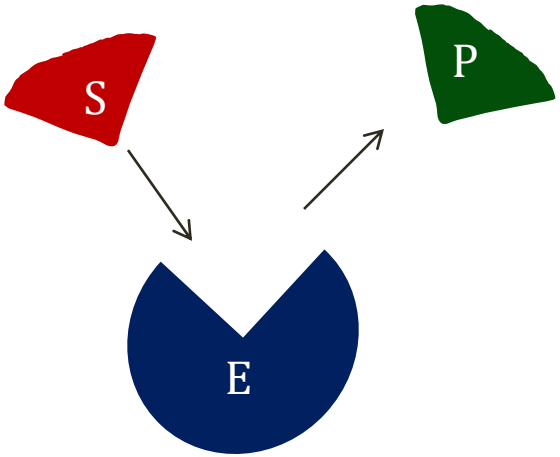
Enzymes

Jason Ryan, MD, MPH

Enzymatic Reactions



$$S + E \rightleftarrows ES \rightleftarrows E + P$$

Enzymatic Reactions

$$S + E \rightleftarrows ES \rightleftarrows E + P$$

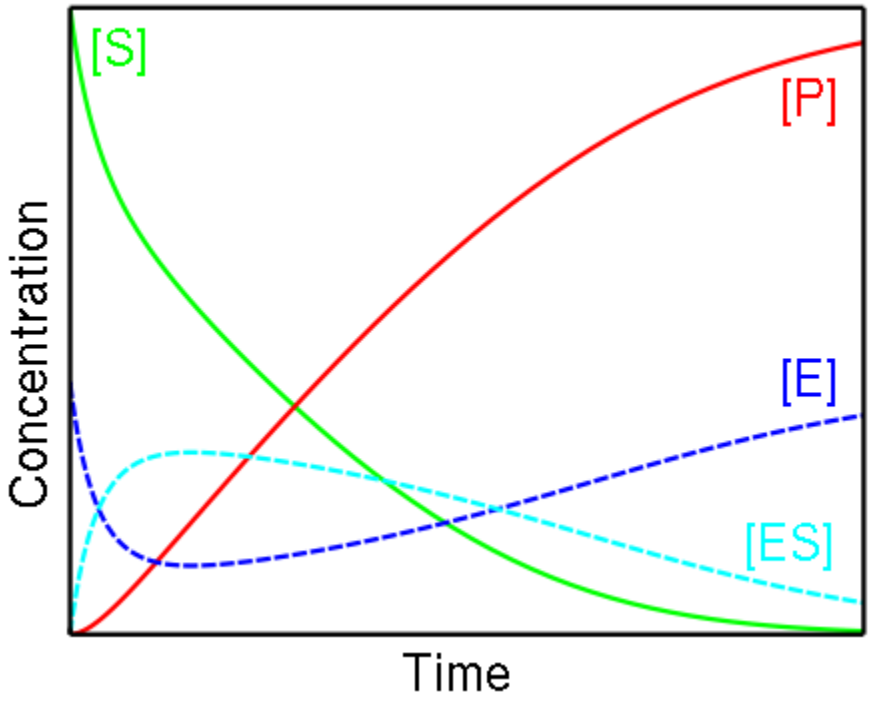


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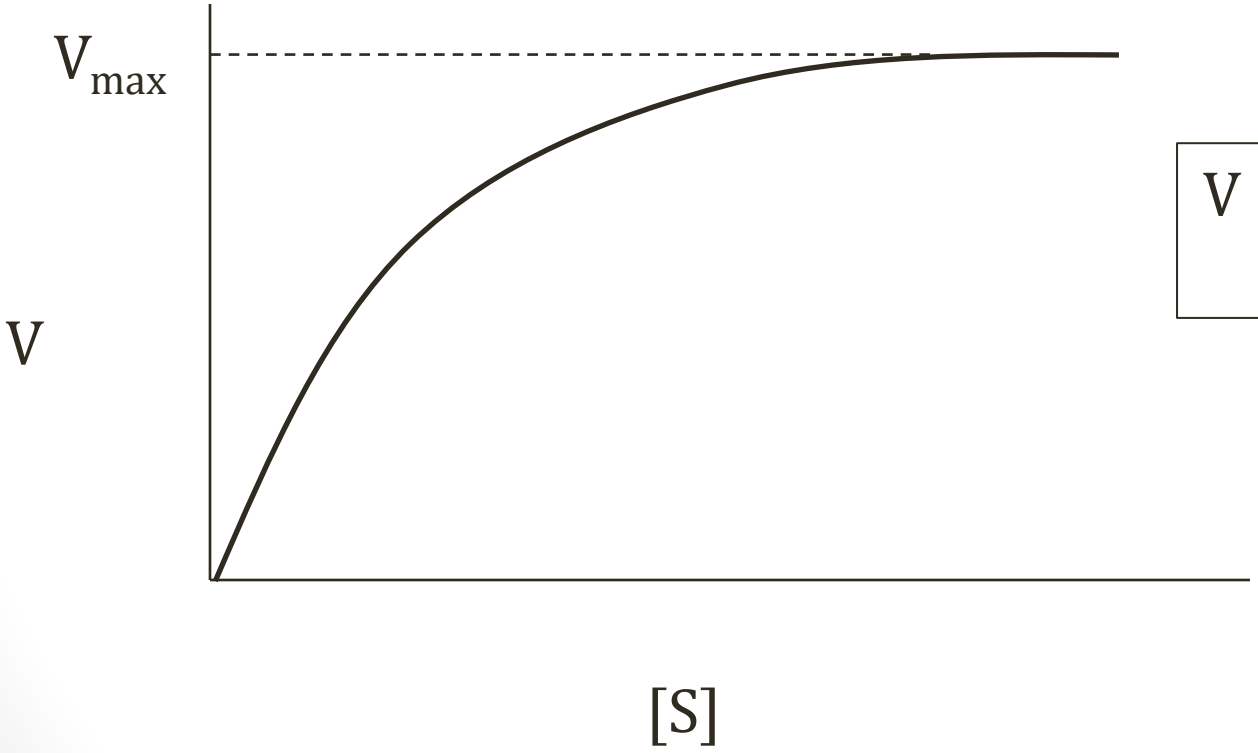
Image courtesy of Wikipedia/U+003F

Michaelis-Menten Kinetics

V = Reaction velocity
Rate of P formation



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

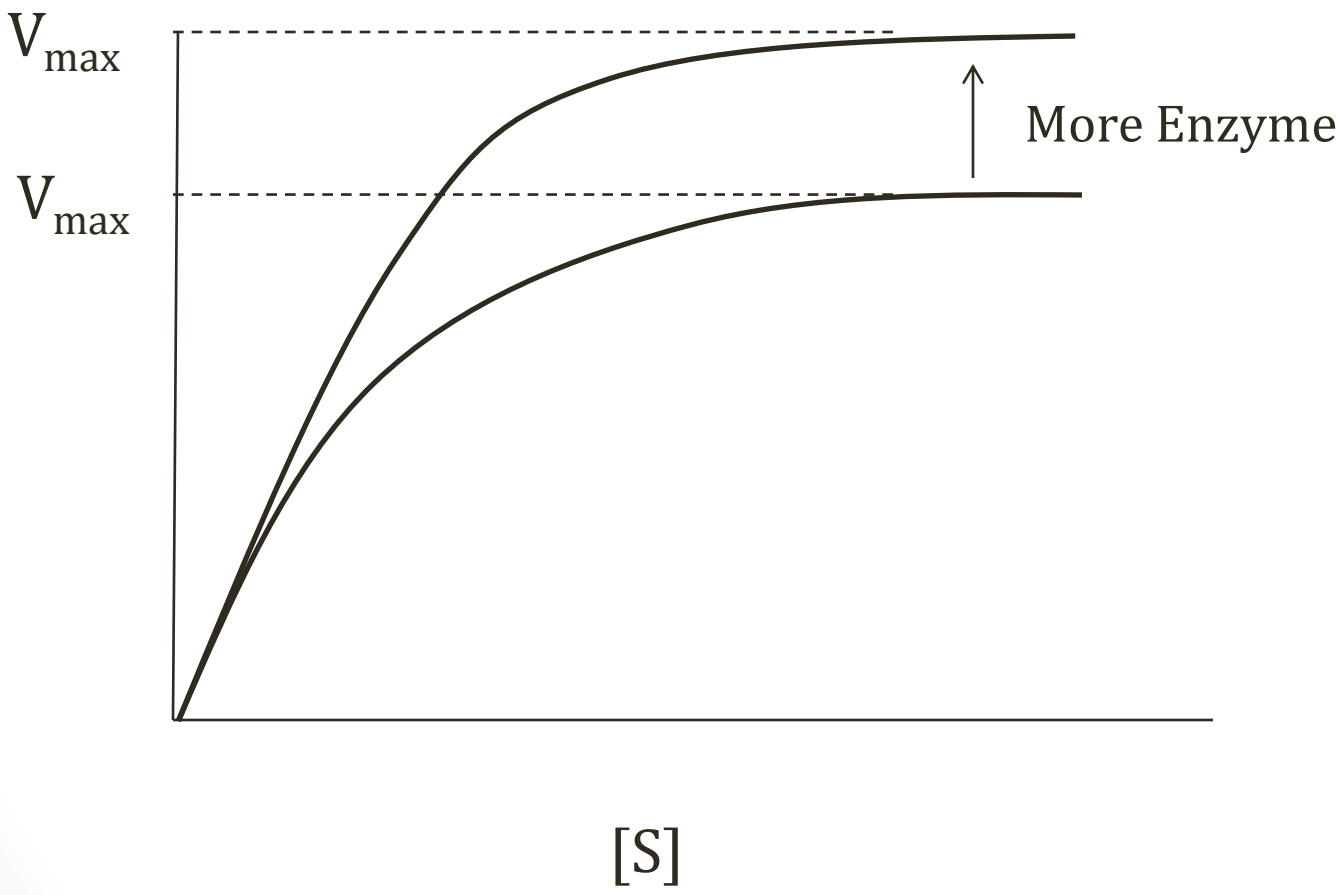
Michaelis-Menten Kinetics

- Adding S → More P formation → Faster V
- Eventually, reach Vmax

Michaelis-Menten Kinetics

- At Vmax, enzymes saturated (doing all they can)
- Only way to increase Vmax is to add enzyme

Enzyme Kinetics



Michaelis-Menten Kinetics

V = Reaction velocity
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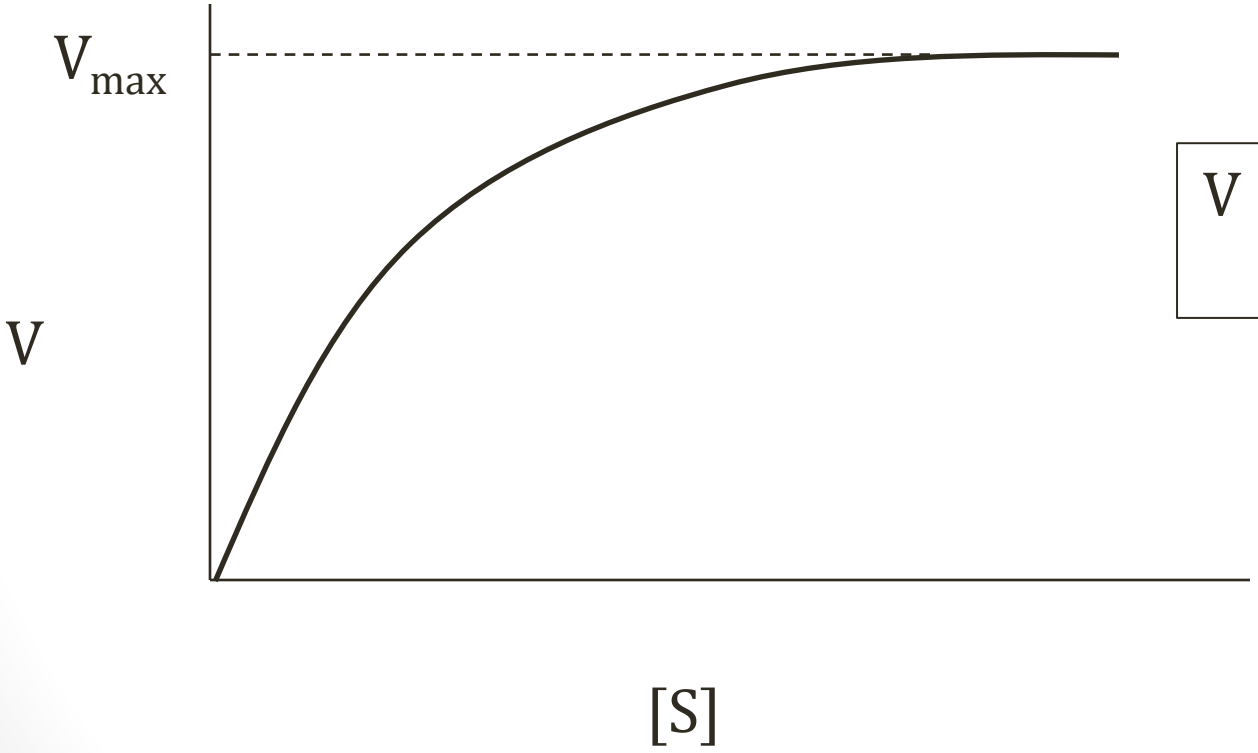


$$V = \frac{V_m * [S]}{K_m + [S]}$$

Michaelis Constant (Km)

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

Key Points:

1. Km has same units as [S]
2. At some point on graph, Km must equal [S]

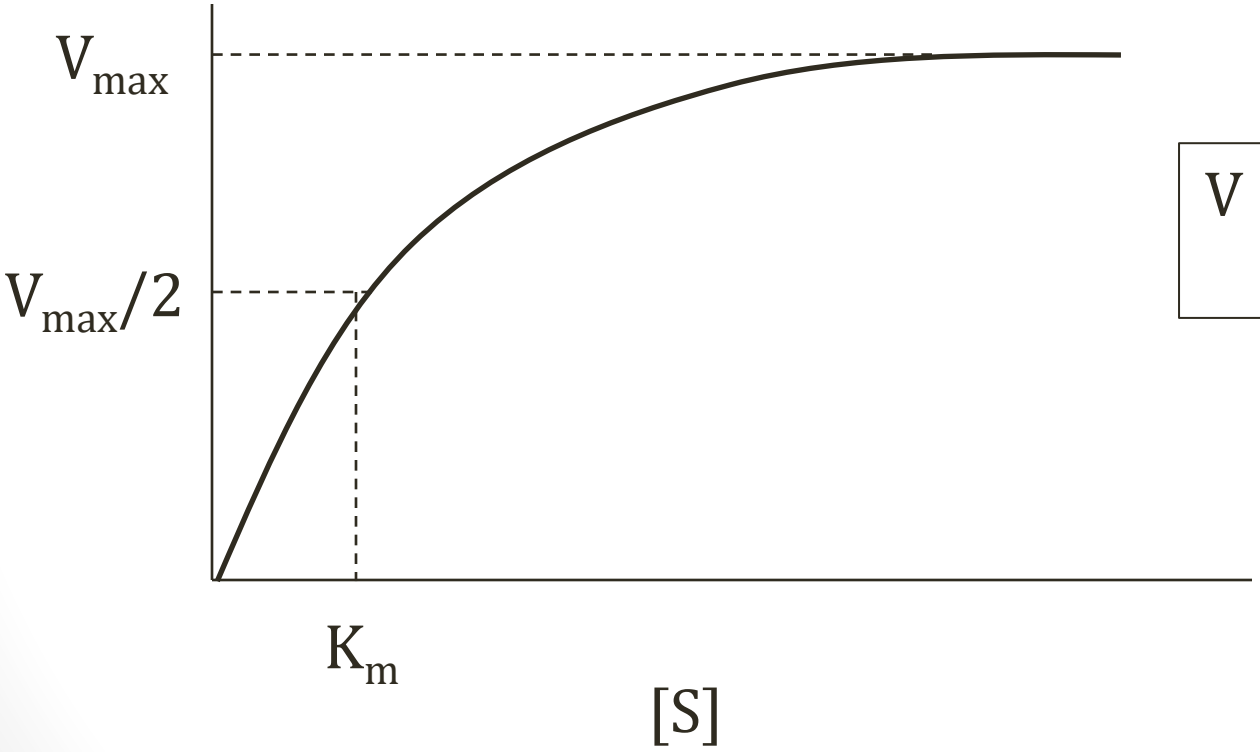
Michaelis Constant (Km)

$$V = \frac{V_m * [S]}{[S] + [S]} = \frac{V_m * [S]}{2\,[S]} = \frac{Vm}{2}$$

When $V = V_m/2$

$[S] = K_m$

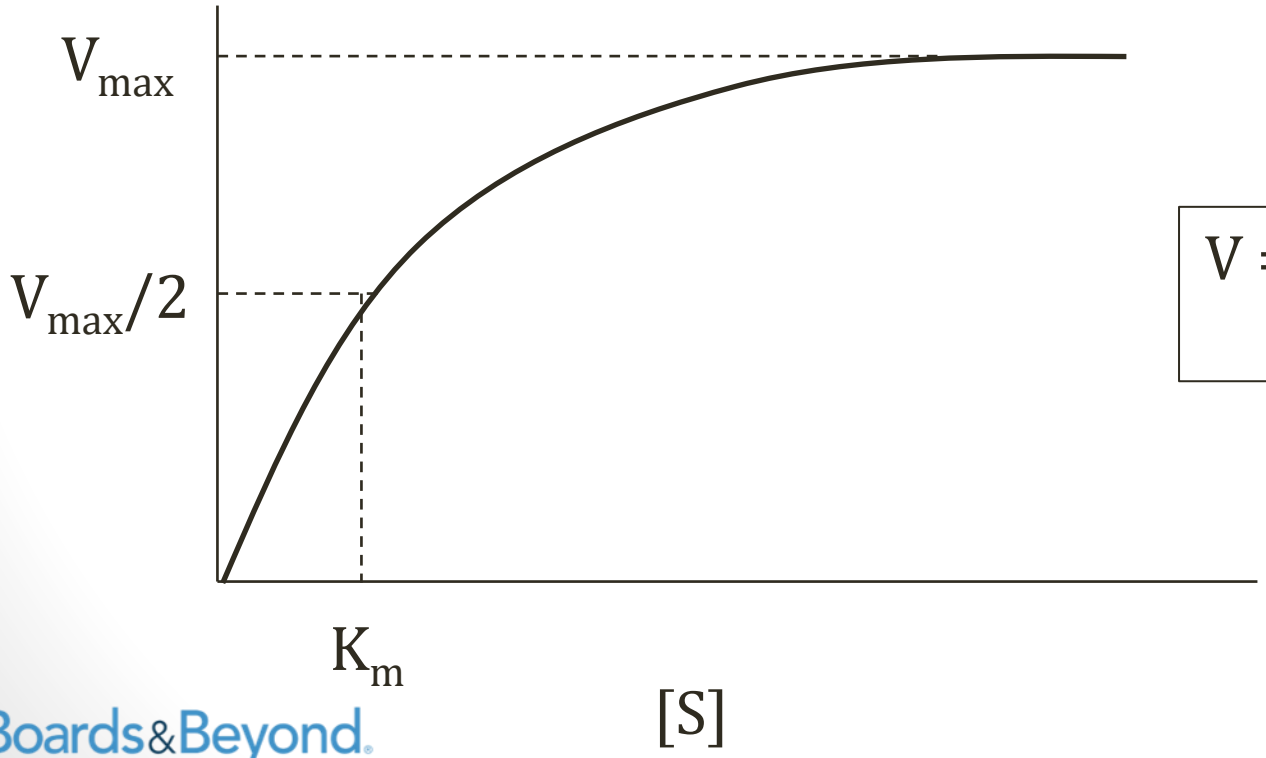
Michaelis Constant (Km)



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

Michaelis Constant (Km)

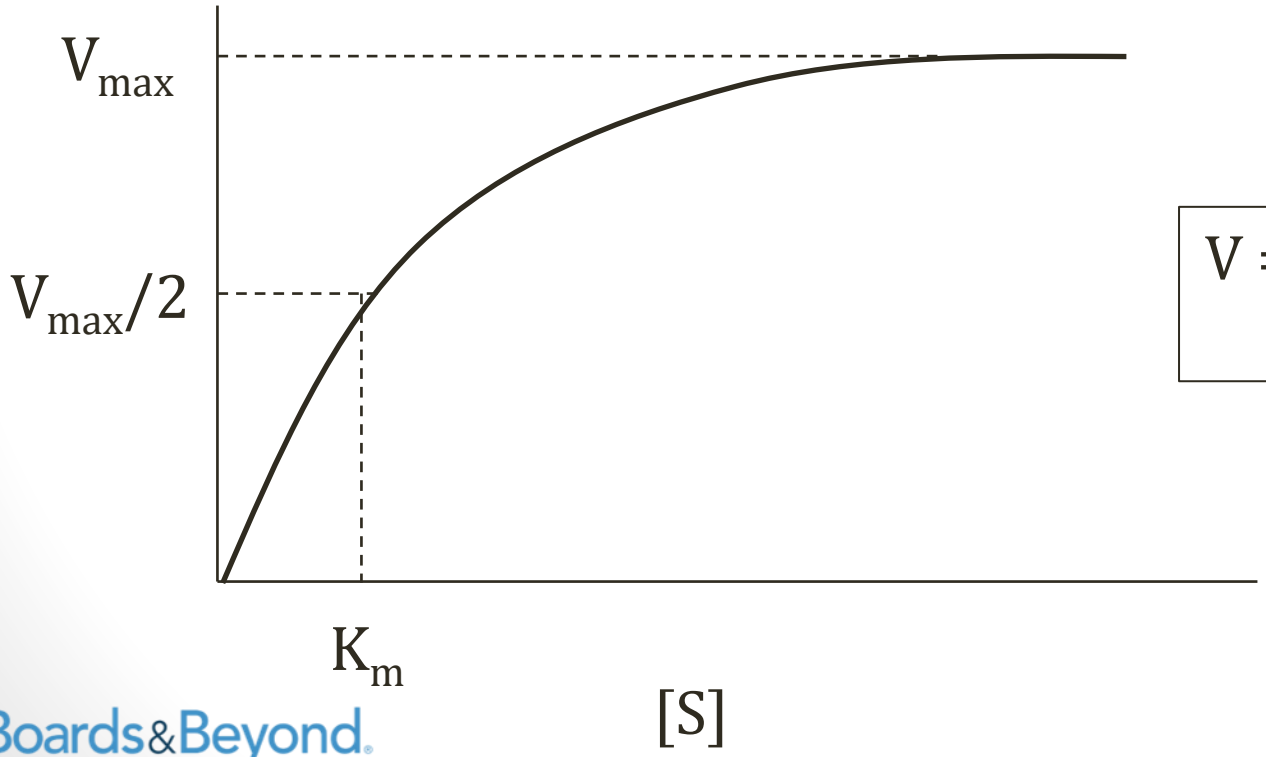
- Small Km → Vm reached at low concentration [S]
- Large Km → Vm reached at high concentration [S]



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

Michaelis Constant (Km)

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



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

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]

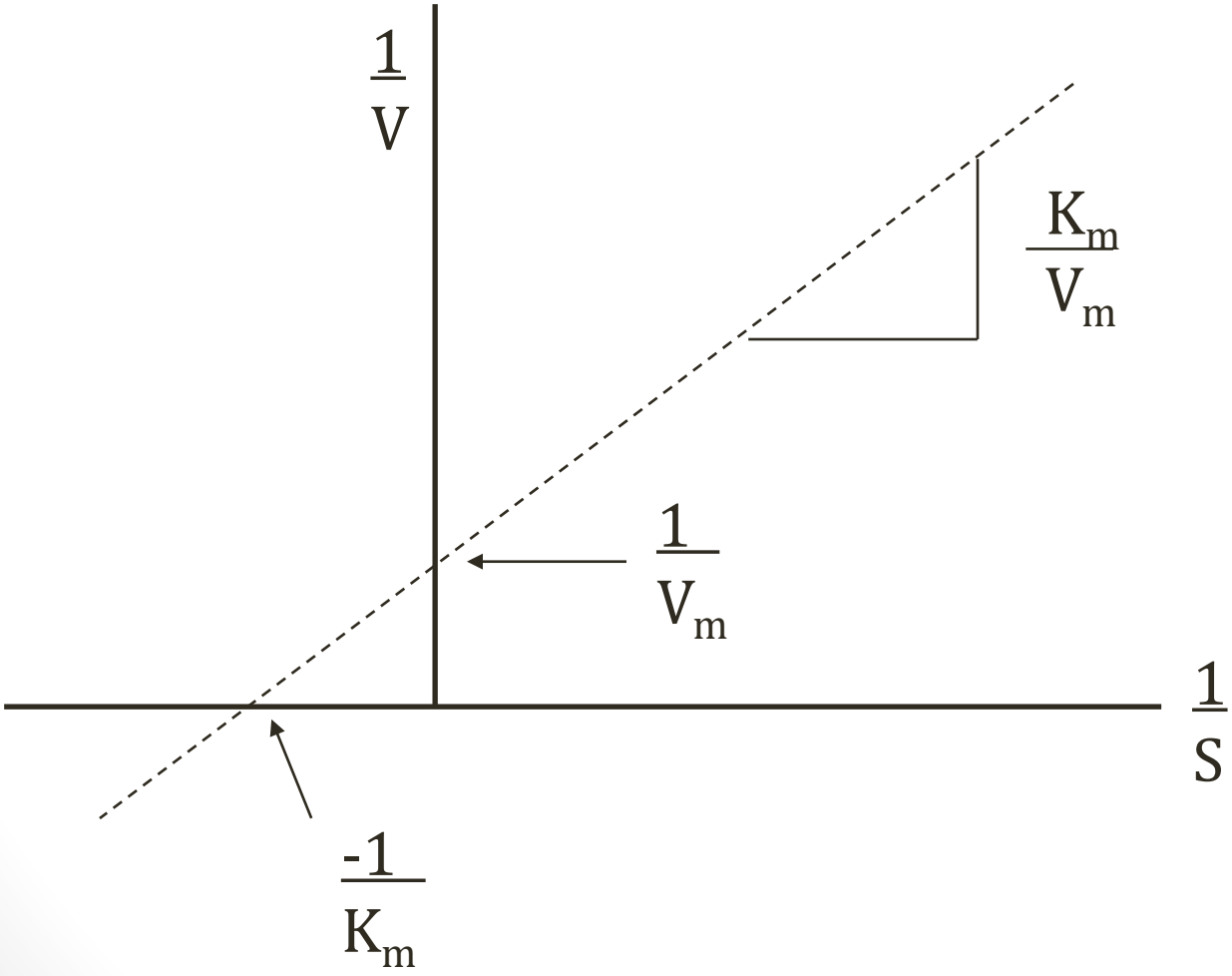
Lineweaver Burk Plot

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

$$\frac{1}{V} = \frac{Km + [S]}{Vm\,[S]} = \frac{Km}{Vm\,[S]} + \frac{[S]}{Vm[S]}$$

$$\frac{1}{V} = C * \frac{1}{[S]} + \frac{1}{Vm}$$

Lineweaver Burk Plot



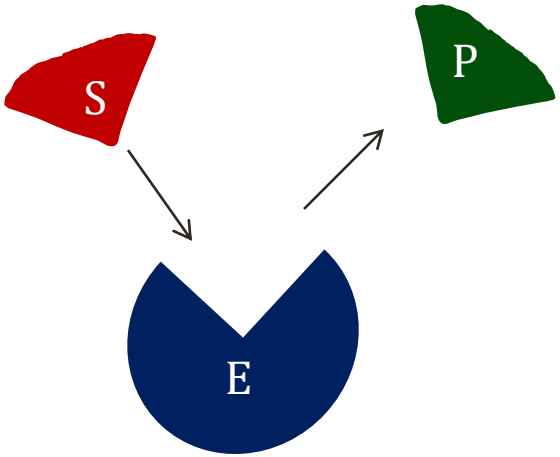
Enzyme Inhibitors

Jason Ryan, MD, MPH

Enzyme Inhibitors

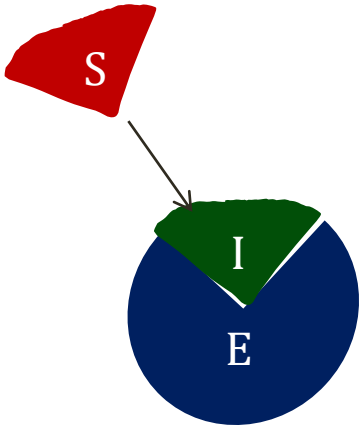
- Many drugs work through enzyme inhibition
- Two types of inhibitors:
  - Competitive
  - Non-competitive

Enzymatic Reactions



$$S + E \rightleftarrows ES \rightleftarrows E + P$$

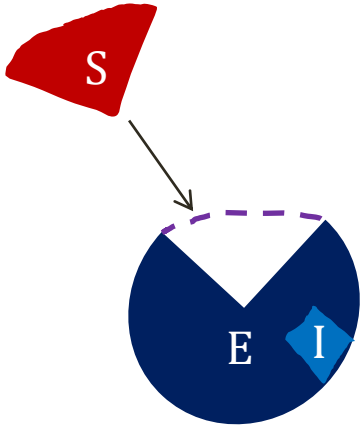
Enzyme Inhibitors



<u>Competitive</u>

Competes for same site as S

Lots of S will overcome this



<u>Non-competitive</u>

Binds different site S

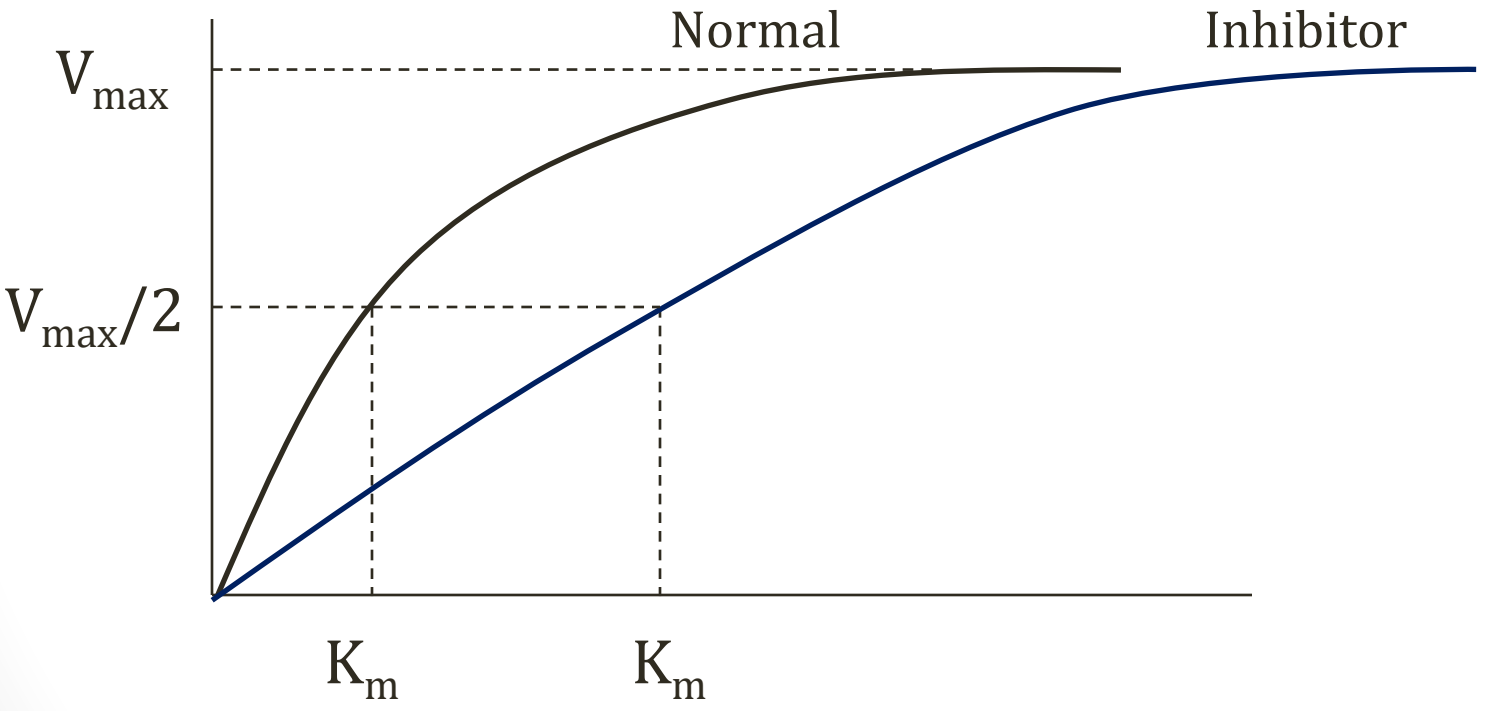
Changes S binding site

S cannot overcome this

Effect similar to no enzyme

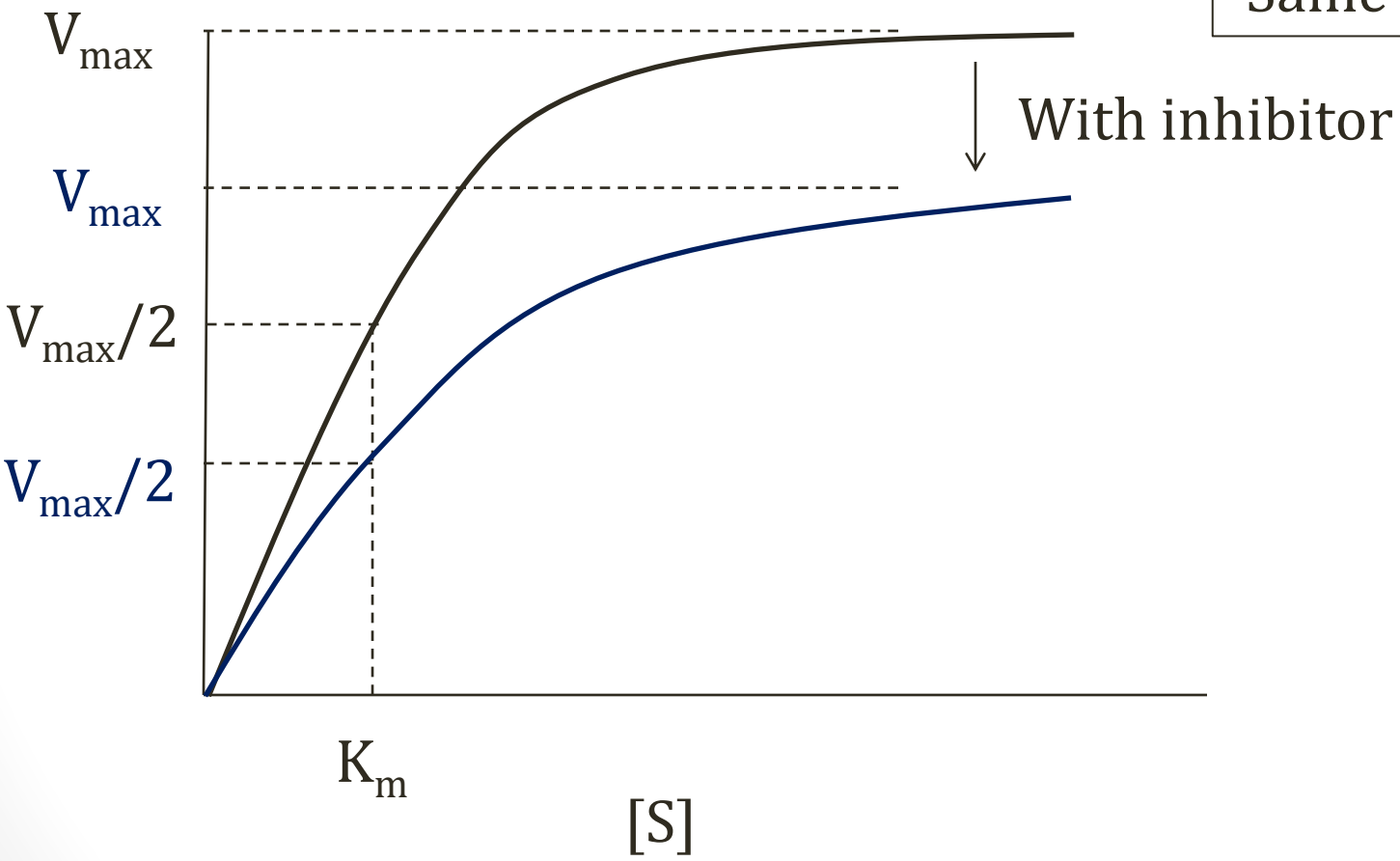
Competitive Inhibitor

Same Vm
Higher Km

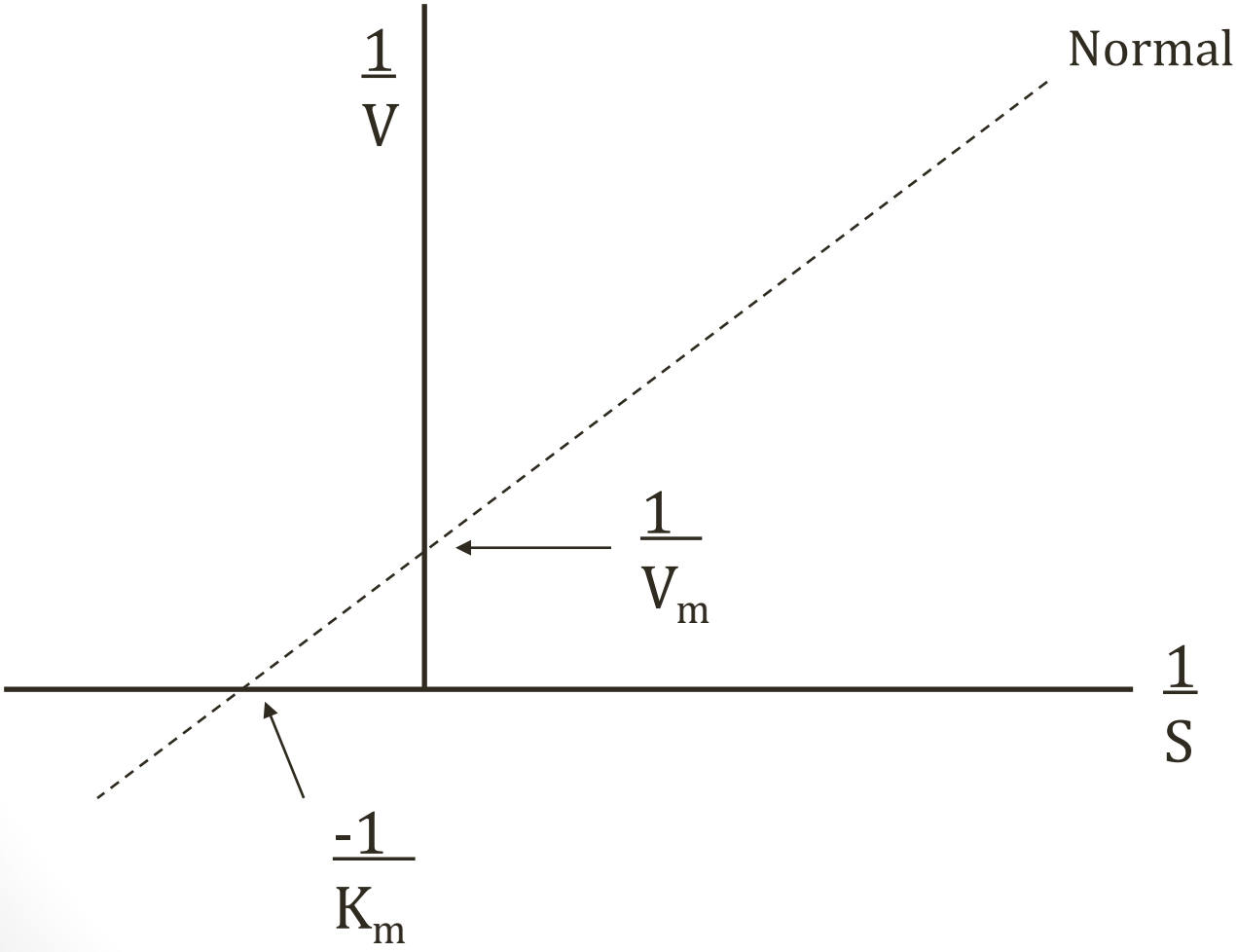


Non-competitive Inhibitor

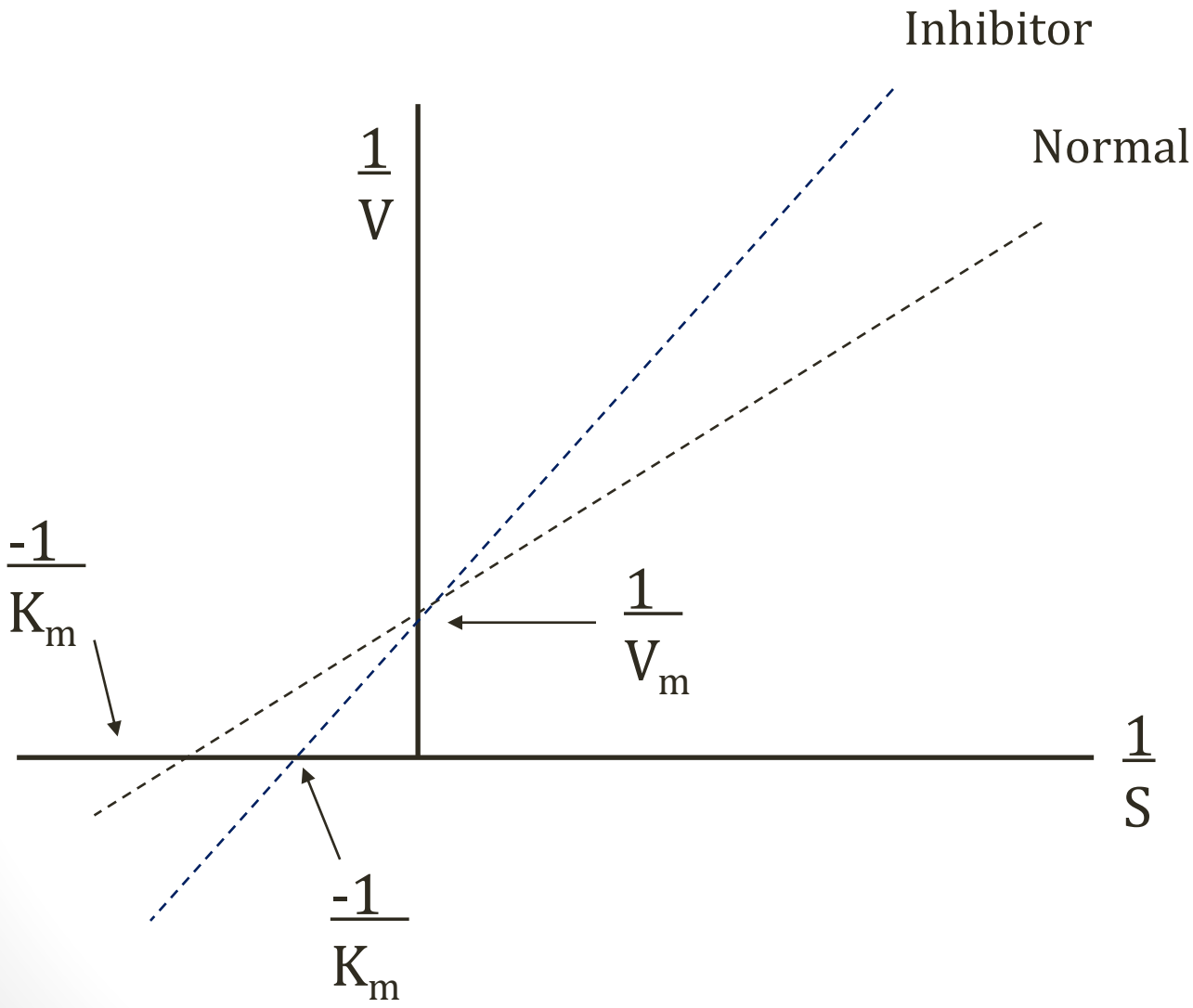
Lower Vm
Same Km



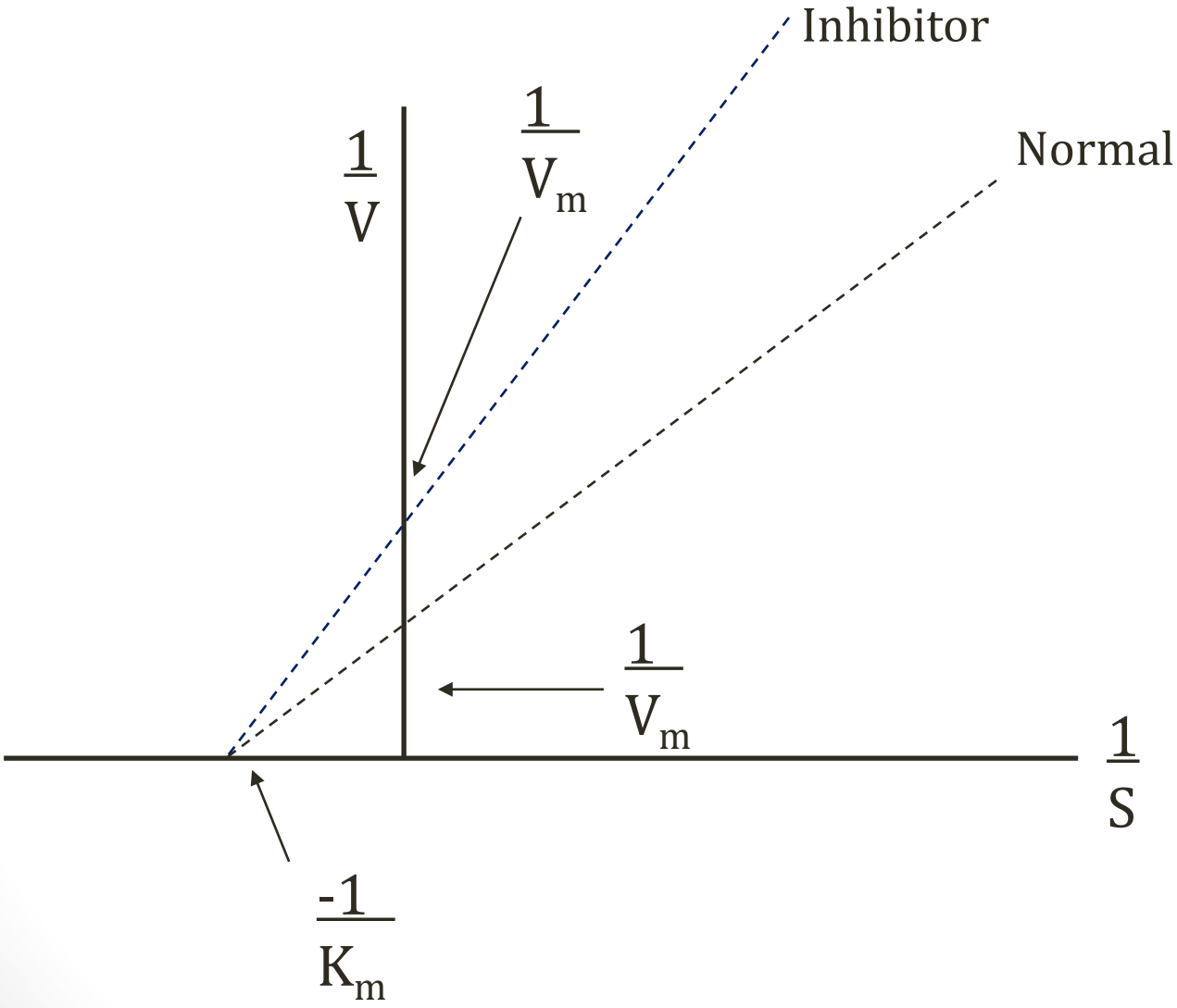
Competitive Inhibitor



Competitive 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

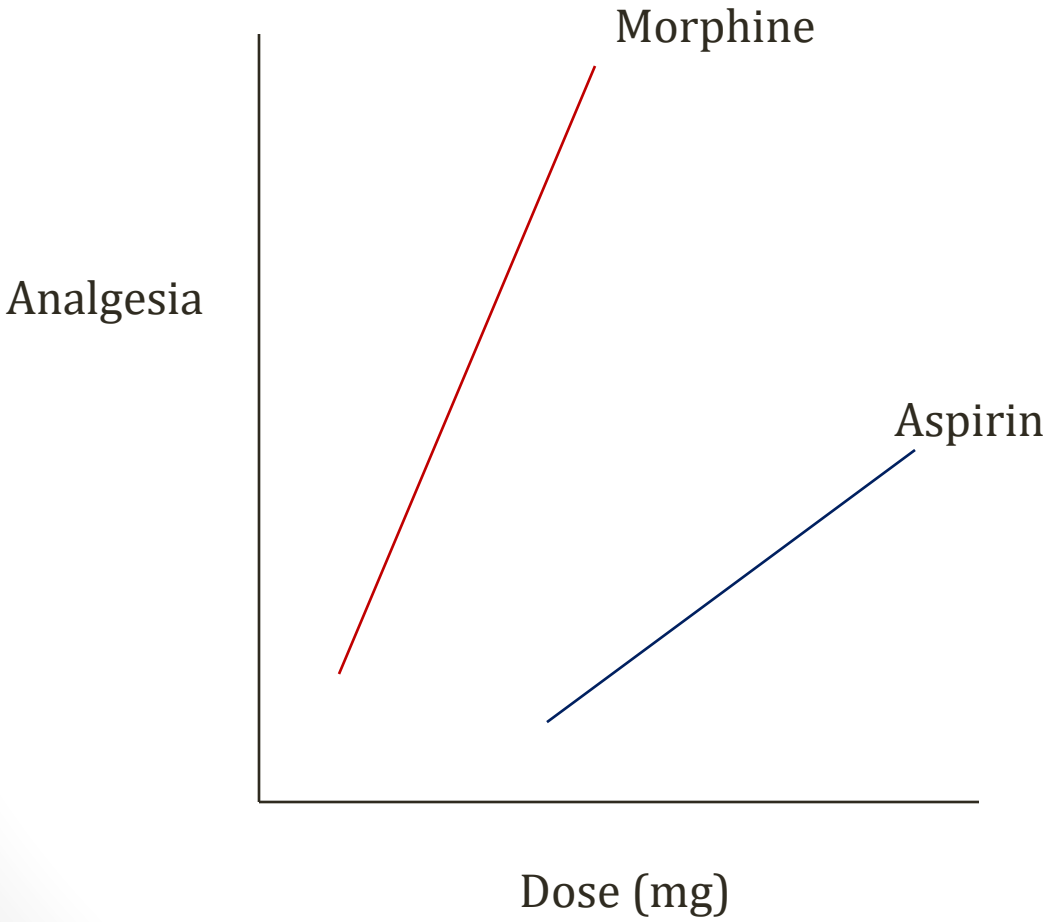
Efficacy

- 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



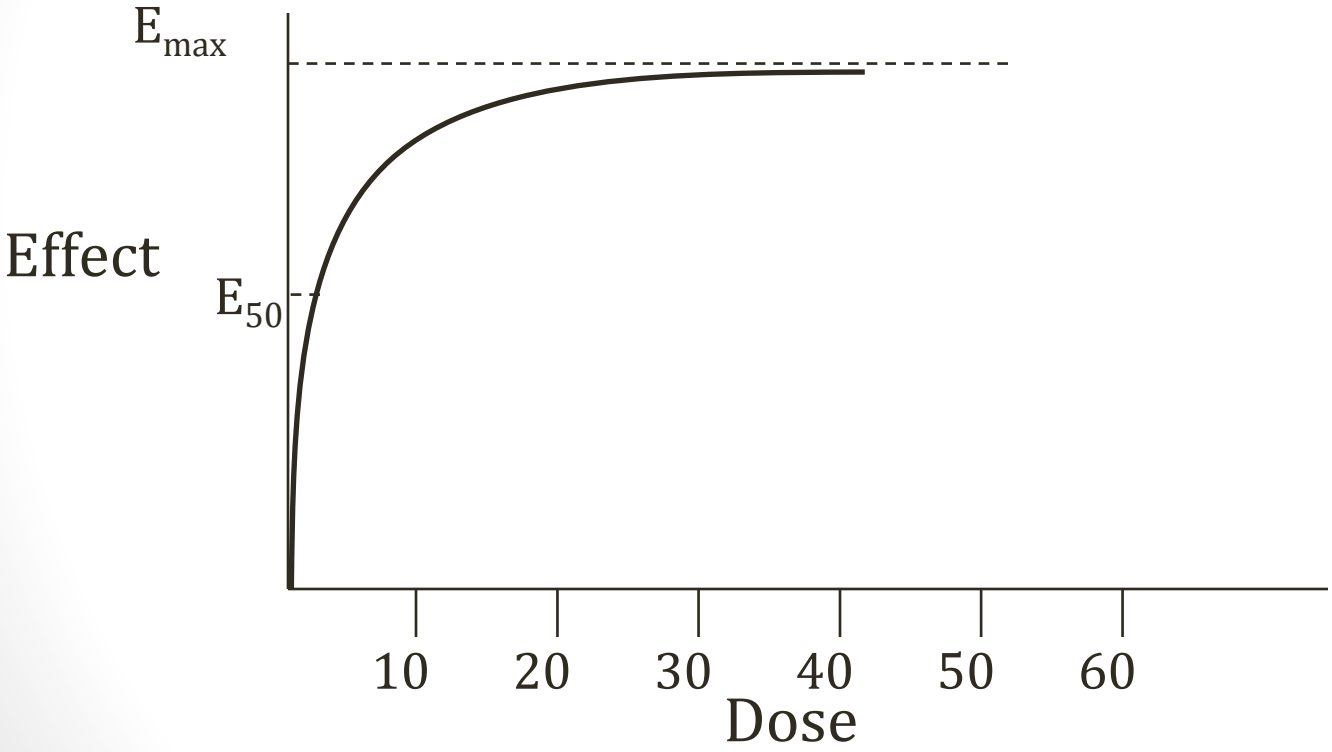
Dose-Response

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

Dose-Response

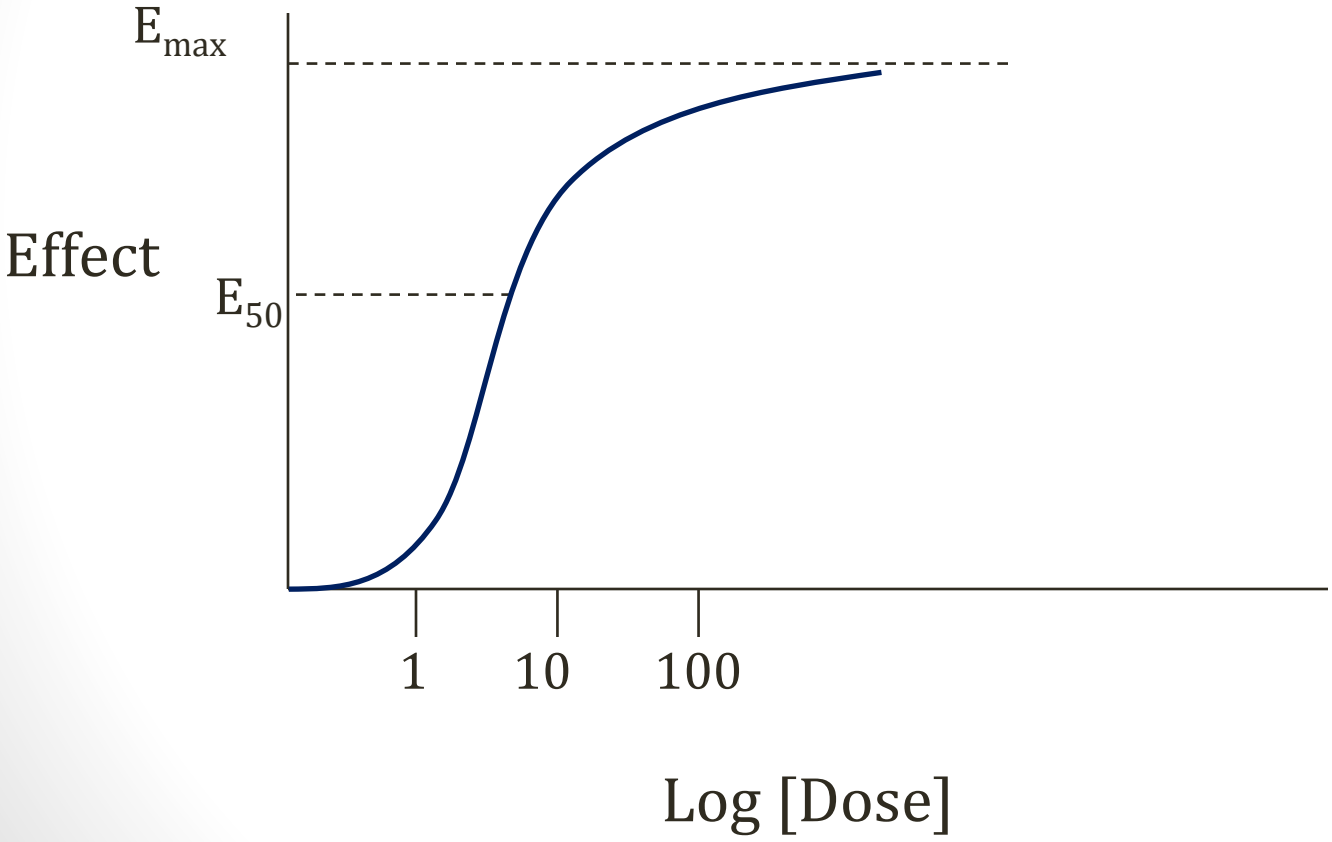
- Graded or quantal responses
- Graded response
  - Example: Blood pressure
  - Can measure “graded” effect with different dosages
- Quantal response
  - Drug produces therapeutic effect: Yes/No
  - Example: Number of patients achieving SBP<140mmHg
  - Can measure “quantal” effect by % patients responding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

Graded Dose Response Curve



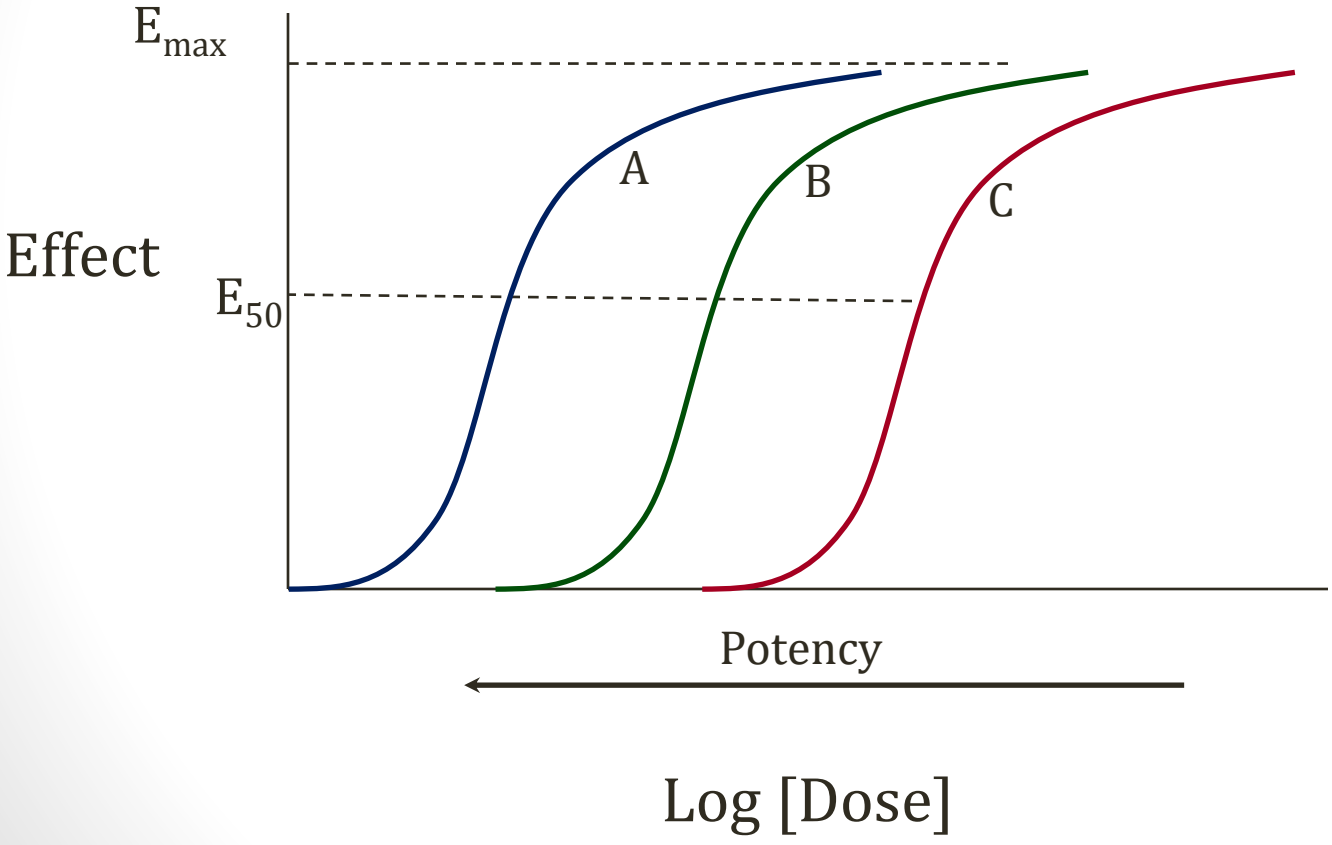
Graded Dose Response Curve

↓EC50 = ↑Potency

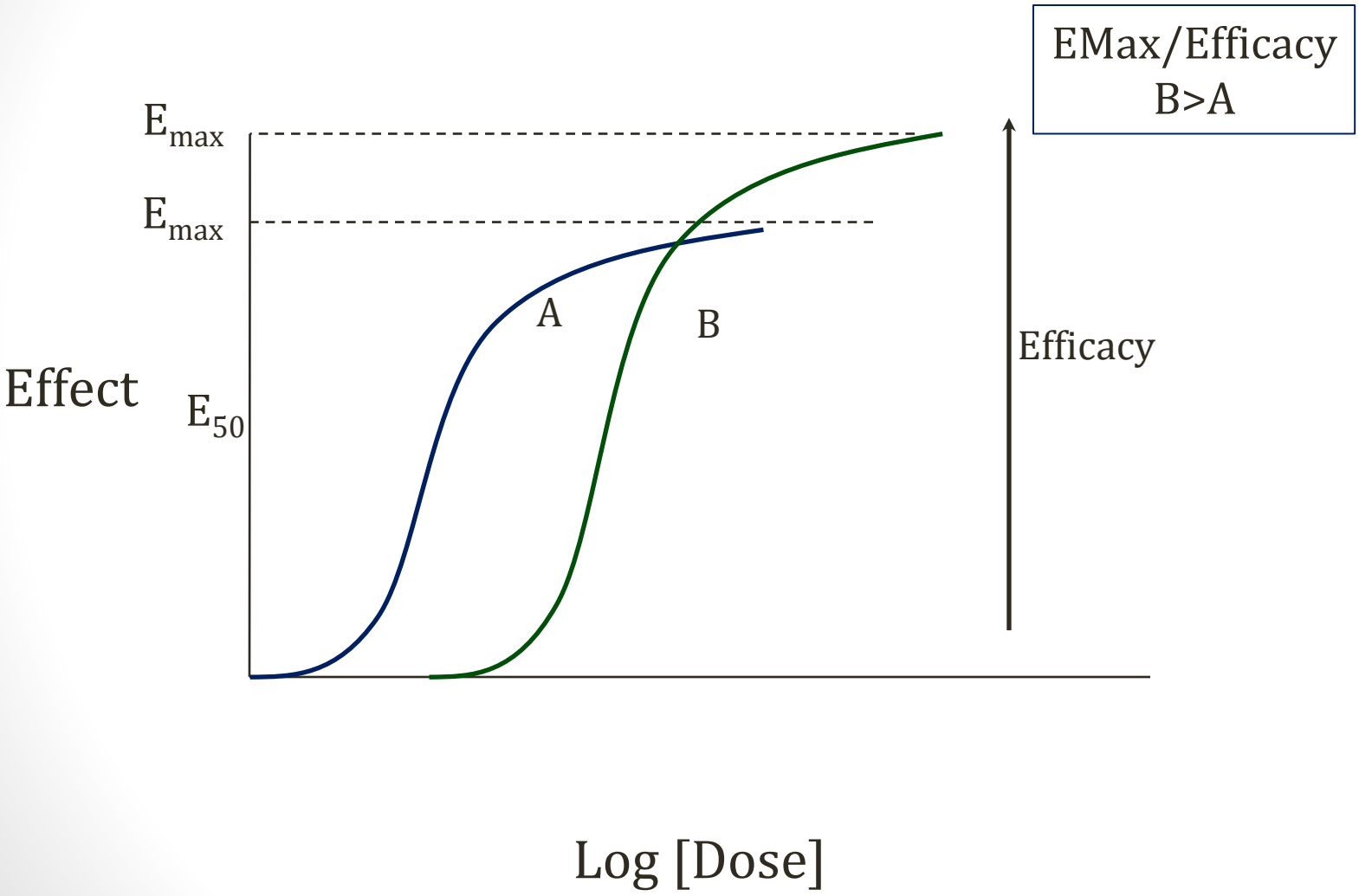


Graded Dose Response Curve

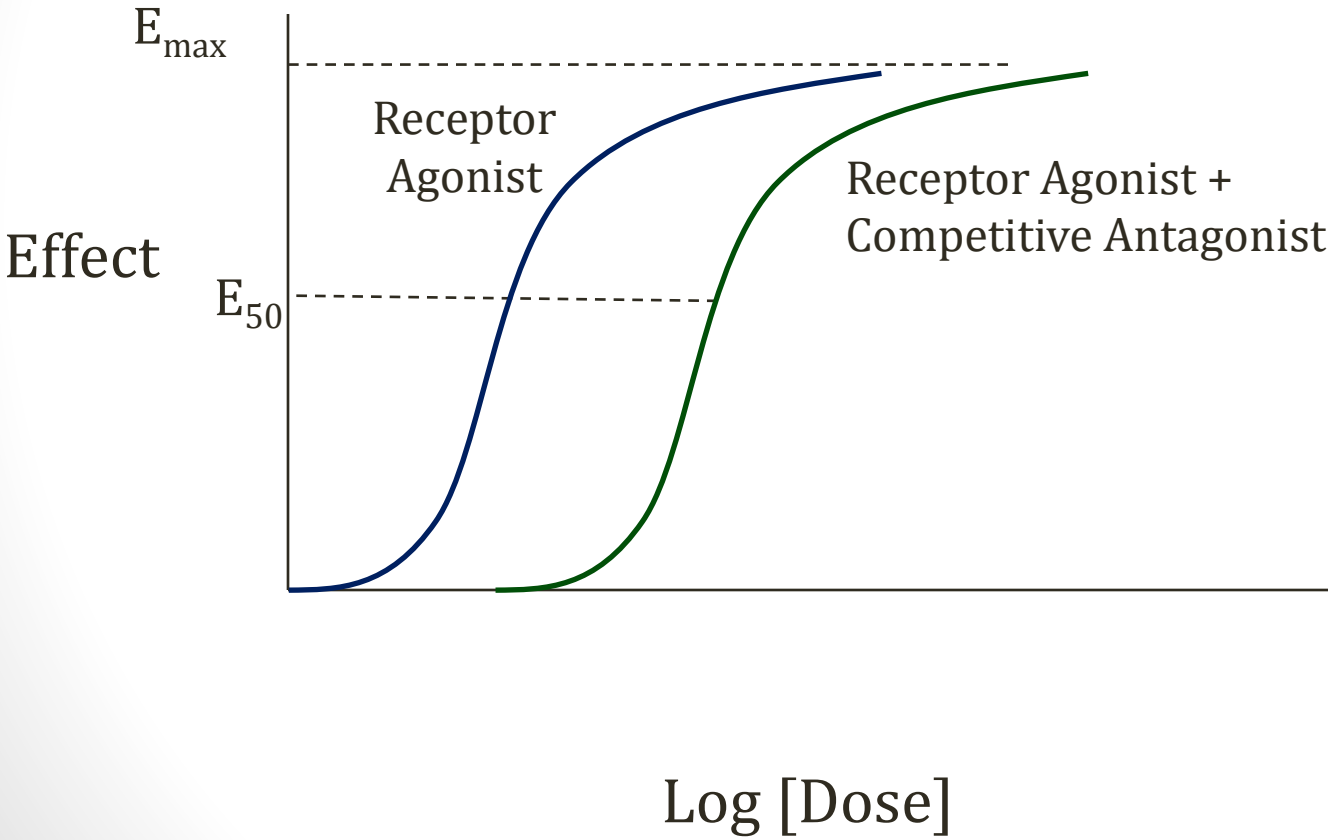
EC50/Potency
A > B > C



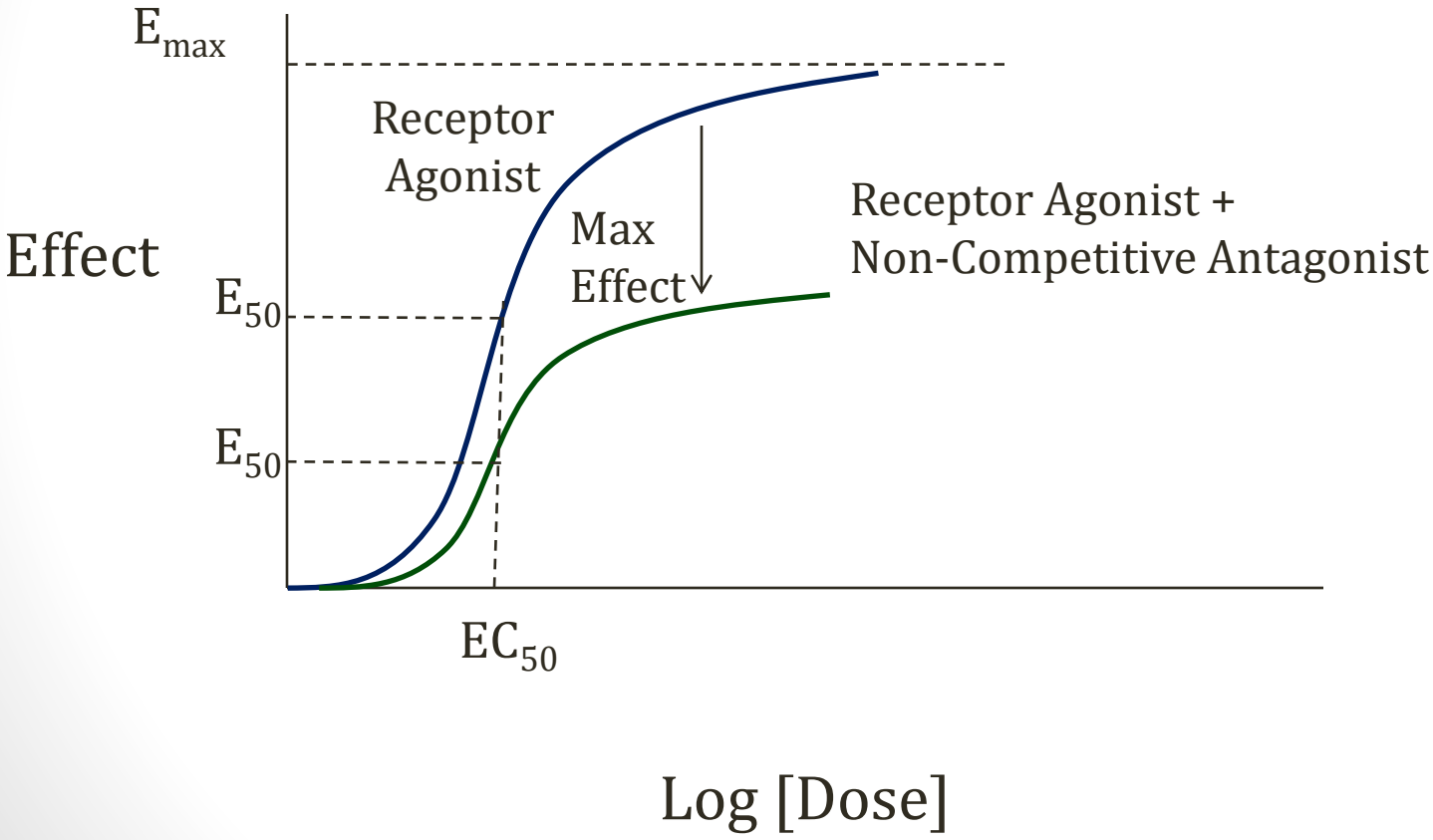
Graded Dose Response Curve



Competitive Antagonists



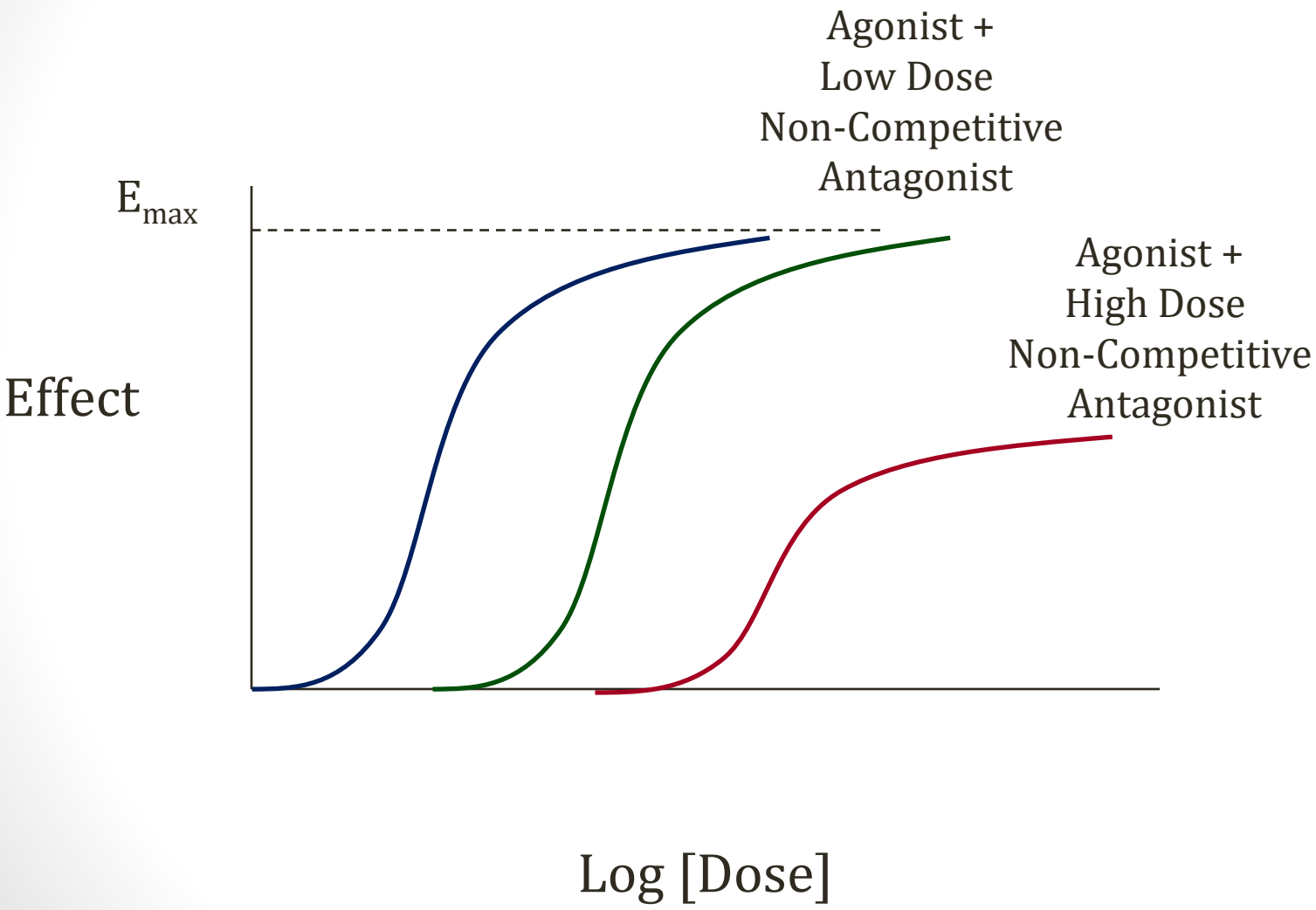
Non-competitive Antagonists



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



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Source: Basic and Clinical Pharmacology, Katzung

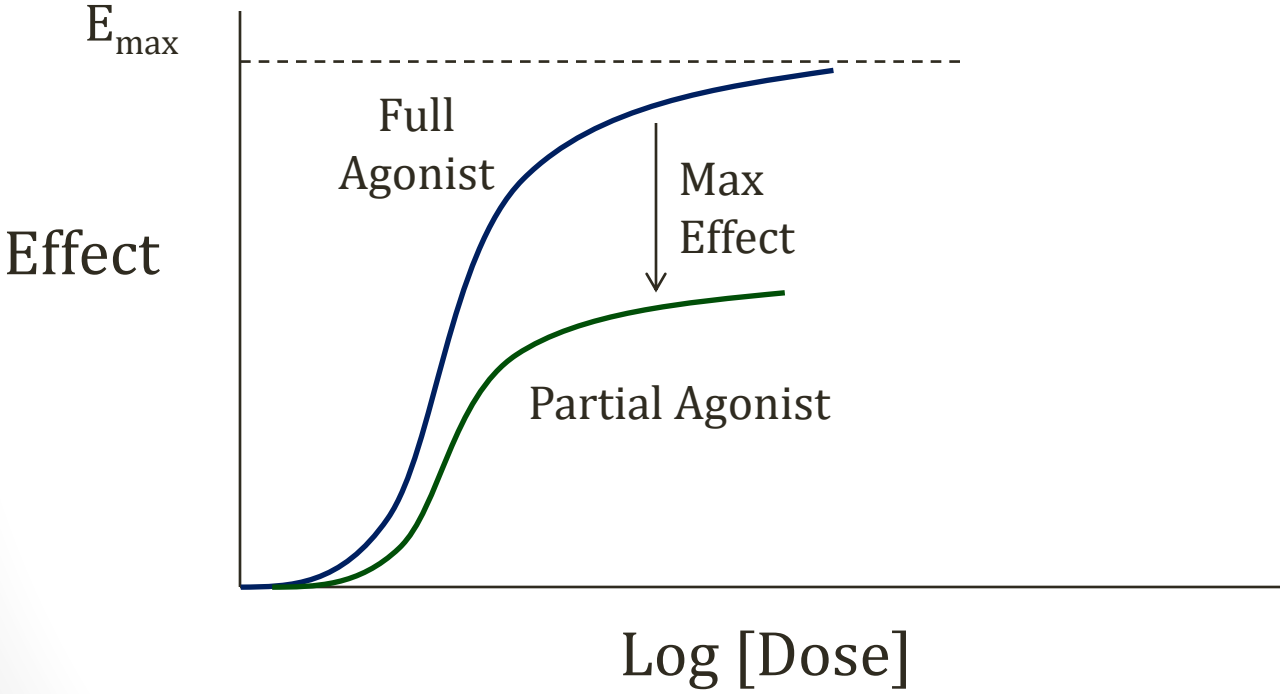
Partial Agonists

- Similar structure to agonists
- Produce less than full effect

Partial Agonists

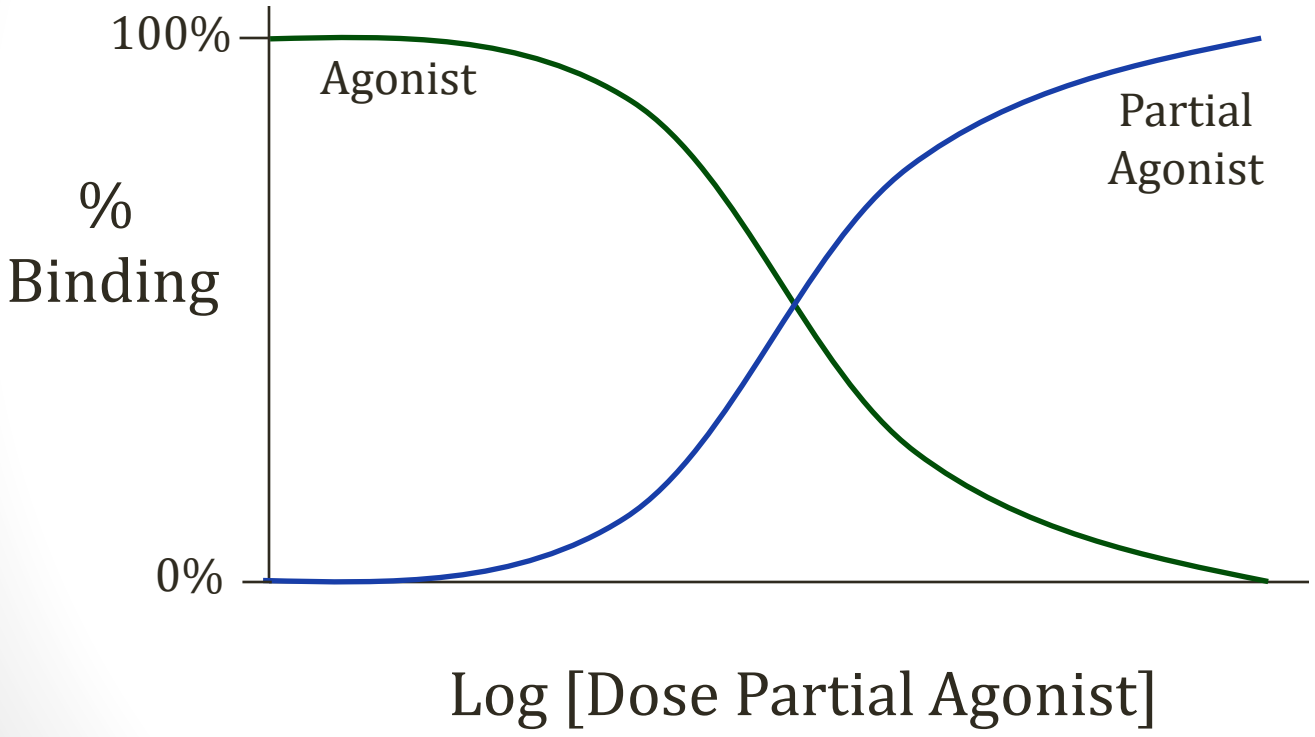
Agonist or Partial Agonist Given Alone

Effect similar to agonist plus NC antagonist



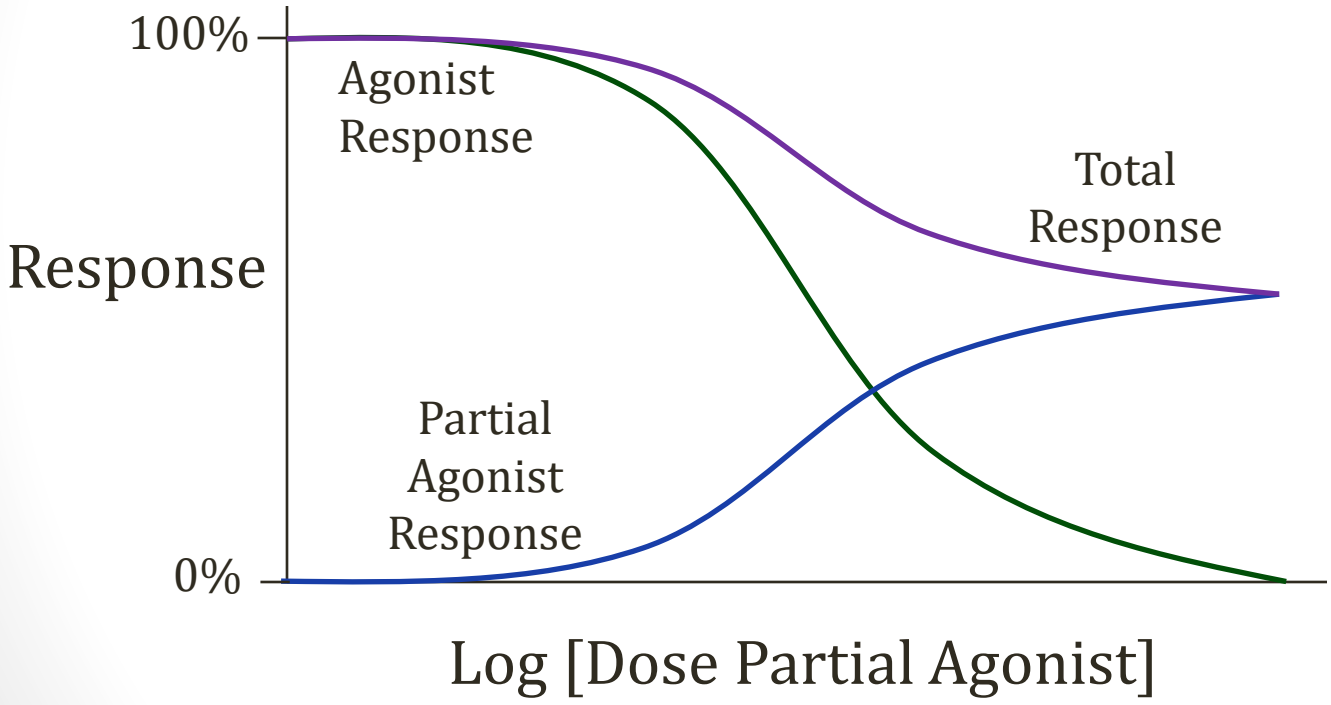
Partial Agonist

Single Dose Agonist With Increasing Partial Agonist



Partial Agonist

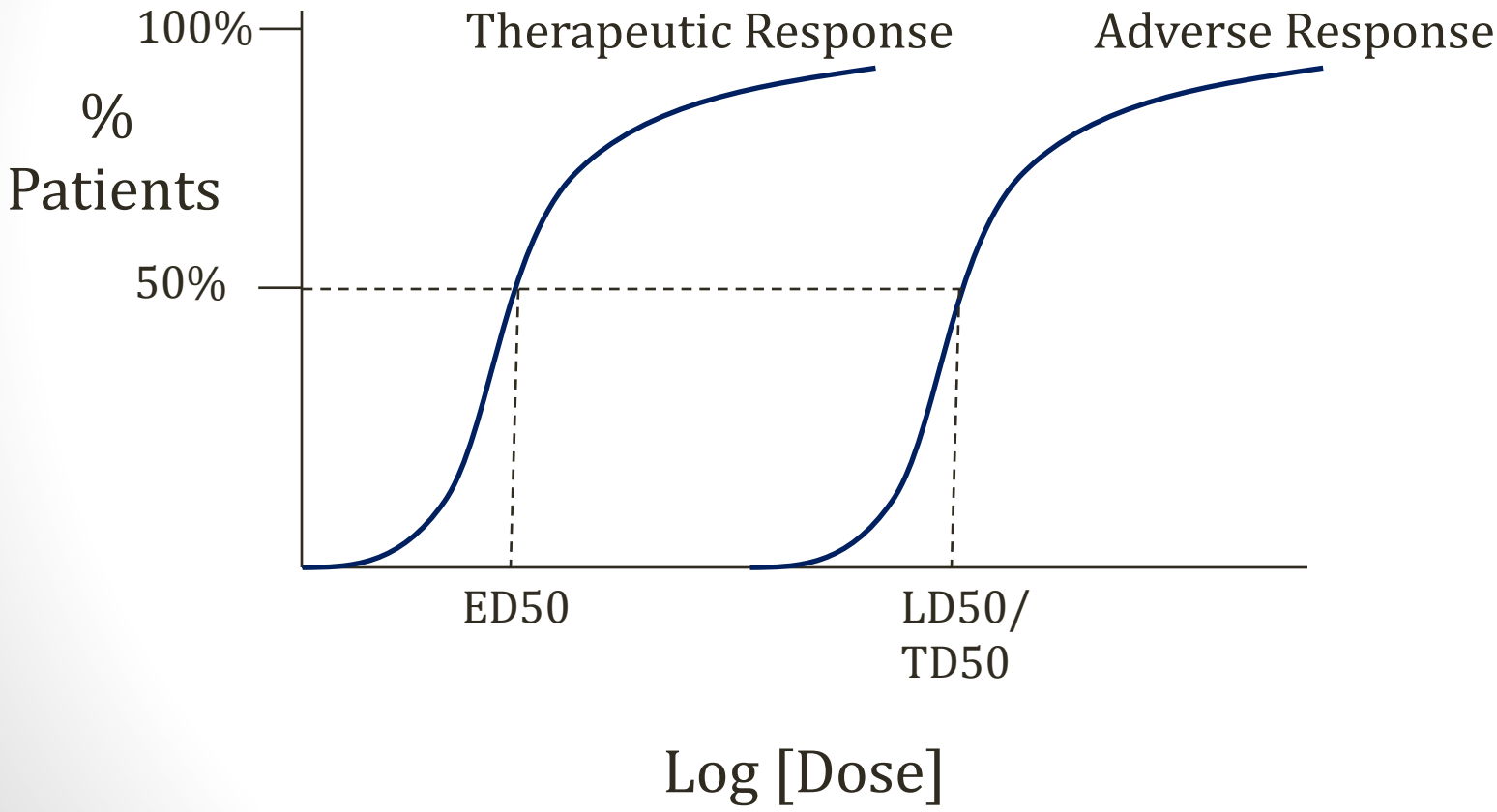
Single Dose Agonist With Increasing Partial Agonist



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

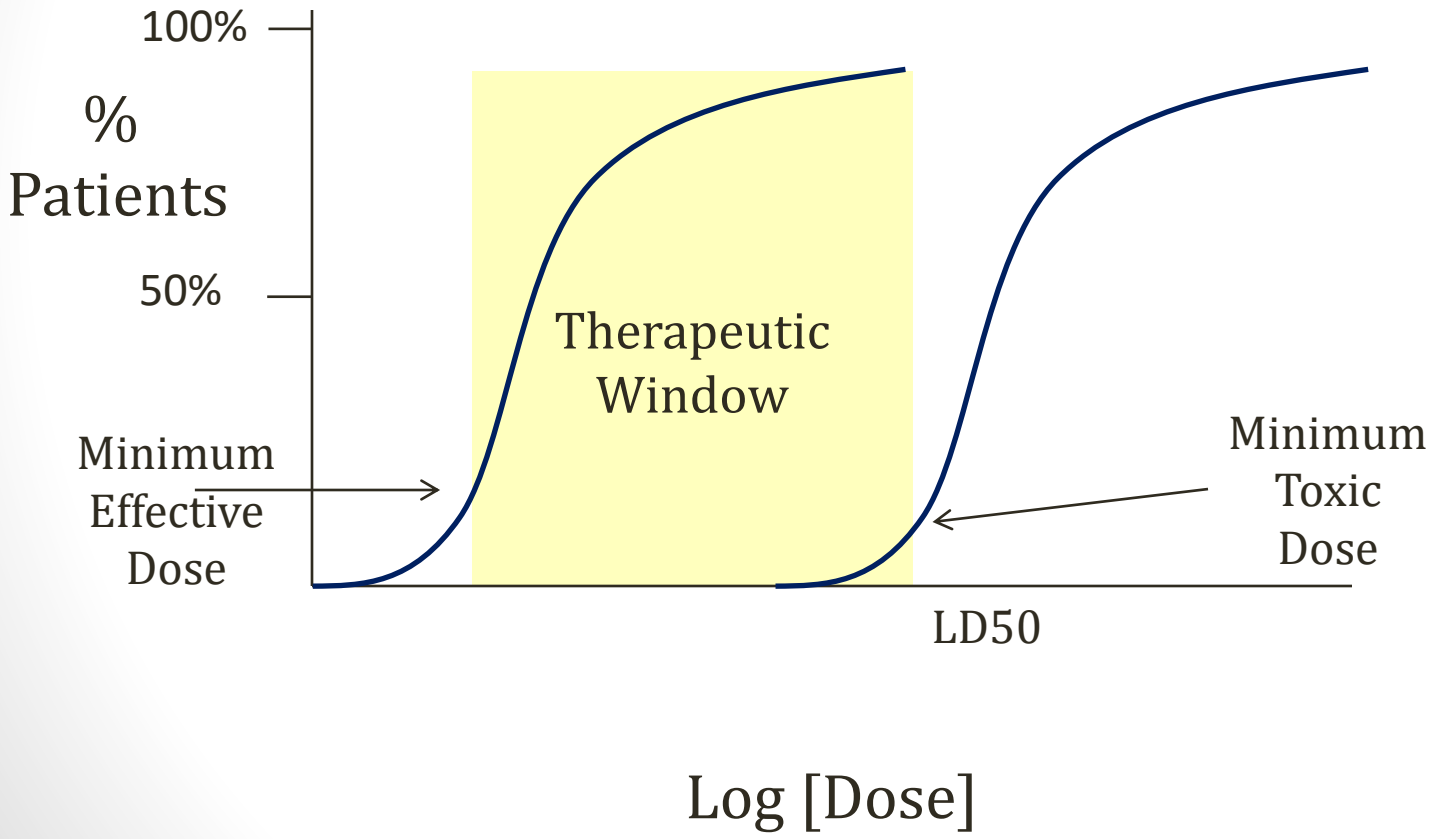


Therapeutic Index

- Measurement of drug safety

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

Therapeutic Window



Low TI Drugs

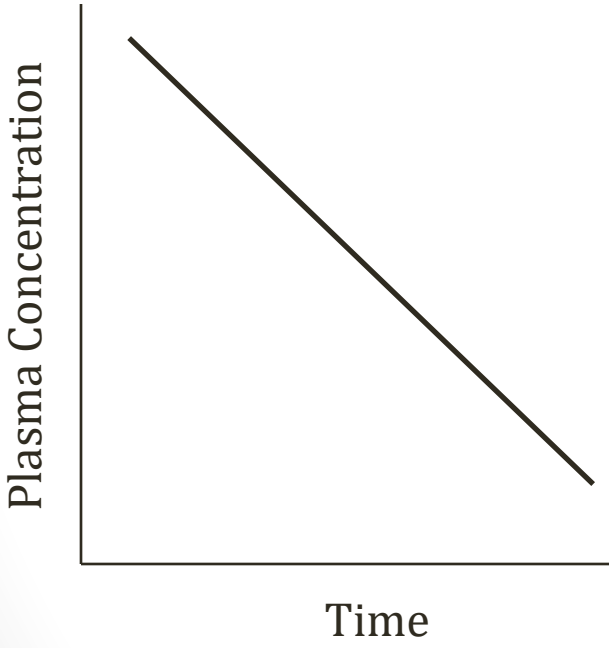
- Often require measurement of levels to avoid toxicity
- Warfarin
- Digoxin
- Lithium
- Theophylline

Drug Elimination

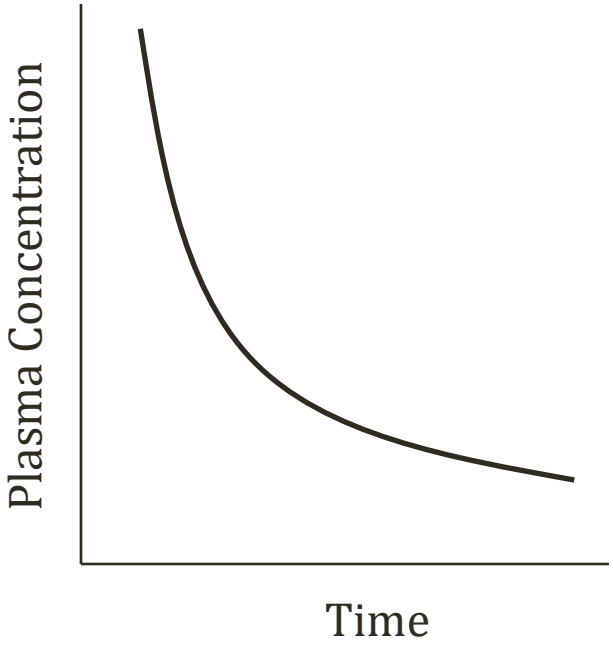
Jason Ryan, MD, MPH

Elimination

Zero Order



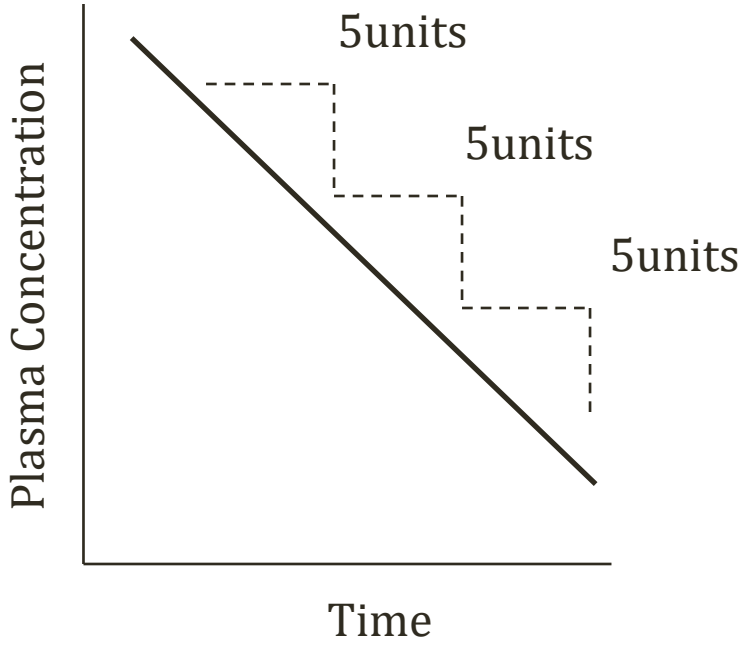
First Order



Zero Order Elimination

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

$$\text{Rate} = 5 * [\text{Drug}]^0$$

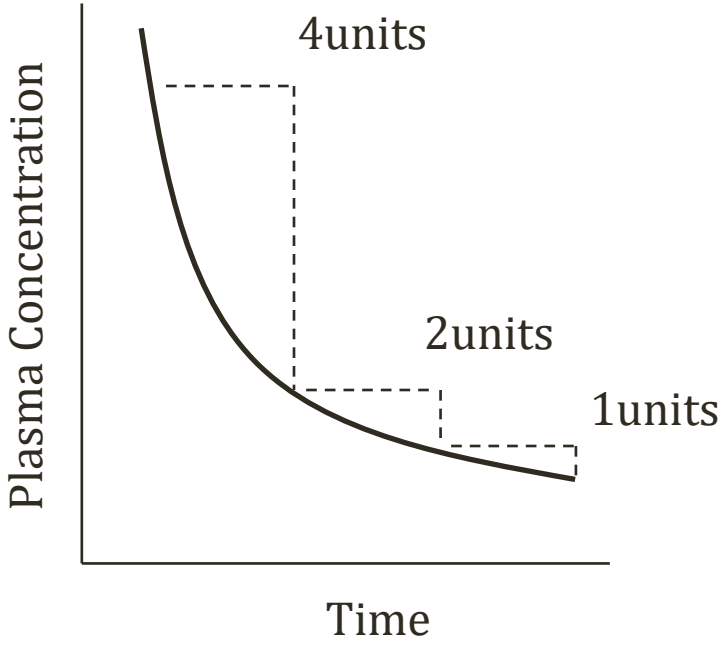


- Ethanol
- Phenytoin
- Aspirin

First Order Elimination

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

$$\text{Rate} = C * [\text{Drug}]^1$$



Zero Order Elimination

Time (hr)	Amount (g)	Change (g)	%
0	20		100
1	15	5	75%
2	10	5	50%
3	5	5	25%

First Order Elimination

Time (hr)	Amount (g)	Change (g)	%
0	10		100
1	5	5	50
2	2.5	2.5	25
3	1.25	1.25	12.5

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 = $V_{max} \cdot C / (K_m + C)$
- "Saturatable" → High C leads to V_{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: $HA <-> A^- + H^+$

Weak Base: $BOH <-> B^+ + OH^-$

Urine pH

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

Weak Acid: $HA <-> A^- + H^+$

Weak Base: $BOH <-> B^+ + OH^-$

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: $HA <-> A^- + H^+$

Weak Base: $BOH <-> B^+ + OH^-$

Examples

- Weak acid drugs
  - Phenobarbital, aspirin
  - Sodium bicarbonate to alkalinize urine in overdose
- Weak base drugs
  - Amphetamines, quinidine, or phencyclidine
  - Ammonia chloride (NH_4Cl) 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)

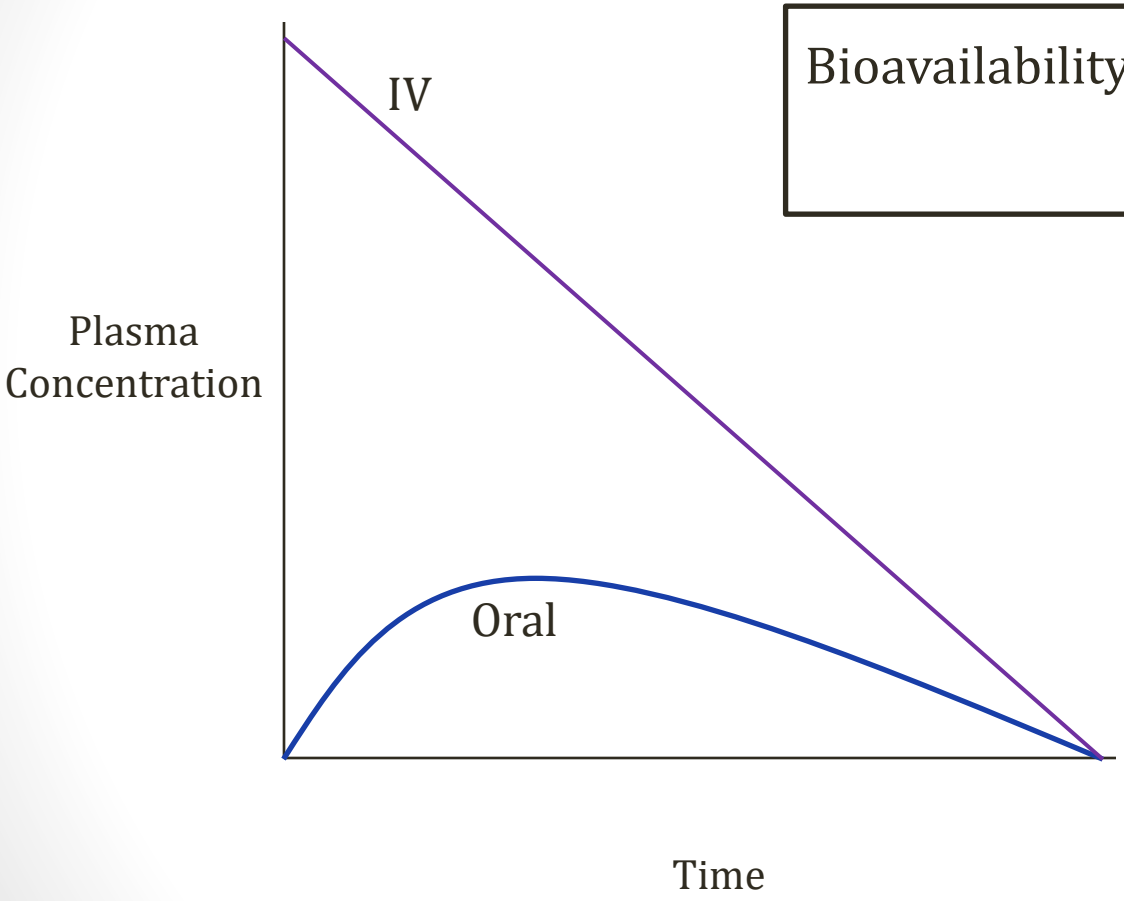
- Intravenous dosing
  - F = 100%
  - Entire dose available to body
- Oral dosing
  - F < 100%
  - Incomplete absorption
  - First pass metabolism

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)

$$\text{Bioavailability} = \frac{\text{AUC oral}}{\text{AUC IV}} \times 100$$



Volume of Distribution (Vd)

- Theoretical volume a drug occupies
- Determined by injecting known dose and measuring concentration

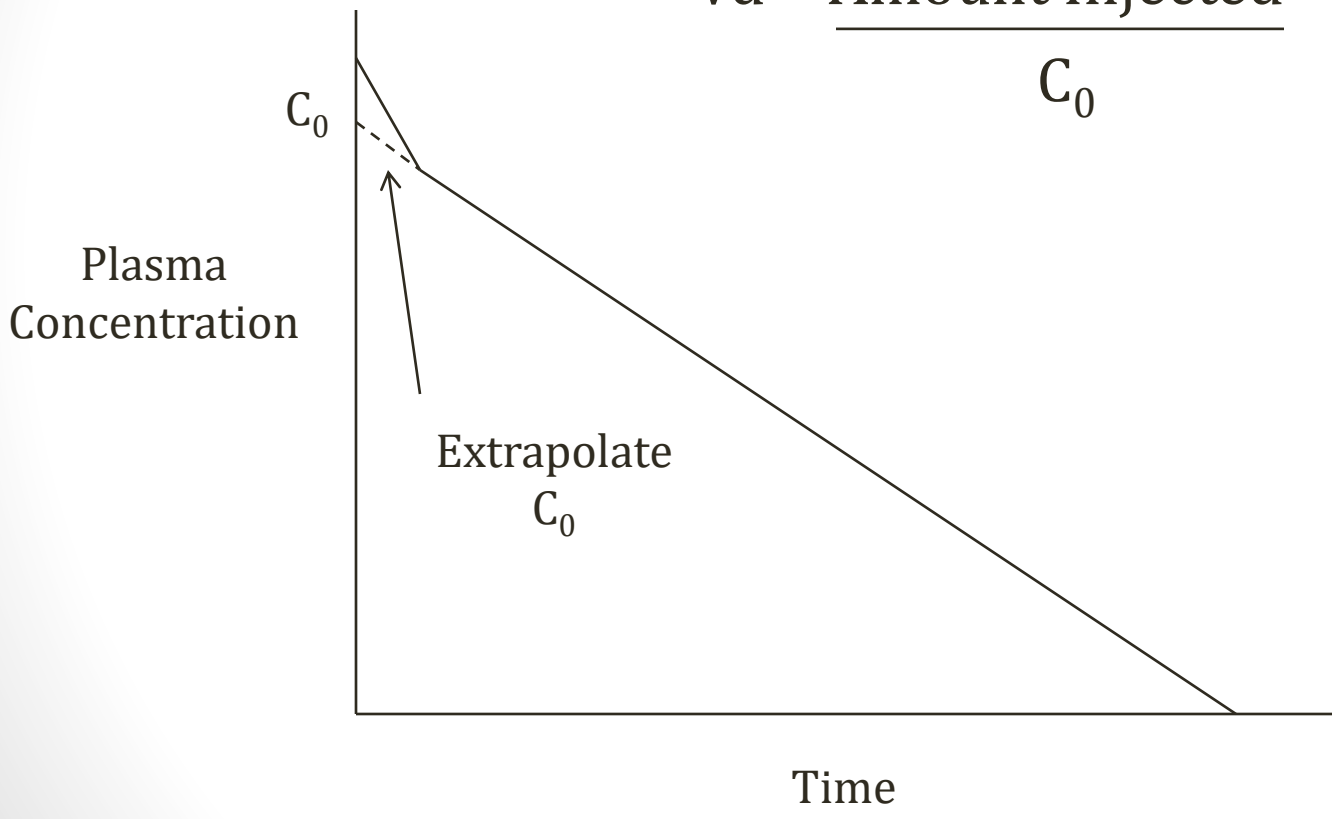
Volume of Distribution (Vd)

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

$$Vd = \frac{10g}{0.5g/L} = 20L$$

Volume of Distribution (Vd)

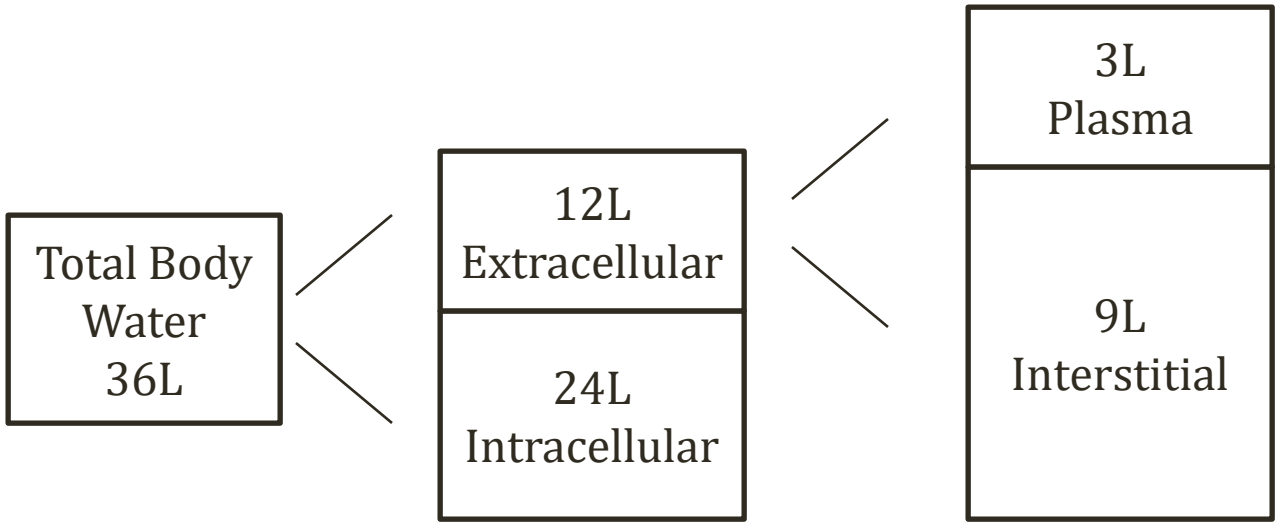
$$Vd = \frac{\text{Amount Injected}}{C_0}$$



Volume of Distribution (Vd)

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

Fluid Compartments



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 → Higher volume holding 10g → Higher clearance

$$C_x = Vd * Ke$$

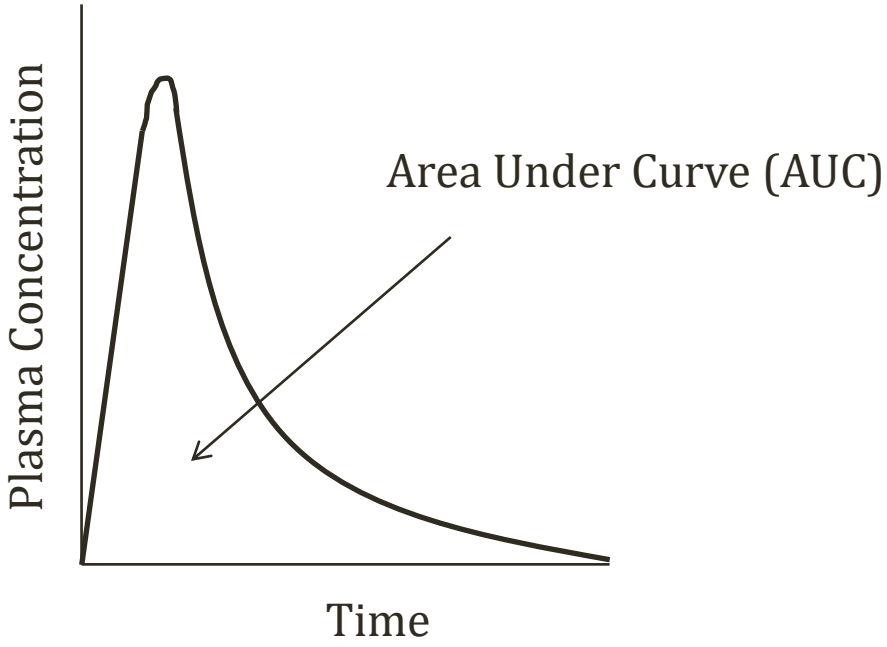
Clearance

$$C_x = Vd * Ke$$

$$Ke = \frac{C_x}{Vd}$$

Clearance

$$Cl\ (l/min) = \frac{Dose\ (g)}{AUC\ (g*min/l)}$$



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)

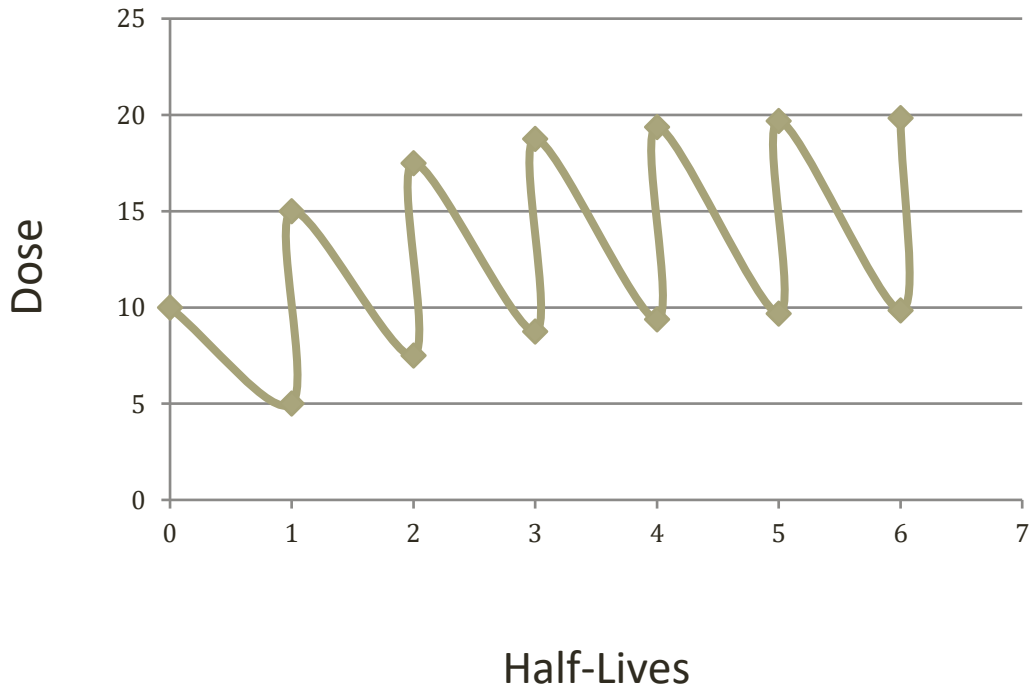
$$t_{1/2} = \frac{0.7 * Vd}{CL}$$

Half-life

No. Half Lives	% Remaining
0	100
1	50
2	25
3	12.5
4	6.25
5	3.12
6	1.56

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%

$$\text{Loading Dose} = \frac{[\text{Drug}] * \text{Vd}}{\text{F}}$$

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