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AN INVESTIGATION OF THE ROLE OF HORMONES AND THE HYPOTHALAMUS IN OVARIAN GROWTH AND VITELLOGENESIS IN *SCELOPORUS CYANOGENYS*

A Thesis

Presented to

The Faculty of the Department of Biology

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Arts

By

C. Gresham Bayne

1970

APPROVAL SHEET

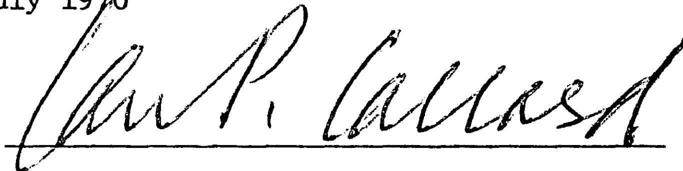
This thesis is submitted in partial fulfillment of

the requirements for the degree of

Master of Arts

C. Gresham Bayne

Approved, July 1970



Ian P. Callard, Ph.D.

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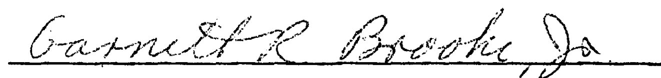

Garnett R. Brooks, Jr., Ph.D.

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ABSTRACT

The normal reproductive and related parameters are described for the iguanid lizard, Sceloporus cyanogenys, from the beginning of vitellogenesis through ovulation. Early vitellogenic animals stimulated with PMS showed marked ovarian growth and related changes. In vitellogenic animals treated with progesterone, ovulation failed to occur and oviduct size was reduced. The effect of prolactin injections was similar to that of progesterone but less pronounced. In near-ovulatory animals, intrahypothalamic and intrapituitary implants of progesterone, and deafferentation and lesions of the hypothalamus failed to inhibit ovulation or show related changes.

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INTRODUCTION

A number of physiologic actions of progesterone in the eutherian mammals are well-documented. These include stimulation of uterine growth following estrogen (Corner and Allen, 1928; Hisaw, 1950), pregnancy maintenance (Corner and Allen, 1928), inhibition of ovulation (Emmens, 1959; Sawyer and Everett, 1959), mammary gland development (Hisaw, 1939; Pearlman, 1948; Emmens, 1959), and certain behavioral changes concerned with post-natal care (Emmens, 1959). All of these actions of the hormone are related to, and essential for, a successful pregnancy and post-natal care of the young. In birds, progesterone is involved in oviduct development (Brandt and Nalbandov, 1956), ovulation (Ralph and Fraps, 1960), and behavioral changes associated with post-natal care (Marshall and Serventy, 1956; Marshall and Coombs, 1957).

In the lower vertebrates, such information is lacking. There is good evidence, however, that the ovaries of reptiles and elasmobranchs can systhesize progesterone *in vitro* (Callard and Leathem, 1964, 1965), and that the delta-5, 3-beta hydroxy steroid dehydrogenase enzyme, essential for the conversion of pregnenolone to progesterone, is present in the corpora lutea of both of these vertebrate groups (Callard, 1966; Lance and Callard, 1969). Further, the presence of progestins in the blood of reptiles has been suggested on the basis of bioassay data (Bragdon *et al.*, 1954). Thus far, however, no physiologic role for this hormone in the

Reptilia or other lower vertebrate class has been clearly demonstrated, although Panigel (1956) found a stimulation of the oviducal muscularis following progesterone treatment in Lacerta vivipara. Ovariectomy has no effect on the course of gestation in a number of different reptilian species (Clausen, 1935, 1940; Rahn, 1939; Bragdon, 1950, 1951; Panigel, 1956; Lien and Callard, 1968).

Recent results from this laboratory have suggested two possible effects of progesterone in reptiles which may be of physiologic importance, McConnell (1970) found that implants of progesterone in the median eminence region of the brain of Sceloporus cyanogenys significantly inhibited gonadal development and ovulation. In addition, progesterone (Zeigler, 1970) and prolactin injections (Callard and Zeigler, 1970) are known to inhibit PMS-stimulated gonadal development in the iguanid lizard, Dipsosaurus dorsalis. Since prolactin is a luteotrophic agent in several mammalian species, most notably the rat (Lahr and Riddle, 1936; Astwood, 1953), it is possible that a similar action in reptiles is responsible for the gonadal inhibition of this hormone reported by Callard and Zeigler (1970).

The present investigation was undertaken to clarify these possibilities in the ovo-viviparous lizard, Sceloporus cyanogenys. In addition, the effect of hypothalamic lesions and deafferentation upon ovulation was studied in an attempt to define more clearly areas of the hypothalamus concerned with ovulation and gonadal growth in this species.

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 from late October to early January. 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 cycle centered on seven o'clock. Animals were fed commercially supplied crickets daily.

B. EXPERIMENTAL GROUPS

Series I: These animals were received during late October and early November and possessed ovaries in the early growth phase. The influence of pregnant mare serum gonadotrophin (PMS) and progesterone was tested. Experiment 1. The following groups were included in this experiment:

a. Controls, start. This group allowed verification of the stage of ovarian development at the beginning of the experiment. (n=5)

b. Controls, end (n=5)

c. PMS injected (n=15)

d. PMS and progesterone injected (n=9)

e. Progesterone injected (n=8)

The experimental period was 14 days and animals were autopsied on day 15.

Experiment 2. On arrival in the laboratory in mid-November autopsy of a control group showed advanced vitellogenesis and ovarian growth. A thirty-day experimental period was allowed as the estimated time required for the completion of ovarian development and ovulation, based upon previous experience in this laboratory. The influence of prolactin and progesterone injections upon ovulation and ovarian development was tested using the following experimental groups:

a. Controls, start (n=5)

b. Controls, end (n=5)

c. Prolactin injected (n=9)

d. Progesterone injected (n=9)

Series II: Animals used for these experiments were received in mid-December and all possessed large ovaries close to ovulation (relative ovarian weight = 10.133 ± .500 g/100 g body weight). The influence of hypothalamic progesterone implants, deafferentation and lesions upon ovulation were tested in the following experiments, lasting thirty days post-operation.

Experiment 1. All animals were laparatomized on Day 1 and the ovaries inspected to insure proper knowledge of reproductive status. The following groups were set up using only preovulatory animals:

a. Controls, end (n=7)

b. Cholesterol implants (n=5)

c. Progesterone intrahypothalamic implants (n=8)

d. Progesterone subcutaneous implants (n=6)

e. Progesterone intrapituitary implants (n=9)

Experiment 2. The following groups were set up:

a. Controls. Eight animals were autopsied on Day 1 to determine the state of the gonads.

b. Hypothalamic deafferentation (n=9)

c. Sham deafferentation (n=5)

d. Hypothalamic lesion (n=10)

e. Sham lesion (n=5)

Since both experiments in Series II were run in parallel using the same shipment of animals, the untreated controls for Experiment 2 were those of Experiment 1.

C. INJECTIONS

PMS (pregnant mare serum gonadotrophin, Ayerst, "Equinex") was injected by the intraperitoneal route, 25 i.u./.05 ml distilled water per day for 14 days. Progesterone (Nutritional Biochemicals Corp.) in the amount of 2 mg/0.1 ml sesame oil was injected subcutaneously daily for either 14 (Experiment 1) or 28 (Experiment 2) days. Prolactin (NIH-P-B-2 Bovine) was administered subcutaneously daily for 28 days at a dose of .01 mg (.25 i.u.) in distilled water per 1 gm body weight.

D. OPERATIVE TECHNIQUES

All operative techniques were performed under Nembutal anesthesia (2.5 mg/100 g body weight) following 30 minutes in a refrigerator. In all hypothalamic operations a small hole was drilled through the roof of the skull 0.5 mm posterior to the pineal eye and the instrument (hormone-laden stainless steel tubing, electrode, or Halasz knife) lowered to a point 0.5

mm above the basisphenoid bone following positioning of the animal in a Kopf small mammal stereotaxic instrument.

1. LESIONS. An electrode of stainless steel insulated with "Insulex" to within 0.5 mm of its tip was used and a lesion produced by using an average of 23 volts for 50 seconds. After lesioning the electrode was withdrawn and the hole plugged with Gelfoam and covered with dental cement. Sham-operated controls were provided by the same method, but no current was passed.

2. DEAFFERENTATION. A knife fashioned after the specifications of Halasz and Pupp (1967) was used for this operation. It was lowered into the brain in the anterior saggital plane to within 0.5 mm of the floor of the skull; the blade was then turned 90° right and left inscribing a semicircular arc anterior to the hypothalamus. The hole was plugged with Gelfoam after removal of the knife from the brain. Sham-operated controls were operated on using the identical technique, but the blade was not rotated in the brain.

3. STEROID IMPLANTATION. The tip of a 32-gauge stainless steel tube was primed with progesterone or cholesterol and lowered into the hypothalamus as described above. In some animals a small amount of progesterone was implanted directly into the adenohypophysis. A small slit was made in the buccal mucosa and a hole made in the basisphenoid bone using a dental pick. The small amount of progesterone was implanted directly into the pituitary and held in place by a Gelfoam plug. Additional controls were provided by animals with progesterone-laden probes being implanted subcutaneously. The progesterone content of the probes was

estimated spectrophotometrically, prior to use; the probes contained 39 ± 7 ug. After removal from the brain, the amount remaining was 11.8 ± 6.5 ug.

E. AUTOPSY

On the last day of each experiment all animals were killed by decapitation, the blood being collected and centrifuged at 2500 rpm for 10 minutes. The plasma was frozen for later analysis. The thyroid gland, adrenals, ovaries, oviducts and livers were dissected out, weighed, and samples preserved in Bouin's fixative for later histological analysis. The brains of all operated animals were also fixed in Bouin's, but were transferred to 70% ethanol after two weeks.

F. LOCATION OF IMPLANTS AND BRAIN DAMAGE

Macroscopic examination of all brains with steroid implants was accomplished by careful removal of the pituitary gland from the ventral side and observation of the probe under a dissecting microscope. Brains were then removed from the skull and, transferred to 20% ethanol prior to embedding in gelatin following which they were sectioned serially at 80u using a cryostat. Serial sections were stained in thionin and observed for microscopic location of implants and brain damage.

The brains of the animals operated on with the Halasz knife were taken from 70% ethanol and embedded in paraffin. They were then sectioned at 10u and every tenth section stained with hematoxylin and eosin, and observed for microscopic location of cut and brain damage.

G. ELECTROPHORESIS

The frozen serum specimens were thawed and separated in a Gelman

Deluxe Electrophoresis Chamber (No. 51170) using Sépraphore III cellulose polyacetate support membranes. The resultant bands were then scanned by the Gelscan Automatic Recording and Integrating Scanner, thus yielding the percentage composition for each protein fraction in the serum. Total serum protein values were obtained in duplicate with the standard Biuret Reagent and spectrophotometric analysis on the Spectronic 20. Simple mathematical comparisons gave the absolute amount of circulating protein in grams per 100 ml blood. In addition, an albumin/total globulin ratio was computed for each sample. Throughout all protein analyses the fractions normally termed Fractions 1,2,3,4, and 5 were assumed to correspond with the typical mammalian distribution of albumin, a_1 globulin, a_2 globulin, B globulin, and gamma globulin. Total globulin was found for each animal and used in statistical comparisons.

H. STATISTICAL METHODS

All organ weight and blood protein data were analyzed for significant differences using the Student's t test for unpaired observations. Ovulation frequency was studied using the Fisher exact probability test for two independent samples. Significance was determined to be the 95% level ($p < .05$), whereas a $p < .001$ was considered highly significant. In the tables, all data are expressed as mean ± standard error.

RESULTS

A. BODY WEIGHT CHANGES (Table 1)

All animals were weighed on arrival in the laboratory and at autopsy. With few exceptions animals increased in weight during the experimental period.

B. THE NORMAL OVARIAN CYCLE AND ASSOCIATED ORGAN CHANGES (Figure 1 and Table 2)

Data from all control groups is presented separately in an attempt to express the changes associated with ovarian growth, ovulation and early gestation. These data represent both starting and finishing controls from each experiment. In addition, animals with cholesterol intrahypothalamic implants are included since these animals were not different from other control groups as far as the parameters tested were concerned.

Ovarian weight and appearance in late October indicated that vitellogenesis had begun. Ovarian weight in animals received during November was somewhat greater, but the change was not significant. However, a marked increase in ovarian weight occurred during the next three weeks as indicated by the control animals killed on December 3 ($p < .001$). A small additional increment in ovarian weight occurred by mid-December, followed by a sharp drop ($p < .001$) after ovulation in the later half of December. Ovaries of pregnant animals consisted of corpora lutea and small follicles and weighed approximately .028 mgs. Oviduct weight changes followed closely those of the ovary, increasing rapidly in weight between mid-November and

and early December ($p < .001$). Oviduct weight remained high following ovulation and during early pregnancy.

Liver weight changes associated with vitellogenesis appeared to precede ovarian growth in onset and reached a peak in early December ($p < .01$ compared to October 22 controls). The cessation of vitellogenesis and ovarian growth was marked by a concomitant decrease in liver weight following ovulation ($p < .001$ between the same groups). Liver weight remained low during early pregnancy. Total serum protein changes correlated well with liver growth, increasing ($p < .05$, November 4 controls versus December 3 controls) during vitellogenesis and decreasing following ovulation. Examination of serum protein patterns by electrophoresis showed quantitative changes correlated with both the liver and total protein changes. However, due to large standard errors, no significant differences were observed except in plasma albumin levels. Albumin increased from October 22-December 3 (p .05) and decreased between December 3 and January 4 ($p < .001$). The a_1 fraction decreased between December 3 and January 15 ($p < .02$).

C. THE EFFECT OF PMS AND PROGESTERONE UPON OVARIAN GROWTH AND FUNCTION (Tables 3 and 4)

The effect of PMS was tested on animals in the early vitellogenic phase as indicated by ovarian weight and appearance. Injection of the hormone markedly increased ovarian, oviduct and mean egg weights, and maximum follicular diameter ($p < .001$ for all comparisons to controls). In addition, the mean number of eggs was increased ($p < .05$).

Liver weight and total plasma proteins were elevated in PMS-injected

animals, but compared to the end controls the differences were not significant. However, the liver weight in PMS-treated animals was significantly higher than that of the starting controls ($p < .02$). Simultaneous injections of progesterone with PMS were highly effective in curtailing the stimulatory effects of PMS on all parameters: ovarian, oviduct and mean egg weights, and maximum follicular diameter ($p < .001$) and relative liver weight ($p < .05$). were all larger in PMS-treated animals than in animals treated with PMS plus progesterone. Injections of progesterone alone appeared to have no effect upon these parameters.

D. THE EFFECT OF PROLACTIN AND PROGESTERONE ON ANIMALS IN THE LATTER STAGES OF VITELLOGENESIS (Tables 5 and 6)

Ovaries were large (9.1 g/100 g body weight) in the starting controls, and ovulation occurred in four out of five animals kept in the laboratory for thirty days. Ovulation frequency for progesterone-injected animals was significantly lower than that in the final controls ($p < .02$); the progesterone injections prevented ovulation in all but one of the nine treated animals. This inhibition was associated with a decrease in ovarian weight compared with the starting controls, although the difference was not significant. Mean egg weight and maximum follicular diameter were not different from those of the controls. However, progesterone injections significantly decreased oviduct weight compared to starting controls, ($p < .001$).

Prolactin was less effective than progesterone in ovulation inhibition, five of nine ovulation successfully ($p < 0.1$). Other measurements in the preovulatory prolactin-injected animals were similar to those of the initial controls, except for the oviduct weight which was decreased ($p < .01$) as with progesterone treatment. Prolactin did not inhibit oviduct

growth in animals which ovulated. Oviducts of these animals were larger than those of preovulatory prolactin-injected animals ($p < .001$).

Liver weights in preovulatory progesterone-treated animals remained at the level of the initial preovulatory controls which was higher than that of the postovulatory final controls ($p < .001$). Liver weights also decreased in postovulatory prolactin-treated animals ($p < .001$ to controls). However, liver weights of preovulatory prolactin-treated animals were also low compared to preovulatory controls and progesterone-treated animals ($p < .001$). No significant differences were observed in total serum protein or individual protein fractions related to hormonal treatment.

E. THE EFFECT OF INTRAHYPOTHALAMIC PROGESTERONE IMPLANTS ON OVULATION (Tables 7 and 8)

This experiment was performed using animals at a stage of the ovarian growth cycle similar to those used in the previous experiments (ovarian weight= 10.13 g/100 g body weight). Location of cholesterol and progesterone implants in the hypothalamus was verified at the time of dissection of the brain from the skull and following thionin staining of frozen sections. All probes ended in the hypothalamus; in some cases the tip of the stainless steel tube carrying the hormone was so close to the surface of the brain so as to make determination of the exact location difficult. Three implants ended in the antero-lateral hypothalamus, and the remaining five terminated in the region of the anterior border of the median eminence. One hundred percent ovulation occurred in all treated groups with the exception of animals with progesterone implants in the anterior pituitary gland. In this group three out of nine

animals failed to ovulate. While ovarian weights of all postovulatory animals were similar, ovaries of animals with progesterone intrapituitary implants showed evidence of some degree of follicular atresia. None of the other parameters measured revealed differences between the various experimental groups.

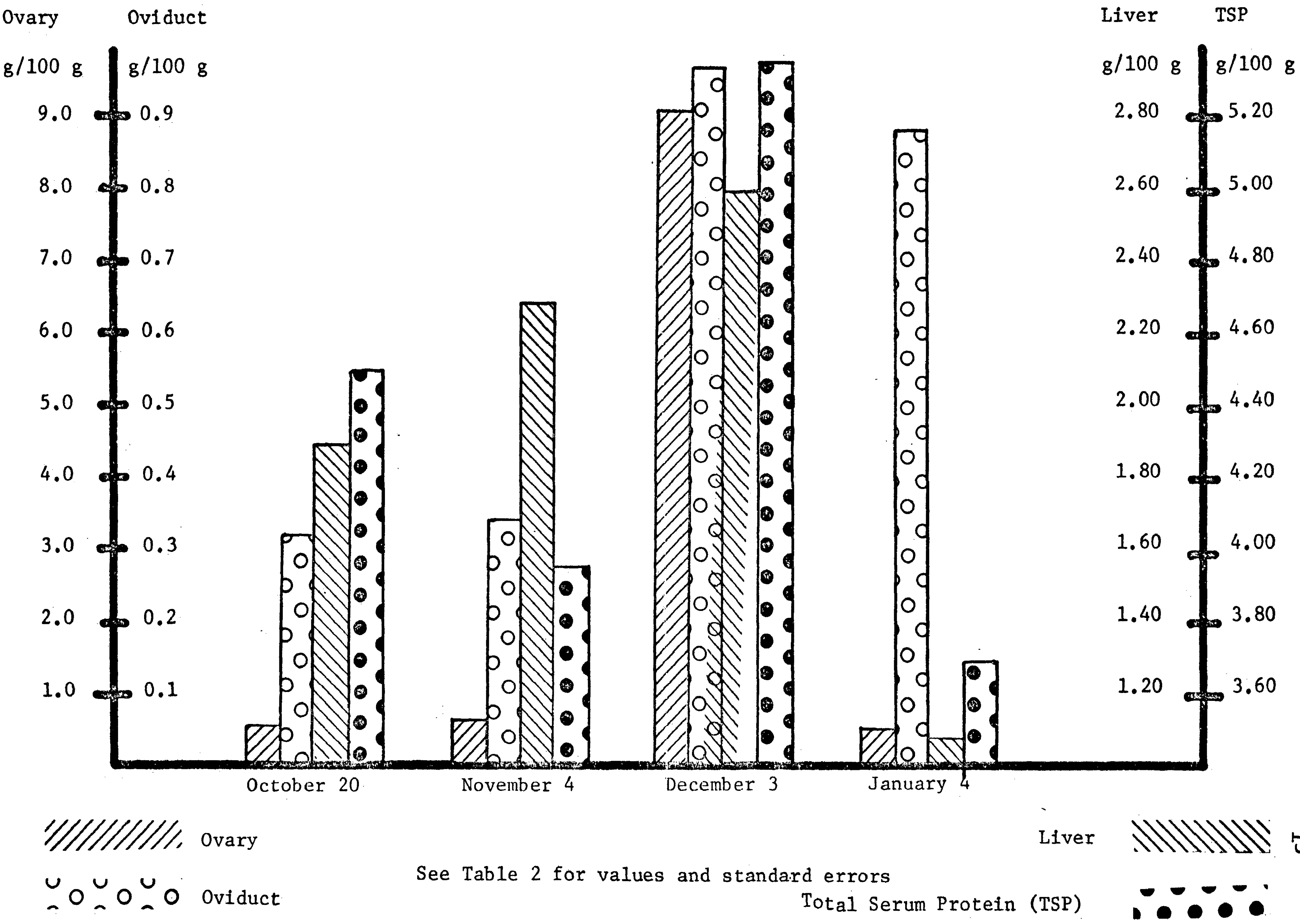
F. THE EFFECT OF HYPOTHALAMIC DEAFFERENTATION AND LESIONS ON OVULATION (Tables 9 and 10)

This experiment was performed on animals similar to those used above for hormonal implants and the same untreated controls served for both experiments. Halasz cuts were located following paraffin embedding and staining with hematoxylin and eosin. Placement of cuts was variable. In four animals, hypothalamic damage was visible at the surface of the anterior hypothalamuc, posterior to the optic chiasma. Three animals had cuts extending across the anterior hypothalamus, midway between the median eminence and the optic chiasma. Two others had extensive lateral hypothalamic damage, with no clear line of deafferentation. No differences related to placement of the cuts were observed, ovulation being unaffected by the operations performed.

Lesions were located following examination of thionin-stained cryostat sections under the microscope. No correlation between lesion location and the occurrence of ovulation could be made. Lesions were seen in seven out of ten animals, the placement of the lesion varying from the antero-lateral and medial regions of the hypothalamus, including the anterior region of the median eminence in two animals. All of these animals ovulated. In three animals which did not ovulate lesions were not visible. Ovarian weight in animals with sham lesions was high due to follicular atresia.

FIGURE 1

THE NORMAL REPRODUCTIVE CYCLE OF SCELOPORUS CYANOGENYS



See Table 2 for values and standard errors

TABLE 1

BODY WEIGHTS OF EXPERIMENTAL ANIMALS

Experimental Groups	n	Weight in Grams Start	End	Mean Weight Change, gm
Start Controls	5	35.6±4.3	-	-
PMS, IP	15	35.0±2.8	33.6±3.5	-1.33
PMS, Progesterone IP	9	29.1±2.3	37.2±2.4	+8.07
End Controls	5	33.8±6.3	39.5±6.4	+5.62
*Progesterone IP	8	36.2±3.8	37.4±3.6	+1.20
Start Controls	5	49.1±6.3	-	-
Progesterone IP	8	43.9±3.1	47.3±3.0	+3.4
Prolactin Preov. IP	4	36.7±2.5	40.9±3.6	+4.2
Prolactin Post. IP	5	43.3±2.2	51.1±4.0	+7.8
End Controls	4	45.6±4.9	51.4±4.6	+5.8
End Controls	7	40.4±3.8	42.9±3.7	+2.5
Progesterone Subcut.	9	42.0±3.6	43.9±3.7	+1.9
Cholesterol Implant	4	46.8±5.5	53.7±5.8	+6.9
Progesterone Implant	8	41.2±3.4	38.1±2.0	-2.1
Progesterone Preov. AP	3	43.2±6.9	36.6±4.1	-6.6
Progesterone Post. AP	6	66.5±8.4	71.3±7.7	+4.8
First Controls	8	44.4±3.4	-	-
Lesions Preov.	3	47.5±4.6	42.3±2.7	-5.2
Lesions Post.	7	45.2±6.8	47.4±7.2	+2.2
Sham Lesions	5	35.1±2.8	37.5±2.5	+2.4
Halasz Cuts	9	44.9±4.7	45.4±4.9	+0.5
Sham Cuts	5	40.7±7.1	39.3±5.8	-1.4
End Controls	4	38.1±5.9	39.0±5.1	+0.9

IP= intraperitoneal injection
Preov.= preovulatory animals
Post.= postovulatory animals
Implant= intrahypothalamic implant
AP= intrapituitary implant
*Data supplied from Dr. Ian P. Callard from animals of similar conditions.

TABLE 2

THE NORMAL REPRODUCTIVE CYCLE OF <u>SCELOPORUS CYANOGENYS</u>

Date Killed	n	Gonad Weight g/100 g	Mean Egg/Embryo Weight, mg	#Eggs	Oviduct Weight g/100 g	Max. Follicular Diameter, cm	Liver Weight g/100 g	TSP g/100 ml
PREOVULATORY ANIMALS								
October 20	5	.612±.122	15.0±6.2	19.0±2.6	.327±.094	.25±.03	.678±.094	4.47±.43
November 4	5	.700±.300	19.4±8.4	14.8±2.7	.348±.061	.31±.08	.896±.161	3.97±.33
November 18	26	-	-	11.2±0.7	-	.71±.02	-	-
December 3	5	9.172±1.073	268±30	17.2±2.9	.975±.038	.78±.02	2.598±.130	5.36±.36
December 15	8	10.133±.500	-	-	-	.85±.03	-	-
POSTOVULATORY ANIMALS								
January 4	4	.052±.007	447±45	15.5±2.2	.888±.081	.96±.02	1.084±.054	3.70±.75
January 15	4	.057±.010	360±44	13.8±2.7	.995±.161	±	1.357±.221	3.85±.32
January 24	4	.113±.023	533±30	16.0±1.5	1.097±.064	-	1.065±.023	3.68±.38
February 8	3	.097±.025	567±44	11.0±3.2	1.200±.025	-	1.156±.044	3.96±.59

TSP= Total Serum Protein

TABLE 3

SERIES I, EXPERIMENT 1: THE EFFECTS OF PMS AND PROGESTERONE INJECTIONS

Treatment	n	Gonad Weight g/100 g	Mean Egg wt. mg	#Eggs	Oviduct wt. g/100 g	Max. Follicular Diameter, cm	Liver wt. g/100 g	TSP g/100 ml
Start Controls Preov.	5	.612±.122	15.0±6.2	19.0±2.6	.327±.094	.25±.03	1.898±.150	4.46±.42
End Controls Preov.	5	.700±.300	19.4±8.4	14.8±2.7	.348±.061	.31±.08	2.307±.348	3.97±.32
PMS Injected Preov.	15	7.796±1.193	86.6±8.9	26.9±2.6	1.537±.158	.66±.03	3.311±.275	5.02±.49
PMS, Progesterone IP Preov.	9	1.641±.341	27.8±4.9	21.0±2.0	.628±.086	.42±.03	2.519±.168	4.11±.43
Progesterone IP Preov.	8	.656±.091	14.8±5.2	16.0±2.5	.362±.092	.28±.04	2.031±.283	4.21±.52

PMS= Pregnant Mare Serum
Preov.= Preovulatory animals
TSP= Total Serum Protein
IP= Intraperitoneal injection

TABLE 4

SERUM PROTEIN DATA FOR SERIES I, EXPERIMENT 1

g/100 ml

Treatment-n	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Total Globulin	Total Serum Protein
First Controls-5	1.18±.06	.96±.09	.80±.20	.72±.06	.75±.07	3.23±.33	4.47±.43
PMS Injected-15	1.16±.11	.85±.10	1.00±.26	.86±.10	.93±.20	3.47±.40	5.03±.49
PMS, Prog. IP-6	1.31±.11	.68±.08	.74±.12	.68±.19	.67±.09	2.77±.44	4.11±.43
End Controls-5	1.27±.17	1.02±.02	.56±.05	.71±.12	.45±.08	2.70±.18	3.97±.33

PMS= Pregnant Mare Serum
Prog.= Progesterone
IP= Intraperitoneal injection

TABLE 5

SERIES I, EXPERIMENT 2: EFFECTS OF PROLACTIN AND PROGESTERONE INJECTIONS

Treatment	n	Gonad wt. g/100 g	Mean Egg/ Embryo wt. mg	#Eggs	Max. Foll. Diameter cm	Oviduct wt. g/100 g	Liver wt. g/100 g	TSP g/100 ml
Start Controls Preov.	5	9.172±1.073	268±30	17.2±2.9	.78±.02	.975±.038	2.598±.130	5.36±.35
End Controls Post.	4	.053±.007	447±45	15.5±2.2	.96±.02	.888±.081	1.084±.058	3.69±.75
Progesterone IP Preov.	8	6.913±1.072	265±23	12.3±2.8	.77±.01	.518±.054	2.283±.236	4.36±.16
Prolactin IP Preov.	4	9.120±.974	282±26	13.0±0.4	.74±.03	.503±.089	1.147±.082	4.60±.15
Prolactin IP Post.	5	.068±.014	541±48	12.2±0.9	1.05±.05	1.056±.053	.986±.032	2.96±.24

Preov.= Prevoulatory animals
Post.= Postovulatory animals
IP= Intraperitoneal injection
TSP= Total serum protein

TABLE 6

SERUM PROTEIN DATA FOR SERIES I, EXPERIMENT 2

g/100 ml

Treatment-n	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Total Globulin	Total Protein
First Controls Preov.-5	1.50±.09	1.30±.11	.77±.09	1.15±.21	.64±.17	3.86±.30	5.36±.36
Progesterone Preov.-8	1.13±.08	.73±.06	.55±.10	.75±.05	1.19±.17	3.32±.15	4.37±.16
Prolactin Preov.-4	1.25±.11	.92±.12	.50±.06	.95±.09	.99±.10	3.36±.13	4.60±.15
Prolactin Post.-5	.80±.06	.62±.06	.39±.03	.71±.11	.43±.09	2.14±.20	2.96±.24
End Controls Post.-4	.75±.20	.81±.21	.50±.14	.59±.06	.56±.09	2.46±.45	3.70±.36

Preov.= Preovulatory animals
Post.= Postovulatory animals

TABLE 7

SERIES II, EXPERIMENT 1: HORMONAL IMPLANTS AND OVARIAN GROWTH AND OVULATION

Treatment	n	Gonad wt. g/100 g	Mean Egg/Embryo wt. mg	#Eggs	Max. Foll. Diameter cm	Oviduct wt. g/100 g	Liver wt. g/100 g	TSP g/100 ml
End Controls (Post.)	7	.074±.013	405±64	12.6±2.0	-	1.082±.131	1.271±.127	3.90±.28
Progesterone SC (Post.)	9	.253±.130	482±8	13.5±1.1	-	1.381±.315	1.188±.085	3.86±.18
Cholesterol IH (Post.)	4	.113±.023	533±29	16.0±1.5	-	1.103±.065	1.065±.032	3.68±.38
Progesterone AP (Preov.)	3	9.55±1.82	331±27	6.3±2.2	.74±.02	2.184±.426	1.793±.137	3.92±.25
Progesterone AP (Post.)	6	.505±.268	530±58	20.5±2.9	-	1.072±.104	1.167±.084	3.99±.36
Progesterone IH (Post.)	8	.101±.020	469±26	10.6±1.1	-	.967±.078	.929±.063	3.58±.38

Preov.= Prevoulatory animals
Post.= Postovulatory animals
SC= Subcutaneous injection
IH= Intrahypothalamic implant
AP= Intrapituitary implant
TSP= Total serum protein

TABLE 8

SERUM PROTEIN DATA FOR SERIES II, EXPERIMENT 1

g/100 ml

Treatment-n	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Total Globulin	Total Protein
Controls-7 Post.	1.05±.12	.89±.08	.49±.07	.68±.07	.78±.11	2.84±.27	3.90±.28
Prog. Subcut. Post.-8	1.21±.07	.72±.10	.56±.11	.72±.11	.61±.09	2.65±.16	3.86±.18
Cholesterol IH Post.-5	1.12±.14	.68±.11	.54±.06	.76±.13	.59±.11	2.57±.30	3.68±.38
Progesterone IH Post.-7	1.02±.08	.84±.12	.43±.06	.68±.17	.57±.04	2.52±.35	3.58±.36
Progesterone AP Preov.-3	1.05±.08	.80±.08	.49±.08	.70±.18	1.04±.30	2.62±.91	3.92±.25
Progesterone AP Post.-4	1.30±.14	.90±.04	.57±.09	.75±.15	.48±.11	2.70±.34	3.99±.36

Preov.= Preovulatory animals
Post.= Postovulatory animals
IH= Intrahypothalamic implant
AP= Intrapituitary implant

TABLE 9

SERIES II, EXPERIMENT 2: HYPOTHALAMIC LESIONS AND DEAFFERENTATION IN *SCELOPORUS CYANOGENYS*

Treatment	n	Gonad wt. g/100 g	Mean Egg/Embryo wt. mg	#Eggs	Max. Foll. Diameter cm	Oviduct wt. g/100 g	Liver wt. g/100 g	Total Serum Protein g/100 ml
First Controls (Preov.)	8	10.133±.500	-	-	.85±.03	-	-	-
End Controls (Post.)	4	.057±.010	360±44	13.7±2.7	-	.995±.161	1.357±.221	3.84±.33
Sham Lesions (Post.)	5	1.656±.866	304±34	8.6±2.2	-	.770±.104	1.296±.176	4.75±.31
Lesions (Preov.)	3	11.864±1.09	361±48	14.0±0.6	.85±.03	.432±.032	2.066±.066	5.70±.85
Lesions (Post.)	7	.064±.007	535±36	13.7±2.7	-	1.031±.176	1.254±.073	4.35±.53
Sham Cuts (Post.)	5	.238±.104	439±6.0	12.4±2.4	-	1.196±.130	1.524±.301	-
Cuts (Post.)	9	.098±.015	508 ±43	14.1±1.3	-	.932±.035	1.092±.035	4.33±.17

Preov.= Preovulatory animals
Post.= Postovulatory animals
Cuts= Halasz deafferentations

TABLE 10

SERUM PROTEIN DATA FOR SERIES II, EXPERIMENT 2

g/100 ml

Treatment-n	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Total Globulin	Total Protein
Controls-4 Post.	.96±.08	.89±.06	.51±.08	.74±.07	.90±.10	3.04±.26	3.85±.32
Lesions-3 Preov.	1.25±.12	1.57±.50	.80±.23	.87±.03	1.24±.11	4.48±.79	5.70±.85
Lesions-7 Post.	1.05±.12	.87±.11	.49±.11	1.02±.16	.94±.15	3.21±.49	4.35±.28
Sham Lesions-4 Post.	1.07±.08	.77±.07	.89±.32	1.28±.13	.73±.04	3.68±.35	4.75±.31
Halasz Cuts-8 Post.	1.13±.07	.73±.10	.62±.05	.88±.23	.84±.17	3.19±.15	4.33±.17

Preov.= Preovulatory animals
Post.= Postovulatory animals

DISCUSSION

On the basis of data collected from animals supplied during the period October through January, it is possible to describe some of the events in the reproductive cycle. of *Sceloporus cyanogenys* leading to ovulation and pregnancy. The data suggest that vitellogenesis and ovarian growth begin during the early fall and by late December the process is complete. Since all animals received prior to mid-December were pre-ovulatory, and those observed during early January were already pregnant, it appears that ovulation occurs in the latter half of December. In support of this observation, most animals which were near ovulation in mid-December as determined by laparotomy, and which were maintained in the laboratory for a four-week period, had ovulated at the end of the month. These observations support previous data from this laboratory relating to the time of ovulation in *Sceloporus cyanogenys* received from Texas over a three-year period (McConnell, 1970) and Callard (personal communication). Oviduct weight changes follow very closely ovarian changes, reflecting ovarian steroid production, probably estrogen (Chieffi, 1966); however, oviduct weights remained high during pregnancy, and do not regress following ovulation.

The process of vitellogenesis is reflected in the observed changes in liver weight and serum proteins. Since ovarian growth is due to the uptake by the ovary of the products of hepatic vitellogenesis, it is natural that hepatic growth correlated with the beginning of vitellogenesis

should precede ovarian growth. It is known that vitellogenesis is dependent upon the production of estrogens by the ovary (Hahn *et al.*, 1969; Follett *et al.*, 1968), and hepatic growth and vitellogenesis have been correlated with ovarian and oviduct growth (Schjeide *et al.*, 1963) and estrogen injections (Dessauer and Fox, 1959; Redshaw *et al.*, 1969). It is considered that the role of estrogen is perhaps one of induction of specific vitellogenic protein production (Hahn *et al.*, 1969; Redshaw *et al.*, 1969), and these specific proteins appear in the blood only in the presence of estrogens or an actively growing ovary. Presumably, ovarian estrogen secretion begins in advance of vitellogenesis.

The onset of vitellogenesis in reptiles is normally correlated with an increase in serum protein fractions. In this study, however, only Fraction 1 showed a significant rise and fall correlated with vitellogenesis, hepatic and ovarian growth, and ovulation. Total serum protein levels also showed the same changes. Although few significant alterations in other plasma protein fractions were observed, all increased during vitellogenesis and decreased follwoing ovulation. In other reptiles a change in Fraction 4 has been well correlated with ovarian growth, and this beta globulin fraction is considered to be the specific vitellogenic protein induced by ovarian estrogen (see Dessauer and Fox, 1959, for snakes). The reason for the absence of significant alterations in these experiments may be due to an increased uptake of beta globulin by the ovary under gonadotrophic stimulation.

Injections of mammalian gonadotrophin (pregnant mare serum) exerted its typical mammalian follicle stimulating effect (Cole and Hart,

1930) in Sceloporus when injected early in the ovarian growth phase. Two weeks of injection of the hormone was adequate to cause near-completion of the ovarian growth cycle thus accelerating ovarian maturation to a great extent. Judging by ovarian appearance, the process was normal. However, hyperstimulation is suggested by an increase in the mean number of eggs maturing for each animal. Oviduct weight increased markedly following PMS injections, and this is presumably a reflection of ovarian estrogen secretion. On the basis of the normal picture of ovarian growth and vitellogenesis, an increase in liver weight and plasma proteins was expected following PMS treatment. While these parameters were elevated, comparison with controls killed at the same time did not reveal significant changes. However, a trend towards an increase of both liver size and plasma proteins is revealed by comparison with the controls killed at the beginning of the experiment. Injections of progesterone concomitantly with PMS inhibited all actions of the PMS noted above. The inhibition was not complete, however, in that some increase in these parameters above the initial control levels was noted with PMS and progesterone treatment.

The results suggest that progesterone acts to inhibit ovarian growth, but the mechanism of action is not clear. There are several possibilities: (a) an inhibition of vitellogenesis, and thus a decrease in the availability of the raw materials required for ovarian growth; (b) an inhibition of ovarian uptake of vitellogenic proteins. Evidence from liver weight and serum protein changes would seem to favor (a), inasmuch as one might expect a further increase in serum protein level if the ovary were not

actively removing the material from the serum. Further, one would not expect an inhibition of liver growth due to inhibition of ovarian uptake of vitellogenic protein. If the action of progesterone is on the liver, preventing vitellogenesis, then it may be acting directly in its own right or by an inhibition of the action of estrogen. In this regard, an inhibitory action of progesterone on oviduct growth (presumably induced by endogenous estrogen) is also suggested by the data. Such an estrogen-progesterone antagonism has been noted in the chick oviduct (Oka and Schimke, 1969) and is a possible explanation of the inhibition of oviduct growth following progesterone administration which is observed in these experiments. It is possible that such an estrogen-progesterone antagonism exists with regard to hepatic vitellogenesis.

A third possibility related to the inhibitory effects of progesterone is present; namely, that it exerts its inhibitory effects at the level of the hypothalamic-pituitary unit, decreasing the output of required gonadotrophins. In this particular experiment this is unlikely, inasmuch as the gonadotrophin is supplied exogenously. However, it is possible that progesterone could effect the production of other hormones of the pituitary necessary for vitellogenesis, and have its inhibitory effect in that way. This possibility will be discussed more fully below.

To a certain extent, the results of Experiment 2 support those of Experiment 1 of the first Series. In this experiment, the effect of progesterone on ovarian growth was not as pronounced as in the previous experiment, as the ovaries were already large and close to ovulation. However, progesterone injections caused an apparent reduction in ovarian size as

compared to the initial control level, and prevented ovulation in all but one animal. The reduction in ovarian size is apparent, and not real, inasmuch as other parameters of ovarian growth (mean egg weight, follicular diameter) were not defferent from initial controls, although mean number of follicles was reduced. In these animals four out of eight showed follicular atresia which could be the cause of reduction in mean number of follicles. However, since ovarian weight usually exceeds 9.0 g/100 g body weight prior to ovulation, (highest ovarian weights recorded in this laboratory were of the order of 12.0 g/100 g body weight), it is likely that progesterone inhibited ovarian growth at the peripheral level. The data suggest that ovarian growth was prevented during the period of the experiment by the injection of progesterone. Ovaries did not thus reach their normal size and ovulation did not occur. It is not possible to determine what the effect of progesterone is upon the process of ovulation *per se* from this experiment. As in the first experiment, progesterone appeared to antagonize oviduct growth, but the effect was more marked, as the oviducts regressed from the level seen in the initial controls.

The effects of prolactin injection were similar to those of progesterone, but the hormone was not as effective in ovulation inhibition. Ovaries of animals injected with prolactin which did not ovulate did not show any further growth from the initial control level. The fact that five animals ovulated successfully when injected with prolactin suggests that the dose of prolactin was not adequate to inhibit fully the processes of final ovarian maturation and ovulation. This may be due to the fact that the animals which ovulated were physiologically more advanced at the onset of

the experiment than those that did not, and that injection of prolactin came too late, and perhaps in too low a dosage, to prevent final ovarian maturation. This is supported by the fact that liver weights of preovulatory animals treated with prolactin are smaller than those of other preovulatory animals, suggesting that vitellogenesis had already begun to decrease in these animals. Of interest with regard to preovulatory versus postovulatory prolactin-injected animals is the fact that oviducts of postovulatory animals were elevated to the control level in spite of the presence of prolactin. This would suggest that the presence of fertilized eggs in the oviducts is sufficient to stimulate the oviduct and to overcome the inhibition of prolactin. This may be due to the presence of quantities of estrogen in the large-yolked eggs. Ovaries of elasmobranchs are known to contain large quantites of estrogen (Wotiz, et al., 1958), and it was suggested that the function of this estrogen is to stimulate the oviducts locally during gestation (Te Winkle, 1950; Hisaw and Hisaw, 1959).

Prolactin injections were also noted to cause oviduct regression, as did progesterone. This data suggests that prolactin may act through the intermediary of progesterone, which is the active material in prevention of ovarian maturation. This concept is supported by recently reported results of Callard and Zeigler (1970), who found that prolactin inhibited PMS-induced ovarian growth in the oviparous lizard, *Dipsosaurus dorsalis*, and that the action of the hormone could be exerted in the absence of the pituitary when the animals were suitably prepared with hormonal supplements. Recent unpublished results (Zeigler, 1970) have shown that progesterone injections have the same effect as prolactin

in inhibiting PMS-induced ovarian growth in Dipsosaurus. These data, and those presented here suggest that prolactin may stimulate the production of progesterone by the ovary of the lizard, and that an action of progesterone is to antagonise ovarian growth in some way. Since the ovaries of both Sceloporus and Dipsosaurus do not contain corpora lutea until after ovulation, the prolactin effect is not strictly a mammalian type luteotrophic effect (Dresel, 1935; Lahr and Riddle, 1936; Astwood, 1941), but less specific, perhaps also stimulating progesterone production by developing follicles.

A possible central nervous action of progesterone deserves discussion. This hormone is known to act at the level of the CNS to influence reproduction in both mammals (Sawyer and Everett, 1959) and birds (Rothchild and Fraps, 1949; Ralph and Fraps, 1959, 1960). In these experiments, implants of progesterone into the anterior hypothalamus and median eminence of the brain did not prevent ovulation, in contrast to progesterone injection. Animals used for implantation experiments were essentially similar physiologically to those used for progesterone and prolactin injections, except that ovarian weights were greater, and the animals were received late in December during the normal ovulation period. It is possible, as with the prolactin-injected animals which ovulated successfully, that these animals were far enough advanced in the cycle to negate an influence of progesterone on vitellogenesis and ovarian growth. Gross localization at the time of autopsy and microscopic examination of fixed brains confirmed the success of the implantation operation, although three of the eight implants were found to terminate in the antero-lateral portions of the hypothalamus.

It is also possible that the hormonal interactions involved in release of ova from the ovary had been set in motion so that no influence of progesterone was noted. However, under similar circumstances, animals with even larger gonads (11.0 g/100 g body weight) when implanted with estrogen in the hypothalamus did not ovulate (McConnell, 1970). This fact would suggest that progesterone does not play any inhibitory role in the process of ovulation in spite of its apparent inhibition of ovarian growth and vitellogenesis.

Attempts to inhibit ovulation by interference at the hypothalamic level using deafferentation and lesion techniques were also unseccessful. Histological examination of the brains showed evidence of lesioning in seven out of ten animals. The fact that the three animals without apparent lesions also failed to ovulate was not believed to be a direct correlation. Microscopic analysis of brain sections from animals operated on with the Halasz knife showed extensive and varied damage to the anterior and lateral regions of the hypothalamus. No significant changes, however, were observed in these experimental animals either. One would expect that the properly placed lesions and Halasz cuts would have an inhibitory effect on ovulation and/or ovarian function. The absence of such effects is evidence that either hypothalamic control is not fundamental to the normal process of ovulation, or the events leading to ovulation had already been set in motion and interference with the hypothalamus could have no retroactive effect. These experiments will have to be repeated using animals at an earlier stage of gonadal development.

In summary, the results of this investigation have shown that PMS is a potent stimulatory agent to the gonads of *Sceloporus cyanogenys*,

and that progesterone is highly effective in inhibiting the stimulatory action of PMS. Progesterone also inhibits normal seasonal ovarian growth in this species, and the effects of progesterone are to a certain extent mimicked by prolactin. The data suggest that both prolactin and progesterone inhibit hepatic vitellogenesis or ovarian uptake of vitellogenic protéin, or both, as well as antagonizing the action of estrogen on the oviduct. It is suggested that prolactin stimulates the production of progesterone by the gonad, and that progesterone antagonizes the stimulatory action of estrogen on vitellogenesis. Prolactin has been found in the reptilian pituitary (Grignon and Herlant, 1959; Nicoll and Bern, 1965; Nicoll, Bern and Brown, 1966) and more specifically the caudal acidophils (Licht and Nicoll, 1969). In addition, it is possible that estrogen produced by the lizard gonad under gonadotrophic stimulation may act to elicit prolactin secretion by the pituitary as has been observed in mammals (Meites and Turner, 1948; Meyer and Clifton, 1956; Cohere and Meunier, 1963), thus bringing about the end of the ovarian cycle.

While progesterone has not yet been chemically identified and quantitated in the blood of the reptiles, there is adequate evidence reviewed above that reptilian gonads have the capacity to synthesize progesterone, and may indeed secrete the hormone. On the other hand, progesterone does not appear to be required for gestation in reptiles, pregnancy being completed successfully in a number of species in the absence of the gonads. A possible physiologic action of progesterone, suggested by these data, is the inhibition of ovarian follicular development. Such a function would be of value to the organism inasmuch as during gestation

the production of a further set of ovarian follicles would be prevented while developing young are *in utero* and functional corpora lutea are present in the ovary.

BIBLIOGRAPHY

Astwood, E. B. (1941). The regulation of corpus-luteum function by hypophysial luteotrophin. Endocrinology 28, 309-320.

Astwood, E. B. (1953). Tests for luteotrophins. Ciba Foundation Colloquia on Endocrinology 5, 74-86.

Bragdon, D. E. (1950). Hormonal control of the reproductive cycle of ovoviviparous snakes as related to the evolution of viviparity. Virginia J. Sci. 1, 391.

Bragdon, D. E. (1951). The non-essentiality of the corpora lutea for the maintenance of gestation in certain live-bearing snakes. J. exp. Zool. 118, 419.

Bragdon, D. E., Lazo-Wasem, E. A., Zarrow, M. X. and Hisaw, F. L. (1954). Progesterone-like activity in the plasma of ovoviviparous snakes. Proc. Soc. exp. Biol., N. Y. 86, 477-480.

Brandt, J. W. A. and Nalbandov, A. V. (1956). Role of sex hormones in albumen secretion by the oviduct of chickens. Poult. Sci. 35, 692.

Callard, I. P. (1966). Reptilian gonadal steroid synthesis. Excerpta Med. Intern. Congr. Ser. 111, 216.

Callard, I. P. and Leathem, J. H. (1964). _In vitro_ synthesis of progesterone by ovaries and adrenals of snakes. Proc. Soc. Exptl. Biol. Med. 115, 567-569.

Callard, I. P. and Leathem, J. H. (1965). _In vitro_ steroid synthesis by the ovaries of elasmobranchs and snakes. Arch. Anat. Microscop. Morphol. Exptl. 54, 35-48.

Callard, I. P. and Zeigler, H. (1970). Inhibitory effects of prolactin upon gonadotrophin - stimulated ovarian growth in the iguanid lizard, _Dipsosaurus dorsalis_. J. Endocrinol. (In Press).

Chieffi, G. (1966). Occurrence of steroids in gonads of non-mammalian vertebrates and sites of their biosynthesis. Exerpta. Med. Intern. Congr. Ser. 132, 1047-1057.

Clausen, H. J. (1935). The effects of ovariotomy and hypophysectomy on parturition in Snakes. Anat. Rec. 70, 97.

Cohere, G. and Meunier, J. M. (1963). Action de l'oestrogène sur l'ultrastructure d'autogreffes de glande pituitaire de Ratte adulte. C. R. Soc. Biol., Paris 157, 1261.

Cole, H. H. and Hart, G. H. (1930). The potency of blood serum of mares in progressive stages of pregnancy in effecting the sexual maturity of the immature rat. Amer. J. Physiol. 93, 57.

Corner, C. W. and Allen, W. M. (1928). Physiology of the corpus luteum. I. The effect of very early oblation of the corpus luteum upon embryos and uterus. Amer. J. Physiol. 86, 74-81.

Dessauer, H. C. and Fox, W. (1959). Changes in ovarian follicle composition with plasma levels of snakes during estrus. Am. J. Physiol. 197, 360-366.

Dresel, L. (1935). The effect of prolactin on the estrous cycle of non-parous mice. Science 82, 173.

Emmens, C. W. (1959). Role of gonadal hormones in reproductive processes. In "Reproduction in Domestic Animals" (H. H. Cole and P. T. Cupps, eds.), pp. 111-154. Academic Press, N. Y.

Follett, B. K., Nicholls, T. J. and Redshaw, M. R. (1968). The vitellogenic response in the South African clawed toad, (Xenopus laevis Daudin). J. Cell. Physiol. 72, 91-102.

Grignon, G. and Herlaut, M. (1959). Sur la presence d'une substance d'activité analogue a celle de la prolactine au niveau de la glands pituitaire de la Tortue terrestre (Testudo mauritarcica). Compt. Rend. Soc. Biol. 153, 2032-2034.

Halász, B. and Pupp, L. (1967). Hormone secretion of the anterior pituitary gland after physical interruption of all nervous pathways to the hypophysiotrophic area. Endocrinology 77, 553-562.

Hahn, W. E., Church, R. B. and Gorbman, A. (1969). Synthesis of new RNA species prior to estradiol induced vitellogenesis. Endocrinology 84, 738-745.

Hisaro, F. L. (1939). In "Sex and Internal Secretions" (E. Allen, ed.). Williams and Wilkins, Baltimore.

Hisaw, F. L. (1950). Factors influencing endometrial growth in monkeys (Macaca mulatta). In "Steroid Hormones" (E. S. Gordon, ed.), pp. 259-276. U. of Wisconsin Press.

Lahr, E. L. and Riddle, O. (1936). Temporary suppression of estrous cycles in the rat by prolactin. Proc. Soc. exp. Biol., N. Y. 34, 880-883.

Lance, V., and Callard, I.P. (1969). A histochemical study of ovarian function in the ovoviviparous elasmobranch, *Squalus acanthias*. Gen. Comp. Endocrinol. 13, (2), 255-267.

Licht, P., and Nicoll, C.S. (1969). Localization of prolactin in the reptilian pars distalis. Gen. Comp. Endocrinol. 12, 526-535.

Lien, J. P., and Callard, I. P. (1968). The effect of gonadectomy and hormonal replacement therapy on reproduction and the reproductive tract of the ovo-viviparous lizard, *Sceloporus cyanogenys*. Virginia J. of Science 19, 179.

McConnell, W. (1970). M. A. Thesis. College of William and Mary. Williamsburg, Virginia.

Marshall, A.J., and Coombs, C.J.F. (1957). The interaction of environmental, internal and behavioural factors in the Rook, *Corvus f. frugilegus Linnaeus*. Proc. zool. Soc. London 128, 545.

Marshall, A.J., and Serventy, D.L. (1956). A case of intersexuality in *Gymnorhina dorsalis*. Emu 56, 207.

Meites, J., and Turner, C.W. (1948). Studies concerning the induction and maintenance of lactation. I. The mechanism controlling the initiation of lactation at parturition. Res. Bull. Mo. agric. Exp. Sta., No. 415.

Meyer, R.K., and Clifton, K.H. (1956). Effect of diethylstilbestrol-induced tumorigenesis on the secretary activity of the rat anterior pituitary gland. Endocrinology 58, 686.

Nicoll, C.S., and Bern, H.A. (1965). Pigeon crop-stimulating activity (prolactin) in the adenohypophysis of lungfish and tetrapods. Endocrinology 76, 156-160.

Nicoll, C.S., Bern, H.A., and Brown, D. (1966). Occurence of mammatrophic activity (Prolactin) in the vertebrate adenohypophysis. J. Endocrinol. 34, 343-354.

Oka, T., and Shimke, R.T. (1969). Progesterone antagonism of estrogen-induced cytodifferentiation in chick oviduct. Science 163, 83-85.

Panigel, M. (1956). Contribution a l'etude de l'ovoviviparite chez les reptiles: gestation et parturition chez le lezard vivipare *Zootoca vivipara*. Ann. Sci. nat. (Zool.) 18, 569.

Pearlman, W.H. (1948). Chemistry and metabolism of progesterone. In "The Hormones." (G. Pincus and K.V. Thimann, eds.). vol. 1, pp. 408-465. Academic Press, New York.

Rahn, H. (1939). Structure and function of placenta and corpus luteum in viviparous snakes. Proc. Soc. exp. Biol. New York 40, 381.

Ralph, C.L., and Fraps, R.M. (1959). Effect of hypothalamic lesions on progesterone-induced ovulation in the hen. Endocrinology 65, 819-824.

Ralph, C.L., and Fraps, R.M. (1960). Induction of ovulation in the hen by injection of progesterone into the brain. Endocrinology 66, 269-272.

Redshaw, M.R., Follett, B.K., and Nicholls, T.J. (1969). Comparative effects of the oestrogens and other steroid hormones on serum lipids and proteins in the _Xenopus laevis_ Daudin. J. Endocrinol. 43, 47-53.

Rothchild, I., and Fraps, R.M. (1949). The induction of ovulating hormone release from the pituitary of the domestic hen by means of progesterone. Endocrinology 44, 141-149.

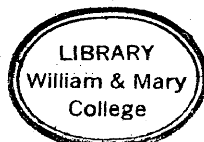
Sawyer, C.H., and Everett, J.W. (1959). Stimulatory and inhibitory effects of progesterone on the release of pituitary ovulating hormone in the rabbit. Endocrinology 65 (4), 644-651.

Schjeide, O.A., Wilkens, M., McCandless, R., Munn, R., Peterson, M., and Carlsen, E. (1963). Liver synthesis, plasma transport, and structural alterations accompanying passage of yolk proteins. Am. Zool. 3, 167-184.

Te Winkel, L.E. (1950). Notes on ovulation, ova and early development in the smooth dogfish, _Mustelus canis_. Biol. Bull., Wood's Hole 99, 474-486.

Wotiz, H.H., Botticelli, C., Hisaw, F.L. Jr., and Ringler, I. (1958). Identification of estradiol-17B from dogfish ova (_Squalus suckleyi_). J. biol. Chem. 231, 589-592.

Zeigler, H. (1970). Personal communication.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