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# *IN VITRO* EFFECTS OF ACTH ON PIGEON CROP-SAC EPITHELIUM

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**ABSTRACT**

ACTH *in vitro* produces non-specific crop-sac epithelium response. Crop-sac maintained in 10 IU.ACTH in isologous serum at 37ºC for 6 hrs. showed an increase of 105 ± 0.95 mg for 12 hrs. 115 ± 1.13mg, for 18 hr. 125 ± 0.95 mg. and for 24 hrs. 145 ± 2.31 mg. The results were analyzed statistically by ANOVA technique. A significant rise in mean weight. In ACTH treated crop-sac, indicates a time related relationship ($P<0.01$, ANOVA, Table 2). Thus present data indicates that there is a correlation between the epithelial response and initiation of ACTH present in the serum.

## INTRODUCTION

Principally, crop in avians is an organ of storage of food. However, the size and shapes of crops may vary according to the eating habits of the species. The pigeons crops are adapted to produce crop-milk and the proliferation of the crop epithelium and its sloughing are induced by prolactin. These birds regurgitate the crop-milk to feed their youngs (Sturkie, 1986). In response to feeding crop produces mucus for lubrication and softening of ingesta and better prepares it for gastric and intestinal digestion. The initial digestion of carbohydrates occurs in crop by salivary amylase, secreted by a source other than crop (Farner, 1960 and Sturkie, 1965). Studies had shown that the presence of crop bacterial growth may cause degradation and digestion of sugars (Bolton, 1965). Different group of birds exhibit a different capacity to produce crop milk as solution to very different problem. The need for protein and fats in the pigeon which they feed very little material to squabs; the need for liquid consumption during development of feeding apparatus (Ehrlich *et al.*, 1988).

Pigeon crop-sac stimulation has been regarded specific for prolactin (PRL) bioassay. The most frequently used methods of quantifying PRL have included the systemic pigeon crop-sac assay of Riddle *et al.* (1933) and the intracutaneous modification of this method as originally described by Lyons and Page (1935). The later method was criticized by Lahr *et al.* (1943) and expressed the opinion that the reaction is non-specific.

However, Bern and Nicoll (1968) reviewed this bioassay and reported that non-specific responses did occur but were not dose response related. Moreover, Raud and Odell (1971) suggested that something in addition to prolactin in the pituitary gland was capable of eliciting a crop-sac response.

Therefore, consistent with the hypothesis that systemic pigeon crop-sac, measures something other than prolactin, the stimulation of crop-sac by adrenocorticotrophic hormone was attempted *in vitro* and the findings are present in this study.

## MATERIALS AND METHODS

*Assay Animals:*

Pigeons 8-10 weeks age belonging to white race weighing 320 ± 35g were used for crop-sac *in vitro* assay. Pigeons were obtained from a local breeder, in group of 12. They were housed separately, one in each cage and were fed millet and water *ad libitum*. They were kept in the laboratory for 2 days. Moreover, they were housed at the same location to ensure similar conditions of temperature, light and air.

*In vitro Assay Procedure:*

A modification of Meites and Nicoll (1965) method of organ culture was employed. Sterile plastic Petri dishes and stainless racks were used. Each pigeon, 6 hrs. after plucking off the feathers (Hall, 1944) of the crop-sac region was killed. The blood collected was used for the separation of serum. 10 I.U. ACTH was added to 0.5 ml of serum. The crop-sac weighed, were introduced into the isologues serum with ACTH for 6 hrs; 12 hrs; 18 hrs. and 24 hrs. at 37$^{o}$C in an incubator. At the end of each culture period, each of the crop-sac was re-weighed and the difference calculated.

## RESULTS

Four groups of twelve pigeons each, were employed for comparative study (Table 1). The mean weight of the control group was 454.42 ± 1.73 mg. 24 hrs. response with ACTH incubation was noted at regular intervals of 6 hrs. Each crop-sac epithelium gave consistent response to 10 I.U. ACTH present in 0.5 ml isologues serum. The mean increase in the weight of *in vitro* 6 hrs. group was 105 ± 0.95 mg; of the 12 hrs was 115 ± 1.13 mg; of the 18 hrs. was 125 ± 0.95 mg and the mean increase in crop-sac weight of 24 hrs. group was 145 ± 2.31 mg. All the values were calculated by ANOVA technique which showed significant increase in mean weight. The results were found to be highly significant at $P < 0.01$, ANOVA, Table 2.

## DISCUSSION

Evidence for the response also derives from plucking off the feathers in the crop-sac region. Since it produces proliferative response within 6 hrs. in the crop-glands (Hall, 1944). Most investigators failed to interrelate this effect with ACTH following stress. Thus, the increase in crop-sac weight following *in vitro* administration of ACTH appears to be in agreement with the *in vitro* effects of ACTH.

It is of interest to note (Raud and Odell, 1971) that systemic pigeon crop-sac assay measures something other than PRL from crude pituitary extract, and something in addition to ACTH is also capable of eliciting a crop-sac response. Moreover, bioassays (Raud and Odell, 1971) of ACTH preparations of high purity demonstrate that ACTH is capable of stimulating crop-sac epithelium to a minor degree, but their crude preparation shows greater stimulation than PRL.

On the other hand, the structure activity relationship of ACTH indicates that the grouping of dibasic amino acids is one of the important affinity sites of the ACTH molecule (Ahmad and Ruqaiya, 2001). Improper purification always leads to removal of one or more dibasic amino acids at different positions and instead of most active ACTH 1-24; ACTH 1-18 and ACTH 1-10 with greatly reduced potency may be obtained (Hoffmann *et al.,* 1962, 1970; Li *et al.*, 1961; Ney *et al.,* 1965). Thus, the loss of ACTH activity, possibly reflects this phenomenon.

The *in vitro* thickening of the epithelium and increased weight with increase in time is also indicative of non-specific response for the systemic assay. Thus, the gross response of the crop-sac to the ACTH preparations demonstrates that materials other than PRL are also capable of stimulating the receptors of crop-sac epithelium.

Table 1
*In vitro* Crop-sac response to 10 I.U*ACTH 1-24 in isologues serum

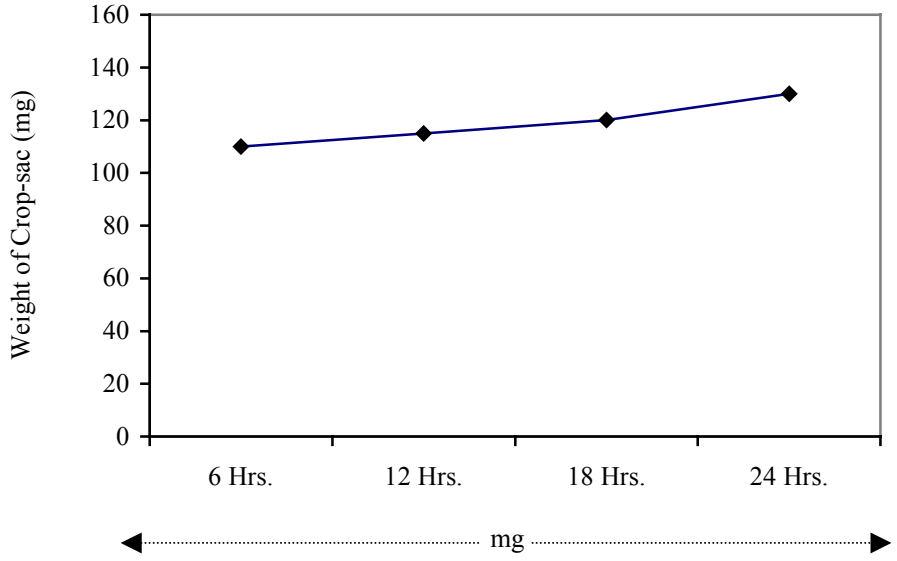
| CROP-SAC | NET WEIGHT GAIN (mg) AT 37°C | | | |
|---|---|---|---|---|
| No. | 6 Hrs. (mg) | 12 Hrs. (mg) | 18 Hrs. (mg) | 24 Hrs (mg) |
| 1 | 105 | 116 | 125 | 147 |
| 2 | 106 | 115 | 124 | 148 |
| 3 | 103 | 114 | 126 | 140 |
| 4 | 104 | 115 | 124 | 145 |
| 5 | 106 | 116 | 124 | 144 |
| 6 | 105 | 114 | 127 | 146 |
| 7 | 105 | 117 | 125 | 143 |
| 8 | 104 | 114 | 125 | 145 |
| 9 | 105 | 115 | 126 | 146 |
| 10 | 106 | 113 | 124 | 148 |
| 11 | 105 | 115 | 125 | 144 |
| 12 | 106 | 116 | 125 | 143 |
| **Average | 105 ± 0.95 | 115 ± 1.13 | 125 ± 0.95 | 145 ± 2.31 |

*ATCH supplied by Fredericksburg Chemical Lab. Ltd. Copenhagen, 1964.
**Each figure is the mean of 24 measurements ± SD.

Table 2
Comparison of effect *in vitro* ACTH 10 I.U. incubation on the pigeon crop-sac

| Source | ANOVA | | | |
|---|---|---|---|---|
| | DF | SS | MS | F |
| Treatment | 3 | 10745 | 3581.67 | *799.48 |
| Error | 44 | -197 | 4.48 | |
| Total | 47 | 10548 | | |

*= $P < 0.01$
Treatment = Time interval (6, 12, 18, and 24 hrs.) after incubation.

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