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Reproduction and Estrogen Induced Vitellogenesis in *Dipsosaurus dorsalis*

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REPRODUCTION AND ESTROGEN INDUCED
VITELLOGENESIS IN DIPSOSAURUS DORSALIS

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

Jinny Franze Gerstle

1971

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TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS	iii
LIST OF TABLES	v
ABSTRACT	vi
INTRODUCTION	2
MATERIALS AND METHODS	4
RESULTS	9
DISCUSSION	19
BIBLIOGRAPHY	25

LIST OF FIGURES AND TABLES

Figure		Page
I.	Percentage of Different Reproductive Stages June Through August in Female <u>Dipsosaurus dorsalis</u>	13
Table		
I.	Relative Organ Weights From Female <u>Dipsosaurus dorsalis</u> in Different Reproductive Stages	14
II.	The Effect of Estrogen Injection and Hypophysectomy on the Organ Weights in Female <u>Dipsosaurus dorsalis</u>	15
III.	Comparison of Plasma Protein Fractions and Total Plasma Calcium	16
IV.	The Effects of Hypophysectomy and Estrogen Injection on the Plasma Proteins and Total Plasma Calcium in Postoviposited <u>Dipsosaurus dorsalis</u>	17
V.	The Effect of Estrogen Injection on the Plasma Protein and Calcium of Male <u>Dipsosaurus dorsalis</u>	18

ABSTRACT

The ovarian cycle of female Dipsosaurus dorsalis was studied during June through October. A peak of vitellogenesis occurred in late June. Ovarian maturation was accompanied by significant increases in oviduct weight, plasma protein fraction 4 (the beta-globulin), and total plasma calcium. Ovulation was followed first by a decrease in calcium and later by a decrease in plasma fraction 4. Oviduct and adrenal weight decreased from peak summer levels by October. A single injection of estradiol 17-beta in late post-oviposited animals and in males was followed by significant increases in plasma protein fraction 4 and calcium. This response did not occur in the absence of the pituitary gland.

REPRODUCTION AND ESTROGEN INDUCED
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INTRODUCTION

Striking increases in the plasma levels of protein, lipid, phosphorus and calcium accompanying ovarian maturation have been well documented in reptiles. Turtles (Clark, 1967), snakes (Dessauer et al., 1956; Dessauer and Fox, 1958, 1959; Dessauer, 1971; Jenkins and Simkiss, 1968), and lizards (Hahn, 1967, 1969) all exhibit characteristic changes in these blood components during the vitellogenic period. Estrogen injection in these and other submammalian vertebrates induces vitellogenesis accompanied by liver hypertrophy, protein synthesis, an increase in serum phosphoprotein and calcium in both nonvitellogenic females and males.

The presence of estrogen in lizard ovaries has been demonstrated by Chieffi (1966). Presumably, estrogen from the developing ovary induces the liver to enlarge and to synthesize a calcium-binding, phospholipoprotein (PLP) complex, plasma vitellin, which moves via the blood stream to the ovary where it is involved in synthesis of yolk (Simkiss, 1961, 1967). Wallace and Dumont (1968) have demonstrated the ability of cultured liver slices to synthesize plasma vitellin in response to estrogen

and studies emphasize the direct action of estrogen on the liver. Further, Follett and Redshaw (1968) have considered the possibility that estrogen acts indirectly via the pituitary gland in the induction of vitellogenesis and concluded that in the amphibian, Xenopus laevis, the hypophysis was not necessary for estrogen-induced vitellogenesis.

In in vivo studies small amounts of estrogen are effective in the induction of vitellogenesis in reptiles (Hahn, 1967; Clark, 1967). However, apart from the work of Dessauer and Fox (1959) in snakes, no studies of vitellogenesis during the normal reproductive cycle in reptiles have been made. The present study was undertaken to study the reproductive cycle of Dipsosaurus dorsalis and to correlate the observed cyclic changes with some parameters of vitellogenesis. In addition, the effect of estrogen injections in both female and male lizards of this species was studied. Since previous work from this laboratory has indicated that the pituitary of Dipsosaurus may be of importance for vitellogenesis (Callard and Zeigler, 1970) the effect of hypophysectomy on the vitellogenic response to estrogen injections was also investigated to support the indications of important differences between the amphibia and the reptilia.

MATERIALS AND METHODS

I. ANIMALS

Adult male and female Dipsosaurus dorsalis, the desert iguana, were obtained from Southwestern Herpetological Research and Sales, Calimesa, California, from June through September. No more than 24 animals were housed in 1.86 sq. meter enclosures for no more than 3 days prior to experimental treatment, male and female animals being kept in separate enclosures. Those maintained for longer periods during experimentation were housed in wire mesh boxes (30x30x60 cm.) with no more than 10 animals per box. Lettuce and water were supplied ad libitum and a bedding of "Sanicel" (Paxton Processing Co.) was used. A temperature gradient having a maximum of 46° C and a minimum of 21° C was available, and a 16 hour light - 8 hour dark lighting schedule was employed.

All animals were laparotomized on arrival during the natural breeding season (Norris, 1953; Mayhew, 1971) to determine the frequencies of different reproductive stages.

Experimental animals were selected at random from the large holding pens and identified by toe clip.

Series A. The Reproductive Cycle

Animals were autopsied at approximately weekly intervals during June, July, August, and October, the reproductive stages of the ovaries determined and classified according to the following criteria and the animals grouped accordingly for analysis.

- I. Early vitellogenic: follicle size = 1.0-4.5 mm. with no corpora lutea present.
Mean body weight was 32.2 ± 1.7 g.
- II. Late vitellogenic: follicle size = 6.0 - 12.0 mm. with no corpora lutea present.
Mean body weight was 40.4 ± 2.3 g.
- III. Early post-ovulatory: follicle size = 0.5 - 5.0 mm. with prominent corpora lutea and eggs in the oviducts. Mean body weight was 47.8 ± 4.5 g.
- IV. Early postoviposited: follicle size = 0.5 - 5.0 mm. with prominent corpora lutea.

IV. Early postoviposited: (continued)

Mean body weight was

34.1 ± 1.6 g.

V. Late postoviposited: follicle size = 0.5 -

5.0 mm. with few or no

small corpora lutea.

Mean body weight was

35.4 ± 3.3 g.

(In the presentation of the data for the ovarian growth cycle (Figure 1) these stages are presented as percentages of the total sample at a given time.)

Series B. The Effect of Estradiol 17-beta in Intact and Hypophysectomized Females.

The effect of hypophysectomy on the response to a small injection of estrogen was studied in post-oviposited females. Animals were given a single subcutaneous injection of estradiol 17-beta (20 ug.) one week after hypophysectomy and autopsied 3 weeks later.

I. Intact + sesame oil. (Mean body weight =

35.3 ± 3.2 g.)

II. Intact + estradiol 17-beta. (Mean body weight =

32.3 ± 2.8 g.)

III. Sham + estradiol 17-beta. (Mean body weight =

34.1 ± 1.5 g.)

IV. Hypophysectomized + sesame oil. (Mean body

weight = 34.5 ± 2.1 g.)

- V. Hypophysectomized + estradiol 17-beta. (Mean
body weight = 36.5 ± 1.5 g.)

Series C. The Effect of Estradiol 17-beta in Males.

The effect of estrogen in male lizards was studied in two groups (intact + estradiol 17-beta and intact + sesame oil vehicle) to compare the response of the male with the female. Estrogen was injected as for females and the mean body weights of these groups were $50.5 \text{ g.} \pm 2.7$ and $47.0 \text{ g.} \pm 2.7$, respectively.

II. SURGERY

Surgery was performed under Nembutal anesthesia with supplemental hypothermia. For hypophysectomy, a ventral approach through the roof of the mouth was used. The sham operated animals received the same treatment as those which were hypophysectomized except that the pituitary was left intact. Laparotomy was performed through a 5 mm. lateral incision and the wound was closed with one surgical clamp.

III. AUTOPSY

All animals were deprived of food and water 24 hours prior to autopsy. All were killed by decapitation and blood collected in centrifuge tubes containing ammonium

heparin. Plasma was removed following centrifugation for 15 minutes in an International Centrifuge at 1500 rpm. and frozen in two aliquots for subsequent analysis. The liver, adrenals and gonads were cleaned of adherent tissue and weighed. In the females, the number and size of ova and corpora lutea, if present, were measured.

IV. PLASMA ANALYSIS

In all cases the plasma was thawed at the time the analysis was conducted. Total protein was estimated using biuret reagent a bovine serum albumin standard. Serum proteins were separated by electrophoresis on cellulose acetate strips and stained with Ponceau S (Briere and Mull, 1964). After clearing, the strips were scanned in a Gelman Model 39297 Scanner and quantified. Total calcium was determined by fluorometry using the Unopette method and calcein as the reagent (Becton-Dickinson, No. 5902), and a Farrand Photoelectric Fluorometer Model A-3 (Kepner and Hercules, 1963).

V. STATISTICAL METHODS

All data were analysed using Student's t test. The 5% level was the chosen level of significance. A probability below 1% was designated as highly significant. All data were presented as means plus or minus the standard error except in figure 1 where only percentages are given.

RESULTS

I. THE OVARIAN CYCLE DURING JUNE, JULY AND AUGUST.

In the third week of June, 42% of the animals were early vitellogenic, 36% late vitellogenic, and 23% had corpora lutea without eggs in the oviducts, i.e., early post-oviposited. The peak in vitellogenesis occurred in the last week of June (45% late vitellogenic) while 32% had corpora lutea and 21% were early vitellogenic without corpora lutea. At this time, 5% of the animals were observed to have prominent corpora lutea with eggs present in the oviducts. These trends continued with 71% of the animals observed having passed through an ovarian cycle by mid-July, the remaining 29% being early vitellogenic (14%), early post-ovulatory (11%), or late vitellogenic (4%). By late August, few animals had not completed an ovarian cycle, and animals with either small follicles alone (33%) or small follicles and corpora lutea (55%) predominated. By mid-October of 49 animals, 63% of the animals autopsied had small follicles only while the balance of the animals had small follicles and regressed corpora lutea (Figure 1).

During vitellogenesis, as follicular size increased,

a concomitant increase in oviduct size was observed ($p < 0.001$, early vs. late vitellogenic animals). Following ovulation and the subsequent decrease in ovarian size, oviduct weight also diminished significantly ($p < 0.02$, late vitellogenic vs. post-oviposited animals). The oviduct and ovaries continued to regress, as shown by a comparison of post-ovulatory animals from June with those of October ($p < 0.005$) (Table I). In preovulatory, postovulatory and post-oviposited animals whether observed in June, July or August, numbers of yolked follicles (4.3 ± 0.2), oviducal eggs (3.1 ± 2.1), and corpora lutea (4.8 ± 0.61) correlated well indicating no egg loss during the ovulation process.

II. CORRELATED CHANGES IN LIVER AND ADRENAL WEIGHT, PLASMA PROTEIN AND CALCIUM LEVELS.

Liver weights in all groups were similar, except for those of post-ovulatory animals with eggs in the oviducts which were smaller than all others ($p < 0.005$). No differences in total plasma protein were observed, and following electrophoretic analysis, only protein fraction 4 was seen to vary significantly. This fraction (the beta globulin) increased during vitellogenesis ($p < 0.005$, early vs. late vitellogenic animals) and remained high in post-ovulatory animals, only decreasing following oviposition.

($p < 0.005$, early post-oviposited vs. early post-ovulatory animals). Plasma calcium changes correlated with those of protein fraction 4, increasing during vitellogenesis ($p < 0.005$, early vs. late vitellogenic animals) and decreasing from the early post-ovulatory stage to the early post-oviposited stage ($p < 0.005$). An increase in plasma calcium was noted in animals autopsied during October after the ovarian cycle was completed and corpora lutea completely regressed ($p < 0.005$, early vs. late post-oviposited animals). However, a concomitant increase in plasma protein fraction 4 was not observed (Table III).

Adrenal weights in all animals during the period June through August were the same regardless of reproductive state. However, in animals autopsied during October (late post-oviposited) a significant decrease in adrenal weight occurred compared to early post-oviposited animals ($p < 0.005$) (Table I).

Significant differences in relative organ weights was independent of changes in body weight resulting from increases in ovarian, oviduct and oviducal egg weights.

III. HYPOPHYSECTOMY AND ESTROGEN TREATMENT IN FEMALES.

A single estrogen injection did not increase liver weight in either intact or hypophysectomized animals. However, hypophysectomy itself reduced liver weight com-

pared to the controls ($p < 0.005$). Total plasma protein was elevated following estrogen injection in controls ($p < 0.05$), but not in hypophysectomized animals. In both groups of estrogen controls (sham-operated and intact), plasma protein fraction 2 was depressed and 4 was elevated ($p < 0.025$ and 0.01 , respectively) compared to oil injected animals. An increase in total plasma calcium was also noted following estrogen injection ($p < 0.005$). Similar changes in plasma proteins and total plasma calcium did not occur in hypophysectomized animals. No significant changes in body weights were observed (Tables II & IV).

IV. THE EFFECT OF ESTROGEN TREATMENT OF INTACT MALES.

The effect of estrogen in males was essentially similar to that in intact females, increases in plasma protein fraction 4 and total plasma calcium ($p < 0.005$) being noted in estrogen injected animals. However, neither plasma protein fraction 4 nor total plasma calcium elevations were as high in the estrogen treated males as those found for the estrogen treated females. No significant changes in body weights were observed (Table V).

FIGURE 1 PERCENTAGE OF DIFFERENT REPRODUCTIVE STAGES JUNE THROUGH AUGUST
IN FEMALE DIPSOSAURUS DORSALIS

JUNE JULY AUGUST
21 28 5 19 26 2 16 23

TABLE I

RELATIVE ORGAN WEIGHTS FROM FEMALE DIPSOSAURUS DORSALIS IN DIFFERENT REPRODUCTIVE STAGES

Reproductive Stage	n	Ovary g/100g	CL Number	Oviduct g/100g	Liver g/100g	Adrenal mg/100g
EV	11	0.14 ± 0.08	0	0.19 ± 0.05	2.50 ± 0.04	20.2 ± 2.6
LV	8	6.48 ± 1.55	0	0.86 ± 0.12	2.64 ± 0.15	26.9 ± 4.1
EP	9	0.19 ± 0.06	4.77 ± 0.61		1.54 ± 0.14	20.4 ± 2.4
EO	15	0.32 ± 0.18	4.13 ± 0.65	0.59 ± 0.50	2.18 ± 0.10	20.4 ± 2.0
LO	7	0.03 ± 0.01	0	0.28 ± 0.09	2.12 ± 0.22	13.6 ± 1.1

EV = early vitellogenic
 LV = late vitellogenic
 EP = early postovulatory
 EO = early postoviposited
 LO = late postoviposited
 CL = corpora lutea

TABLE II

THE EFFECT OF ESTROGEN INJECTION AND HYPOPHYSECTOMY ON THE ORGAN WEIGHTS IN FEMALE DIPLOSAURUS DORSALIS

Treatment	n	Ovary g/100g	Follicle Size (mm.)	Oviduct g/100g	Liver g/100g	Adrenal mg/100g
Intact + O	7	0.03 ± 0.01	1.9 ± 0.20	0.28 ± 0.09	2.12 ± 0.22	13.63 ± 1.2
Intact + E	8	0.05 ± 0.01	1.6 ± 0.20	0.41 ± 0.05	2.18 ± 0.13	13.38 ± 1.8
Sham + E	12	0.06 ± 0.01	2.3 ± 0.30	0.36 ± 0.05	2.07 ± 0.10	15.48 ± 1.4
Hypox + O	7	0.10 ± 0.06	2.0 ± 0.30	0.33 ± 0.07	1.22 ± 0.08	14.72 ± 5.9
Hypox + E	8	0.16 ± 0.09	1.9 ± 0.02	0.43 ± 0.09	1.36 ± 0.25	15.41 ± 0.7

O = oil treated

E = estrogen treated

Hypox = hypophysectomized

TABLE III
COMPARISON OF PLASMA PROTEIN FRACTIONS AND TOTAL PLASMA CALCIUM

Reproductive Stage	Total Protein g%	Protein fraction number and corresponding fraction from mammalian plasma.					Ca mM/l
		1 (Albumin) %	2 (Globulin α) %	3 (Globulin α) %	4 (Globulin β) %	5 (Globulin γ) %	
EV n	3.53 $\pm$ 0.14 10	49.3 $\pm$ 1.6 7	17.6 $\pm$ 1.9 7	13.2 $\pm$ 1.4 7	11.6 $\pm$ 1.3 7	8.3 $\pm$ 1.5 7	2.67 $\pm$ 0.2 11
LV n	3.56 $\pm$ 0.28 8	46.9 $\pm$ 1.2 6	16.6 $\pm$ 2.1 6	11.4 $\pm$ 1.6 6	21.4 $\pm$ 2.0 6	3.7 $\pm$ 0.8 6	3.73 $\pm$ 0.6 9
EP n	3.44 $\pm$ 0.41 8	46.8 $\pm$ 4.6 6	14.5 $\pm$ 3.4 6	12.3 $\pm$ 0.8 6	21.2 $\pm$ 3.2 6	5.3 $\pm$ 1.8 6	2.69 $\pm$ 0.4 8
EO n	3.89 $\pm$ 0.21 14	51.9 $\pm$ 2.2 9	15.8 $\pm$ 1.4 9	15.6 $\pm$ 2.3 9	10.1 $\pm$ 1.2 9	6.6 $\pm$ 0.1 9	1.84 $\pm$ 0.2 11
LO n	4.20 $\pm$ 0.30 10	47.5 $\pm$ 1.9 6	18.0 $\pm$ 1.5 6	15.3 $\pm$ 2.7 6	12.8 $\pm$ 2.1 6	6.5 $\pm$ 1.2 6	2.79 $\pm$ 0.2 9

EV = early vitellogenic
LV = late vitellogenic
EP = early postovulatory
EO = early postoviposited
LO = late postoviposited

TABLE IV

THE EFFECTS OF HYPOPHYSECTOMY AND ESTROGEN INJECTION ON THE PLASMA PROTEINS AND TOTAL PLASMA
CALCIUM IN POSTOVIPOSITED DIPSOSAURUS DORSALIS

Treatment	Total Protein g%	Protein fraction number and corresponding fraction from mammalian plasma					Ca mM/l
		1 (Albumin) %	2 (Globulin α) %	3 (Globulin α) %	4 (Globulin β) %	5 (Globulin γ) %	
Intact + O n	4.2 $\pm$ 0.3 10	47.5 $\pm$ 1.9 6	18.0 $\pm$ 1.5 6	15.3 $\pm$ 2.7 6	12.8 $\pm$ 2.1 6	6.5 $\pm$ 1.2 6	2.79 $\pm$ 0.2 9
Intact + E n	5.8 $\pm$ 0.6 8	33.0 $\pm$ 2.9 8	9.2 $\pm$ 2.7 8	11.8 $\pm$ 2.3 8	35.9 $\pm$ 4.1 8	10.1 $\pm$ 1.8 8	10.10 $\pm$ 0.8 8
Sham + E n	5.6 $\pm$ 0.8 12	35.3 $\pm$ 3.4 12	10.5 $\pm$ 1.8 12	21.2 $\pm$ 8.2 12	33.6 $\pm$ 4.5 12	7.6 $\pm$ 1.5 12	9.60 $\pm$ 0.7 12
Hypox + O n	3.7 $\pm$ 0.2 7	49.9 $\pm$ 1.7 7	17.0 $\pm$ 1.1 7	15.6 $\pm$ 1.6 7	10.6 $\pm$ 1.3 7	7.0 $\pm$ 0.7 7	2.52 $\pm$ 0.3 6
Hypox + E n	4.9 $\pm$ 0.9 7	44.4 $\pm$ 3.5 7	17.4 $\pm$ 1.3 7	23.4 $\pm$ 5.7 7	9.9 $\pm$ 2.2 7	11.1 $\pm$ 0.7 7	2.52 $\pm$ 0.3 7

O = oil treated

E = estrogen treated

Hypox = hypophysectomized

TABLE V

THE EFFECT OF ESTROGEN INJECTION ON THE PLASMA PROTEIN FRACTIONS AND TOTAL PLASMA CALCIUM OF
MALE DIPSOSAURUS DORSALIS

Treatment	Total Protein g%	Protein fraction number and corresponding fraction from mammalian plasma					Ca mM/l
		1 (Albumin) %	2 (Globulin α) %	3 (Globulin α) %	4 (Globulin β) %	5 (Globulin γ) %	
E n	4.8 $\pm$ 0.2 19	36.0 $\pm$ 1.5 18	21.4 $\pm$ 1.6 18	12.3 $\pm$ 1.2 18	24.6 $\pm$ 1.2 18	5.7 $\pm$ 0.8 18	5.79 $\pm$ 0.3 19
O n	4.3 $\pm$ 0.4 15	45.9 $\pm$ 2.8 13	25.7 $\pm$ 2.2 13	11.9 $\pm$ 2.9 13	8.0 $\pm$ 1.0 13	7.8 $\pm$ 1.2 13	2.53 $\pm$ 0.3 15

E = estrogen treated

O = oil treated

DISCUSSION

In all essential points, the ovarian cycle described here correlates well with that described for the same species by Mayhew (1971). Both the present study and that of Mayhew (1971) agree that the period of ovarian activity extends from June through August. However, the latter study did not demonstrate a peak in reproductive activity during late June which declined during July and August. Observations of the numbers of mature ovarian follicles, numbers of corpora lutea, and eggs in the oviducts showed good correlation. Mayhew (1971) noted that corpora lutea disappeared shortly after eggs were laid, but the present observations indicate that although corpora lutea regress rapidly following oviposition, they may remain visible in the ovaries for a considerable period of time. This is indicated by the fact that 35% of the animals autopsied in October had visible corpora lutea even though only 11% of the animals autopsied during late August had eggs in the oviducts.

At the start of the reproductive cycle increasing titers of gonadotropin initiate ovarian estrogen synthesis during the spring months as indicated by increasing oviduct

weight. Estrogen is considered to act on the liver to induce the synthesis of a specific PLP which circulates in the blood to the ovary (Urist and Schjeide, 1961) where it is absorbed by the ovarian follicles under gonadotropic stimulation (Wallace and Jared, 1969). This sex hormone-dependent plasma protein has been demonstrated during follicular maturation and following estrogen injection in a number of species (fish: Chung-Wai and Vanstone, 1961; Urist and Schjeide, 1961; amphibia: Follett and Redshaw, 1968; Urist and Schjeide, 1961; Wallace and Jared, 1968; reptiles: Clark, 1967; birds: Heald and Rookledge, 1965; Greengard et al., 1964).

In the present study, alterations in hepatic weight, the plasma protein spectrum, and plasma calcium were noted during the period of seasonal ovarian growth as a result of ovarian estrogen secretion. While no alterations in total plasma protein were noted, an increase in the beta globulin fraction, presumably due to increased synthesis of PLP, occurred at the expense of small decreases in other plasma protein fractions. In addition to its role as a yolk precursor, PLP is a calcium-binding protein. Calcium itself is a critical electrolyte and structural

component of the embryo. As early as 1928, Hess et al. recognized the relationship of hypercalcemia to reproduction in the puffer cod-fish. In 1936, Laskowski correlated an increase in serum PLP with ovarian follicle maturation in a number of egg-laying animals and more recently Urist and Schjeide (1961) have demonstrated that plasma PLP binds Ca in birds. Dessauer and Fox (1959) observed that ^{45}Ca migrates with PLP following electrophoresis of snake plasma. The increase in plasma PLP in these experiments was accompanied by a marked increase in plasma calcium supporting previous suggestions that this plasma constituent is a calcium-binding agent responsible for calcemia during ovarian maturation. A decrease in plasma calcium without a concomitant drop in plasma PLP was noted at the time of ovulation. It is possible that this may coincide with an exchange of plasma calcium with oviducal components concerned with the deposition of calcium in the egg shell during its passage down the oviduct.

In previous investigations of estrogen induced vitellogenesis in reptiles, emphasis has been placed on a direct action of estrogen on the liver and the role of the pituitary gland in the process of vitellogenesis has not been considered (Clark, 1967; Dessauer et al., 1958; Jenkins and Simkiss, 1968; Wallace and Jared, 1968). Our experiments demonstrate that plasma protein changes typical of

those that occur during the normal ovarian cycle can be induced by a single injection of estradiol 17-beta and that these changes persist 3 weeks after the injection. Although liver hypertrophy is a typical sequel of estrogen injection in reptiles (Callard and Banks, 1970), no hepatic hypertrophy was observed in these experiments as in the normal animal. The explanation for this probably lies in the fact that only a single injection of 20 ug was administered and that any liver hypertrophy would probably have diminished in the three weeks that followed injection. The correlated changes in plasma PLP and calcium indicate that the hormone had induced hepatic synthesis of PLP. Although no studies on the half life of PLP were done in these experiments, it has been observed that the half life of Xenopus PLP is 40 days (Wallace and Jared, 1969) in animals without gonadotropin treatment, but that the half life is 2 days if gonadotropin is injected. This is due to the fact that the ovarian follicles are only able to accept the PLP from the blood if exposed to gonadotropin. Thus, in the absence of ovarian uptake of PLP in my experiments, the protein remains in the blood after its initial synthesis following estrogen injection, and a concomitant elevation of plasma calcium is observed.

Alterations in plasma composition of a similar

nature following a single estrogen injection were noted in male Dipsosaurus. However, the response was quantitatively less than that which occurred in the female. This may be due to the presence of androgens which have been shown to antagonize estrogen-induction of PLP synthesis in the bird (Hawkins, et al., 1970). Following hypophysectomy in female lizards, injection of estrogen did not have a stimulatory effect on either plasma PLP or plasma calcium, suggesting a role for the pituitary gland in the biosynthetic or induction process. A similar dependence of androgen induced plasma protein changes on an intact pituitary has been noted in rats (Kumar, 1969).

Although no further attempt to delineate the role of the pituitary in the estrogenic response was made in this study, previous work by Callard and Zeigler (1970) and Callard and Banks (1970) has indicated that ovarian growth in response to gonadotropin and liver hypertrophy in response to estrogen are both dependent upon an intact pituitary gland and these experiments further indicate that growth hormone is the active pituitary principle. Thus, it appears that normal vitellogenesis cannot occur in the absence of this hormone. While the exact mode of action of growth hormone in this system has not been elucidated, the hormone may increase cell permeability to pro-

tein precursors while estrogen causes stimulation of DNA & RNA synthesis, as shown on cells of rat uterus by Monterrat and Claude (1971), or activation of adenyl cyclase (Sutherland and Rall, 1969).

Thus, this study has described the ovarian cycle of Dipsosaurus dorsalis, a necessary prerequisite for further investigation on the reproductive process of this species. In addition, a requirement for the pituitary gland in the hepatic response, as exhibited by hepatic weight, plasma protein and calcium levels, to estrogen has been demonstrated. This is a significant observation which supports previous studies from this laboratory.

BIBLIOGRAPHY

- Briere, R. O. and Mull, J. D. (1964). Electrophoresis of serum protein with cellulose acetate. *Am. J. Clin. Path.* 42, 197.
- Callard, I. P. and Banks, W. L. (1970). Effects of hypophysectomy on the hepatic response to estrogen in the female iguanid lizard, Dipsosaurus dorsalis. *Am. Zoo.* 10, 111.
- Callard, I. P. and Zeigler, Jr., H. (1970). Inhibitory effects of prolactin upon gonadotropin-stimulated ovarian growth in the iguanid lizard, Dipsosaurus dorsalis. *J. Endocr.* 47, 131-32.
- Chieffi, G. (1966). Occurrence of steroids in gonads of non-mammalian vertebrates and sites of synthesis. *Proc. 2nd Internat. Congress on Hormonal Steroids*, No. 132. Milan.
- Chung-Wai Ho, F. and Vanstone, W. E. (1961). Effect of estradiol monobenzoate on some serum constituents of maturing Sockeye salmon (Oncorhynchus nerka). *J. Fish. Res. Bd. Can.* 18, 859-63.
- Clark, N. B. (1967). Influence of estrogens upon serum calcium, phosphate and protein concentrations of fresh-water turtles. *Comp. Biochem. Physiol.* 20, 823-34.
- Dessauer, H. C. (1970). In "Biology of Reptilia". (C. Gans and T. S. Parsons, Eds.). Academic Press, London. pp. 1-15.
- Dessauer, H. C., and Fox, W. (1958). Geographic variation in plasma protein patterns of snakes. *Proc. Soc. Exp. Biol. Med.* 98, 101-05.
- Dessauer, H. C. and Fox, W. (1959). Changes in ovarian follicle composition with plasma levels of snakes during estrous. *Am. J. Physiol.* 197, 360-66.

- Dessauer, H. C., Fox, W. and Gilbert, N. L. (1956). Plasma calcium, magnesium and protein of viviparous colubrid snakes during estrous cycle. Proc. Soc. Exp. Biol. Med. 92, 299-301.
- Follett, B. K. and Redshaw, M. R. (1968). The effects of oestrogen and gonadotrophins on lipid and protein metabolism in Xenopus laevis (Daudin). J. Endocrinol. 40, 439-56.
- Greengard, O., Gordon, M. and Smith, M. A. (1964). Studies on the mechanism of diethylstilbestrol-induced formation of phosphoprotein in male chickens. J. Biochem. 239, 2079-82.
- Hahn, W. E. (1967). Estradiol-induced vitellogenesis and concomitant fat mobilization in the lizard Uta stansburiana. Comp. Biochem. Physiol. 23, 83-93.
- Hahn, W. E., Church, R. B. and Gorbman, A. (1969). Synthesis of new RNA species prior to estradiol induced vitellogenesis. Endocrinol. 84, 738-45.
- Hawkins, R. A., Heald, P. J., Swain, M., and Taylor, P. (1970). The effects of some antioestrogens on the uptake of (6, 7-³H) 17-beta-oestradiol by tissue of the domestic fowl. Acta Endocrin. 63, 253-64.
- Heald, P. J. and Rookledge, K. A. (1964). Effect of gonadal hormones, gonadotrophins and thyroxine on plasma free fatty acids in the domestic fowl. J. Endocrinol. 30, 115-28.
- Hess, A. F., Bills, C. E., Weinstock, M. and Revikin, H. (1928). Difference in calcium level of blood between the male and female cod. Proc. Soc. Exp. Biol. Med. 25, 349-50.
- Jenkins, N. K. and Simkiss, K. (1968). The calcium phosphate metabolism of reproducing reptiles with particular reference to the adder (Vepra berus). Comp. Biochem. Physiol. 26, 865-76.
- Kepner, B. L. and Hercules, D. M. (1968). In "Turner Manual of Fluorometric Clinical Procedures". G. K. Turner Associates, Palo Alto, California.

- Kumar, M. (1969). Androgenic induction of alfa 2u-globulin in the rat: requirement of an intact pituitary. *Nature* 223, 399-400.
- Laskowski, M. (1936). In "Estrogen induces lipophosphoprotein in serum of male Xenopus laevis". Wallace, R. A. and Jared, D. W. *Sci.* 160, 91-92.
- Mayhew, W. W. (1971). Reproduction in the desert lizard, Dipsosaurus dorsalis. *Herpetol.* 27, 57-77.
- Montserratt, M. F. and Claude, A. V. (1971). Effect of RNA from estradiol-treated immature rats on protein synthesis in immature uteri. *Endocrinol.* 88, 279-285.
- Norris, K. S. (1953). The ecology of the desert iguana Dipsosaurus dorsalis. *Ecology* 34, 265-87.
- Simkiss, K. (1961). Calcium metabolism and avian reproduction. *Biol. Rev.* 36, 321-67.
- Simkiss, K. (1967). "Calcium in Reproduction Physiology". Reinhold, New York. pp. 73-250.
- Urist, M. R. and Schjeide, O. A. (1961). The partition of calcium and protein in the blood of oviparous vertebrates during estrus. *J. Gen. Physiol.* 44, 743-56.
- Sutherland E. W. and Rall, T. W. (1969). In "Biological Phosphorylations". (H. M. Kalckar, Ed.). Prentice Hall, Inc. New York, pp. 692-707.
- Wallace, R. A. and Dumont, J. R. (1968). The induced synthesis and transport of yolk proteins and their accumulation by the oocyte in Xenopus laevis. *J. Cell Physiol.* 72 (Suppl. 73), 73-102.
- Wallace, R. A. and Jared, D. W. (1968). Estrogen induces lipophosphoprotein in serum of male Xenopus laevis. *Sci.* 160, 91-92.
- Wallace, R. A. and Jared, D. W. (1969). Studies on amphibian yolk. VIII. The estrogen induced hepatic synthesis of a serum pipophosphoprotein and its selective uptake by the ovary and transformation into yolk platelet proteins in Xenopus laevis. *Devel. Biol.* 19, 498-503.