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(Crotaphytus wislizenii)

PHILIP A. MEDICA, FREDERICK B. TURNER, AND DONALD D. SMITH

LABORATORY OF NUCLEAR MEDICINE AND RADIATION BIOLOGY

900 VETERAN AVENUE

UNIVERSITY OF CALIFORNIA, LOS ANGELES, CALIFORNIA 90024

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Hormonal Induction of Color Change in Female Leopard Lizards

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Philip A. Medica, Frederick B. Turner and Donald D. Smith

Laboratory of Nuclear Medicine and Radiation Biology

University of California, Los Angeles, 90024

ABSTRACT -- Progesterone is the direct cause of the red-orange pigmentation in gravid Crotaphytus wislizenii. Follicle stimulating hormone (FSH) will induce the color change in leopard lizards in 7-21 days depending upon the season. Estrogen alone had no apparent effect on color change, but when administered before progesterone it appeared to have a priming effect.

INTRODUCTION

During the reproductive season female Crotaphytus wislizenii develop red-orange coloration on the sides of the face, body and ventral surface of the tail (VanDenburgh, 1922; Smith, 1946; Stebbins, 1954; and Mertens, 1960). The red-orange color develops in females in southern Nevada when yolked follicles reach a size of 12 mm. (Turner et al., 1969). The color fades after the eggs are deposited but returns to its full brightness if a second clutch of eggs is produced. Similarly, female collared lizards (Crotaphytus collaris) develop orange spots during the reproductive season. Cooper and Ferguson (1972a, b) have shown that this coloration is induced by progesterone, the production of which is mediated by follicle-stimulating hormone (FSH).

The primary purpose of this paper is to examine the mechanism of color change among female leopard lizards. We postulate that the sequence of events is as follows: FSH stimulates the ovary to produce progesterone and estrogen during the growth and development of follicles (Ferguson, 1966; Licht, 1970; Licht and Hartree, 1971), and the color change is then induced by the ovarian hormones. Hence, we would expect the administration of FSH to induce red-orange pigmentation in females with ovaries, but to have no visible effects on females lacking ovaries. We first attempted to determine whether FSH would induce color change in normal female leopard lizards. We then tested whether females lacking ovaries would undergo color change if treated with FSH. Finally, the effects of progesterone and estrogen upon color change were evaluated.

METHODS

All lizards were collected in Nye County, Nevada. They were placed in 10-gallon reptile cages which were one meter below a 20-w ultra-violet

lamp, and 50 cm below a 250-w sunlamp. These lights provided heat and ultra-violet light and were set on a 14-hour day cycle. Air temperatures during the day were 29° C - 34° C (and did not fall below 21° C at night), but soil temperatures were higher and lizards were able to maintain normal body temperatures.

Lizards were maintained on a diet of Uta stansburiana, and crickets (Acheta domestica). Experimental animals were injected intraperitoneally with 250 ug NIH-FSH-S6 in 0.25cc saline three times weekly (on Monday, Wednesday, and Friday). Progesterone was injected intramuscularly (0.1mg/0.1cc), and estrogen at 1 ug per intramuscular injection. These hormones were administered on a daily basis. The study was conducted in four experiments.

Expt. I: Eight C. wislizenii were maintained from September 1, 1969, until November 24, 1969. Injections of FSH were initiated with four experimental animals on October 20, 1969, and continued until November 24, 1969. These four Crotaphytus were sacrificed on November 26, 1969, and ovaries and oviducts removed and weighed.

On November 27, 1969, the four control animals were anesthetized with chloroform and ovaries and oviducts removed and weighed. Three of these individuals died within a few days. The other survived until January 13, 1970, and between December 1, 1969 and January 9, 1970, received 17 injections of FSH. This phase of the experiment was repeated in June of 1972 using two ovariectomized females and one normal female. Injections were administered beginning June 13, 1972 and ended June 30, 1972. Ether was used as an anesthesia with the 1972 lizards.

Expt. II: This portion of the study began on June 28, 1970, using six female Crotaphytus wislizenii. One was an apparently sterile 70-month old female which had been subjected to continuous gamma irradiation since

birth (Turner and Lannom 1968, Turner et al., 1972). Of these six lizard, three (two normal and the presumably sterile female) were injected with FSH beginning June 28, 1971. The other three females were maintained as controls.

Expt. III: Administration of progesterone to four female Crotaphytus wislizenii, two ovariectomized and two normal, began on May 2, 1972. Two additional females were maintained as controls with no injections given.

Expt. IV: Estrogen was administered to two ovariectomized females starting June 13, 1972 and continued for as long as 12 days. A third lizard was given five injections of estrogen and then concurrent injections of estrogen and progesterone.

RESULTS

Expt. I: All four of the experimental lizards changed color, and the four controls remained as they had been at the time of capture.

All of the experimental C. wislizenii contained yolked follicles, ranging from 3-12 mm in size. Three lizards (1, 2, and 3) contained large amounts of yolk in the coelom suggesting rupture of the follicles. The ovaries of two females (1, 2) contained corpora lutea, indicating that follicles had been ovulated. One lizard (1) contained eggs in the oviduct which were 7 mm long (approximately 1/3 normal size). A comparison of the weights and numbers of follicles is given in Table 1. The ovaries removed from the control lizards contained unyolked follicles 2 mm in size. The ovariectomized female experienced no change in color during the 38 days studied. The experiment conducted in June 1972 produced a color change in 7 days in the normal female and no change after 17 days in the ovariectomized females.

The time required for color change varied according to the time of

year at which the experiments were conducted. Table 2 summarizes results of the FSH experiments.

Expt. II: Of the three female leopard lizards injected with FSH, the two normal females began to change color after three injections (8 days). The irradiated female never showed any indication of color change. By July 26, 1971, a total of thirteen injections had been administered over a period of 28 days. Simultaneously, three other normal females were kept under the same light and temperature conditions and no color change occurred.

Expt. III: Of the four female C. wislizenii injected with progesterone (two ovariectomized and two normal) all began to change color between 5 and 10 days. The two control females never changed color.

Expt. IV: The two ovariectomized females injected with estrogen never changed color. One survived 6 days, the other 13 days. However, the female pretreated with estrogen and then given concurrent estrogen and progesterone injections began to turn red-orange after only one injection of progesterone.

DISCUSSION

Evidently, follicle-stimulating hormone injected three times a week will induce color change in normal female Crotaphytus wislizenii within seven days in June, eight days in July, and 16 to 21 days in October-November. Progesterone is the direct cause of the red-orange color in C. wislizenii as is true of Crotaphytus collaris (Cooper and Ferguson, 1972 a,b). The priming effect of estrogen, serving to increase the rapidity of color change, was also observed by Cooper and Ferguson (in press).

The control lizards in all phases of the experiment were maintained

under conditions of light and temperature which might have been expected to promote some follicular development. Yet no such growth occurred. It is possible that, following the normal period of reproduction, female C. wislizenii experience a refractory period similar to that observed in Uta (Tinkle and Irwin, 1965) and Sceloporus spp. (Marion, 1970; Callard et al., 1972) during which reproduction cannot be stimulated by light or temperature.

Other authors have speculated as to the functional significance of the red-orange coloration of female C. wislizenii during the breeding season (Montanucci, 1965; Fitch, 1967; and Mayhew, 1968). We have observed brightly colored females copulating in southern Nevada, as has been observed elsewhere (Montanucci, 1965; and Mayhew, 1968). Cooper and Ferguson (1972 b) observed courting behavior in C. collaris when the orange pigmentation was present. The coloration may be an attractant to male lizards and serve to insure fertilization of the maturing ova, because the pigmentation apparently does not develop in females with follicles (of the first clutch) <12 mm in size. Ovulation takes place when yolked follicles are between 15-16 mm. In other genera, e.g., Holbrookia, Callisaurus, and Cophosaurus, the breeding coloration of gravid females is displayed to the courting male and appears to deter his advances (Clarke, 1965).

The results of these experiments have bearing on a continuing study of leopard lizards occupying three 8-ha fenced areas in Rock Valley, Nevada. One of these areas has been continuously exposed to gamma radiation from a centrally located source of ¹³⁷Cs since 1964 (French, 1964). Leopard lizards in the irradiated area have not reproduced since the summer of 1966, and females in this area do not experience normal color changes during the spring. Three females taken from the irradiated

area in 1969 showed no apparent ovarian tissue (Turner et al., 1972), and this appeared to be the basis of female sterility. It was possible, however, that undetected ovarian tissue remained and that the sterility was due to some more proximal failure, e.g., in pituitary function.

To examine this question, Licht experimented with three female Cnemidophorus tigris from the irradiated area (Turner et al., 1972). Laparotomies prior to hormone treatment revealed no detectable ovarian tissue in these females. FSH was then administered to these females and to five controls. Among the controls yolk follicles were produced and the oviducts hypertrophied. Examination of the three irradiated females following hormone injections revealed no ovarian tissue and oviducts comparable to those of non-reproductive females. This experiment confirmed the absence of competent ovarian tissue in some of the irradiated whiptails and indicated that the sterility was owing to absence of competent ovarian tissue. The failure of the apparently sterile female leopard lizard to change color when treated with FSH (Expt. II) indicates the absence of ovaries, which is again in accord with anatomical observations (Turner et al., 1972).

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Laboratory of Nuclear Medicine and Radiation Biology

University of California, Los Angeles, 90024

TABLE 1. COMPARISONS OF FEMALE Crotaphytus wislizenii INJECTED
WITH FSH (1-4) AND CONTROL FEMALES (5-8) IN OCTOBER-NOVEMBER 1969.
(Weight of oviducts in females 5-8 is left oviduct X 2).

Animal Number	Body Weight (g)	Oviducts (g)	Ovaries (g)	Corpora Lutea	Number and Size of Yolked Follicles Present	
					3-7mm	8-12mm
1	25.5	0.48	2.17	3	7	5
2	63.1	0.70	4.56	3	1	7
3	64.4	0.55	3.54	-	15	5
4	33.2	0.56	4.14	-	17	-
5	39.3	0.05	0.07	-	No yolked foll- icles present	
6	20.2	0.04	0.04	-	"	
7	14.7	0.03	0.02	-	"	
8	12.7	0.01	0.03	-	"	

TABLE 2. DAYS ELAPSED BEFORE COLOR CHANGE IN NORMAL FEMALE
Crotaphytus wislizenii INJECTED WITH FSH DURING JUNE, 1972;
 JUNE-JULY, 1971; AND OCTOBER-NOVEMBER, 1969.

Animal Number	June 1972	July 1971		October-November 1969			
	1	1	2	1	2	3	4
Days to begin changing color	7	8	8	18	21	16	18
Days to become brightly colored	13	18	16	23	31	21	27