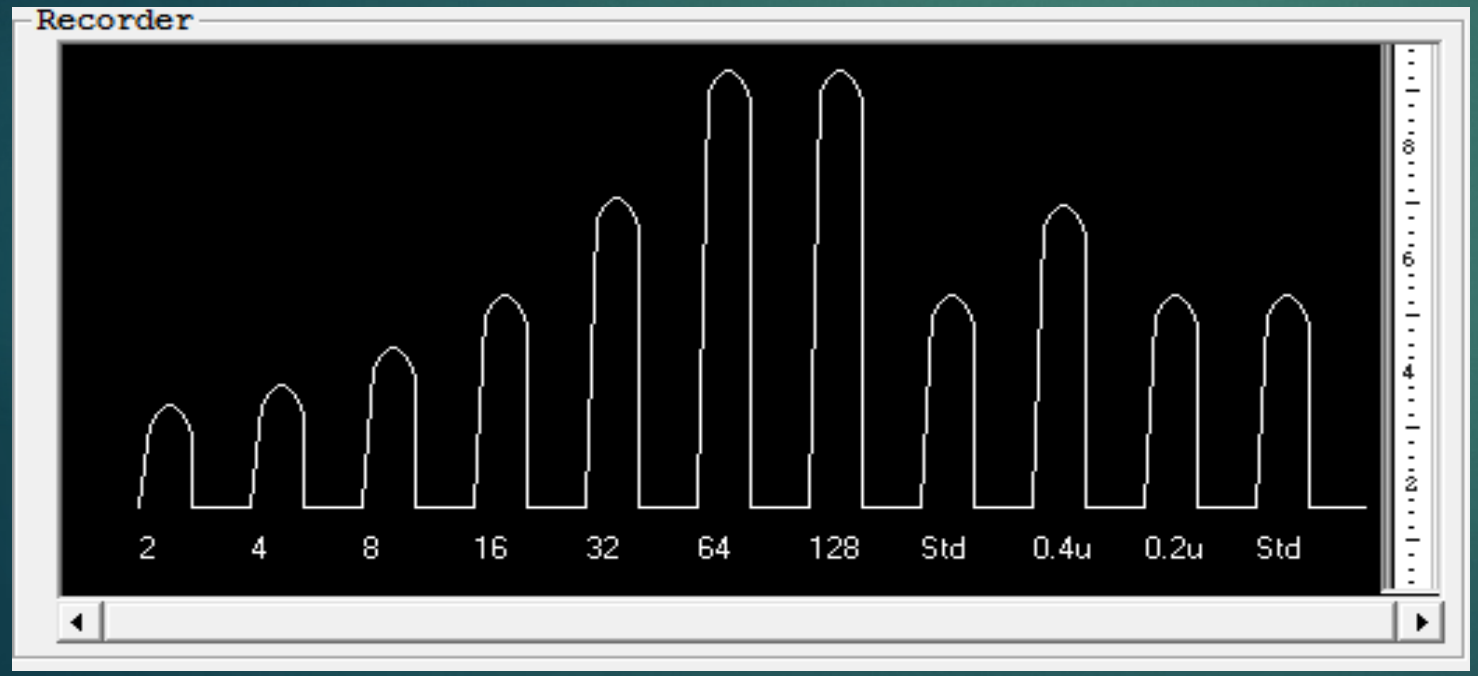
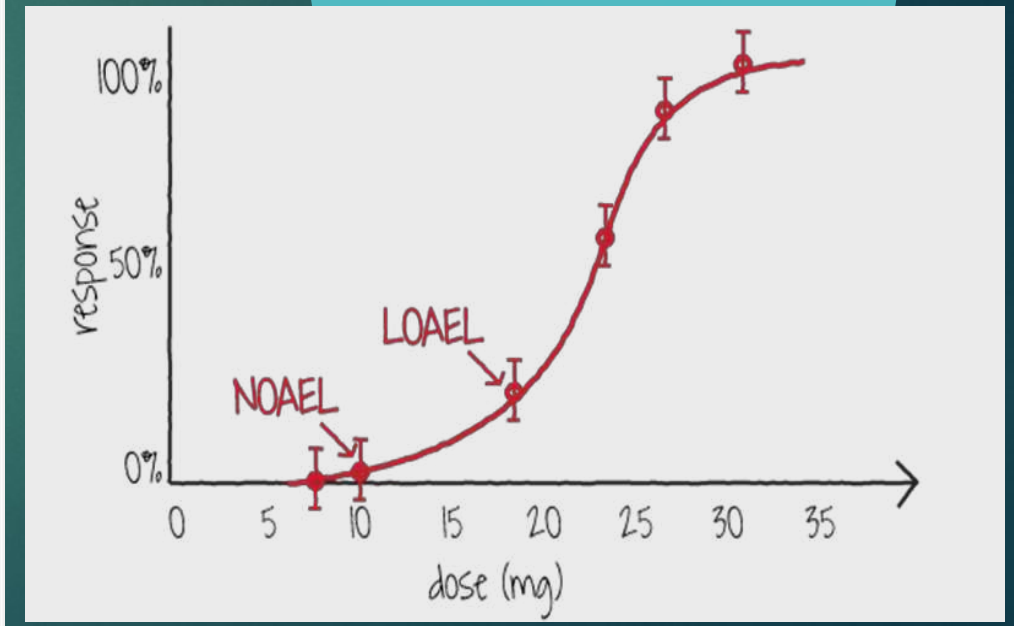
# Bio-Assay Pharmacology
# &
# Dose Response Relationship

Pharmacology Concepts
By Rajesh Choudhary



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# BIOASSAY

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Definition: Assay of the drug or chemical by using Biological System.

Biological System: In-Vitro (living Tissues, cells, microbes) & In-Vivo (Animals)

Types

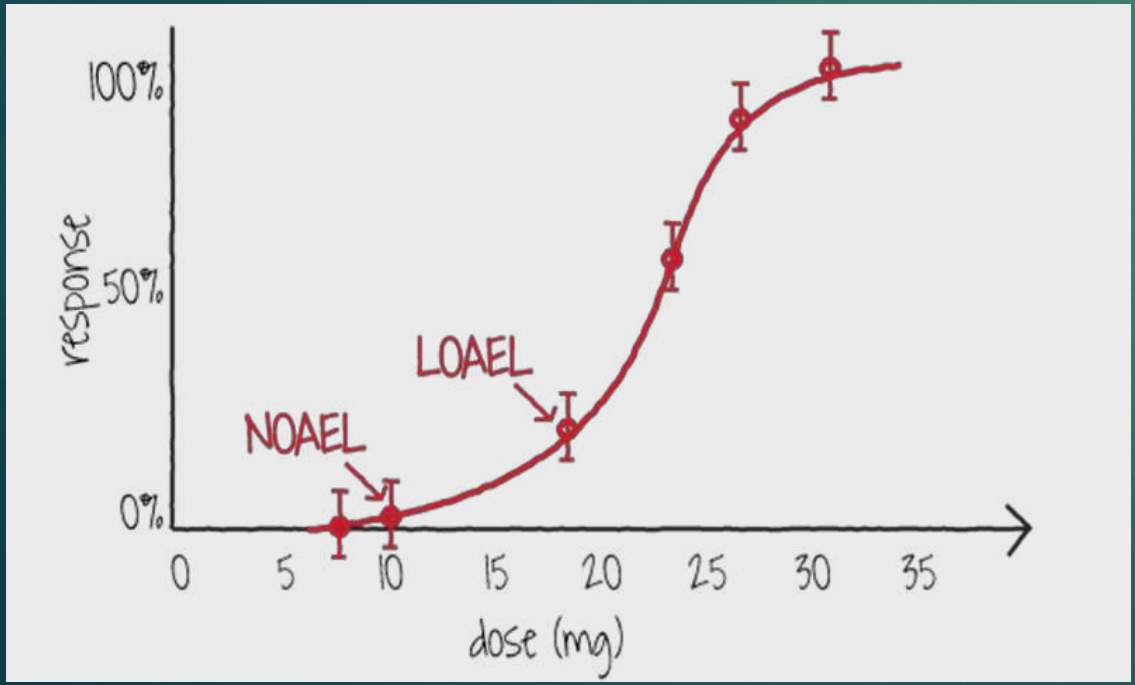
1. Qualitative Bioassay: Qualitative estimation of biological response or pharmacological effect of a drug.

- ✓ Examination of response +ve or –ve only
- ✓ Not related to dose/concentration of a drug or chemical
- ✓ E.g. Behavioral effects, Analgesic effects, Contraction or Relaxation

# BIOASSAY

2. Quantitative Bioassay (Quantitative estimation): Estimation of the concentration / potency of a drug/ chemical by measuring its biological response in living systems

- ✓ Examination of biological response in respect to dose or conc.
- ✓ It is related to dose/concentration of a drug or chemical




E.g. Dose estimation of a toxic agent for toxic response.

NOAEL: No observed adverse effect level

LOAEL: Lowest observed adverse effect level

# Importance/ Reason of Bio-Assay



- Chemical Assay is either:
  - ✓ not available
  - ✓ if available, too complex,
  - ✓ insensitive to low doses e.g. Histamine can be bioassayed in microgram conc.
- Active principle of drug is unknown and cannot be isolated, e.g. insulin, posterior pituitary extract etc.
- Unknown Chemical composition, e.g. long acting thyroid stimulator.
- Chemical composition of drug variable but has same pharmacological action e.g. cardiac glycosides isolated from diff sources, catecholamines etc.

# Application of Bioassay



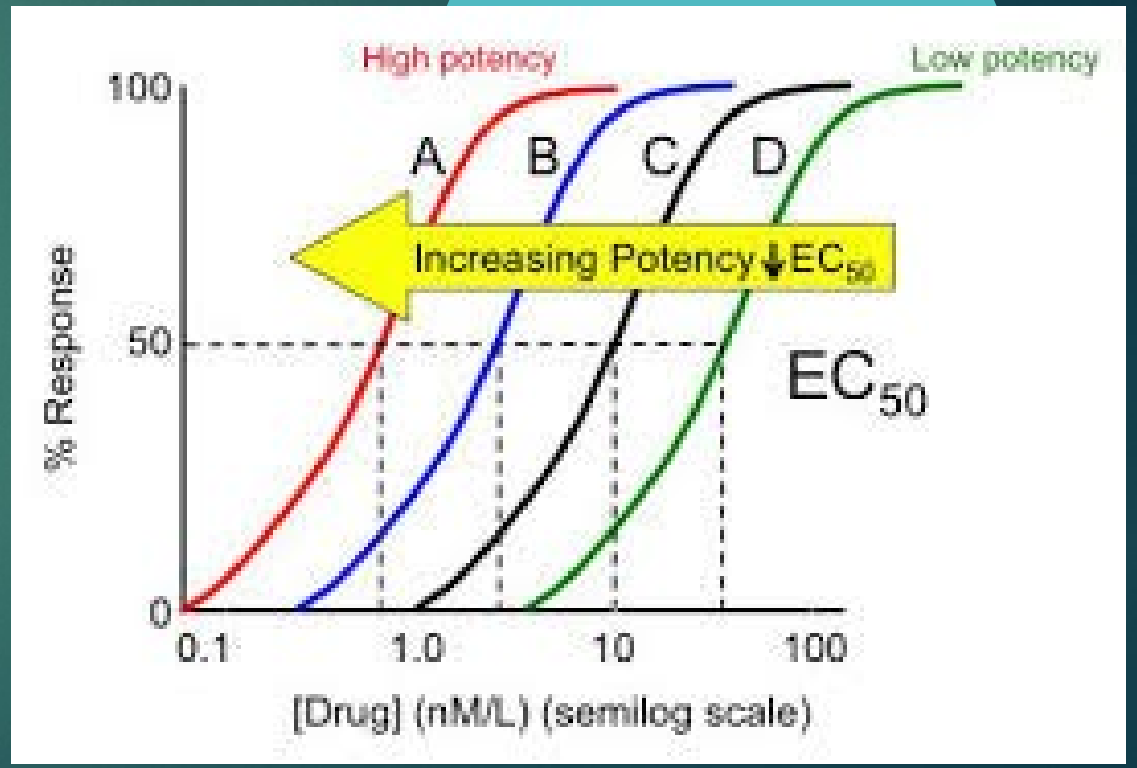
- Used in determination of drugs potency
- Screenings of new agents isolated from plants, animals or chemical labs and find their field of activities
- Establishment of SAR (structure activity relationship)
- Essential in monitoring environmental pollutants
- Determination of the pharmacological activities of a new drug
- Determine the therapeutic advantage of one drug over another treatments
- Useful in study of new hormonal or other chemically mediated control systems

# Principles of Bio-Assay

- The basic principle of bioassay is to compare the test substance with the International Standard preparation of the same and to find out how much test substance is required to produce the same biological effect, as produced by the standard.

Potency: concentration or dose of drug, in which it produces 50% response ($EC_{50}$ and $IC_{50}$)

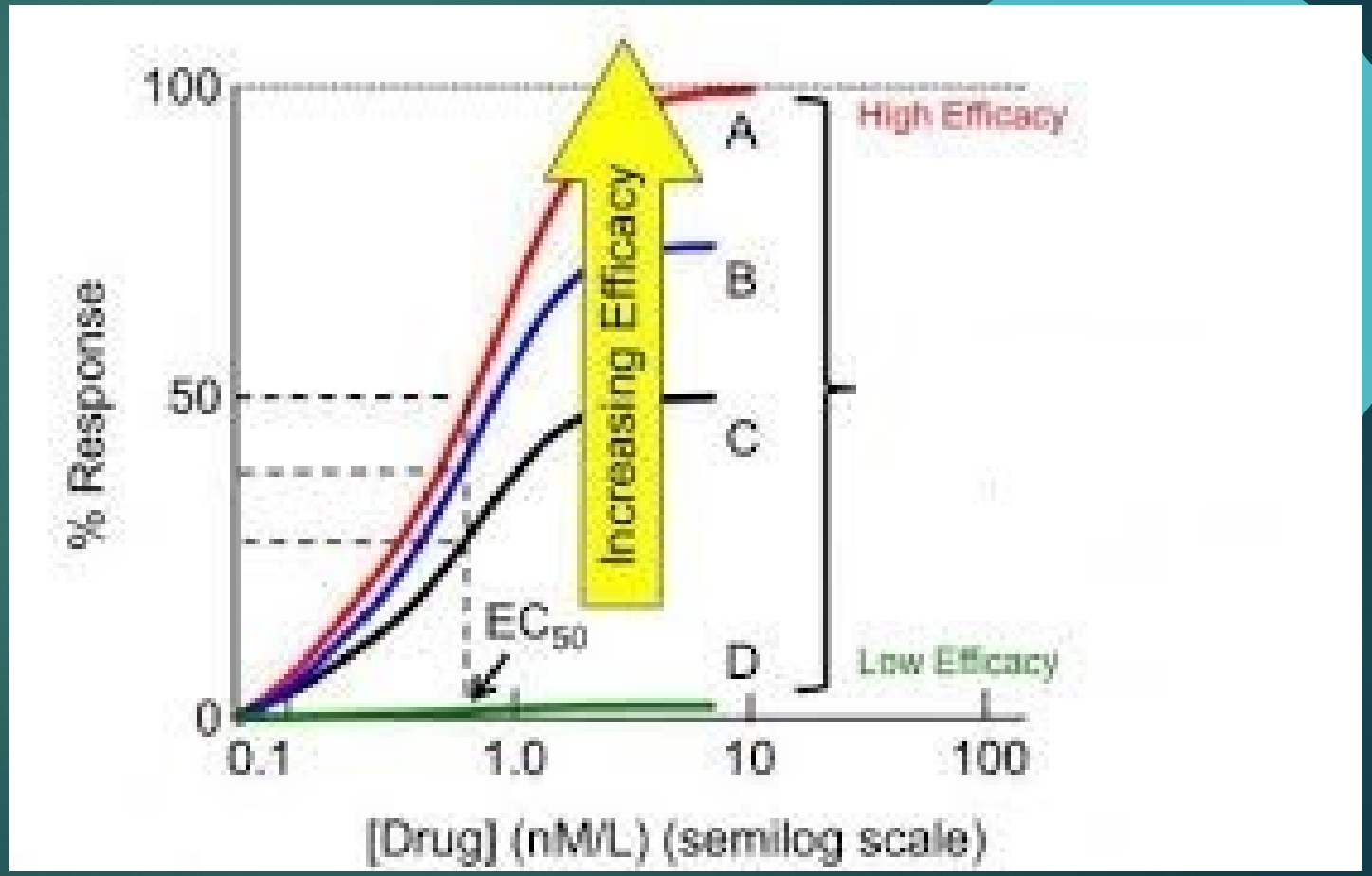
Potency: A>B>C>D
Efficacy: A=B=C=D



# Principles of Bio-Assay

Efficacy: concentration or dose of drug, in which it produces 100% response (maximum/ceiling Effects)

Efficacy: A>B>C>D



# Bioassay of histamine on the guinea-pig ileum - Tutorial Mode

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**Direction**

**You are given a histamine solution of unknown concentration. Find out the concentration of histamine using a guinea-pig ileum preparation. Obtain a Dose-Response curve and carry out matching assay.**

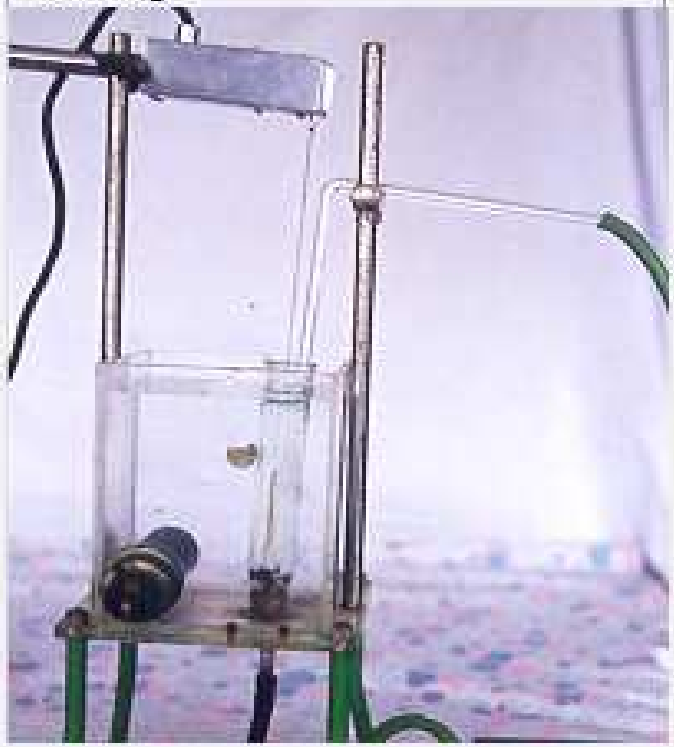
**Help**

Instruction

Choose a dose & Inject

**Recorder**

**Set up**



**Dose selection**

Dose µg 0.1 + −

Histamine-Known

Inject

**Do**

Print

Matching Assay

**Exit**

NeXt

Return

New

# Bioassay Methods

1. Quantal Bioassay (Direct Method): End Point Bioassay, All or None' response in different animals. E.g.,
   - Digitalis induced cardiac arrest in guinea pigs
   - Insulin induced Hypoglycemic convulsions in mice.
   - Calculation of LD50 in mice or rats
2. Graded Assay: Response to varying dose
   - Matching assay
   - Bracketing assay
   - Interpolation assay
   - Multiple point assay (3 point, 4 point, 6 point bioassay)

# Quantal Bioassay

## Digitalis Induced Cardiac Arrest:

- The extract containing digitalis (standard or unknown) infused into vein of cats till the heart stops to beat. Volume of fluid passed into the vein noted (threshold dose).
- Potency is calculated as follows:

Conc of Unknown = (Threshold dose of std/ Threshold dose of test) x Conc of std

*Advantages of Quantal Bioassay:*

- Drug effects appears rapidly and easily recognized.
- Effect directly proportional to drug dose.
- Rapid end point detection.

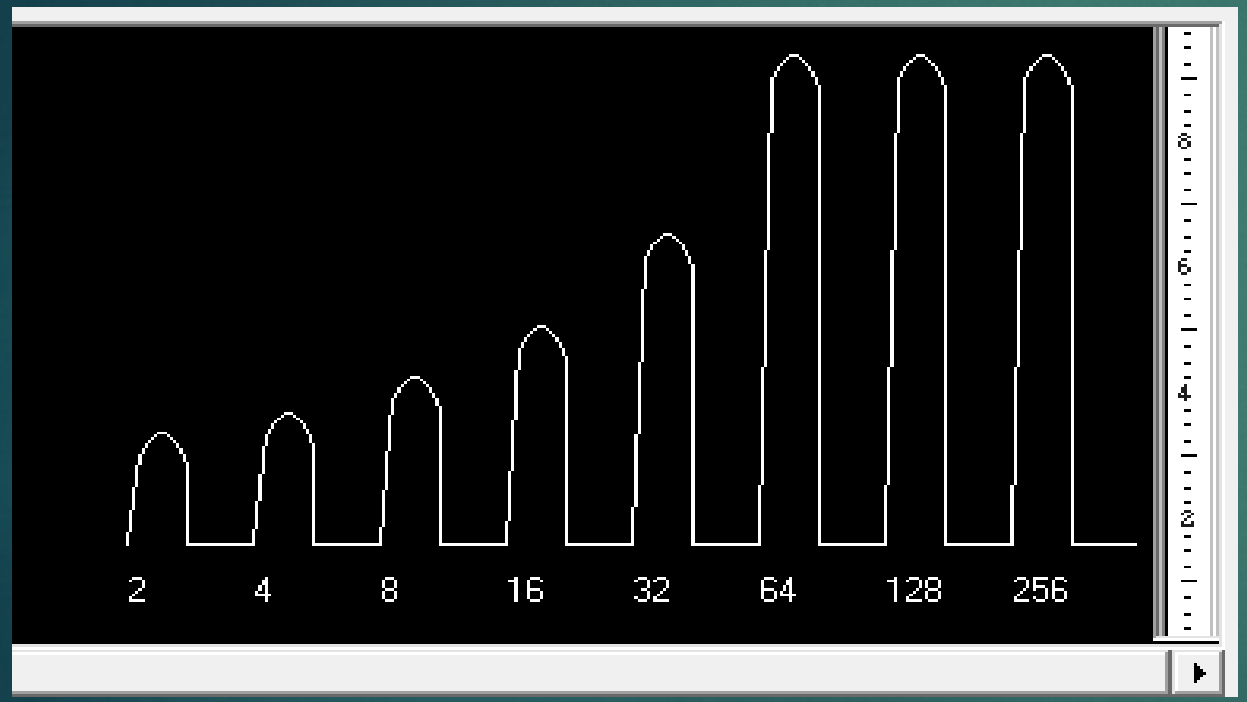
*Disadvantage of Quantal Bioassay:*

- Only toxicity or high dose study possible.
- Dose ranging study can‘t be done.

# Graded Bioassay

## An assay may be based on a graded response with dose

e.g. change in blood glucose concentration, contraction of a strip of smooth muscle, change in the time taken for a rat to run a maze.



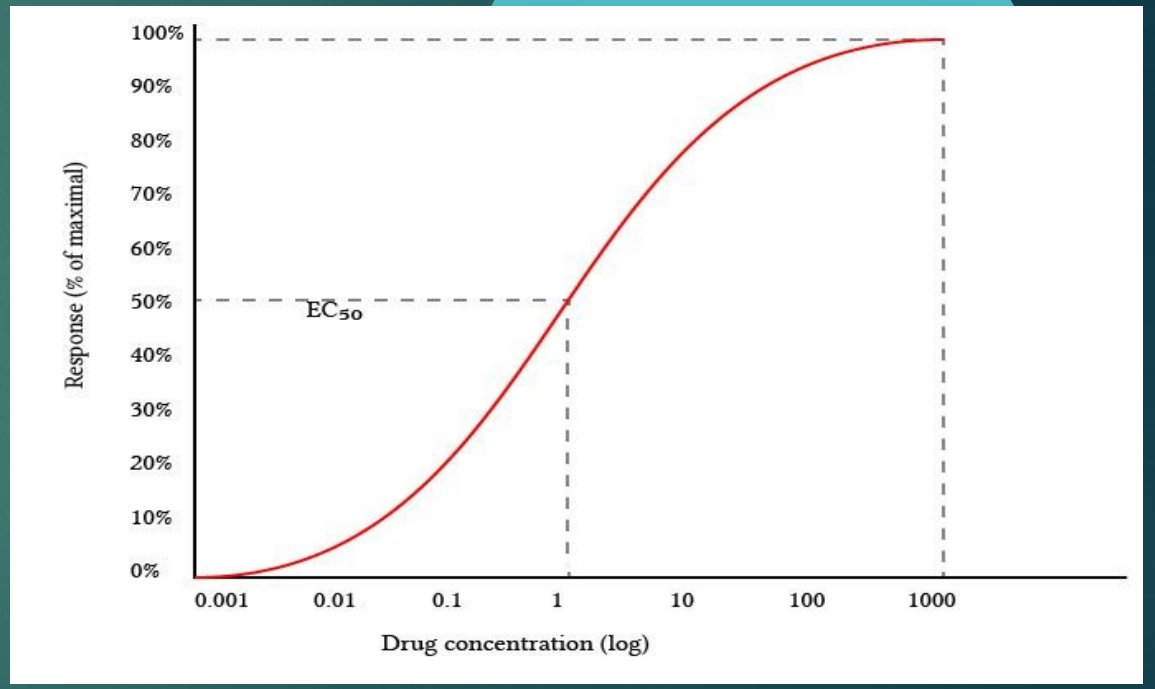




Figure. Dose response curve (DRC) of histamine on ileum

# Matching Bioassay

- Test response is matched with the standard on same tissue

e.g. Std dose (mcg) - 16 mcg,  Test dose (ml) = 0.2 ml

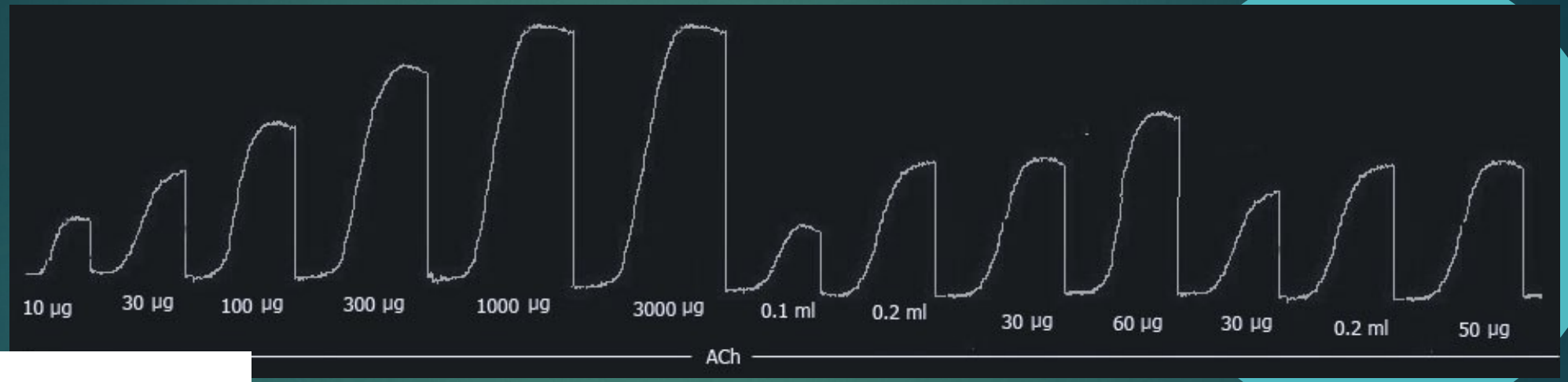
Conc. of unknown= Std dose (mcg)/ Test dose (ml)

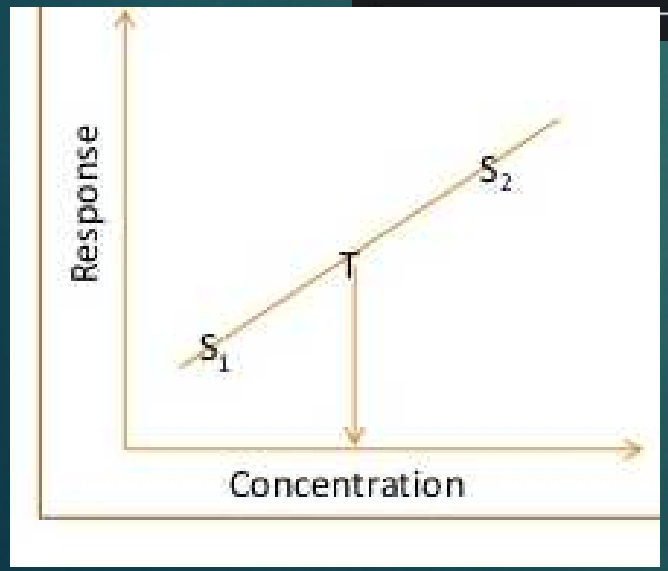
16/0.2 = 80 mcg/ml

# Bracketing Bioassay

- Test drug response is bracketed between lower and higher response of standard.

S1 (30 ug), T(0.2 ml), S2 (50ug)

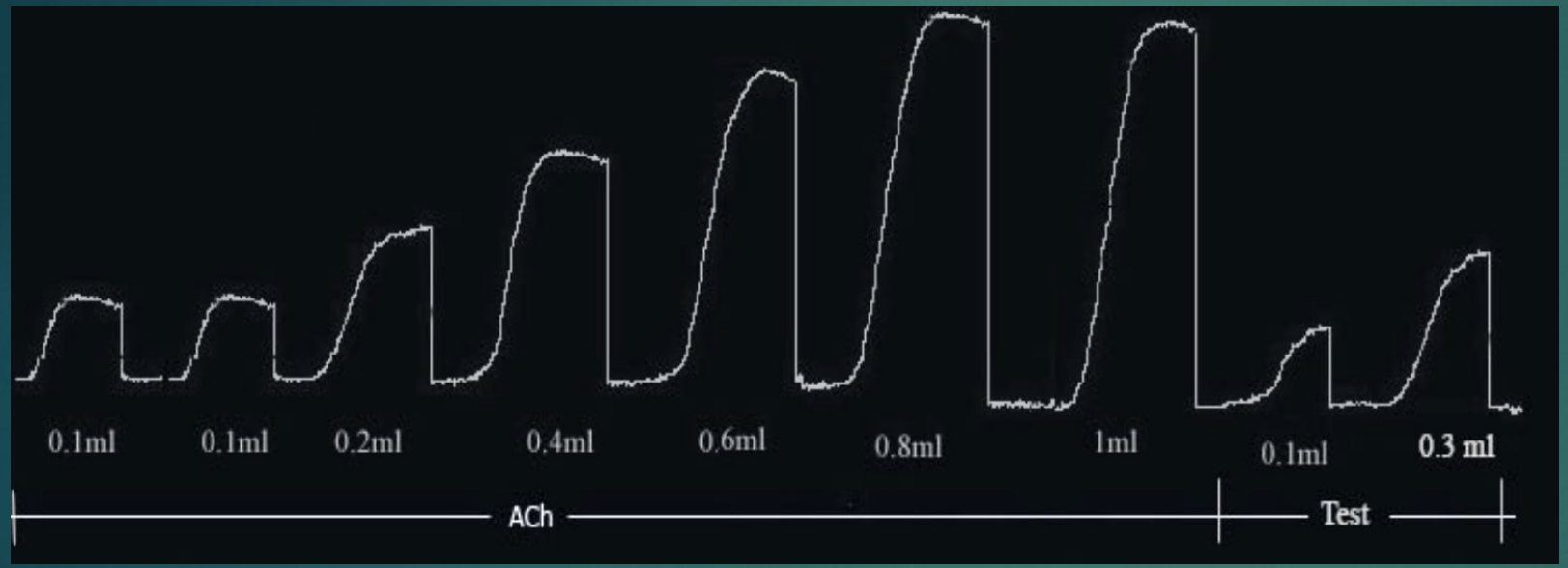


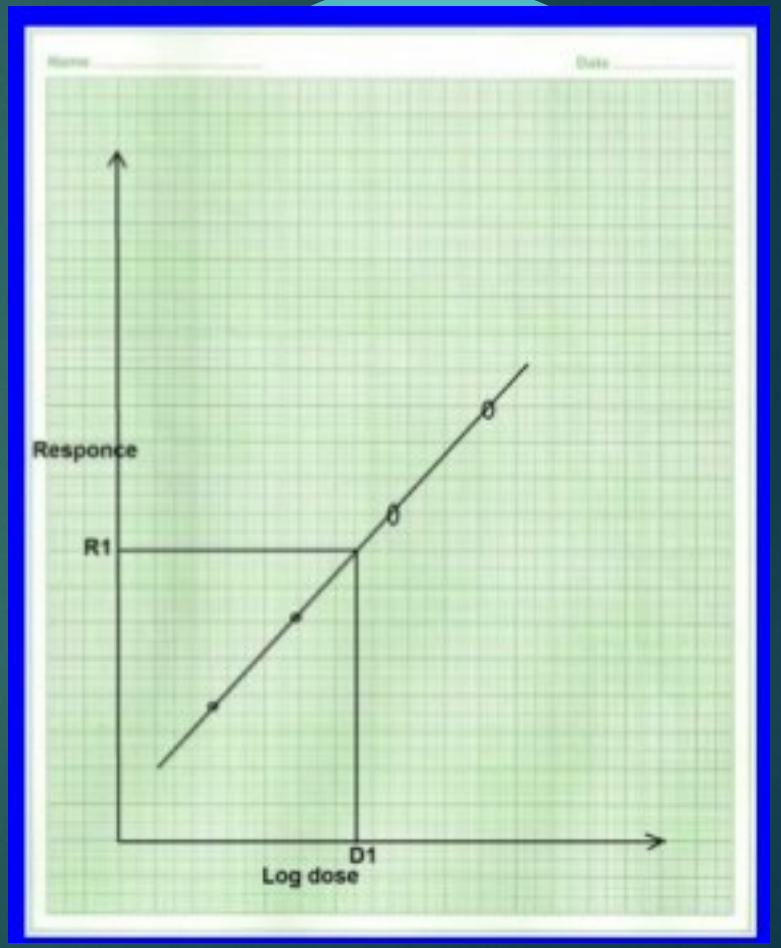




Bracketing assay of ACh on Frog Rectus Abdominus muscle

# Interpolation Bioassay

- Conc. of unknown is read from a standard plot of a log dose response curve of at least 2-3 sub maximal concentrations

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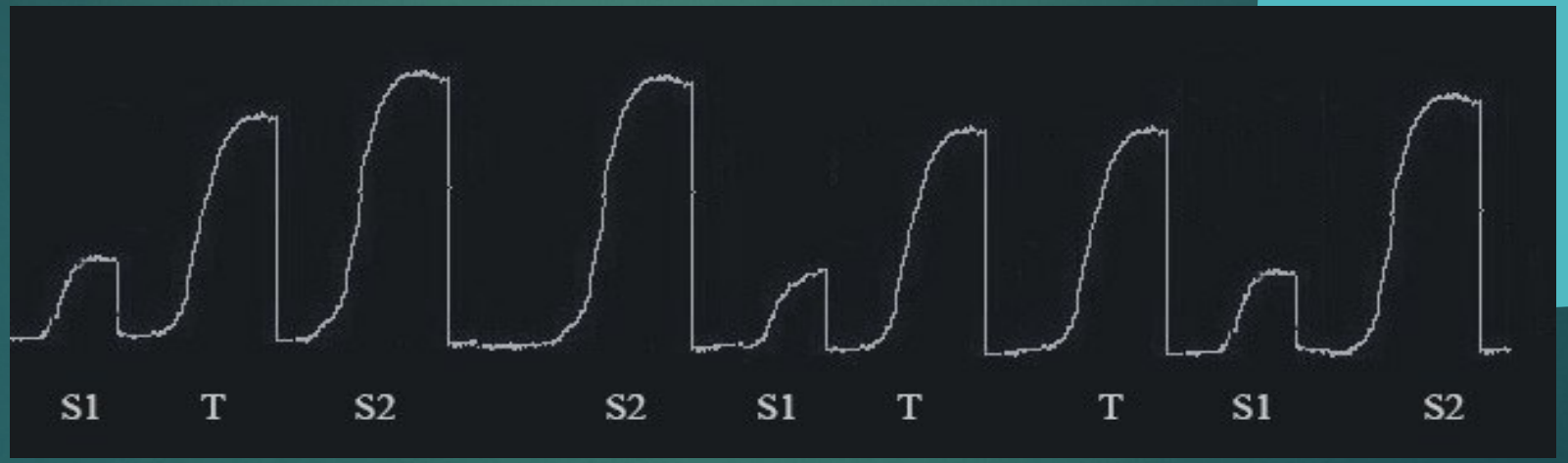
# Multiple Point Bioassay

## Three Point Bioassay : 2 std + 1 Test

- DRC of std is plotted. Select two std doses s1& s2 [ in 1:2 dose ratio] from linear part of DRC
- Choose a test dose t with a response T between S1 & S2
- Record 3 sets data as follows:




Unknown conc. Can determine by:

1. Graphical : Plot mean of S1, S2 and T against dose.

# Multiple Point Bioassay

Mathematically:

$$\textbf{Conc. of Unknown} = \frac{\mathbf{n1}}{\mathbf{t}} \times antilog \left\{ \frac{T - S1}{S2 - S1} \times log \frac{n2}{n1} \right\} Cs$$

- Where, n1 = Lower Standard dose
  n2 = Higher Standard dose
  t = Test dose
  S1 = Response of n1
  S2 = Response of n2

PC

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