

The Effect of Very Low doses of Estrogen on the Growth of Anuran Tadpoles

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ABSTRACT

The study deals with the effects of very low doses of estrogen on the tadpole of two anuran species. In experiment I (T-I), 1 µg/ml estrogen retarded 0.90 mm length of *Rana cyanophlyctis* test as compared to control on day 21. In experiment II (T-II) group 1 and 2 of test tadpoles of *Bufo melanostictus* exposed to 2 µg/ml and 5 µg/ml estrogen were 0.70 mm and 1.2mm shorter than their counterparts on day 21 respectively.

Keywords: *Estrogen, Tadpole Growth, Growth hormone antagonist*

1. INTRODUCTION

No therapeutic modality is known to lower the uniformly effective concentration of growth hormone. There are also no universally accepted criteria for the interpretation of changes in the concentration of growth hormone following the treatment. Therefore the decrease in the concentration of growth hormone after its therapy is not an absolute indicator of a main criterion [1,2, 3]. Therapeutic modalities for the treatment of excess of growth hormone include, (a) External radiation (b) transphenoidal techniques and (c) drug therapy. The last of the three includes a variety of pharmacological agents used to lower concentration of growth hormone, however estrogen does not lower growth hormone concentration in the plasma; but it probably acts by antagonizing the action of growth hormone [4].

Therefore, all important rationale of this study is to evaluate the growth inhibiting activity of estrogen in the tadpoles of two anuran species.

2. MATERIALS AND METHODS

Tadpoles from field ponds belong to several simultaneous hatchings. Collection of such population indicates variability in the standard of experimental parameters. In order to avoid variation eggs belonging to a single spawn of *Bufo melanostictus* and that of *Rana cyanophlyctis* was collected.

After the collection each of the spawns along with sufficient amount of pond water was introduced into polythene bags. They were then transported to the laboratory and were divided into several batches in tanks filled with tap water, kept standing for a day. After hatching they were allowed to grow for some days. With the perforation of mouth; the tadpoles were fed with a refined suspension of parboiled spinach [5]. Thus larvae were maintained till they reached a desired length suitable for starting the experiments.

a. Method of Measurement

The method employed for the measurement was similar to that adopted by Ahmad and Siddiqui [6] and Ahmad et al. [7, 8]. In order to measure, each tadpole was introduced into the 6cm diameter Petri dish in the absence of water. No sooner the tadpole stopped its wriggling movements; the Petri dish was slightly tilted towards the head side. By sliding the tadpole; body was shaped into a straight line. The Petri dish was arranged on a glass bearing a millimeter grid and the length of the tadpole from head to tail was noted [9, 10].

b. Design of Experiment

Following the measurement, they were divided at random into two groups of 23 tadpoles of *Rana cyanophlyctis* for experiment I. One of these groups was kept as control and the other as test. Whereas in experiment II there were three groups; each comprised of 25 tadpoles of *Bufo melanostictus*. One group was kept as control while the other two as test 1 and test 2 [11].

c. Drug Concentration

500 ml of tap water containing 1 µg/ml estrogen was used each day; as the aquatic medium for the test group of experiment I. Where as in experiment II 500 ml tap water contained 2 µg/ml estrogen for the test 1 and 500 ml of water containing 5 µg/ml estrogen for the test 2.

d. Care

Each day 500ml of water or the specific solution of corresponding concentration of estrogen per group was introduced into each tank, thus all animals were maintained in identical condition of volume of water or estrogen solution per individual per group. All groups were kept under constant temperature during the course of experiment [6, 12, 13].

e. Cleaning of Tanks

It is known that algae grown on the internal surfaces of tanks; from growth inhibiting substances and tadpole exudates are known to inhibit the growth. Therefore cleaning of plastic tanks, changing of water controls and estrogen solution of test of experiment I and test 1 and test 2 of experiment II were done every day. The water refilling of control tanks and preparation of estrogen solutions used was always a day old.

f. Feeding

For feeding parboiled spinach was liquidized with small quantity of aged water and the volume was made up to 500 ml by further addition of water. On every day 1 ml of this refined suspension per tadpole was added to each tank [5, 14, 15].

3. RESULTS

An examination of data of experiment I indicates that the test group of *Rana cyanophlyctis* tadpoles maintained in solution of 1µg/ml of estrogen showed 0.90 mm less body length compared to tadpoles of control group on day 21 (Table- I). Whereas the data of experiment II indicates that the *Bufo melanostictus* of test 1 and test 2 maintained in solutions of 2 µg/ml and 5 µg/ml estrogen respectively were 0.7mm and 1.2mm less in body length than their counterparts (Table- II).

4. DISCUSSION

Very high doses of estrogen can ameliorate some of the clinical features of diseases; especially severe elevations in blood glucose [1, 16, 17, 18]. The effect is most likely caused by the action of estrogen at the target cells.

In the present study low doses of estrogen were used to investigate their antagonist effect on the secretions of endogenous growth hormone. Similarly a typical regimen consisting of giving medication daily for 21 days followed by one week without therapy showed that 5-10 µg /ml ethyl estradiol were sufficient to reduce the elevated growth hormone levels.

Data collected in the study indicates that tadpoles maintained in 1µg/ml estrogen concentration showed a nominal antagonizing effect on tadpole endogenous growth hormone. However, the results of experiment I and II exhibited more antagonistic effect at 2µg/ml concentrated dose and still greater growth suppressing effect of 5µg/ml concentration.

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Table 1: The effect of estrogen on the growth of *Rana cyanophlyctis* tadpoles. Values are body lengths (mm)

Days	Controls	Test – 1 $\mu\text{g/ml}$
0	23.00 \pm 0.27	23.00 \pm 0.27
1	24.70 \pm 0.25	24.00 \pm 0.21
2	24.70 \pm 0.25	24.30 \pm 0.21
3	25.40 \pm 0.20	25.30 \pm 0.21
4	25.40 \pm 0.20	25.30 \pm 0.28
5	25.55 \pm 0.20	25.30 \pm 0.33
6	25.55 \pm 0.42	25.80 \pm 0.33
7	25.56 \pm 0.256	25.90 \pm 0.25
8	25.57 \pm 0.24	25.30 \pm 0.26
9	25.58 \pm 0.22	25.30 \pm 0.24
10	25.59 \pm 0.26	25.30 \pm 0.22
11	26.00 \pm 0.24	25.30 \pm 0.22
12	26.30 \pm 0.21	25.30 \pm 0.22
13	26.33 \pm 0.28	25.90 \pm 0.21
14	26.40 \pm 0.33	25.90 \pm 0.34
15	26.45 \pm 0.36	25.20 \pm 0.25
16	26.50 \pm 0.20	25.20 \pm 0.25
17	26.60 \pm 0.25	25.20 \pm 0.25
18	26.80 \pm 0.26	25.20 \pm 0.28
19	29.90 \pm 0.28	25.20 \pm 0.28
20	27.70 \pm 0.30	26.30 \pm 0.28
21	28.20 \pm 0.26	27.30 \pm 0.27

Each figure is the mean of 23 measurements with \pm S.D.

Table 2: The effect of estrogen on the growth of *Bufo melanostictus* tadpoles.
Values are body lengths (mm)

DAYS	CONTROLS	TEST -1 2µg/ml	TEST - 2 5µg/ml
0	7.0±0.00	7.0±0.00	7.0±0.00
1	7.9±0.00	7.3±0.00	7.7±0.03
2	8.3±0.04	8.1±0.03	8.2±0.04
3	8.5±0.02	8.2±0.04	8.3±0.04
4	9.6±0.03	9.4±0.03	9.5±0.03
5	11.0±0.03	10.2±0.04	10.4±0.04
6	11.7±0.03	10.9±0.03	11.2±0.09
7	11.9±0.05	11.2±0.09	11.5±0.16
8	12.0±0.05	12.0±0.05	12.1±0.05
9	12.5±0.04	12.1±0.05	12.2±0.04
10	13.4 ±0.01	13.0±0.05	13.2±0.06
11	13.6±0.04	13.4±0.01	13.6±0.04
12	14.3±0.07	14.0±0.01	14.2±0.01
13	15.2±0.01	14.6±0.01	15.1±0.02
14	16.2±0.08	15.8±0.11	16.2±0.08
15	16.4±0.06	16.2±0.08	16.5±0.03
16	17.0±0.16	16.4±0.03	17.5±0.06
17	17.6±0.16	17.5±0.06	17.6±0.16
18	18.5±0.13	17.6±0.06	18.0±0.13
19	18.6±0.11	18.5±0.13	18.4±0.12
20	19.3±0.01	18.7±0.11	18.9±0.02
21	20.1±0.19	19.4±0.19	18.9±0.02

Each figure is the mean of 30 measurements with ±S.D.