve

- **Effect**
  - $E_{\text{max}}$
  - $E_{50}$

- **Log [Dose]**

- **Potency**

- **EC50/Potency**
  - $A > B > C$
Graded Dose Response Curve

Effect

Log [Dose]

E_{max}

E_{50}

EMax/Efficacy

B>A
Competitive Antagonists

- $E_{\text{max}}$
- $E_{50}$
- Receptor Agonist
- Receptor Agonist + Competitive Antagonist

Log [Dose]
Non-competitive Antagonists

Effect

$E_{\text{max}}$

$E_{50}$

$E_{50}$

$EC_{50}$

Log [Dose]

Receptor Agonist

Receptor Agonist + Non-Competitive Antagonist

Max Effect
Spare Receptors

- “Spare” receptors: Activate when others blocked
- Maximal response can occur even in setting of blocked receptors
- Experimentally, spare receptors demonstrated by using irreversible antagonists
  - Prevents binding of agonist to portion of receptors
  - High concentrations of agonist still produce max response
Spare Receptors

Agonist + Low Dose Non-Competitive Antagonist

Agonist + High Dose Non-Competitive Antagonist

Source: Basic and Clinical Pharmacology, Katzung
Partial Agonists

- Similar structure to agonists
- Produce less than full effect
Partial Agonists
Agonist or Partial Agonist Given Alone

Effect similar to agonist plus NC antagonist

$E_{\text{max}}$

Effect

Log [Dose]
Partial Agonist

Single Dose Agonist With Increasing Partial Agonist

Log [Dose Partial Agonist]

% Binding

100%

0%

Agonist

Partial Agonist
Partial Agonist
Single Dose Agonist With Increasing Partial Agonist

Response

Log [Dose Partial Agonist]
Partial Agonists

- **Pindolol/Acebutolol**
  - Old antihypertensives
  - Activate beta receptors but to less degree that norepinephrine
  - “Intrinsic sympathomimetic activity” (IMA)
  - Lower BP in hypertensive patients
  - Can cause angina through vasoconstriction

- **Buprenorphine**
  - Partial mu-opioid agonist
  - Treatment of opioid dependence

- **Clomiphene**
  - Partial agonist of estrogen receptors hypothalamus
  - Blocks (-) feedback; ↑LH/FSH
  - Infertility/PCOS
Quantal Dose Response Curve

Log [Dose]

% Patients

100%

50%

ED50

LD50/ TD50

Therapeutic Response

Adverse Response
Therapeutic Index

- Measurement of drug safety

Therapeutic Index = $\frac{LD_{50}}{ED_{50}}$
Therapeutic Window

- 100% Patients
- 50% Patients
- Minimum Effective Dose
- Minimum Toxic Dose

Log [Dose]

Therapeutic Window
Low TI Drugs

- Often require measurement of levels to avoid toxicity
- Warfarin
- Digoxin
- Lithium
- Theophylline
Drug Elimination

Jason Ryan, MD, MPH
Elimination

Zero Order

First Order

Plasma Concentration vs. Time
Zero Order Elimination

- Constant rate of elimination per time
- No dependence/variation with [drug]
- No constant half life

Rate = 5 * [Drug]^0

- Ethanol
- Phenytoin
- Aspirin
First Order Elimination

- Rate varies with concentration of drug
- Percent (%) change with time is constant (half life)
- Most drugs 1st order elimination

\[ \text{Rate} = C \times [\text{Drug}]^1 \]
# Zero Order Elimination

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Amount (g)</th>
<th>Change (g)</th>
<th>%</th>
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First Order Elimination

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<td>50</td>
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<td>25</td>
</tr>
<tr>
<td>3</td>
<td>1.25</td>
<td>1.25</td>
<td>12.5</td>
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</table>
Special Types of Elimination

- Flow-dependent
- Capacity-dependent
Flow-dependent Elimination

- Some drugs metabolized so quickly that blood flow to organ (usually liver) determines elimination
- These drugs are “high extraction” drugs
- Example: Morphine
- Patients with heart failure will have ↓ clearance
Capacity-dependent Elimination

• Follows Michaelis-Menten kinetics
• Rate of elimination = $V_{\text{max}} \cdot C / (K_m + C)$
• “Saturatable” $\rightarrow$ High C leads to $V_{\text{max}}$ rate
• When this happens zero order elimination occurs
• Three classic drugs:
  • Ethanol
  • Phenytoin
  • Aspirin
Urine pH

- Many drugs are weak acids or weak bases

Weak Acid: $HA \leftrightarrow A^- + H^+$

Weak Base: $BOH \leftrightarrow B^+ + OH^-$
Urine pH

- Drugs filtered by glomerulus
- Ionized form gets “trapped” in urine after filtration
- Cannot diffuse back into circulation

**Weak Acid:** HA $\leftrightarrow$ A$^-$ + H$^+$

**Weak Base:** BOH $\leftrightarrow$ B$^+$ + OH$^-$
Urine pH

• Urine pH affects drug excretion
• Weak acids: Alkalinize urine to excrete more drug
• Weak bases: Acidify urine to excrete more drug

Weak Acid: HA $\leftrightarrow$ A$^-$ + H$^+$

Weak Base: BOH $\leftrightarrow$ B$^+$ + OH$^-$
Examples

• Weak acid drugs
  • Phenobarbital, aspirin
  • Sodium bicarbonate to alkalinize urine in overdose

• Weak base drugs
  • Amphetamines, quinidine, or phencyclidine
  • Ammonia chloride (NH₄Cl) to acidify urine in overdose
  • Historical: Efficacy not established, toxicity severe acidosis
Drug Metabolism

• Many, many liver reactions that metabolize drugs
• Liver “biotransforms” drug
• Usually converts lipophilic drugs to hydrophilic products
  • Creates water-soluble metabolites for excretion
• Reactions classified as Phase I or Phase II
Phase I Metabolism

• Reduction, oxidation, or hydrolysis reactions
• Often creates active metabolites
• Two key facts to know:
  • Phase I metabolism can slow in elderly patients
  • Phase I includes cytochrome P450 system
Cytochrome P450

• Intracellular enzymes
• Metabolize many drugs (Phase I)
• If inhibited → drug levels rise
• If induced → drug levels fall
Cytochrome P450

- Inhibitors are more dangerous
  - Can cause drug levels to rise
  - Cyclosporine, some macrolides, azole antifungals
- Luckily, many P450 metabolized drugs rarely used
  - Theophylline, Cisapride, Terfenadine
- Some clinically relevant possibilities
  - Some statins + Inhibitor → Rhabdomyolysis
  - Warfarin
P450 Drugs
Some Examples

**Inducers**
- Chronic alcohol
- Rifampin
- Phenobarbital
- Carbamazepine
- Griseofulvin
- Phenytoin

**Inhibitors**
- Isoniazid
- Erythromycin
- Cimetidine
- Azoles
- Grapefruit juice
- Ritonavir (HIV)
Phase II Metabolism

• Conjugation reactions
  • Glucuronidation, acetylation, sulfation
• Makes very polar inactive metabolites
Slow Acetylators

- Genetically-mediated ↓ hepatic N-acetyltransferase
- Acetylation is main route isoniazid (INH) metabolism
- Also important sulfasalazine (anti-inflammatory)
- Procainamide and hydralazine
  - Can cause drug-induced lupus
  - Both drugs metabolized by acetylation
  - More likely among slow acetylators
Pharmacokinetics

Jason Ryan, MD, MPH
Pharmacokinetics

- Absorption
- Distribution
- Metabolism
- Excretion
- All impact drug’s ability to achieve desired result
Drug Administration

- **Enteral**
  - Uses the GI tract
  - Oral, sublingual, rectal

- **Parenteral**
  - Does not use GI tract
  - IV, IM, SQ

- **Other**
  - Inhalation, intranasal, intrathecal
  - Topical
Bioavailability (F)

- Fraction (%) of drug that reaches systemic circulation unchanged
- Suppose 100mg drug given orally
- 50mg absorbed unchanged
- Bioavailability = 50%
Bioavailability (F)

- Intravenous dosing
  - $F = 100\%$
  - Entire dose available to body
- Oral dosing
  - $F < 100\%$
  - Incomplete absorption
  - First pass metabolism
First Pass Metabolism

- Oral drugs absorbed → liver
- Some drugs rapidly metabolized on 1st pass
- Decreases amount that reaches circulation
- Can be reduced in liver disease patients
Bioavailability (F)

Bioavailability = $\frac{AUC_{\text{oral}} \times 100}{AUC_{\text{IV}}}$
Volume of Distribution (Vd)

- Theoretical volume a drug occupies
- Determined by injecting known dose and measuring concentration
Volume of Distribution (Vd)

\[
Vd = \frac{\text{Total Amount In Body}}{\text{Plasma Concentration}}
\]

\[
Vd = \frac{10g}{0.5g/L} = 20L
\]
Volume of Distribution (Vd)

\[ Vd = \frac{\text{Amount Injected}}{C_0} \]
Volume of Distribution (Vd)

- Useful for determining dosages
- Example:
  - Effective [drug] = 10mg/L
  - Vd for drug = 10L
  - Dose = 10mg/L * 10L = 100mg
Fluid Compartments

- Total Body Water: 36L
  - Extracellular: 24L
  - Intracellular: 12L
  - Plasma: 9L
  - Interstitial: 3L

Vd ↑ when drug distributes to more fluid compartments (blood, ECF, tissues)
Volume of Distribution (Vd)

• Drugs restricted to vascular compartment: ↓Vd
  • Large, charged molecules
  • Often protein bound
  • Warfarin: Vd = 9.8L

• Drugs that accumulate in tissues: ↑↑Vd
  • Small, lipophilic molecules
  • Often uneven distribution in body
  • Chloroquine: Vd = 13000L
Protein Binding

• Many drugs bind to plasma proteins (usually albumin)
• This may hold them in the vascular space
• Lowers Vd
Hypoalbuminemia

- Liver disease
- Nephrotic syndrome
- Less plasma protein binding
- More unbound drug → moves to peripheral compartments
- ↑Vd
- Required dose of drug may change
Clearance

- Volume of blood “cleared” of drug
- Volume of blood that contained amount of drug
- Number in liters/min (volume flow)

\[ C_x = \frac{\text{Excretion Rate}}{P_x} \]
Clearance

• Mostly occurs via liver or kidneys
• Liver clearance
  • Biotransformation of drug to metabolites
  • Excretion of drug into bile
• Renal clearance
  • Excretion of drug into urine
Clearance

- In liver or kidney disease clearance may fall
- Drug concentration may rise
- Toxicity may occur
- Dose may need to be decreased
Clearance

- Can also calculate from Vd
- Need elimination constant (Ke)
- Implications:
  - Higher Vd, higher clearance
  - Supposed 10g/hour removed from body
  - Higher Vd $\rightarrow$ Higher volume holding 10g $\rightarrow$ Higher clearance

$$C_x = Vd \times Ke$$
Clearance

\[ C_x = Vd \times Ke \]

\[ Ke = \frac{C_x}{Vd} \]
Clearance

\[
\text{Cl (l/min)} = \frac{\text{Dose (g)}}{\text{AUC (g*min/l)}}
\]

**Plasma Concentration**

**Area Under Curve (AUC)**

**Time**
Half-Life

- Time required to change amount of drug in the body by one-half
- Usually time for [drug] to fall 50%
- Depends on Vd and Clearance (CL)

\[ t_{1/2} = \frac{0.7 \times Vd}{CL} \]
# Half-life

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<th>% Remaining</th>
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<td>3.12</td>
</tr>
<tr>
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Steady State

- Dose administered = amount drug eliminated
- Takes 4-5 half lives to reach steady state
Calculating Doses

• **Maintenance dose**
  • Just enough drug to replace what was eliminated

• **Loading dose**
  • Given when time to steady state is very high
  • Get to steady state more quickly
  • When t1/2 is very high

• **In kidney/liver disease, maintenance dose may fall**
  • Less eliminated per unit time
  • Less needs to be replaced with each dose

• **Loading dose will be unchanged**
Maintenance Dose

Dose Rate = Elimination Rate
= [Drug] * Clearance

Dose Rate = [5g/l] * 5L/min
= 25 g/min
Maintenance Dose

* If Bioavailability is <100%, need to increase dose to account for this

\[
\text{Dose Rate}_{\text{oral}} = \frac{\text{Target Dose}}{F}
\]

Target Dose = 25g/min
Bioavailability = 50%
Dose Rate = 25/0.5 = 50g/min
Loading Dose

- Target concentration * Vd
- Suppose want 5g/l
- Vd = 10L
- Need 5 * 10 = 50grams loading dose
- Divide by F if bioavailability <100%

\[
\text{Loading Dose} = \frac{[\text{Drug}] \times Vd}{F}
\]
Steady State

- Dose administered = amount drug eliminated
- Takes 4-5 half lives to reach steady state
Key Points

- Volume Distribution = Amt injected / [Drug]
- Clearance = 0.7 * Vd / t12
- 4-5 half lives to get to steady state
- Maintenance dose = [Steady State] * CL / F
- Loading dose = [Steady State] * Vd / F