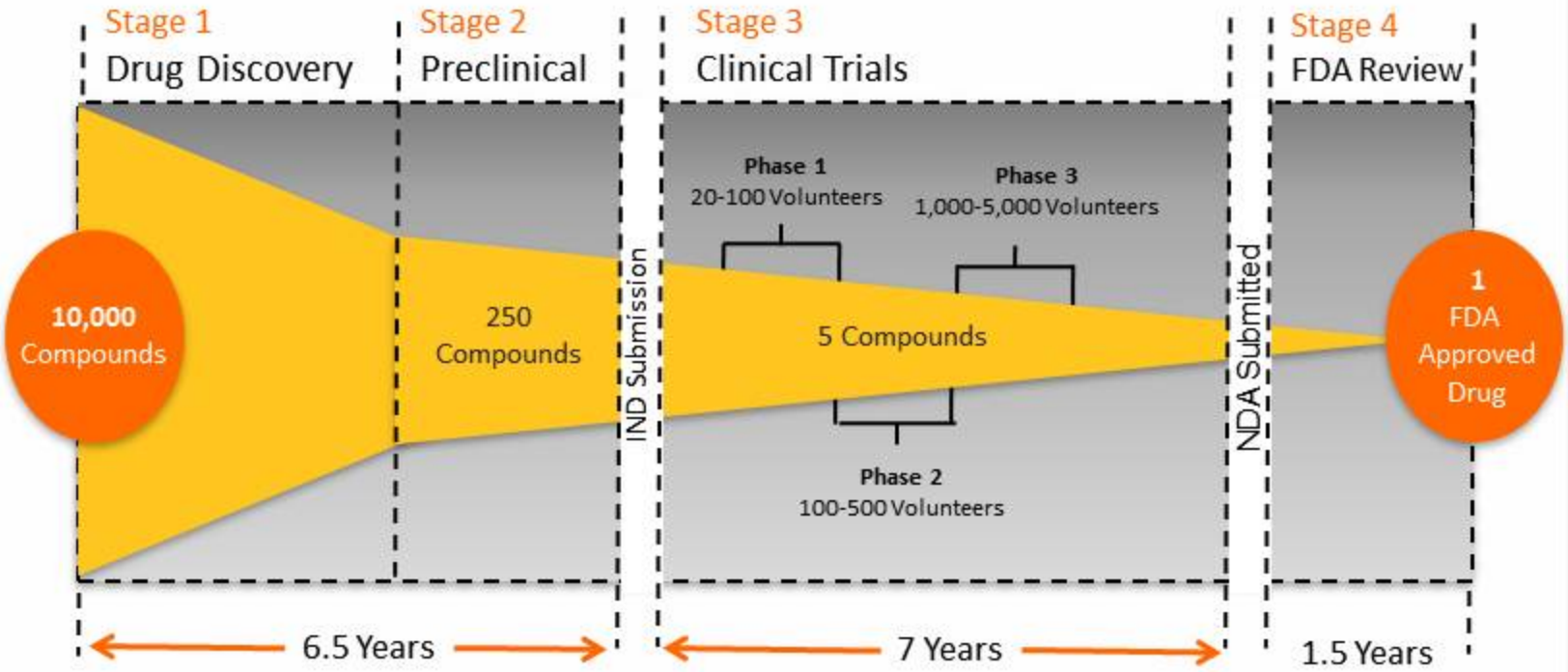
Pharmacological Studies

Module 3 Topic 6

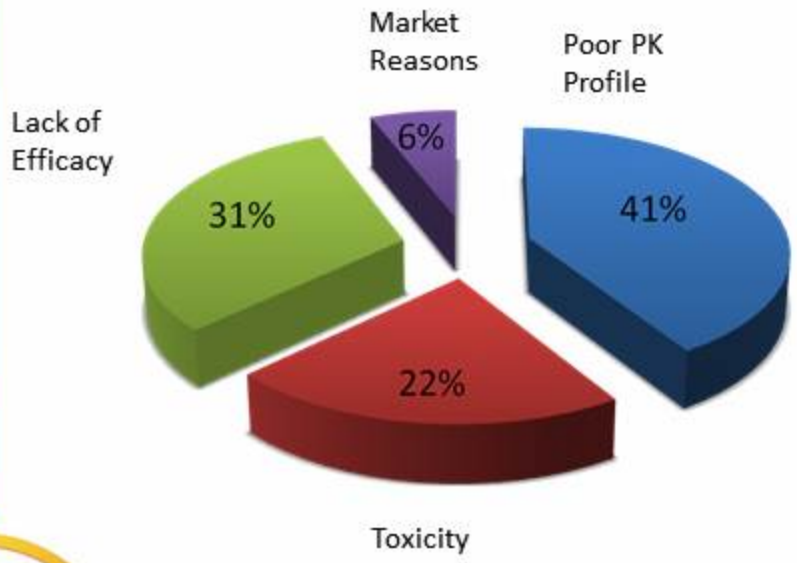
A Slow and Costly Process



Source: Pharmaceutical Research and Manufacturers of America

Why Compounds Fail or Slow Down in Development

FAILURE



SLOWED DEVELOPMENT

- Synthetic Complexity
- Low Potency
- Ambiguous Toxicity Finding
- Inherently Time-Intensive Target Indication
- Poor pharmaceutical Properties

Tufts Center for the Study of Drug Development, Tufts University

Drug Discovery to IND

Target Identification and Validation	Hit Identification	Lead Identification	Lead Optimization	Preclinical Development
Molecular target proposed/ identified	High-Throughput Screening of compound library	Medicinal chemistry effort to turn "hits" into "leads"	Extensive med chem to improve potency and selectivity	GLP-compliant toxicology and PK studies
Biological hypothesis relevant to disease	Virtual or *in silico* screening	Potency and selectivity	*In vivo* efficacy in additional models	GLP-compliant safety pharmacology studies

Drug Discovery to IND

Target Identification and Validation	Hit Identification	Lead Identification	Lead Optimization	Preclinical Development
Genetic models to demonstrate proof of concept	Confirm potency and selectivity of "hits" in 1° and 2° assays	*In vitro* PK: CYP inhibition, metabolic stability	*In vivo* PK characterization	Drug formulation
	Initial *in vitro* pharmacokinetic (PK) assessment	*In vivo* efficacy in relevant animal models of disease	Initial dose-ranging toxicology studies	Pre-IND meeting with FDA

Identifying Drug Targets

Drug Targets

- Enzymes, receptors, protein-protein interactions (e.g. gene, key enzyme, receptor, ion-channel, nuclear receptor)
- Biological system, signalling pathways

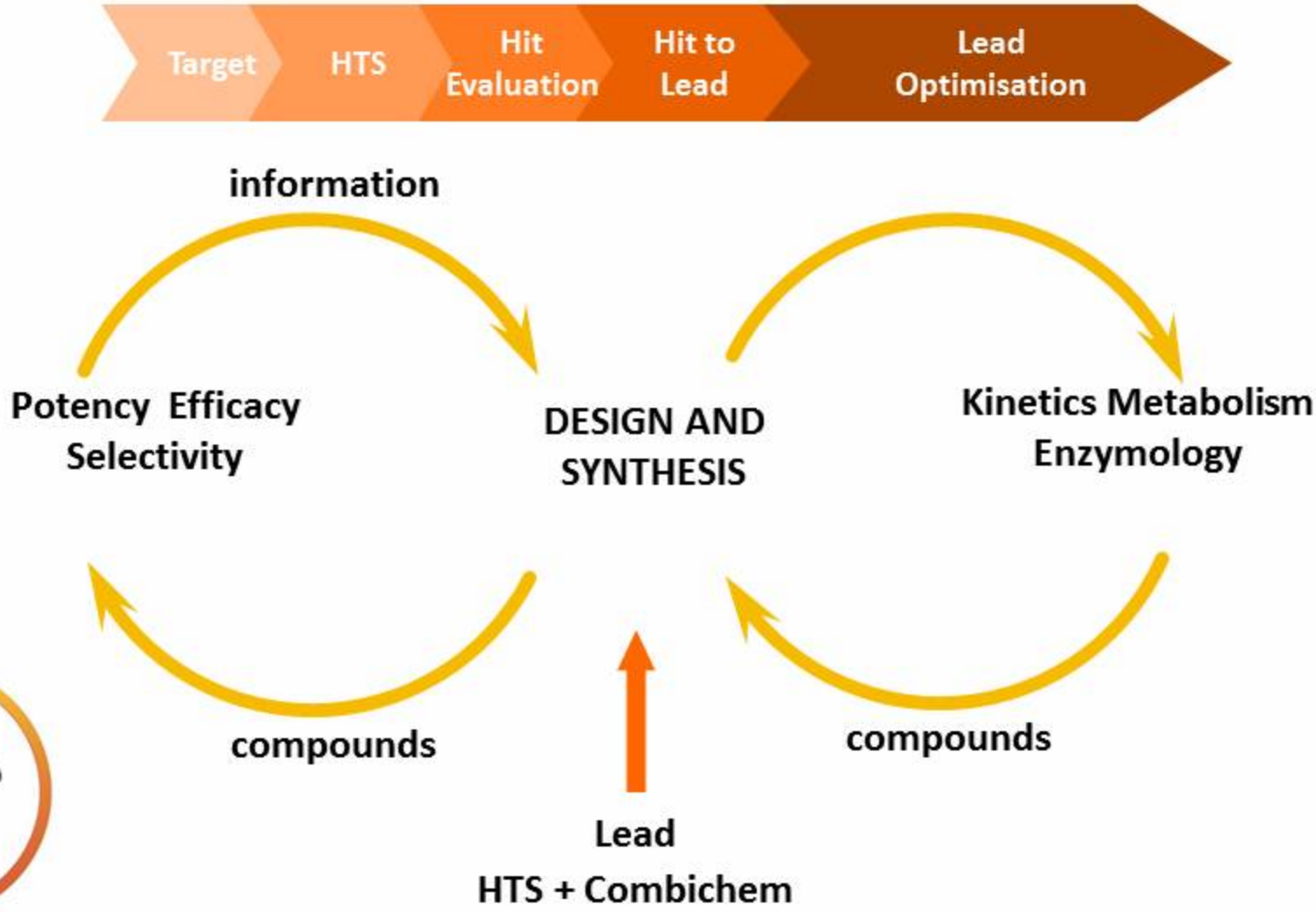
Discovery Biology

- Disease of Interest (unmet medical need): understand the mechanism of disease and its progression
- Identify a viable therapeutic target and validate
  - Knock-out studies in whole cell or animal models
  - RNAi, antibodies, tool compounds
- Develop and validate robust biological assays for testing compounds
- Whole cell, transformed cells, cell-free biochemical

Screening for Hit Identification

- State-of-the-art technology is available to screen large libraries of compounds against various types biological assays
- Compounds which affect the assay in a favourable manner are called “Hits”
- Each set of hits are tested separately against the target assay to identify validated hits

Fastest - first and best



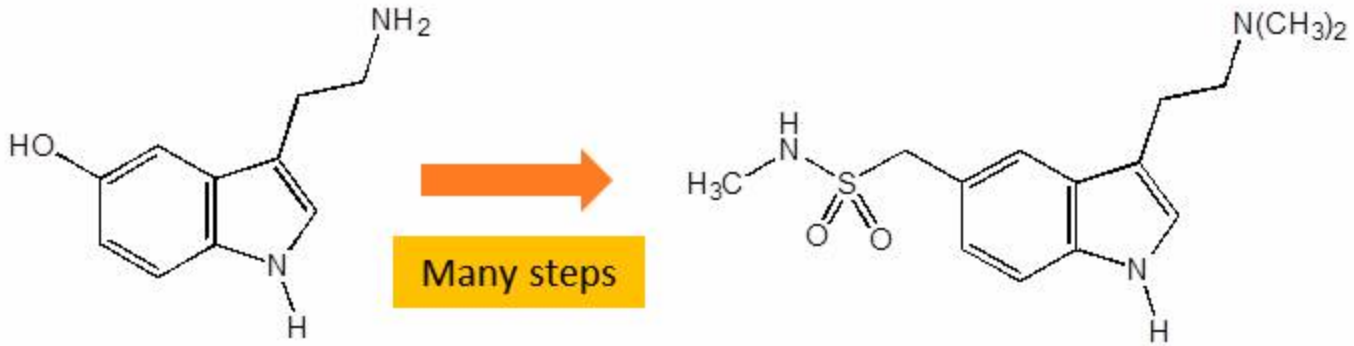
Discovery Chemistry or Medicinal Chemistry

- Start with hits and create lead compounds (a.k.a., Hit-to-Lead, H2L)
  - Synthesize and test analogs of hits to optimize certain properties: biological activity (potency), identify off target effects, selectivity (related or unrelated targets to avoid side effects or toxicity)
  - Use drug design to create novel compounds and optimal compounds
    - Computer-aided drug design (CADD)
    - Intuitive drug design based on medicinal chemistry experience
  - Novel compounds could result in valuable intellectual property (IP)

Discovery Chemistry or Medicinal Chemistry (Contd)

- In vivo ADME characterization of optimized compounds
- Explore proof of concept efficacy in animal models (non GLP)

Another Way to Find Leads – Modify Existing Active Compound



5-Hydroxytryptamine (5-HT)
Serotonin (a natural neurotransmitter synthesized in certain neurons in the CNS)

Sumatriptan (Imitrex)
Used to treat migrain headaches known to be a 5-HT_1 agonist

- Design structural changes and create compounds to:
  - Improve biological activity and selectivity for the target
  - Eliminate side effects
  - Carve out patent space (IP)
  - Improve physicochemical properties
  - Improve therapeutic index (TI) (effectiveness vs. unfavorable side effects)

Lead Optimization – Iterative Process

- Identify optimal compound(s) for preclinical studies with favourable parameters
  - Synthetic scalability
  - In *vitro* potency and selectivity
  - In vivo efficacy in proof of concept and diseases models
  - Toxicology (dose-ranging toxicology studies in vivo)
  - Patentability
  - In vivo pharmacokinetics (PK: half-life, Cmax, Tmax, etc.)
  - In *vitro* ADME characterization
  - Optimal mode of delivery
- Highly collaborative work with pharmacology, toxicology, biology, process chemistry, patent law, etc.

Investigational New Drug Application (IND)

Primary goal - present the data package to the FDA to allow the initiation of the clinical program:

- To present data to justify that the compound exhibits pharmacological activity to meet an unmet need,
- To support the product being reasonably safe for initial use in humans,
- To justify exposing humans to reasonable risks when used in limited, early-stage clinical studies.

http://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/InvestigationalNewDrugINDApplication/default.htm#FDA Guidances for Investigational New Drugs

Investigational New Drug Application (IND)

The IND application must contain information in three broad areas:

- **Manufacturing Information (GMP)** - Information pertaining to the composition, manufacturer, stability, and controls used for manufacturing the drug substance and the drug product. This information is assessed to ensure that the company can adequately produce and supply consistent batches of the drug.

http://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/InvestigationalNewDrugINDApplication/default.htm#FDA Guidances for Investigational New Drugs

Investigational New Drug Application (IND) (Contd)

- **Animal Pharmacology and Toxicology Studies (GLP)**
  - Preclinical data to permit an assessment as to whether the product is reasonably safe for initial testing in humans. Also included are any previous experience with the drug in humans (often foreign use).

http://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/InvestigationalNewDrugINDApplication/default.htm#FDA Guidances for Investigational New Drugs

Investigational New Drug Application (IND) (Contd)

- **Clinical Protocols and Investigator Information (GCP)** - Detailed protocols for proposed clinical studies to assess whether the initial-phase trials will expose subjects to unnecessary risks. Also, information on the qualifications of clinical investigators - professionals (generally physicians) who oversee the administration of the experimental compound - to assess whether they are qualified to fulfil their clinical trial duties. Finally, commitments to obtain informed consent from the research subjects, to obtain review of the study by an institutional review board (IRB), and to adhere to the investigational new drug regulations

http://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/InvestigationalNewDrugINDApplication/default.htm#FDA Guidances for Investigational New Drugs

IND Enabling Preclinical Studies

- Efficacy pharmacology (animal models)
- Safety pharmacology
- General toxicology – oral and specific route of administration
- Genetic toxicology
- Pharmacokinetics (PK- local and systemic)
- ADME (absorption, distribution, metabolism, excretion)
- Reproductive toxicology
- Carcinogenicity (ask for a waiver based on genotoxicity)
- Special studies

IND Enabling Studies Needed

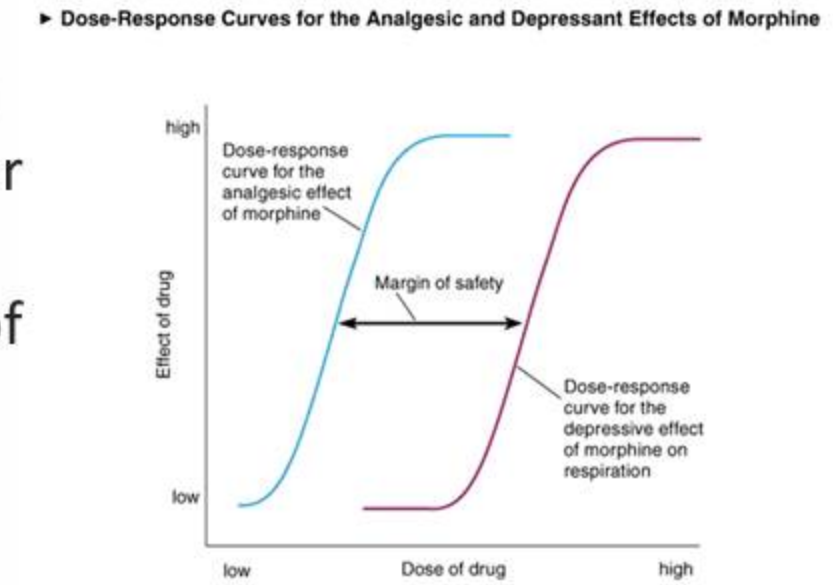
- Will determine with FDA during the pre IND meeting
- What studies are needed – dependent on product & indication
  - Who are the patients ?
  - What is the unmet need ?
  - What is the expected dosing – acute or chronic?
  - Where will it be delivered – systemic or local?
- Need to demonstrate safety pre-clinically for the First in Human Study (FIH)

Pharmacology

- In vitro studies on animal tissues - to reveal the effect of the compound and possible mechanism of action
- In Vivo animal models to demonstrate efficacy
  - Efficacy studies are conducted more for candidate selection/ prioritization
- Understanding that pharmacology impacts interpretation of toxicology studies

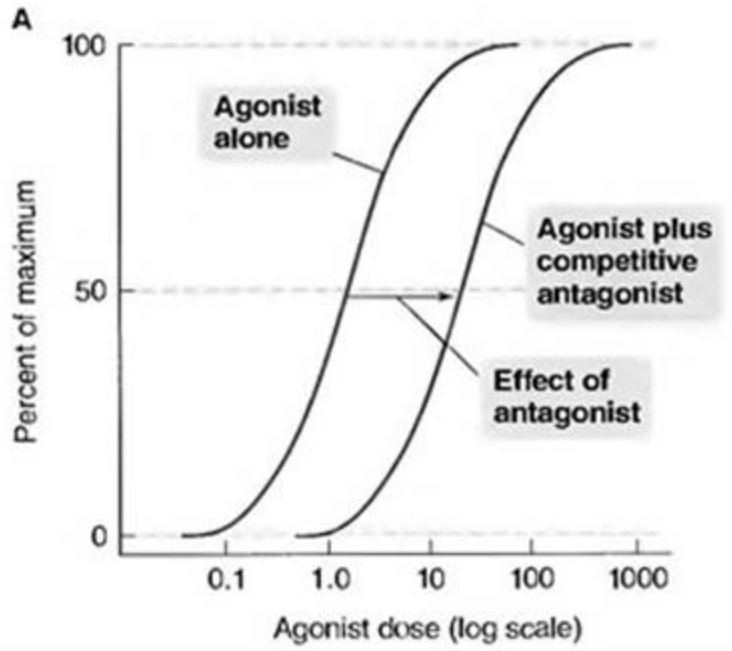
Dose response curve

- The dose response curve produced with respect to a particular effect is the most important property of a drug.
- This needs to be studied on almost every model to understand the action of the drug



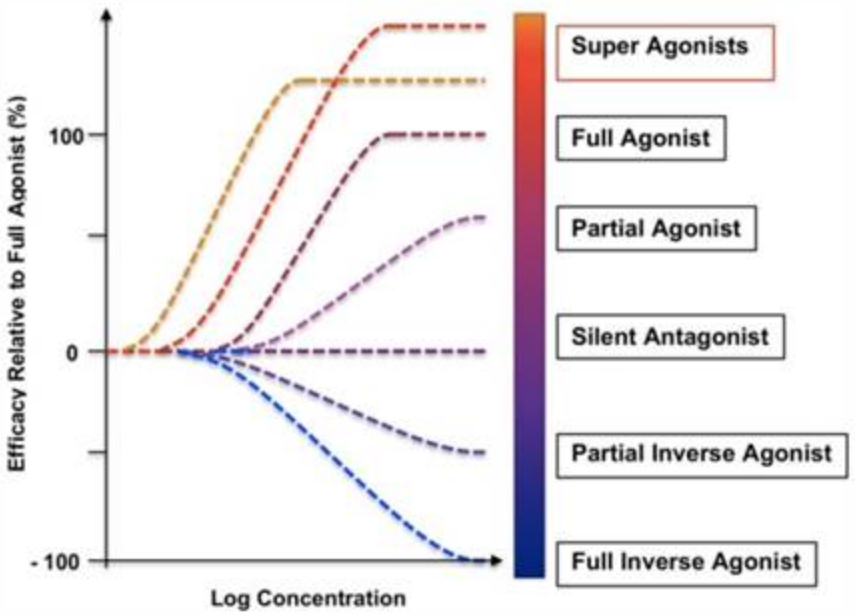
Study of antagonists

- A number of drugs used clinically are antagonists of endogenous compounds.
- Their interaction with the endogenous compounds is studied on different pharmacological models to understand the nature of antagonism.



Drug Receptor interaction

- Most drugs act on receptors, to produce an effect, some block the receptor to prevent the effect of an endogenous compound.
- Receptor antagonism is an important mechanism by which drugs act.

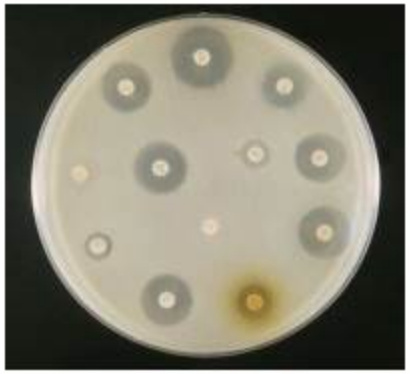


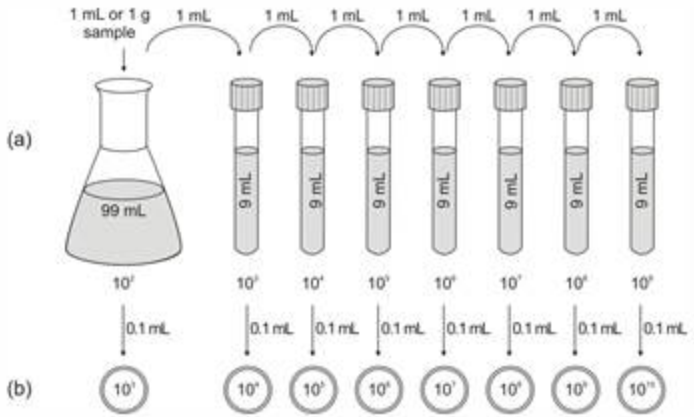
Studies on Organisms

- Studying effects on organisms gives a clue about their antimicrobial effects.
- Using large number of strains of different Gram +ve and –ve organism one can unravel the spectrum of the antibiotic.
- The same theory can be extended to antifungal and anti protozoal agents.
- Single compounds and combinations may be studied to to reveal additive effects too.

Antibiotic spectrum

- Using either Agar well method, or the serial dilution method. Both method are suitable to study antibiotic combinations too.
- One may study different concentrations of a single compound or different compounds against a particular species.



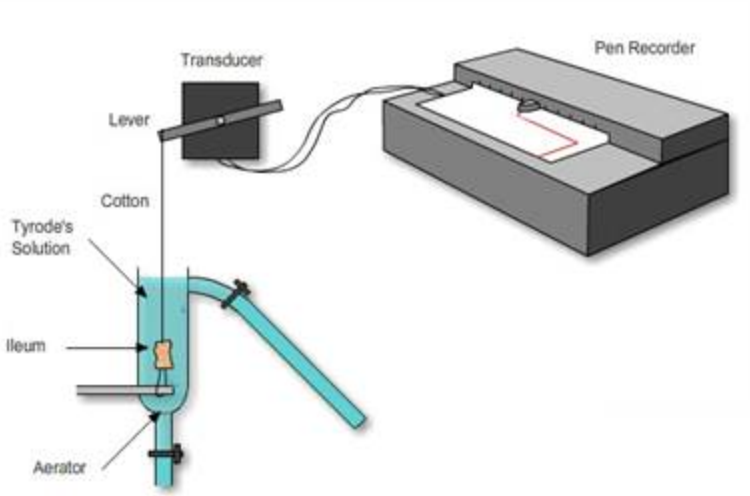


Cell Lines

- A cell line is a permanently established cell culture that will proliferate indefinitely given appropriate fresh medium and space.
- Attempts have been made to culture almost every tissue, including neuronal cells, bone, cartilage, hair cells, etc.
- The HeLa cell line was established in 1951 from a biopsy of a cervical tumour taken from Henrietta Lacks, a working-class African-American woman living near Baltimore. The cells from the tumour became the first human cells to grow well in a lab. They contributed to the development of a polio vaccine, the discovery of human telomerase and countless other advances.

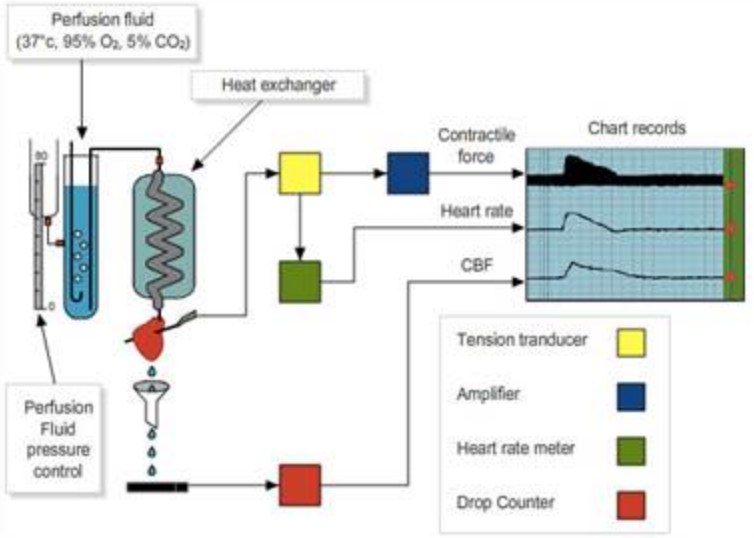
In vitro Studies (smooth muscle)

- Certain experiments on animal isolated tissues help understanding basic pharmacology of the compound and its mechanism of action.
- The tissue responds to a number of drugs with a contraction that can be inhibited by specific antagonists.

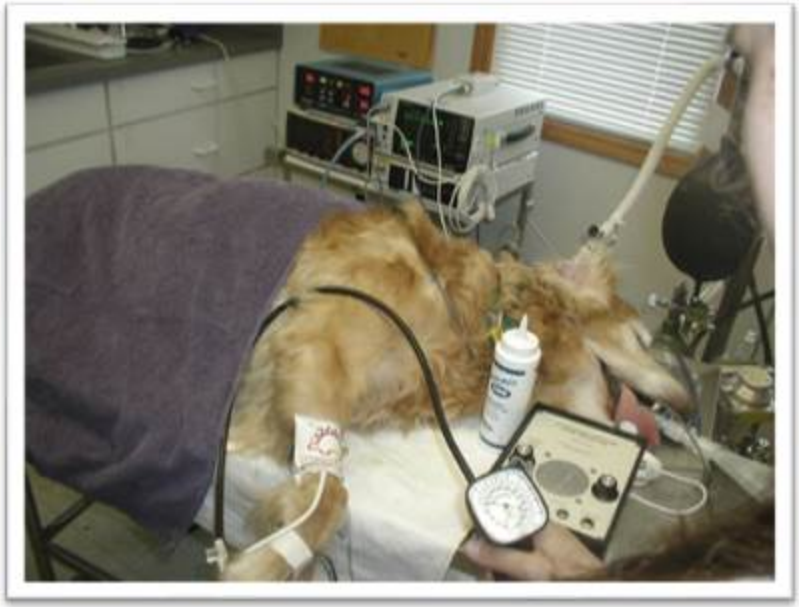


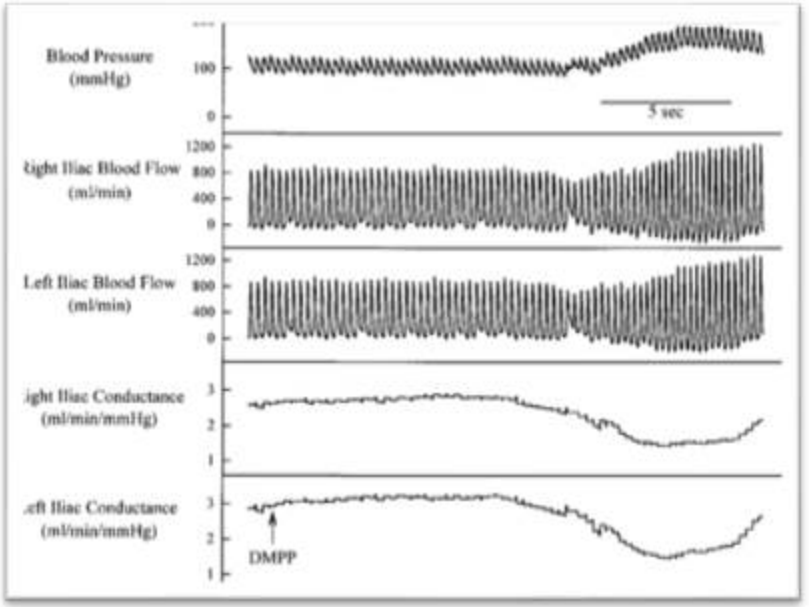
In vitro Studies (Cardiac tissue)

- Isolated rat/rabbit heart has been used to study the effect of drugs on cardiac tissue.
- A carefully isolated heart provided the required perfusion and oxygenation can work for up to 6 to 8 hours, and be used for testing half a dozen compounds.



Whole animal experiments

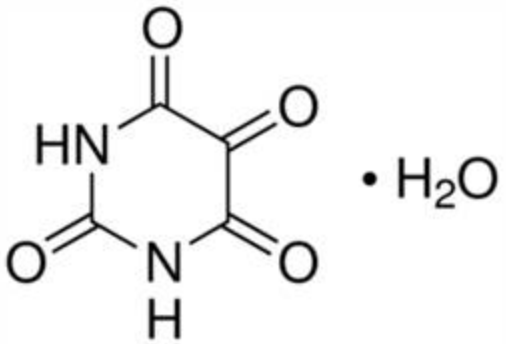




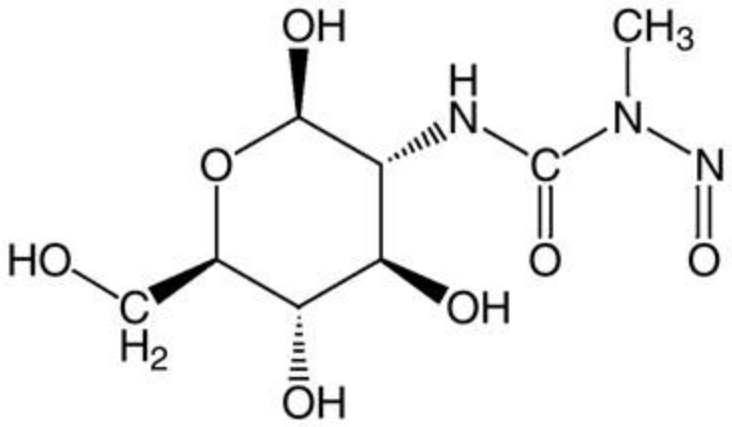
In vivo Studies (antidiabetic agents)

- The effect of antidiabetic agents may be studied on rabbits. However many agents may not affect an animal with normal blood glucose level.
- The animal may be rendered hyperglycemic using surgical procedures or compounds that damage the insulin forming cells

Experimental Hyperglycemia



Alloxan Monohydrate
100 mg/kg



Streptozotocin 150 mg/kg

Animal Models

Induction mechanism	Model	Main features	Possible uses
Spontaneous autoimmune	NOD mice	Beta cell destruction due to an autoimmune process	Understanding genetics of type 1 diabetes
	BB rats		Understanding mechanism of type 1 diabetes
	LEW.1AR1/-iddm rats		Treatments that may prevent beta cell death
			Treatments that may manipulate autoimmune process

Animal Models (Contd)

Induction mechanism	Model	Main features	Possible uses
Genetically induced	AKITA mice	Beta cell destruction due to ER stress. Insulin dependent.	New formulations of insulin
			Transplantation models.
			Treatments to prevent ER stress
			(could also be used in type 2 diabetes research)

Co relation with clinical disease

- Many animal models and even human models have been studied for asthma. None of the models were like clinical asthma cases, with respect to response to anti allergic compounds.

- These results throw doubt on the use of models of mast cell degranulation in the search for anti-allergic drugs and, possibly, on the relative importance of mast cell degranulation in the pathogenesis of asthma.

Church and Gradidge (1980)

Safety Pharmacology

- A very large number of drugs have no effect on cardiovascular, nervous or respiratory systems.
- In fact effects on these systems turn out to be adverse effects for these drugs.
- It is necessary to establish that drugs have no effects on these systems.
- These potentially harmful effects are studied on the systems in laboratory animals.

Safety Pharmacology

- Investigate potential undesirable pharmacodynamic effects on the physiological function of vital organs
  - Generally given at higher than indicated dose orally
- Core battery
  - Cardiovascular system
  - Respiratory system
  - Central nervous system

Introduction

Risk Assessment

- Hazard identification
- Dose Response Assessment
- Exposure Assessment
- Risk Characterization

Introduction

- **RISK** - the probability that harm will occur under specified conditions
- **SAFETY** - the probability that harm will not occur under specified conditions

Introduction

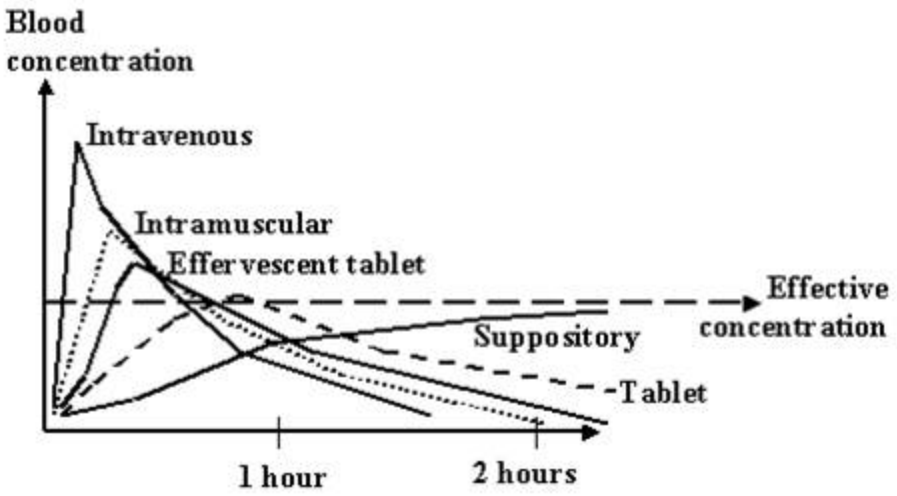
Major Factors

- Route of administration
- Duration and frequency of exposure
- Dose or concentration

Introduction to Toxicology

Rapidity of Response

- Intravenous
- Inhalation
- Intraperitoneal
- Subcutaneous
- Intramuscular
- Topical
- Intradermal



Introduction to Toxicology

Spectrum of Undesired Effects

- Allergic reactions
  - Chemical allergies
- Idiosyncratic reactions
- Immediate vs. delayed toxicity
- Reversible vs. irreversible toxicity
- Local vs. systemic toxicity

Interactions

- Additive
- Synergistic
- Potentiation
- Antagonism (functional, chemical, dispositional, receptor)

Tolerance

State of decreased responsiveness to a toxic effect of a chemical, resulting from previous exposure

- Dispositional tolerance: a decreased amount of drug reaching the site
- Cellular: reduced responsiveness of a tissue
- Rapid tolerance: occurring rapidly, within a few doses

Dose response

Assumptions

- Response is due to chemical administered
- The response is related to the dose
- Repeated doses cause similar response
- The degree of response is related to the concentration at the site
- The concentration at the site is related to the dose administered
- Has a quantifiable method of measuring and a precise means of expressing the toxicity
- There is a receptor site with which the chemical interacts

Table 2–2. TOXICITY RATING CHART

TOXICITY RATING OR CLASS	PROBABLE LETHAL ORAL DOSE FOR HUMANS	
	Dosage	*For Average Adult*
1. Practically nontoxic	> 15 g/kg	More than 1 quart
2. Slightly toxic	5–15 g/kg	Between pint and quart
3. Moderately toxic	0.5–5 g/kg	Between ounce and pint
4. Very toxic	50–500 mg/kg	Between teaspoonful and ounce
5. Extremely toxic	5–50 mg/kg	Between 7 drops and teaspoonful
6. Supertoxic	< 5 mg/kg	A taste (less than 7 drops)

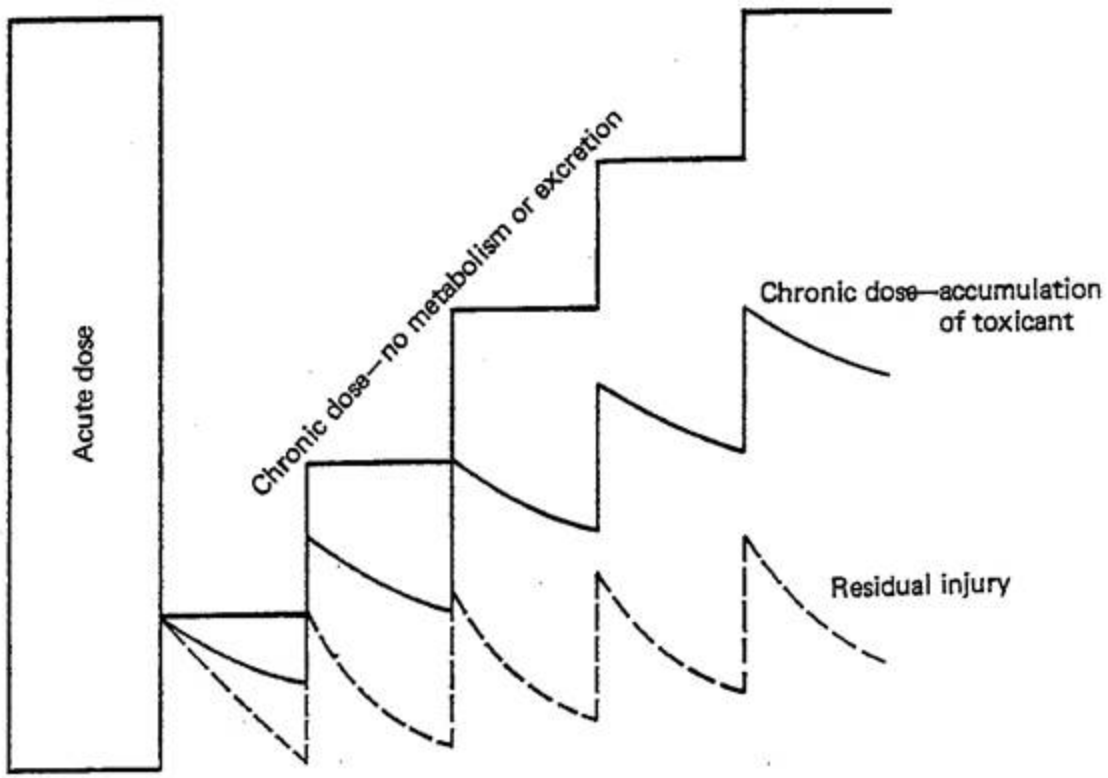


Figure 2–1. Diagrammatic view of dose and corresponding measure of effect. Acute dose is compared to the cumulative dose after multiple administration of a chemical that has limited elimination and thus accumulates, and one that produces injury, which accumulates with multiple dosing.

CASARETT AND DOULL'S TOXICOLOGY, THIRD EDITION

Table 2–1. APPROXIMATE ACUTE LD50'S OF SOME REPRESENTATIVE CHEMICAL AGENTS

AGENT	LD50 (mg/kg)*
Ethyl alcohol	10,000
Sodium chloride	4,000
Ferrous sulfate	1,500
Morphine sulfate	900
Phenobarbital sodium	150
Picrotoxin	5
Strychnine sulfate	2
Nicotine	1
d-Tubocurarine	0.5
Hemicholinium-3	0.2
Tetrodotoxin	0.10
Dioxin (TCDD)	0.001
Botulinum toxin	0.00001

* LD50 is the dosage (mg/kg body weight) causing death in 50 percent of the exposed animals.

From Casarett & Doull's, Toxicology
3rd Edition, 1986