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The Influence of Hypothalamic Steroid Implants on Ovulation and Ovarian Growth and Function in the Iguanid Lizard, Sceleporus cyanogenys

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THE INFLUENCE OF HYPOTHALAMIC STEROID IMPLANTS ON OVULATION AND OVARIAN GROWTH AND FUNCTION IN THE IGUANID LIZARD, SCELOPORUS CYANOGENYS

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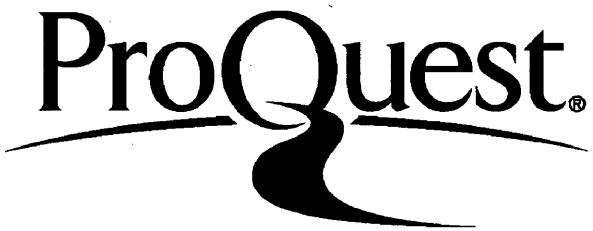

APPROVAL SHEET

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ABSTRACT

The influence of intrahypothalamic, intrapituitary and subcutaneous steroid implants on ovulation, ovarian growth and function were studied in Sceloporus cyanogenys. Implants of crystalline estrogen in the median eminence region of the hypothalamus were highly effective in inhibiting ovulation, but did not influence ovarian growth. Of the three estrogens tested, only estradiol 17 β was 100% effective in ovulation inhibition. In addition, implants of estradiol benzoate and estradiol undecylate inhibited ovarian steroid production, as indicated by oviduct growth. Subcutaneous and intrapituitary implants of estradiol 17 β did not influence ovulation, ovarian growth or function. Intrahypothalamic implants of progesterone inhibited ovarian growth and prevented ovulation in 50% of the experimental animals. Of animals implanted with cholesterol, only 25% did not ovulate. No marked changes in liver or adrenal weight that could be clearly correlated with the experimental treatment were observed. However, intrahypothalamic and intrapituitary estrogen depots significantly increased total plasma protein due primarily to an increase in fraction number three.

THE INFLUENCE OF HYPOTHALAMIC STEROID IMPLANTS ON OVULATION AND OVARIAN GROWTH AND FUNCTION IN THE IGUANID LIZARD, SCELOPORUS CYANOGENYS

INTRODUCTION

Evidence concerning the role of the hypothalamus in the control of the adenohypophysis has been summarized by Harris (1948, 1955). Since that time a large body of evidence relating to specific hypothalamic areas concerned with gonadal control has been revealed by lesion and hormone implantation techniques in mammals. Lesions involving the median eminence result in not only anestrus but ovarian and uterine atrophy in guinea pigs (Dey et al., 1940; Dey, 1941, 1943), rats (D'Angelo, 1959; Cooke, 1959; Flerko and Bardos, 1959), cats (Laqueur et al., 1955), and in rabbits (Flerko, 1953). Lesions placed between the optic chiasm and the median eminence result in constant vaginal estrus and polyfollicular ovaries in the guinea pig (Dey et al., 1940; Dey, 1941, 1943). In addition, repeated periods of prolonged diestrus with hyperluteinized ovaries, occur in rats with dorsally placed lesions involving parts of the paraventricular and dorso-medial nuclei (Flerko and Bardos, 1959). Hormone and ovarian autograft implantation experiments have revealed the importance of steroid sensitive units within the hypothalamus in gonadal feedback control (Flerko and Szentagothai, 1957; Holhweg and Daume, 1959; Lisk, 1960, 1963).

Observations on mammals have been extended to birds by Rothchild and Fraps (1949), Ralph and Fraps (1959, 1960), Assenmacher (1957 a, b, 1958), and Kordon and Gogan (1964). Also Dierickx (1965, 1966, 1967)

has indicated that the gonadotrophic center is present in the middle hypothalamus and that hypothalamic structures necessary for ovulation may be located in the pre-optic nucleus in the amphibian Rana temporaria. A single report demonstrates the importance of specific hypothalamic areas for gonadal development in the goldfish (Peters, 1970).

In reptiles, a report by Lisk (1967) suggested the presence of steroid sensitive hypothalamic areas important in the onset of seasonal gonadal development in Dipsosaurus dorsalis, the desert iguana. No studies extending this observation have been made. The present experiment is an attempt to clarify some of the interactions of gonadal steroids with the hypothalamus in the control of ovarian growth and subsequent ovulation in the ovoviviparous lizard, Sceloporus cyanogenys.

MATERIALS AND METHODS

A. ANIMALS

Adult female Sceloporus cyanogenys, the ovoviviparous blue spiny lizard, were obtained in two groups from a commercial supplier in Texas during the month of December. Animals were housed in 20 sq. ft. enclosures on a bedding of "Sanicel" (Paxton Processing Co.). Room temperature was maintained at 28° ± 2°C during the day and fell to 22° ± 2°C during the night. A 250 watt heat lamp was suspended at the edge of the pen which allowed a maximum of 37°C at the floor with a decreasing gradient across the pen. Shade was supplied and water was available ad libitum. Heat lamps and overhead fluorescent lights were automatically controlled on a 12 hour light - 12 hour dark regime. Animals were fed commercially supplied crickets daily.

The animals were divided into the following experimental groups for implantation of steroids:

Series 1 (Received and implanted early December)

A. Control start (autopsied on day 0).

B. Cholesterol intrahypothalamic implants.

C. Progesterone intrahypothalamic implants.

D. Estradiol 17 β intrahypothalamic implants.

E. Estradiol 17 β subcutaneous implants.

F. Estradiol 17 β intrapituitary implants.

G. Sham pituitary implants.

Series 2 (Received and implanted late December)

Twenty-three animals of this series were laparotomized at the start of the experiment to determine the extent of gonadal development. Four (17%) of these animals had ovulated and possessed developing embryos in the oviduct. Five of these animals were autopsied as beginning controls, 3/5 being preovulatory.

A. Control start (autopsied on day 0).

B. Control end (autopsied on day 21).

C. Cholesterol intrahypothalamic implants.

D. Estradiol benzoate intrahypothalamic implants.

E. Estradiol undecylate intrahypothalamic implants.

The experimental period was 21 days with day 0 being the time of implantation.

B. HORMONAL IMPLANTS

Implants were prepared from 32 gauge stainless steel tubing dipped into the steroid heated to its melting point and stereotaxically placed according to Callard and Willard (1969). Quantities of steroid lodged in the tubes were estimated spectrophotometrically both prior to and following three weeks implantation as follows: 1) Before implantation: Estradiol 17 β 24 ± 1.0 µg (n=10), progesterone - 39 ± 7.5 µg (n=8). 2) After implantation: Estradiol 17 β 12 ± 2.0 µg (n=8), progesterone - 11.8 ± 6.5 µg (n=7).

Subcutaneous implants were made by inserting a short piece of hormone laden tubing through an incision in the lateral body epidermis.

After three weeks implantation 15 ± 2.0 μg (n=5) steroid was found remaining in the tube. Implants of steroid in the adenohypophysis were made by exposing the gland ventrally through a hole made in the basisphenoid with a dental pick. The steroid was implanted directly into the anterior lobe tissue and the hole in the basisphenoid plugged with Gelfoam (Upjohn Company). Sham pituitary implants were performed in the same manner, but no steroid was implanted in the gland.

At autopsy animals were killed by decapitation and the blood collected in a heparinized centrifuge tube. Plasma was removed following centrifugation and frozen for later analysis. The liver, adrenals, thyroid, ovaries and oviducts were cleaned of adherant tissue and weighed. The numbers of ova and developing embryos were recorded. Heads were fixed in 10% formol and after 48 hours transferred to 20% ethanol and the brains removed. Gross localization of the probe in situ was made if possible. Whole brains were mounted in 5% gelatin, sectioned at 40 microns in a cryostat and stained with thionin and subsequently examined microscopically for accurate localization of the implant placement. Serum proteins were separated on cellulose acetate strips and stained with oil red O. After clearing the strips were scanned in a Gelman Model 39272 Scanner and quantified. Total plasma proteins were estimated using the Biuret reagent.

C. STATISTICAL METHODS

All data were analyzed using Student's t test with the exception of ovulation frequency (Table I) which was analyzed using confidence

intervals for binomial proportions (Steel and Torrie, 1960). All data in the tables are given as means plus or minus the standard error (except Table I).

The 5% level is the chosen level of significance. However, where a probability level above 5% occurs and there is reason to suspect biological significance the level is included in the text. A probability level of 1% is designated as highly significant.

Where pre- and postovulatory animals occurred in a single group (cholesterol, and estradiol 17 β intrapituitary in series 1 and estradiol benzoate implanted animals in series 2) average values for total plasma proteins, liver and adrenal weights, were calculated for both pre- and postovulatory animals within each group. Since no significant differences were observed, these values were then combined to give a mean which included both pre- and postovulatory animals for these groups.

RESULTS

A. LOCATION OF IMPLANTS

All brain implants were located in the hypothalamus. Estrogen implants ending in the median eminence region were most effective in inhibiting ovulation, a total of 14/15 of such animals being preovulatory. All estradiol 17 β implants ended in the median eminence. Of the 5 estradiol benzoate implanted animals which ovulated, one implant was in the lateral hypothalamus and another through the surface of the antero-medial region of the hypothalamus. Of the other three, one implant ended in the median eminence, and two in the antero-medial hypothalamus. Since 17% of the control animals of series 2 had ovulated, it is possible that these last 3 animals had ovulated prior to arrival in the laboratory. However, comparisons of the extent of embryonic development in pregnant animals were not made, and it is not possible to assess the validity of this possibility. In the estradiol undecylate group, one animal ovulated and the implant in this animal was through the surface of the antero-medial hypothalamus.

No attempt was made to recover estrogen implants from the adenohypophysis, although pituitaries were extirpated after fixation of the whole head. Pituitaries from both sham-implanted and implanted animals were misshapen due to pressure exerted by the Gelfoam inserted to plug the hole in the floor of the skull.

B. OVULATION, OVARIAN AND OVIDUCT WEIGHTS

Series 1. (Tables I and II).

All starting controls were in the follicular growth stage. Three weeks after implantation ovulation occurred in 60% of the cholesterol group and 50% of the progesterone group. Animals with subcutaneous implants of estradiol 17 β all ovulated. In contrast, none of the animals with intrahypothalamic implants of estradiol 17 β ovulated during the three weeks. The difference in ovulation frequency between animals with subcutaneous implants and beginning controls is significant, as is the difference between subcutaneous and intrahypothalamic estrogen implants. Neither adenohypophyseal estradiol 17 β implants nor the sham operation significantly affected ovulation frequency.

Ovaries of animals arriving in the laboratory were in the growth phase. All animals receiving subcutaneous estradiol 17 β implants ovulated and hence the ovaries of these animals were quite small at autopsy consisting principally of corpora lutea (see Table II). Ovaries of preovulatory animals with implants of cholesterol and intrahypothalamic estradiol 17 β were significantly larger than initial controls. Ovaries from progesterone implanted animals that did not ovulate were not significantly different from initial controls, but were significantly smaller than those of estrogen implanted animals.

Oviducts of start controls, estradiol 17 β intrahypothalamic, and cholesterol implants that were preovulatory were similar in weight. Animals with estradiol 17 β subcutaneous implants were all postovulatory

after three weeks. After intrahypothalamic steroid implantation, oviducts of postovulatory animals with cholesterol and progesterone implants were significantly smaller than those of animals with subcutaneous estradiol 17 β implants. Ovarian and oviduct weights of postovulatory animals with adenohypophyseal estrogen depots were similar to those of other postovulatory animals. No meaningful comment regarding these parameters in the sham-operated animals can be made due to mortality in this group.

No differences in follicular numbers were observed. However, animals with subcutaneous estrogen implants had significantly smaller oviducal eggs than either cholesterol or progesterone animals.

Series 2. (Tables I and III).

Three weeks after intrahypothalamic steroid implantation, 33% of the estradiol benzoate and 9% of the estradiol undecylate animals were postovulatory. In contrast, animals with cholesterol implants were 90% ovulated, and unoperated controls, 100%. The ovulation frequencies for estradiol undecylate treated animals were significantly lower than those for both the end controls and the cholesterol treated animals.

In this series, ovaries of start control preovulatory animals were significantly larger than those of start controls in series 1. At autopsy, ovaries of animals with estradiol benzoate and estradiol undecylate implants that were not ovulated were not different from those of the start controls. Weights of postovulatory ovaries of all experimental groups showed a significant decline during the experimental period when compared

to the start controls.

Three weeks after steroid implantation, oviducts of preovulatory animals with estradiol benzoate and estradiol undecylate implants were significantly smaller than the beginning controls. In contrast, postovulatory beginning controls had oviducts which were not different from postovulatory estradiol benzoate animals, cholesterol animals, or the three week controls. No differences in either follicular number or mean oviducal egg weight were observed.

C. LIVER WEIGHTS AND PLASMA PROTEINS (Tables IV and V)

Liver weights in series 1, of start controls and those with estradiol 17 β intrapituitary implants are larger than those of all other experimental groups, both pre- and postovulatory. The difference is highly significant. Comparison of liver weights from hypothalamic estrogen implanted animals and those of subcutaneous estrogen implanted animals revealed a highly significant difference. However, liver weights of animals with estradiol implants in the hypothalamus are significantly larger than those of either the cholesterol or progesterone intrahypothalamic groups ($.1 < p > .05$).

All animals in series 2 which were preovulatory have similar liver weights regardless of the treatment. As indicated by a comparison of beginning and final controls, or beginning controls and cholesterol implanted animals, liver weight decreased significantly following ovulation. This is further supported by the comparison of liver weights of pre- and postovulatory animals within the estradiol benzoate group where those of

preovulatory animals are significantly higher. Comparison of start control liver weights from series 1 and series 2 reveals that those of series 1 are significantly larger.

The total plasma protein of animals with either intrahypothalamic or intrapituitary estradiol 17 β implants is higher than that for either starting controls, progesterone, or cholesterol implanted animals ($p < .001$ for all three comparisons). Total plasma protein of animals with subcutaneous estrogen implants and intrahypothalamic progesterone implants is similar to that for the starting controls. However, animals with cholesterol implants have a lower total plasma protein than the control animals.

In experimental series 2, although the estrogen implanted animals have higher total plasma protein values than the start controls, the cholesterol treatment, or the end controls, no statistically significant differences are observed. It is suggested, however, that this trend is biologically important since it parallels the effects in series 1.

Quantitative examination of the plasma protein fractions (Table V) indicates that implantation of estradiol 17 β in the hypothalamus causes a significant diminution in all protein fractions with the exception of fraction 3, (equivalent to mammalian alpha 2 globulin) which increases. Control, progesterone, and cholesterol treated animals are similar. In series 2 no marked quantitative changes in plasma protein fractions are observed. However, trends similar to those noted in series 1 are seen, particularly in fraction 3. No qualitative changes are noted in any

group, only 5 protein bands being observed.

D. ADRENAL WEIGHTS (Table VI)

Adrenal weights are highly variable. No significant differences are observed, except in series 2 where start controls are larger than all other groups.

TABLE I

THE INFLUENCE OF HORMONAL IMPLANTS ON OVULATION IN SCELOPORUS CYANOGENYS

Experimental Groups	Ovulation No.	Ovulation %	Confidence Interval (.95 Level)	
Series 1				
Control start	0/13	0.0	0.0	26.0
Cholesterol, IH	6/10	60.0	26.0	87.0
Progesterone, IH	5/10	50.0	18.0	82.0
Estradiol 17 β, IH	0/10	0.0	0.0	32.0
Estradiol 17 β, SC	5/5	100.0	63.0	100.0
Estradiol 17 β, IP	5/7	71.5	32.0	96.0
Sham Pituitary Implant	6/6	100.0	63.0	100.0
Series 2				
Control start	4/23	17.0	5.0	37.0
Control end	5/5	100.0	63.0	100.0
Cholesterol, IH	9/10	90.0	47.0	100.0
Estradiol benzoate, IH	3/9	33.0	8.0	67.0
Estradiol undecylate, IH	1/11	9.0	0.0	40.0

IH = intrahypothalamic
SC = subcutaneous
IP = intrapituitary
No. = $\frac{\text{number ovulated}}{\text{total in treatment}}$

TABLE II

OVARIAN AND OVIDUCT WEIGHTS FROM PRE- AND POSTOVULATORY SCELOPORUS CYANOGENYS, SERIES 1

Treatment	n	Preovulatory Animals			Postovulatory Animals		
		Ovary g/100 g	No. Follicles	Oviduct g/100 g	Ovary g/100 g	Mean Egg Wt. (g)	Oviduct g/100 g
Control start	13	6.79 ± 0.66	—	0.68 ± 0.07	—	—	—
Cholesterol, IH	11	10.13 ± 0.99[5]	13.0 ± 1.2[5]	0.61 ± 0.08[5]	0.39 ± 0.13[6]	0.18 ± 0.01[6]	0.79 ± 0.05[6]
Progesterone, IH	10	8.27 ± 1.08[5]	15.4 ± 3.1[5]	0.63 ± 0.13[5]	0.28 ± 0.07[5]	0.21 ± 0.02[6]	0.73 ± 0.03[5]
Estradiol 17 β, IH	10	11.24 ± 0.76	13.7 ± 0.5	0.71 ± 0.04	—	—	—
Estradiol 17 β, SC	5	—	—	—	0.21 ± 0.07	0.12 ± 0.01	0.94 ± 0.05
Estradiol 17 β, IP	7	13.94[2]	10[2]	0.80[2]	0.46 ± 0.22[5]	—	0.98 ± 0.16[5]
Sham Pituitary Implant	2	—	—	—	2.38[2]	—	1.126[2]

IH = intrahypothalamic
SC = subcutaneous
IP = intrapituitary
Superscript numbers represent actual 'n' when group is split into pre- and postovulatory animals.

TABLE III

OVARIAN AND OVIDUCT WEIGHTS FROM PRE- AND POSTOVULATORY SCELOPORUS CYANOGENYS, SERIES 2

Treatment	n	Preovulatory Animals			Postovulatory Animals		
		Ovary g/100 g	# Follicles	Oviduct g/100 g	Ovary g/100 g	Mean Egg Wt. (g)	Oviduct g/100 g
Control start	5	11.99 ± 0.41[3]	14.3 ± 2.9[3]	0.86 ± 0.12[3]	0.46[2]	0.17 ± 0.02[2]	1.04[2]
Control end	5	—	—	—	0.09 ± 0.01	0.25 ± 0.07	0.90 ± 0.09
Cholesterol, IH	10	11.66[1]	12[1]	0.89[1]	0.20 ± 0.07[9]	0.25 ± 0.02[9]	0.89 ± 0.02[9]
Estradiol benzoate, IH	11	11.98 ± 0.88[6]	14.7 ± 1.7[6]	0.57 ± 0.02[6]	0.22 ± 0.11[5]	0.27 ± 0.04[5]	0.93 ± 0.08[5]
Estradiol undecylate, IH	10	11.30 ± 0.75[10]	15.7 ± 0.8[10]	0.56 ± 0.04[10]	0.11[1]	—	0.89[1]

IH = intrahypothalamic
SC = subcutaneous
IP = intrapituitary
Superscript numbers represent actual 'n' when group is split into pre- and postovulatory animals.

TABLE IV

LIVER WEIGHTS AND TOTAL PLASMA PROTEINS IN FEMALE SCELOPORUS CYANOGENYS

Treatment	n	Liver (g/100 g)	n	Plasma Protein (g/100 g)
Series 1				
Control start	13	2.49 ± 0.08	7	5.46 ± 0.16
Cholesterol, IH	10	1.65 ± 0.18	7	4.05 ± 0.19
Progesterone, IH	10	1.67 ± 0.23	9	4.93 ± 0.22
Estradiol 17 , IH	10	2.07 ± 0.10	9	7.04 ± 0.55
Estradiol 17 , SC	5	1.38 ± 0.09	4	5.95 ± 0.41
Estradiol 17 , IP	7	2.92 ± 0.27	5	9.37 ± 1.09
Sham Pituitary Implant	2	1.74	2	3.05
Series 2				
Control start	5	1.81 ± 0.21	3	4.08 ± 0.59
Control end	5	1.00 ± 0.04	4	4.34 ± 0.65
Cholesterol, IH	10	1.25 ± 0.11	8	4.14 ± 0.20
Estradiol benzoate, IH (Pre)	5	1.86 ± 0.20	5	5.58 ± 1.12
Estradiol benzoate, IH (Post)	5	1.18 ± 0.04	5	3.98 ± 0.28
Estradiol undecylate, IH	11	1.71 ± 0.12	11	5.18 ± 0.30

IH = intrahypothalamic
SC = subcutaneous
IP = intrapituitary

TABLE V

QUANTITATIVE CHANGES IN PLASMA PROTEIN FRACTIONS IN SCELOPORUS CYANOGENYS*

Treatment	n	(A/G)	Protein Fraction Number and Corresponding Fraction from Mammalian Plasma 1 (Albumin)	2 (Globulinα)	3 (Globulinα)	4 (Globulinβ)	5 (Globulinγ)
Series 1							
Control start	7	.398 ± .025	.283 ± .013	.253 ± .006	.120 ± .011	.174 ± .017	.170 ± .016
Cholesterol, IH	7	.400 ± .033	.283 ± .016	.249 ± .026	.122 ± .006	.186 ± .009	.160 ± .012
Progesterone, IH	9	.386 ± .027	.280 ± .011	.224 ± .016	.135 ± .009	.190 ± .017	.171 ± .014
Estradiol 17 β, IH	9	.250 ± .025	.198 ± .015	.181 ± .019	.394 ± .055	.127 ± .012	.101 ± .028
Estradiol 17 β, SC	4	.248 ± .080	.190 ± .049	.313 ± .035	.218 ± .033	.157 ± .020	.122 ± .015
**Estradiol 17 β, IP	6	.065 ± .010	.061 ± .010	.807 ± .020		.068 ± .011	.056 ± .007
Sham Pituitary Implant	2	.321 ± .043	.243 ± .043	.228 ± .012	.278 ± .007	.140 ± .040	.113 ± .002
Series 2							
Control start	3	.438 ± .080	.300 ± .039	.230 ± .004	.114 ± .009	.211 ± .065	.145 ± .039
Control end	4	.428 ± .031	.299 ± .016	.231 ± .004	.135 ± .015	.141 ± .012	.194 ± .025
Cholesterol, IH	8	.481 ± .045	.320 ± .023	.220 ± .009	.152 ± .010	.156 ± .024	.152 ± .009
Estradiol benzoate, IH	10	.373 ± .046	.265 ± .023	.206 ± .005	.178 ± .030	.171 ± .016	.180 ± .013
Estradiol undecylate, IH	11	.332 ± .027	.246 ± .015	.215 ± .010	.175 ± .029	.191 ± .017	.173 ± .011

IH = intrahypothalamic
SC = subcutaneous
IP = intrapituitary
A/G = Albumin to Globulin ratio
** In this group fractions 2 and 3 were inseparable and appear as a single value.
* Quantities of plasma proteins are expressed as a fraction of one (1).

TABLE VI

ADRENAL WEIGHT CHANGES IN FEMALE SCELOPORUS CYANOGENYS

Treatment	n	Body Wt. (g)	Adrenal mg/100 g
Series 1			
Control start	13	37.3 ± 3.4	11.26 ± 2.96
Cholesterol, IH	10	43.8 ± 1.2	10.97 ± 0.79
Progesterone, IH	9	57.7 ± 5.2	8.68 ± 1.58
Estradiol 17 β, IH	10	47.7 ± 2.5	10.03 ± 2.09
Estradiol 17 β, SC	5	29.6 ± 2.0	13.35 ± 3.02
Estradiol 17 β, IP	7	48.0 ± 4.7	7.75 ± 0.73
Sham Pituitary Implant	2	45.3	5.25
Series 2			
Control start	4	46.3 ± 5.5	14.99 ± 2.18
Control end	5	49.7 ± 8.7	7.77 ± 1.57
Cholesterol, IH	10	52.2 ± 4.1	8.50 ± 0.73
Estradiol benzoate, IH	11	48.8 ± 3.1	8.01 ± 0.68
Estradiol undecylate, IH	11	47.5 ± 2.6	10.84 ± 0.81

IH = intrahypothalamic
SC = subcutaneous
IP = intrapituitary

TABLE VII

STATISTICAL COMPARISONS OF PLASMA PROTEIN FRACTIONS
(COMPARED TO CONTROL START)

Treatment	(A/G)	1 (Albumin)	2 (Globulinα)	3 (Globulinα)	4 (Globulinβ)	5 (Globulinγ)
Series 1						
Cholesterol, IH	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Progesterone, IH	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Estradiol 17 β, IH	.001↓	.001↓	.01↓	.001↑	.05 ↓	.1 <p> .05↓
Estradiol 17 β, SC	n.s.	.1 <p> .05↓	n.s.	.05 ↑	n.s.	.1 <p> .05↓
Estradiol 17 β, IP	.001↓	.001↓	——	——	.001↓	.001↓
Sham Pituitary Implant	n.s.	.05 ↓	n.s.	.01 ↑	.05 ↓	n.s.
Series 2						
Control end	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cholesterol, IH	n.s.↑	n.s.↑	n.s.	.05↑	n.s.	n.s.
Estradiol benzoate, IH	n.s.↓	n.s.↓	n.s.	.1 <p> .05↑	n.s.	n.s.
Estradiol undecylate, IH	n.s.↓	n.s.↓	n.s.	.1 <p> .05↑	n.s.	n.s.

IH = intrahypothalamic
SC = subcutaneous
IP = intrapituitary
(↓↑) indicates direction of change away from control

DISCUSSION

In the first series of experiments, ovarian growth continued in the laboratory and was not influenced by estradiol implants. Ovaries of both cholesterol and progesterone implanted animals which did not ovulate are smaller than those of the estrogen implanted animals. Further, gonads of progesterone implanted animals are not significantly larger than those of the controls. In contrast to the apparent absence of an effect on ovarian growth, intrahypothalamic estrogen had a pronounced inhibitory effect on ovulation. In addition, both cholesterol and progesterone implants had some effect on ovulation frequency. Since none of the control animals had ovulated at the start of the experiment, and all but 2/18 of the other control groups (estradiol 17 β subcutaneous, pituitary implants, and sham pituitary implants) ovulated before the end of the experimental period, the influence of intrahypothalamic estradiol on ovulation appears to be an important one. The argument that estrogen acts at the pituitary level (Bogdanove, 1963), carried to the adenohypophysis by the portal vessels, is dispelled to a certain extent by the relative ineffectiveness of estrogen implants into adenohypophyseal tissue. However, it could still be argued that such implants are likely to be less effective than hypothalamic implants if one assumes that distribution of the hormone from the hypothalamus via the hypophysial portal system is more effective than diffusion from the site of

location in the anterior lobe. This assumption is difficult to verify, but it could be tested by using autoradiographic techniques.

Since subcutaneous implants were without effect on ovulation, a systemic (as opposed to intrahypothalamic) effect of the hormone on ovulation can be discounted. The amounts of hormone released from both subcutaneous and intrahypothalamic implants (about 17 μg/3 weeks or 0.8 μg/day) are in fact quite large considering the potency of this hormone, if one uses mammalian criteria.

If follicular growth is dependent principally upon hypophyseal FSH and ovulation upon LH or a combination of FSH and LH, then it is difficult to escape the conclusion that the estrogen implants interfered with release of pituitary LH, but not FSH. However, in mammals, LH is involved with steroid secretion by the gonad also (Chester Jones and Ball, 1962). This activity appears to be unaffected in series 1 but inhibited in series 2, indicated by oviduct growth. It is possible that ovarian steroid production (estrogen perhaps) (Prisco et al., 1968) and concomitant ovarian growth in the lizard is dependent solely upon FSH and that LH is involved primarily with ovulation. It is also possible that prolactin may be involved in ovarian steroid production (Callard and Zeigler, 1970). Licht (1970) has suggested that there may be one gonadotrophic complex responsible for both ovarian growth and function. If so, ovarian growth, ovulation, and steroid production may require different titers of this gonadotropic complex.

The influence of cholesterol implants on ovulation in series 1 deserves

comment. Since all but one of such animals in series 2 ovulated, the influence of cholesterol in the first group does not appear to be due to an effect on ovulation per se. It is possible, as with progesterone, that certain of these animals had a slower rate of follicular growth which prevented follicles from maturing to the size at which they would normally ovulate. The absence of preovulatory animals with ovarian weights in excess of 13.94 gm/100 gm, suggests that this weight is about the upper limit for egg size prior to ovulation. Preovulatory animals implanted with progesterone had smaller ova and therefore it is possible in this group that vitellogenesis (as expressed in ovarian growth) or ovarian uptake of vitellogenic protein is diminished. Evidence from the literature indicates that progesterone can have a biphasic effect, either stimulating or inhibiting ovulation in the rat (Sawyer and Everett, 1959). Ralph and Fraps (1960) report that progesterone causes premature ovulation of the hen's first follicle.

Data from the second series of experiments yielded essentially similar results. However, since ovaries of these animals upon arrival in the laboratory were already large and perhaps close to ovulation (17% of these animals had ovulated upon arrival) no influence of estrogen upon ovarian growth was seen. However, both estradiol benzoate and estradiol undecylate inhibited ovulation when implanted in the hypothalamus while cholesterol was ineffective. In contrast to series 1 animals, both groups of estrogen implanted Sceloporus in the second series showed significant inhibition of oviducal growth. In fact, oviducts regressed. This result suggests

an influence of estrogen implantation upon ovarian steroid production. These data may be reconciled with those of series 1 if one considers the smaller initial size of their oviducts. Further, estrogen implanted series 2 animals had oviducts more similar in weight to those of series 1 than the controls of series 2. This result might suggest that the absence of an effect of estrogen upon oviducal growth in series 1 was due to the presence of relatively low titers of steroid which maintained a certain degree of oviduct development. However, further oviduct growth (seen in series 2) may require higher titers of estrogen which are not available in animals with estrogen implants.

Examination of implant locations revealed that median eminence implants were most effective in inhibiting ovulation, only 1/16 animals ovulating when implants were in the median eminence. All estradiol 17 β implants were in the median eminence and, of the remaining animals (either estradiol benzoate or estradiol undecylate implants), only 6 ovulated. Two of these had implants just through the antero-medial hypothalamus, one implant was lateral to the median eminence and three terminated in the antero-medial hypothalamus. Thus it appears that implants in the median eminence region and to a certain extent in the antero-medial hypothalamus are effective in ovulation inhibition. These results suggest the importance of this region in the control of ovulation in lizards, as demonstrated in mammals and birds (Dey et al., 1940; D'Angelo, 1959; Flerko, 1953; Lisk, 1960, 1963; Kordon and Gogan, 1964; Ralph and Fraps, 1959). In Amphibia, Dierickx (1967) has suggested that the preoptic nucleus may contain structures necessary for ovulation. Previous

results from this laboratory (Callard and Willard, 1969; Callard and Chester Jones, 1970) have indicated the importance of this area of the lizard central nervous system in the control of the reptilian adrenal gland. In the lizard, Dipsosaurus dorsalis, one animal with an estradiol implant in the median eminence failed to show follicular development (Lisk, 1967).

Relevant to any discussion of ovarian growth in reptiles is a consideration of vitellogenesis. Ovarian growth in reptiles and amphibia principally dependent upon estrogen-induced hepatic synthesis of specific vitellogenic proteins (Hahn, et al., 1969 and Follett et al., 1968) and gonadotropin-induced protein uptake by the gonad. It is known that this phenomenon is dependent upon ovarian estrogen production. Hepatic size in vitellogenic (preovulatory animals) was greatest in the beginning controls and decreased markedly in postovulatory controls in both series. Further, liver weights of the beginning controls of the first series were larger than those of the second, perhaps correlated with a decrease of active vitellogenesis in series 2 (animals in this group had maximally enlarged ovaries). Hypothalamic estrogen, cholesterol, and progesterone implants appeared ineffective in influencing liver size; any differences in liver weights in implanted animals are probably due to a decrease in vitellogenesis. However, implants of estrogen in the pituitary may have increased liver weight.

Total protein levels are highest in animals with estradiol 17 β depots in the pituitary and hypothalamus. In animals with subcutaneous

estrogen implants, total plasma protein is the same as that of initial controls. Examination of plasma protein fractions in series 1 reveals a marked effect of estradiol 17 β, increasing fraction 3 and decreasing all others. A protein fraction with a similar electrophoretic mobility increases following estrogen injection and during the ovarian growth phase in snakes (Dessauer and Fox, 1959). Although similar trends are noted in series 2 and are probably important, significant differences in total plasma protein were not detected.

This observation of an influence of intrahypothalamic and intrapituitary estradiol 17 β on the plasma protein picture is important when it is noted that subcutaneous estradiol 17 β did not influence plasma proteins to a similar extent. However, the quantity of estradiol 17 β released from the subcutaneous depot was sufficient to have a stimulatory effect upon the oviducts, whereas intrahypothalamic estrogen did not. These data suggest an action of estradiol at both hypothalamic and pituitary loci. Estrogen stimulates hepatic vitellogenesis directly and the only report of hypophysectomy concludes that the hypophysis is not necessary for hepatic response to estrogen.

Relevant to the effects observed in these treatments are the differential effects of estradiol in series 1 and 2. There are at least two possibilities. First, the fact that estradiol 17 β is a natural endogenous hormone, while estradiol benzoate and undecylate are synthetic esters, may explain the greater effect of estradiol 17 β on plasma protein levels. It is also a possibility that the differential effects are a reflection of hepatic sensitivity changes

dependent upon the stage of vitellogenesis. Since the series 2 animals were probably non-vitellogenetic at the time of steroid implantation, while those of series 1 were actively vitellogenic, estradiol benzoate and undecylate might be expected to have only a slight effect on the plasma proteins.

In summary, it is suggested that the effects of estradiol on both ovulation (inhibitory) and plasma protein levels (stimulatory) may be linked. If estrogen is the principal agent concerned with vitellogenesis, either through a direct action on the liver or via the pituitary-hypothalamic unit, or both, it may stimulate vitellogenesis while inhibiting ovulation. Such a dual action would allow ovarian growth to proceed without premature ovulation. The inhibitory effect of estradiol 17 β implants on ovulation is consistent with mammalian findings. However, such implants in mammals also appear to inhibit ovarian growth and this effect was not seen in the lizard. On the contrary, the stimulatory effect of estradiol on plasma protein levels might suggest the opposite.

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