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Changes in Frog Water-Balance Activity of Hen's Blood

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Frog water-balance assay was applied to determine the level of vasotocin-like factor in the blood of laying hens. The water-balance activity in blood during oviposition was significantly higher than it was one or two hours before oviposition. However, the activity rapidly decreased to the level of pre-oviposition one hour after lay.

INTRODUCTION

It has been reported that the administration of neurohypophysial hormones induces premature oviposition in the hen (Riddle, 1921; Burrows and Byerly, 1942 ; Burrows and Fraps, 1942). Tanaka and Nakajo of Gifu University (1960, 1962) reported a decrease in the content of vasotocin in the posterior pituitary of hens coincident with oviposition and suggested that the hormone was released into the blood and caused oviposition. Douglas and Sturkie (1964) did not find a significant increase in the vasotocin level two to twenty minutes before oviposition, and suggested that vasotocin does not cause oviposition but that oviposition induces its release. On the other hand, Niezgoda ***et al.*** (1973) reported that 10 minutes before oviposition, the vasotocin level in blood increased significantly and showed the highest value during oviposition. They concluded that the hormone was involved in expulsion of the egg.

The purpose of the present experiment was to determine the level of vasotocin-like substance in the blood of hens by applying a frog water-balance assay.

METERIALS AND METHODS

Blood samples (10 ml) were obtained from the wing vein by a heparinized syringe with a 23 gauge needle. The plasma was deproteinized and neutralized according to the method of Sturkie and Lin (1966) ; after centrifugation of the blood at ***3,000*** rpm for 15 minutes, the plasma was deproteinized with 0.2g of perchloric acid, followed by centrifugation as above and the supernatant was neutralized by sodium carbonate.

This neutralized supernatant was then placed in a cellophane bag and dialyzed for two hours against running water. The content of the cellophane bag was freeze-dried and dissolved in 0.5 ml of 0.9% NaCl solution. Frog water-balance assay was carried out according to the method described by Tanaka and

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Nakajo (1962), except that the bladder was not emptied prior to weighing the frog after immersed in water for an experimental period of two hours.

RESULTS AND DISCUSSION

In a preliminary experiment the neutralized supernatant prepared by the method of Sturkie and Lin (1966) was lyophilized and dissolved in 0.5 ml of 0.9 % NaCl solution. Several frogs were subjected to the intraperitoneal injection of the preparations (0.5 ml/frog). All of the frogs so treated died with tetanic spasms immediately after the administration. This was not unexpected since it was considered that a trace of free perchloric acid, highly toxic substance, might be present in the preparations. However, such toxic effect on the frog could be eliminated by dialyzing the treated plasma against water. Figure 1 illustrates the water-balance activity of the plasma extract, represented by percent increase in body weight. Each point indicates the mean value of 5 to 14 hens. The activities during and immediately after oviposition were significantly higher than it was one or two hours before oviposition. However, the activity rapidly decreased to the level of pre-oviposition one hour after lay. This agreed with the results reported by previous investigators (Douglas and Sturkie, 1964 ; Sturkie and Lin, 1966 ; Niezgoda et *al.*, 1973), suggesting that the present method could be used for the assay of vasotocin-like or oxytocic activity in the blood of laying hen.

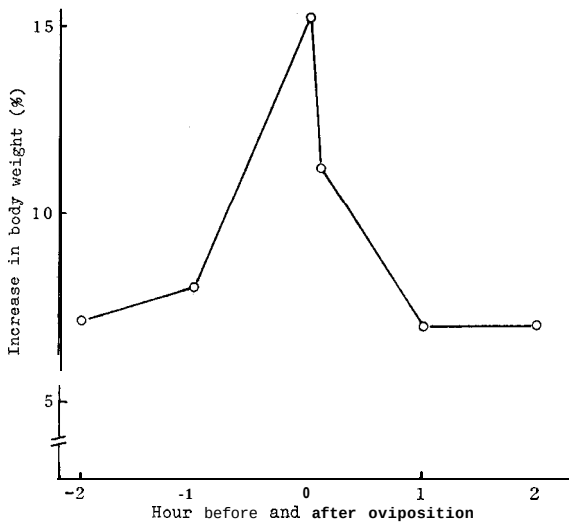

Fig. 1. The water-balance activity of the plasma extract,

It is evident that vasotocin-like activity in the blood of laying hens changes in association with oviposition, although such active factor in the hen's blood has not been identified. Sturkie and Lin (1967) suggested that the release of vasotocin bore no causal relation to oviposition, but it might be related to ovulation.

On the other hand, Rothchild and Fraps (1944) have reported that the ovarian ruptured follicle of the hen is an important factor in determining the time of lay of the egg. Recently, Tanaka and Nakada of Kyushu University (1974) have successfully extracted an active factor for inducing premature oviposition from ovarian remnants of ruptured follicles. Accordingly, additional work will be needed before the details of a mechanism of oviposition are understood.

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