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BIOASSAY OF PITUITARY PROLACTIN OF THE LIZARD *UROMASTIX HARDWICKII*

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ABSTRACT

The present study has been designed to evaluate the pituitary prolactin content in the lizard *Uromastix hardwickii*.

Pituitary glands were removed from 24 lizards and a suspension was prepared. This suspension was injected in the crop sac glands of a group of 12 pigeons. Another group of 12 pigeon was injected distilled water instead.

Results of the study indicate a mean crop gland diametric response of 0.8cm following pyrogen free distilled water administration. Whereas following the administration of pituitary suspension the response was of 2.12cm. The first response was due to injection stress and the latter response was due to PRL.

INTRODUCTION

Prolactin (PRL) is a hormone secreted by acidophilic cells of the Anterior pituitary gland (Smeller, 1941; Evertt, Daker, 1947; and Harfant 1956b, Mezzi *et al.,* 1979 and Kelly *et al.,* 1984). It is also called mammotropin or lactogenic hormone. The size and number of cells secreting prolactin increase during pregnancy and lactation.

Evidence indicates that PRL is secreted by 2 separate classes of acidophils in Cat (Dawson 1946; Harlent and Racodot, 1957), rabbits (Pearse, 1951) monkeys (Dawson, 1948) and bat (Harlant, 1956a). Carminophils (a type of acidophil cells in anterior pituitary) possess PRL containing granules that secrete the hormone. The secretory activity of these cells vary according to the physiological status of the animal (increasing in pregnancy and lactation).

It is also known that besides the pituitaries of mammals and birds, PRL is also present in their blood, urine, liver and placenta. It is also found in cow manure (Meites and Turner, 1948).

PRL is a protein hormone and its identity as crop gland stimulating factor in birds was established long before (Riddle and Braucher, 1931; Riddle *et al.,* 1932, 1933). Pituitary PRL levels have also been taken as an index of physiological activity (Meites and Turner, 1948b). Its levels have been determined in several rodents besides other animals but in *Uromastix hardwickii* either in inactive (hibernating) or active physiological state the level of PRL remains unknown.

The aim of present investigation is to determine pituitary PRL content of *Uromastix hardwickii* qualitatively in terms of its effect on crop sac gland of Pigeon. Crop sac glands are the out pouching of esophagus in pigeons.

MATERIALS & METHODS

Twenty-four pigeons 8-10 wks of age belonging to white race were used for crop sac test. The birds were obtained from local breeders and housed in the laboratory for 5 days keeping one pigeon in one cage. They were fed with millet and water ad libitum.

Twenty-four lizards Uromastix were used in this study to get pituitary glands. They were obtained from local supplier and kept in

the departmental lab (Ahmad and Taqawi 1978a, 1978b, 1979 and 1980). The lizards used were both females and males.

Removal of lizard pituitaries

The lizards were decapitated without using an anesthetic. Their skulls were cleaned of skin and muscles and the vaults (skull cap) were removed with the help of a pair of large scissors. The brain was scooped out of each skull and the pituitary glands extracted from sella turcica.

Pituitary suspension

A pituitary suspension was made by grinding the extracted pituitaries in mortar and adding a small quantity of pyrogen free distilled water. The suspension so formed measured 5m1. It was transferred into a serum bottle and stored in refrigerator to be injected subsequently to birds during bioassay period.

The birds were divided into two groups. One group to receive injections of pituitary suspension for prolactin assay and the other to receive distilled water injections for "Sham" assay.

Assay procedure

This was essentially the same as that of Grosvenor & Turner (1958). Feathers were plucked off the skin overlying the crop sac 68 hrs before starting injections. An amount of 0.1 ml of pyrogen free distilled water was given to each of the 12 birds for "Sham" experiment and 0.1 ml of pituitary suspensions to the birds of other group for bioassay.

These injections were given for a period of four days with a 1ml hypodermic syringe bearing 27-gauge needle. The injection was given at the centre of each crop sac, which was marked previously with a nontoxic dye. The injections were made such that an intradermal bleb was always formed.

The birds were killed on 5^{th} day, a day after the last injection. The skin was removed from the crop sac and then the sac was removed and bisected. After removing the fat, the lining of each half of crop sac was rinsed *with tap* water and stretched against the light of a table lamp bearing a 100watt bulb. There was a proliferated area, which appeared opaque and circular. This proliferated epithelium was measured in cm with a caliper upon stretching against light.

Following arrangements were made to ensure the uniformity of results.

1. Birds were uniform in age (8-10 wks) and of uniform weight (340 ± 35 gms).
2. All birds were housed at the same location to ensure equivalent conditions of temperature and light.
3. Constant volume of 0.1ml was used for each injection.
4. The injections were made always to form an intradermal bleb on the follicles from which feathers have been plucked off six hours earlier.
5. The sites of injections were not varied.
6. The needle was withdrawn carefully to prevent reflux of fluid.
7. The diameters of the proliferated crop-gland were measured at two or three places and the average was taken to represent the response level in each pigeon.
8. The diameters were measured independently by more than one person to increase the objectivity of the results.

RESULTS

The mean crop sac response area i.e. the diameter of proliferated epithelium of crop gland in birds that received pyrogen free distilled water was found to be 0.85 cm. The minimum value was 0.66cm and the maximum value was 0.91 cm in 24 measurements from 12 birds (Table 1).

The mean crop sac response area i.e. the diameter of proliferated epithelium of crop gland in birds that received Uromastix

Table-1
Crop-sac diametric response following intradermal injections of pyrogen free double distilled water

No.	Pigeon Wt. gm	Crop-Sac *Diameter (cm)
1	320	0.66
2	340	0.85
3	332	0.88
4	345	0.82
5	328	0.78
6	341	0.82
7	352	0.91
8	320	0.89
9	335	0.88
10	328	0.93
11	350	0.89
12	350	0.86
Average ± SD:	336.7 +11.36	0.85 ± 0.072

*Each figure is the average of right and left crop-sac diametric measurements with SD ±.

pituitary suspension was 2.12cm. The minimum response was 2.08cm and the maximum 2.16cm in 24 measurements in 12 birds (Table 2).

DISCUSSION

PRL secretion from anterior pituitary gland is influenced by so many factors. The largest factor is a suckling stimulus or breast manipulation in nursing mother. PRL concentration can rise 10-100 times within 30 minutes of stimulation.

Stresses such as psychic and/or physical are reported to cause a considerable increase in concentration of plasma PRL (Frantz *et al.*, 1972, Friesen 1973, Krulich *et al.*, 1974 & Euker *et al.*, 1975). Stress induced increases are also known to occur during experimentation (Seggi and Brown 1975, Mattheij and Van Pijkeren 1977). Evidence in support of such reaction may be derived from plucking off feathers from crop sac region. Such stress produces some proliferative response in crop glands within six hours (Halls 1944a).

Hypertonicity have also been reported to result in large elevation of plasma PRL (Buckman *et al.*, 1973). PRL release is also evocable after administration of pyrogen free distilled water as seen in the present study.

The crop gland diametric response to PRL however has only been considered valid within certain limits. Accordingly a response of less than 2cm and more than 3.85cm are

Table-2
Crop-sac diametric response following intradermal injections of normal *Uromastix hardwickii* pituitary suspension

No.	Pigeon Wt. gms	Crop-Sac *Diametric (cm)
1	345	2.12
2	350	2.16
3	332	2.08
4	345	2.11
5	352	2.13
6	338	2.12
7	355	2.16
8	352	2.08
9	327	2.15
10	341	2.10
11	346	2.14
12	355	2.09
Average with ± SD:	344.8 ± 8.95	2.12 ± 0.028

*Each figure is the average of right and left crop-sac diametric measurements with SD+

unreliable to rate (Qazi 1962) and will be regarded as source of error.

In the present study the average diametric response of crop glands following administration of the Uromastix hardwickii pituitary suspension has been 2.12cm. According to the aforementioned criteria values of our study are well within the suggested limit and therefore valid.

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