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# Reproduction and Estrogen Induced Vitellogenesis in Dipsosaurus dorsalis

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REPRODUCTION AND ESTROGEN INDUCED

VITELLOGENESIS IN DIPSOSAURUS DORSALIS

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A Thesis

Presented to

The Faculty of the Department of Biology

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In Partial Fulfillment

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## ACKNOWLEDGMENTS

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# ABSTRACT

The ovarian cycle of female <u>Dipsosaurus dorsalis</u> 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

VITELLOGENESIS IN DIPSOSAURUS DORSALIS

# INTRODUCTION

Striking increases in the plasma levels of pro-
tein, lipid, phosphorus and calcium accompanying ovarian
maturation have been well documented in reptiles. Tur-
tles (Clark, 1967), snakes (Dessauer et al., 1956: Des-
sauer and Fox, 1958, 1959; Dessauer, 1971; Jenkins and
Simkiss, 1968), and lizards (Hahn, 1967, 1969) all ex-
hibit characteristic changes in these blood components
during the vitellogenic period. Estrogen injection in
these and other submammalian vertebrates induces vitel-
logenesis accompanied by liver hypertrophy, protein syn-
thesis, 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 <u>Dipsosaurus</u> <u>dorsalis</u>, 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 <u>ad</u> <u>libitum</u> and a bedding of "Sanicel" (Paxton Processing Co.) was used. A temperature gradient having a maximum of $46^{\circ}$ C and a minimum of $21^{\circ}$ 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 $\pm$ 1.7 g.

II.   Late vitellogenic: follicle size = 6.0 - 12.0 mm. with no corpora lutea present. Mean body weight was 40.4 $\pm$ 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 $\pm$ 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-ovi-

posited females.  Animals were given a single sub-

cutaneous 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 g. ± 2.7 and 47.0 g. ± 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.  Laporotomy was performed through a 5 mm. lat-
eral 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 vitello-
genic (14%), early post-ovulatory (11%), or late vitello-
genic (4%). By late August, few animals had not com-
pleted 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). Fol-
lowing 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 ob-
served 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 dif-
ferences in total plasma protein were observed, and follow-
ing electrophoretic analysis, only protein fraction 4 was
seen to vary significantly. This fraction (the beta globu-
lin) increased during vitellogenesis (p < 0.005, early vs.
late vitellogenic animals) and remained high in post-ovula-
tory 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).
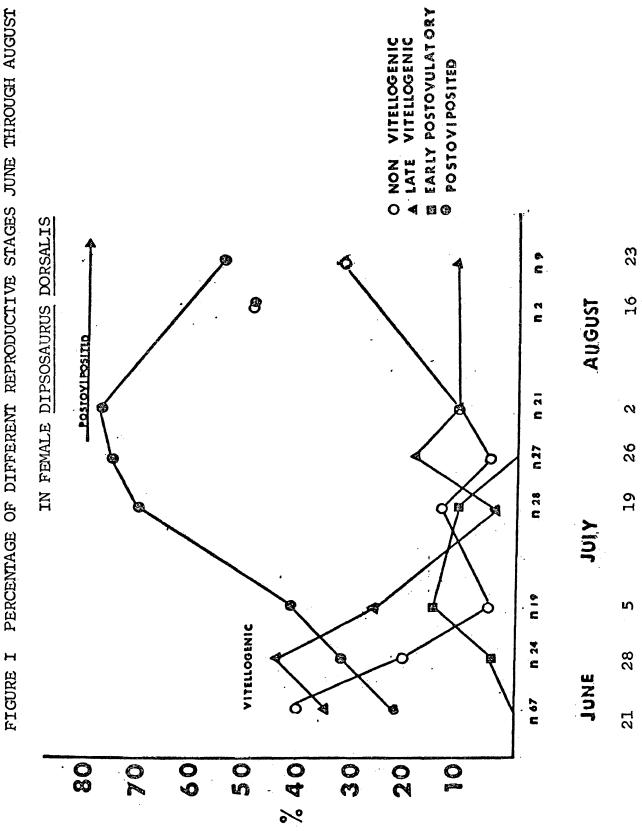
Significant differences in relative organ weights was independent of changes in body weight resulting from increases in ovarian, oviduct and oviducal egg weights.

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 I   PERCENTAGE OF DIFFERENT REPRODUCTIVE STAGES JUNE THROUGH AUGUST

IN FEMALE _DIPSOSAURUS DORSALIS_

## 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 DIPSOSAURUS DORSALIS

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

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 $\alpha$) % | 3 (Globulin $\alpha$) % | 4 (Globulin $\beta$) % | 5 (Globulin $\gamma$) % | |
| EV n | 3.53 + 0.14 10 | 49.3 + 1.6 7 | 17.6 + 1.9 7 | 13.2 + 1.4 7 | 11.6 + 1.3 7 | 8.3 + 1.5 7 | 2.67 + 0.2 11 |
| LV n | 3.56 + 0.28 8 | 46.9 + 1.2 6 | 16.6 + 2.1 6 | 11.4 + 1.6 6 | 21.4 + 2.0 6 | 3.7 + 0.8 6 | 3.73 + 0.6 9 |
| EP n | 3.44 + 0.41 8 | 46.8 + 4.6 6 | 14.5 + 3.4 6 | 12.3 + 0.8 6 | 21.2 + 3.2 6 | 5.3 + 1.8 6 | 2.69 + 0.4 8 |
| EO n | 3.89 + 0.21 14 | 51.9 + 2.2 9 | 15.8 + 1.4 9 | 15.6 + 2.3 9 | 10.1 + 1.2 9 | 6.6 + 0.1 9 | 1.84 + 0.2 11 |
| LO n | 4.20 + 0.30 10 | 47.5 + 1.9 6 | 18.0 + 1.5 6 | 15.3 + 2.7 6 | 12.8 + 2.1 6 | 6.5 + 1.2 6 | 2.79 + 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 ± 0.3 10 | 47.5 ± 1.9 6 | 18.0 ± 1.5 6 | 15.3 ± 2.7 6 | 12.8 ± 2.1 6 | 6.5 ± 1.2 6 | 2.79 ± 0.2 9 |
| Intact + E n | 5.8 ± 0.6 8 | 33.0 ± 2.9 8 | 9.2 ± 2.7 8 | 11.8 ± 2.3 8 | 35.9 ± 4.1 8 | 10.1 ± 1.8 8 | 10.10 ± 0.8 8 |
| Sham + E n | 5.6 ± 0.8 12 | 35.3 ± 3.4 12 | 10.5 ± 1.8 12 | 21.2 ± 8.2 12 | 33.6 ± 4.5 12 | 7.6 ± 1.5 12 | 9.60 ± 0.7 12 |
| Hypox + O n | 3.7 ± 0.2 7 | 49.9 ± 1.7 7 | 17.0 ± 1.1 7 | 15.6 ± 1.6 7 | 10.6 ± 1.3 7 | 7.0 ± 0.7 7 | 2.52 ± 0.3 6 |
| Hypox + E n | 4.9 ± 0.9 7 | 44.4 ± 3.5 7 | 17.4 ± 1.3 7 | 23.4 ± 5.7 7 | 9.9 ± 2.2 7 | 11.1 ± 0.7 7 | 2.52 ± 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 <u>DIPSOSAURUS DORSALIS</u>

| Treatment | Total Protein g% | Protein fraction number and corresponding fraction from mammalian plasma | | | | | Ca mM/1 |
| | | 1 (Albumin) % | 2 (Globulin α) % | 3 (Globulin α) % | 4 (Globulin β) % | 5 (Globulin γ) % | |
|---|---|---|---|---|---|---|---|
| E n | 4.8 + 0.2 19 | 36.0 + 1.5 18 | 21.4 + 1.6 18 | 12.3 + 1.2 18 | 24.6 + 1.2 18 | 5.7 + 0.8 18 | 5.79 + 0.3 19 |
| O n | 4.3 + 0.4 15 | 45.9 + 2.8 13 | 25.7 + 2.2 13 | 11.9 + 2.9 13 | 8.0 + 1.0 13 | 7.8 + 1.2 13 | 2.53 + 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 cir-
culates 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 <u>Dipsosaurus</u>. 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, <u>et al</u>., 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 cy-
clase (Sutherland and Rall, 1969).

Thus, this study has described the ovarian cycle of
Dipsosaurus dorsalis, a necessary prerequisite for fur-
ther 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 observa-
tion which supports previous studies from this labora-
tory.

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