

Bio-Assay of Histamine & Serotonin (5-HT)

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BIOASSAY

Bioassay: Assay of the drug or chemical by using **Biological System (Cells, Tissues, Organs, whole animals)**

Types:

1. **Qualitative Bioassay:** estimation of biological response or pharmacological effect of a drug. Examination of response +ve or –ve only.
2. **Quantitative Bioassay (Quantitative estimation):** Estimation of the concentration / potency of a drug/ chemical by measuring its biological response in living systems.

Bioassay of Histamine & Serotonin

Principle of Bioassay: 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

Model:

- **Histamine:** Bioassay of Histamine by using Guinea pig ileum
- **Serotonin:** Bioassay of Serotonin by using rat fundus strip

Method: Graded Assay: Response to varying dose

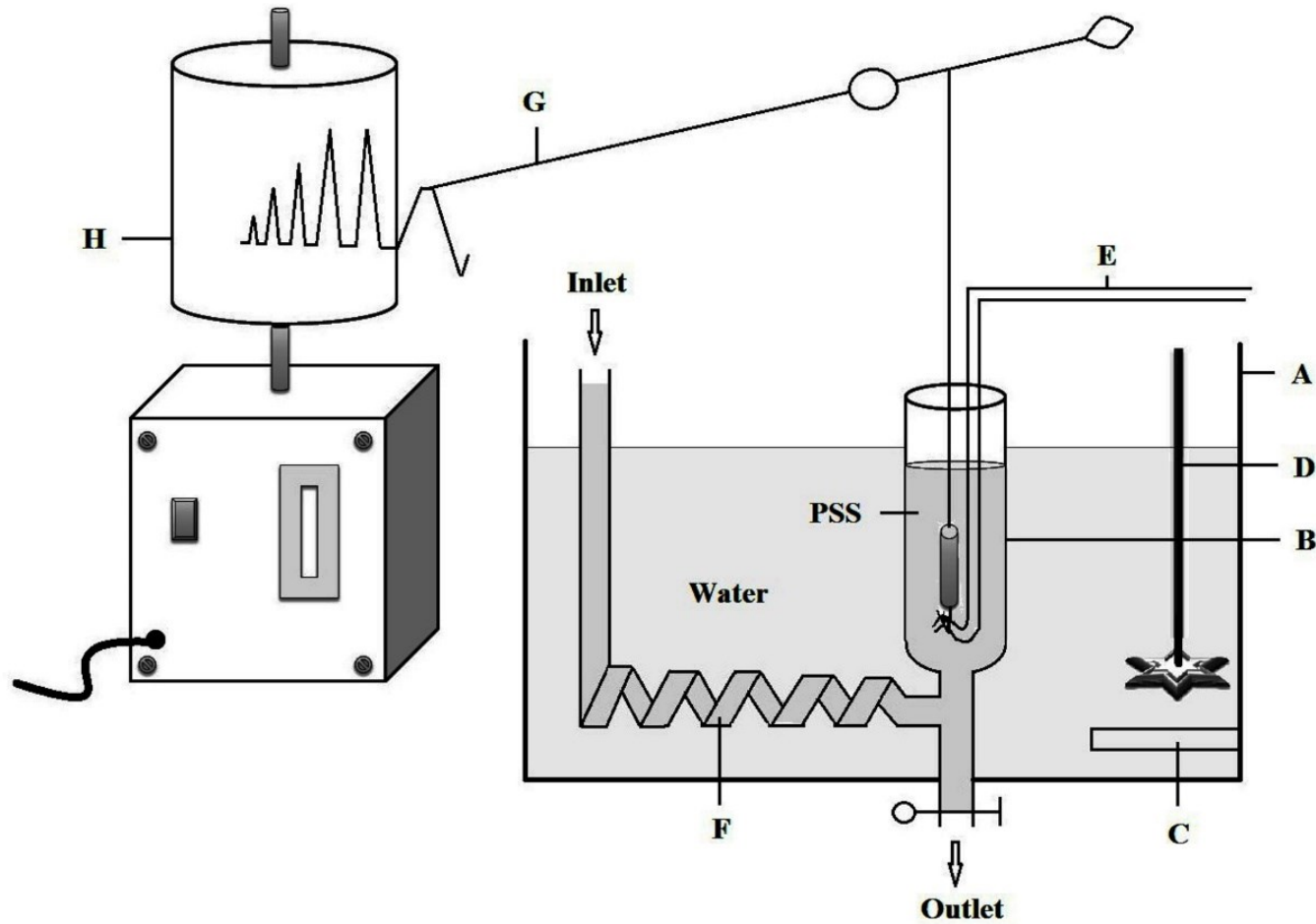
- ✓ Matching assay
- ✓ Bracketing assay
- ✓ Interpolation assay
- ✓ Multiple point assay (3 point, 4 point, 6 point bioassay)

Bioassay of Histamine & Serotonin

Requirements:

1. **Std Drug Solution:** **Histamine** (10 & 100 ug/ml) and **Serotonin** (10 & 100 ug/ml)
2. Test solutions of histamine and serotonin
3. **Instrument:** Student Organ Bath
4. **PSS:** Tyrode
5. **Tissues:**
 1. Guinea pig ileum (for histamine)
 2. Rat fundus strips (for serotonin)
6. **Temp:** 35-37 °C
7. **Time Frame :** 30 sec Baseline + 30-60 Sec. Contact time + 3 min Wash*3

Student Organ bath Setup



A: Water bath

B: Organ Bath

C: Thermostat

D: Propeller

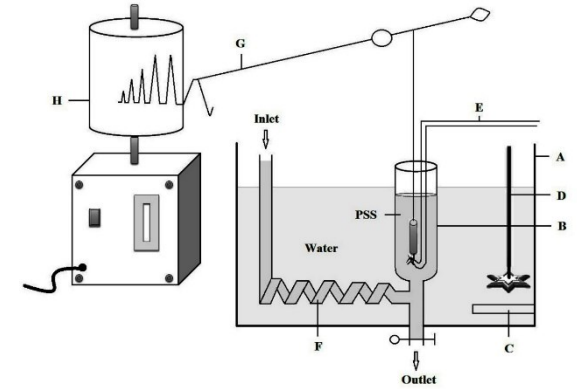
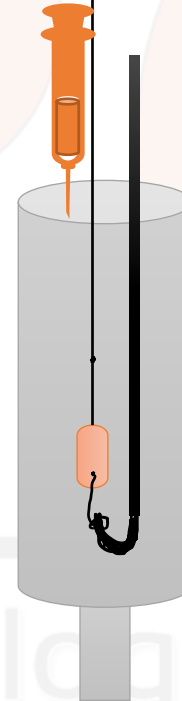
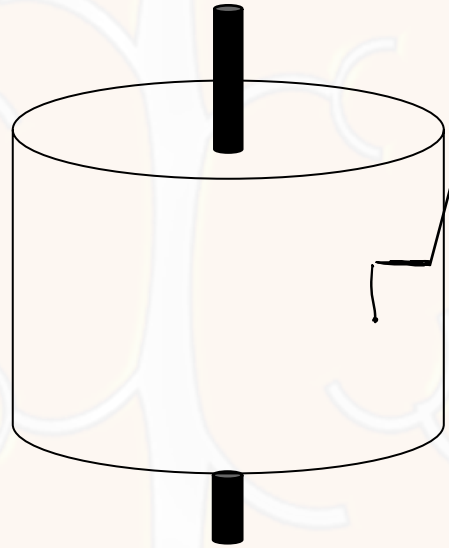
E: Tissue holder tube

F: Inlet coiled tube

G: Liver

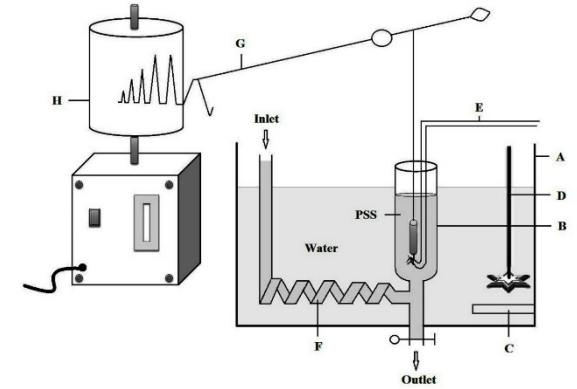
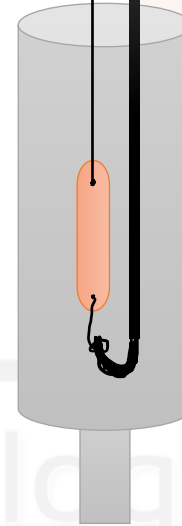
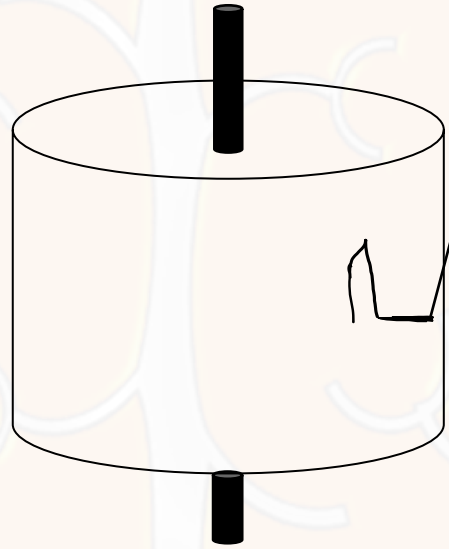
H: Rotating drum

Student Organ bath Setup



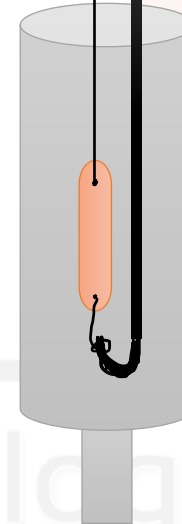
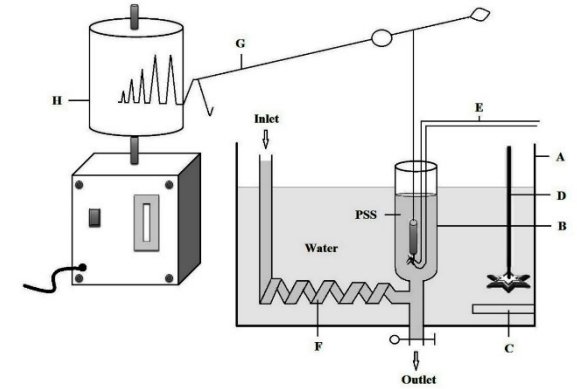
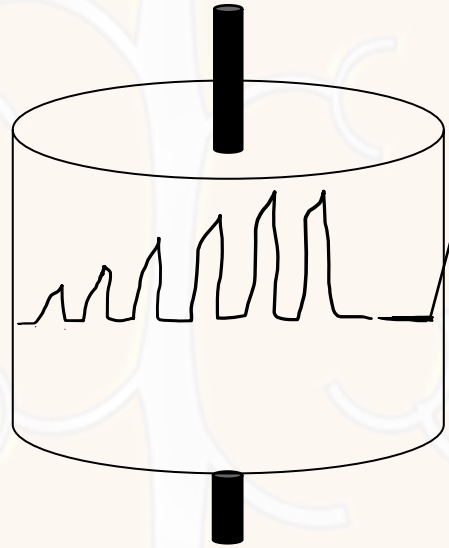
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Student Organ bath Setup



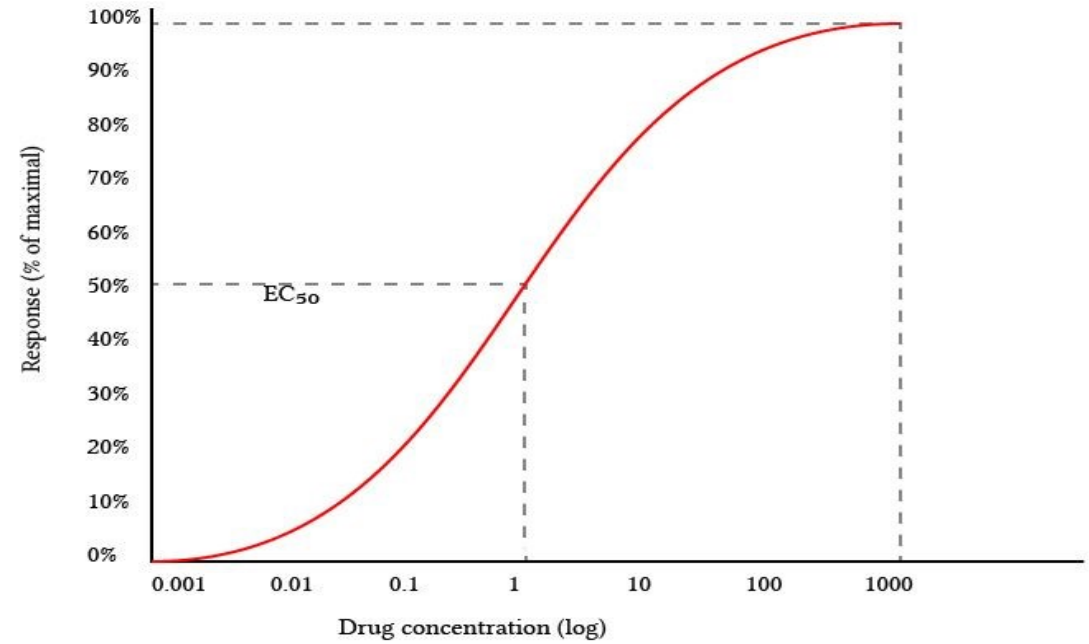
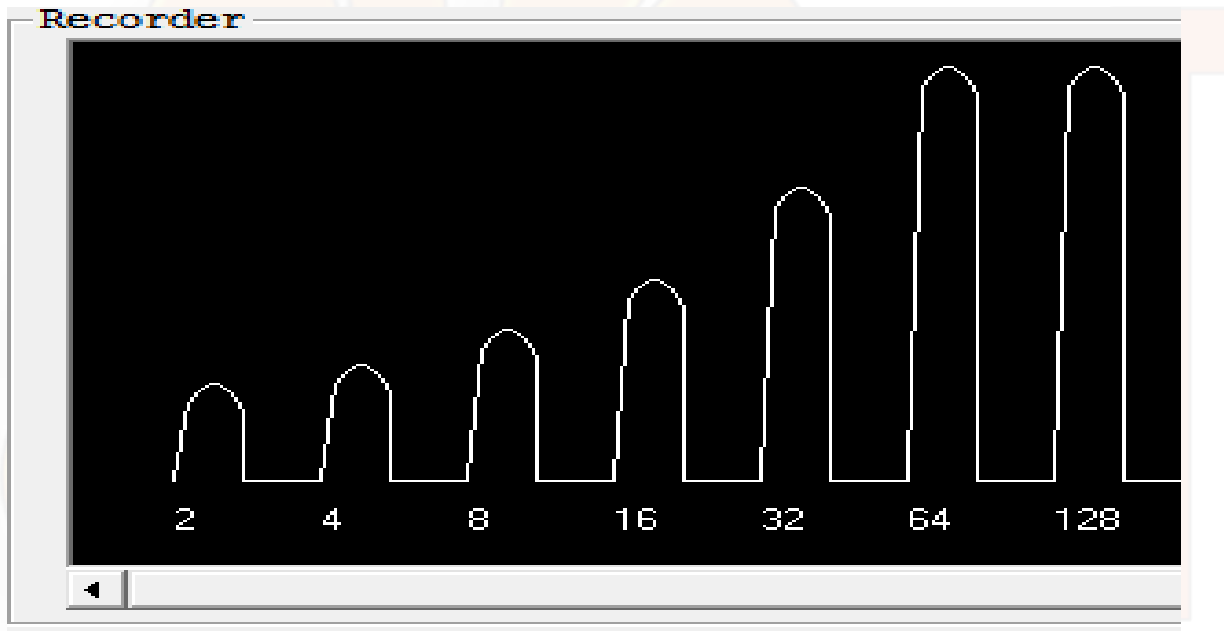
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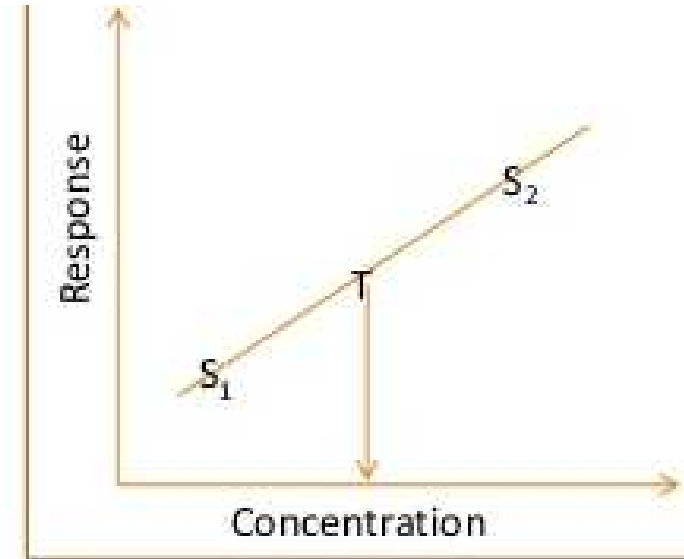
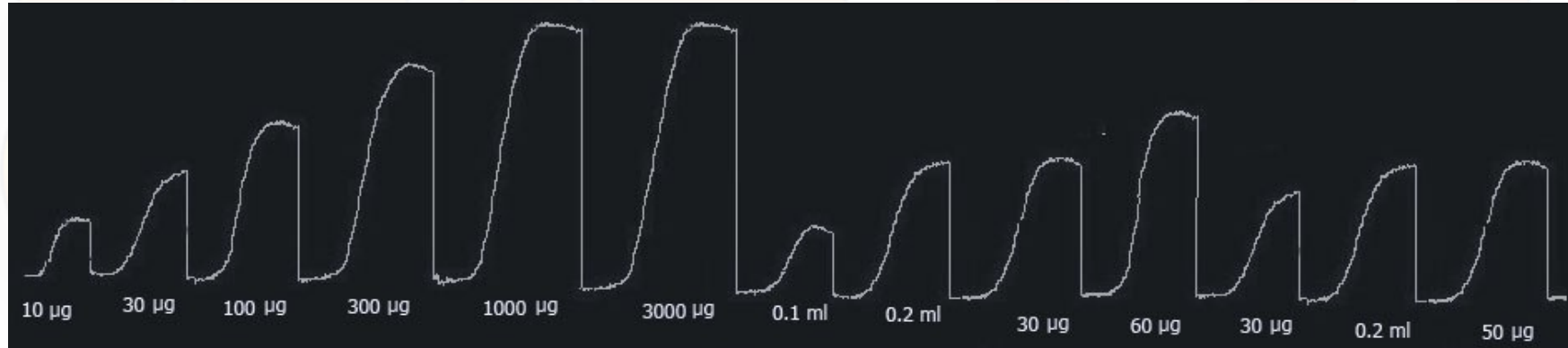
Dose Response Curve



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Bracketing Bioassay

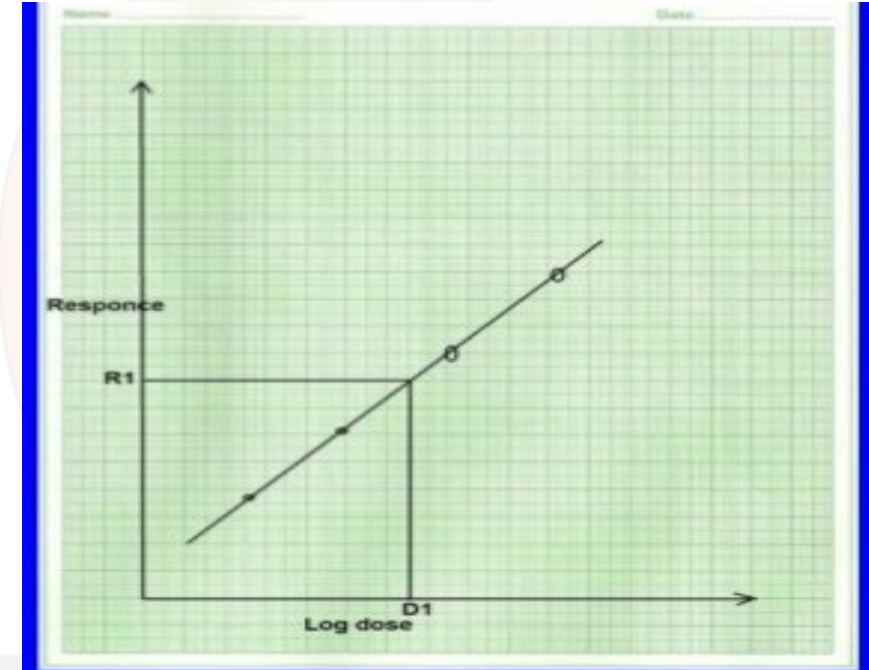
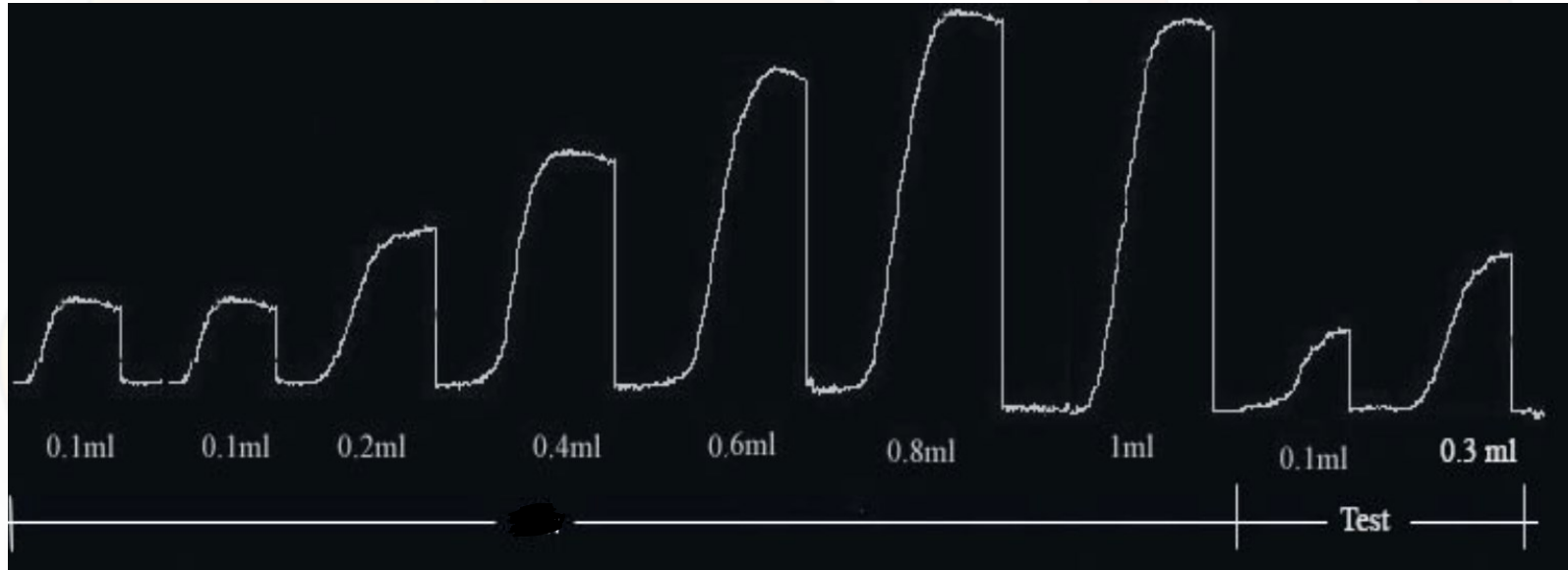
- Test drug response is bracketed between lower and higher response of standard.
- S1 (30 μg), T(0.2 ml), S2 (50 μg)



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Interpolation Bioassay

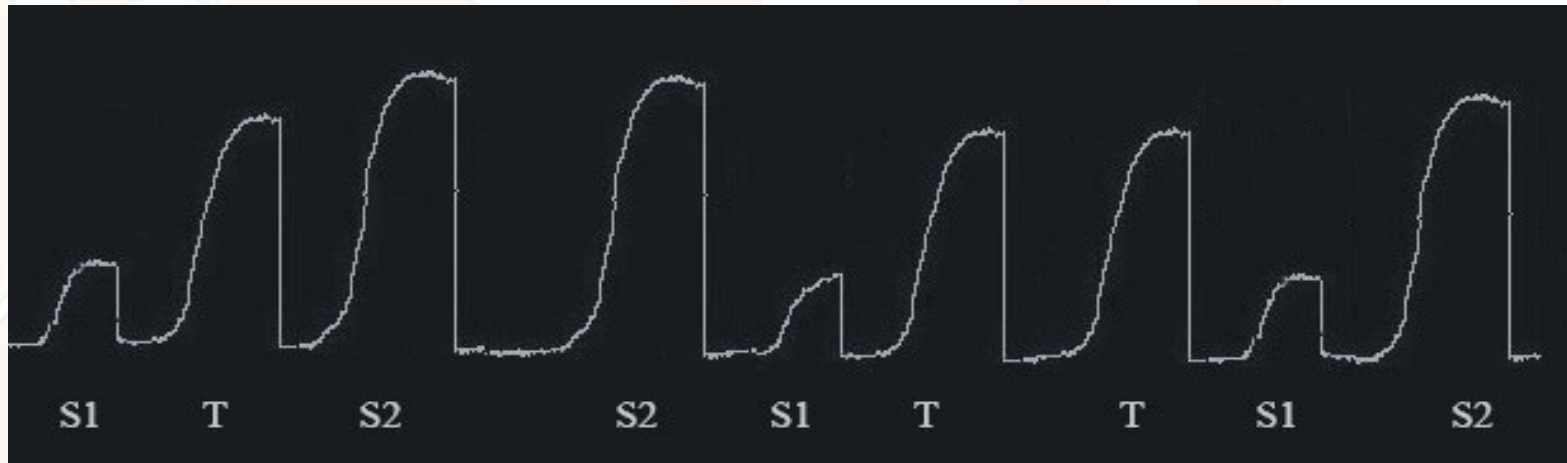
- Conc. of unknown is read from a standard plot of a log dose response curve of at least 2-3 sub maximal concentrations



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Three Point Bioassay

- 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: S1 T S2.....S2 T S1.....T S1 S2



Unknown conc. Can determine by:

1. **GRAPHICAL** : Plot mean response of S1, S2 and T against their log dose.

2. MATHEMATICALLY:

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

• where,

n1 = lower standard dose = 16 ug

n2 = higher standard dose = 32 ug

t = test dose = 0.4 ml

S1 = response of n1 = 3 cm

S2 = response of n2 = 4.25

T = response of t = 3.25

2. MATHEMATICALLY:

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

$$\text{CONC} = 16/0.4 \times \text{Antilog} [(3.25-3)/(4.25-3) \times \log (32/16)]$$

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

$$\text{CONC} = 16/0.4 \times \text{Antilog} [(3.25-3)/(4.25-3) \times \log (32/16)]$$

$$= 40 \times \text{Anti log} [(0.25/1.25) \times \log 2]$$

$$= 40 \times \text{Antilog} [(0.2) \times 0.3]$$

$$= 40 \times \text{Antilog} (0.06)$$

$$= 40 \times 10^{0.06}$$

$$= 40 \times 1.14$$

$$= 45.6 \text{ ug/ml}$$



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