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Corticosterone as Inhibitor of Bufo Melanostictus Tadpole Growth

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ABSTRACT

This investigation reports the effect of corticosterone on the growth of *Bufo melanostictus* tadpoles. Test tadpoles of experiment I (T-I), maintained in 1µg/ml solution of corticosterone, were 2.39mm shorter than controls by day 11. Test tadpoles of experiment II (T-II), maintained in 5µg/ml solution were 4.32mm shorter than their counterparts. Growth inhibition was dose related and became significant ($p<0.05$) when the larger amount was administered.

Keywords: *Metamorphosis, Macrophages, Proteolysis, Anuran tadpoles, Growth inhibitor.*

1. INTRODUCTION

Only a minute amount of corticosterone has been detected [1] in the blood of *Amphiuma*. Equally, a very small quantity has been found in the blood of the adult American bullfrog, *Rana catesbeiana* [2]. Despite these demonstrations, it has not yet been shown that corticosterone is present in the blood of amphibian larvae [3]. However, several studies have demonstrated the influence of exogenous corticosterone on the growth and development of larval amphibians [4, 5, 6].

Kobayashi [4] studied the effects of deoxycorticosterone acetate on metamorphosis of thyroxine induced tadpoles. He also claimed that corticosteroids synergise the destructive phase of shrinking of tails and a shortening of structures.

The purpose of the present investigation is to investigate the destructive action of corticosterone in premetamorphic *Bufo melanostictus* tadpoles.

2. MATERIALS AND METHODS

Animals:

Bufo melanostictus tadpoles belonging to a single hatching were collected from the rainwater ponds of the University Campus in the month of August 2011. After collection, tadpoles of similar size were divided into several groups of equal number. They were soon given refined suspension of parboiled spinach [7, 8]. They were then allowed to acclimatize for a number of days. On attaining premetamorphic period, tadpoles were used to determine the effect of corticosterone; since physiological connections between pituitary and hypothalamus are established when they enter premetamorphic period of stage 52 [9, 10, 11]. The tadpoles were selected with the help of a binocular microscope; and after measurement, individuals of the same size were randomly divided into comparable groups of control and test.

Growth correlations:

It may be stated that during development, there appears to be gradual unfolding with time; of anterior pituitary and receptor-gland hormones that regulate the final size, form and body parts. This elaboration of growth is indicative of a second phase of specific constitutional growth potential, provided by the genotype of the egg. During this phase the growth regulating hormones gradually take over the function of the coordination and regulation of final size [12]. However, growth is also dependent upon environmental factors [13]; since nutrients, oxygen supply [14] and temperature have an effect on the physico-chemical background in which the synthetic processes of growth take place. Thus, growth follows the expression of size and weight. Therefore, growth of *Bufo melanostictus* tadpoles was worked out by measuring its body size and decisive evidence of growth has also been sought through the use of these measurements [15].

Method of measurement:

The method adopted for this purpose was similar to that described by Ahmad and Mukarram [16]; and was later adapted by Ahmad et al., [15, 17, 18, 19]. In order to measure each tadpole, nylon net was used to introduce tadpoles into a 6 cm diameter glass Petri dish, in the absence of water. As soon as the tadpole ceased its wriggling movements, the Petri dish was tilted slightly 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 from the head to tail was noted.

Design of experiment:

Space is a major factor in the growth rate of tadpoles, and crowding has been reported to accelerate metamorphosis by operating through the hypothalamic

mechanism in anuran tadpoles [20, 21]. Therefore, animals thus measured were divided at random into three groups of fifty tadpoles each and kept in separate tanks [22]. One group was kept as control and the other two as test.

Care:

All measures, as pointed out previously, regarding the healthy management of animals during the experimental period, in relation to volume of surrounding water in tanks; its temperature and feeding procedure were followed essentially as suggested by Ahmad et al. [15, 20]. Thus each of the control tanks was cleaned daily, filled with 500ml of water [10]. Whereas, the test tanks I and II were filled with 1µg/ml and 5µg/ml corticosterone solution, respectively. The test and control groups were kept in similar healthy conditions. For feeding, 6% parboiled spinach in dechlorinated water was prepared and 1ml of this suspension per tadpole was introduced into each tank every day [7, 8, 22, 23].

It was also in the interest of the investigation to maintain the external temperature at which tadpoles of *Bufo melanostictus* normally live. Since higher temperatures favour thyroid stimulating hormone (TSH) over the growth factor production in the pituitary and accelerate metamorphosis [24]. Therefore, the tadpoles were kept under fairly constant temperature (28°C ± 1°C) during the experimental period [25].

Dose administered in concentrations of 1µg/ml and 5µg/ml corticosterone were prepared fresh each time before use. It is known that corticosterone has a short biological half-life of 8 to 12 hours. Since it is absorbed from sites of local application i.e. skin [26], tadpoles remained immersed and the solution was changed at 12 hour intervals. Thus prolonged administration through the whole skin may be sufficient to cause all systemic effects, even adrenocortical suppression.

3. OBSERVATIONS AND RESULTS

A consideration of Fig. I indicates that *Bufo melanostictus* tadpoles maintained in 1µg/ml corticosterone (T-I) for a period of 11 days showed a retarded growth. The effect of the dose was obvious from the second day of treatment. The difference of growth between the control and test became more significant with the advancement of days ($p = 0.2585$). By the 11th day, T- I tadpoles were 2.39mm shorter than controls.

The test tadpoles of experiment II (T-II), maintained in a solution of 5µg/ml of corticosterone, showed slower growth than the test tadpoles of T- I. The effect of the hormone was obvious from the 2nd day of treatment, and these tadpoles were significantly ($p<0.05$) shorter than control group. Thus, test tadpoles of T-II were 1.93mm and 4.32mm shorter than test tadpoles of T- I and control tadpoles, respectively.

Hind limbs were visible in 65 percent of controls by day 9; in 70 percent by day 10; and 100 percent by day 11. Moreover, fore limbs emerged in 10 percent of controls by day 11. On the other hand, neither tadpoles of T- I, nor tadpoles of T- II, showed hind limbs.

Animals of both the test groups remained much smaller than their controls. All test tadpoles showed similar retardation of head, trunk and tail, indicating that all test tadpoles were evenly sensitive to the hormones.

Skin pigmentation was badly affected. Test tadpoles of T- I and T- II showed similar loss of colour. Thus all test tadpoles were white, or cream coloured, and tiny.

4. DISCUSSION

It has been shown that corticosterone plays an important role in amphibian metamorphosis along with the thyroid hormones; thyroxine, triiodothyronine and prolactin [27] but the real nature of action of thyroxine and corticosterone is different. Thyroxine activates through the macrophage system, composed of leucocytes and the lymphoid tissue actually destroying the tail protein by the invading process of phagocytosis; and by forming antibodies and sensitized lymphocytes to combat and destroy the toxic agents invading from the aquatic environment, during metamorphic climax.

Corticosterone not only decreases the circulating concentration of T-lymphocytes by inhibiting the clonal expansion of immunocytes through lymphokines [28] but monocytes, eosinophils and basophils also. Corticosterone inhibits the functions of leucocytes and tissue macrophages. The effect on macrophages is noticeably marked. It limits their ability to phagocytose tail proteins exactly due to direct consequences of the stabilization of lysosomal membranes; there upon reducing the concentration of proteolytic enzymes.

Therefore, the cause of the tail and trunk shortening, as observed in this study, was due to the continued inhibition of DNA synthesis, with the suppression of mitosis causing arrest of tadpole growth.

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