

AIM: To study the bioassay of oxytocin using rat uterine horn by interpolation method/graphical method.

APPARATUS:

- Reservoir, tubing, hemostatic forceps, isolated organ bath, aeration tube, isotonic frontal writing lever and recording drum.

EXPERIMENTAL CONDITION:

- Physiological Salt solution (PSS) : De Jalon
- Animal : Female rat [120-150 gm]
- Temperature : 37 (+ or -) 10C
- Aeration : Carbogen (95% O₂ and 5% CO₂)
- Basal tension on the tissue : 500 gm
- Magnification of the response : 10 times
- Drug : Oxytocin (1, 10 or 100 µg/mL)

PRINCIPLE:

Oxytocin is a hormone secreted by the posterior pituitary gland. The rat uterine preparation is commonly used for the bioassay of oxytocin. The sensitivity of the uterus to oxytocin depends on the oestrous cycle. The various stages of oestrous cycle can be identified by preparing vaginal smears and observing under microscope. Rat uterus is highly sensitive. An adult female rat (2-3 months old) has an oestrous cycle of five days. The oestrous cycle is divided into different stages.

- ✓ Dioestrous – characterized by presence of leukocytes in vaginal smear.
- ✓ Proestrous /estrous – characterized by the presence of large number of nucleated epithelial cells.
- ✓ Frank oestrous – Presence of cornified epithelial cells.
- ✓ Meta oestrous or late oestrous – presence of mixture of nucleated, cornified epithelial cells and leucocytes.

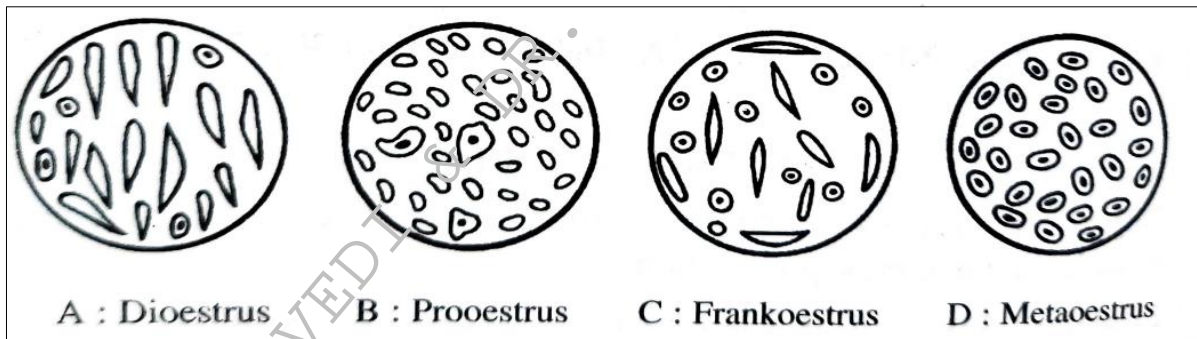
If the rat is not in frank oestrous stage, it can be induced by the administration of estrogen preparation, stilbestrol (0.1 mg/kg, sc:24 hrs before) Frank oestrus uterus is highly sensitive

to oxytocin and hence preferred for bioassay than the dioestrous uterus which is relatively less sensitive.

PROCEDURE:

A. Preparation of Animal:

1. Examine the vaginal smear under microscope to know about the proper stage of oestrus cycle. If the rat is not in frank oestrus, inject 0.1 mg/kg of Stilbestrol and wait for 24 hr. (Vaginal smear is prepared by taking a drop of vaginal wash and putting on the glass slide).
2. If the epithelial cells are present in the smear, it is said to be in frankoestrous phase.



B. Isolation of tissue:

1. Animal is sacrificed by cervical dislocation.
2. Cut open the pelvic region and expose both the horns of uterus. Separate them gently from the surrounding fatty material and transfer them into a dish containing De Jalon's solution. When the rat is in oestrus generally the uterus is fleshy and pink in colour.
3. Then the uterus is cut longitudinally and a tissue portion of 2-3 cm long is taken and both ends are tied with the thread.

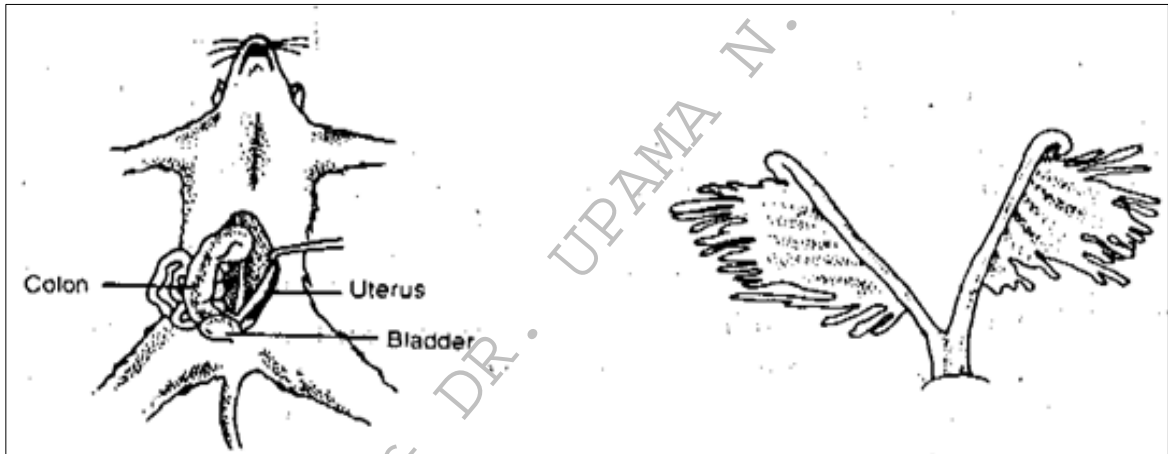
C. Mounting the tissue:

1. About 2-3 cm long tissue is mounted in organ bath containing De Jalons solution at 32° C along with proper aeration.
2. A tension of about 500 mg (0.5g) is applied and tissue is allowed to equilibrate for 45 min.

D. Recording of the response:

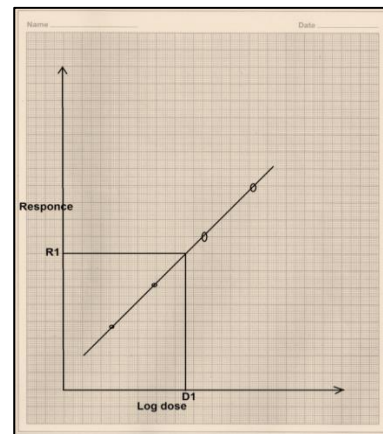
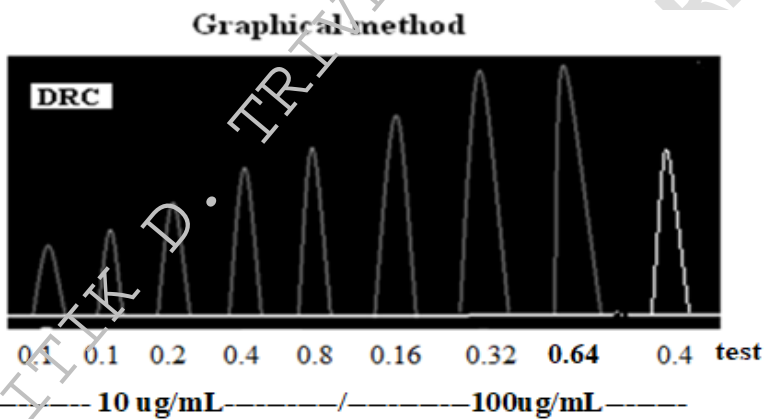
1. Record the DRC for the standard oxytocin solution is taken.
2. Record responses due to 0.1, 0.2, or 0.4 ml of the test substance. See that these responses would fall on the linear portion of the concentration –Response curve for the stand solution
3. Label and fix the tracing.

4. Plot the concentration response curve due to standard acetyl choline solution. Measure the heights of the contractions (response) due to different doses (A and B) of test solution .read the corresponding concentration from the standard curve.



Two Horns of the uterus in a female rat

GRAPH



DRAW GRAPH

OBSERVATION TABLE

Sr. No	Drug Name	Conc. of drug	Dose of drug in mL	Response in mm	% Response
1.	Oxytocin	10 µg/mL	0.1		
2.			0.2		
3.			0.4		
4.			0.8		
5.		100 µg/mL	0.16		
6.			0.32		
7.			0.64		
8.	Test	?	0.4		

Plot a graph X-Y-----Dose of drug (mL) Vs % of response

Find From Graph: Dose of standard _____

Dose of Test _____

CALCULATION

Dose of std.

Concentration of unknown = ----- x conc. of std. x dil. factor

Dose of test

=

RESULT: The concentration of given unknown sample is _____ µg/ml.

INFERENCE: Rat uterus is highly sensitive to oxytocin.

QUESTIONS:

1. Give the principle of graphical/interpolation method.
2. Discuss mechanism of Oxytocin.