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Plasma Steroid Hormone Levels of Female Red-Sided Garter Snakes, *Thamnophis sirtalis parietalis*: Relationship to Mating and Gestation

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Plasma levels of progesterone (P), testosterone (T), estradiol (E_2), and corticosterone (B) of female red-sided garter snakes were measured during the period of ovarian development. Differences in hormone levels were analyzed with respect to three factors: (1) whether the female mated in the spring, (2) ovarian condition, and (3) time after emergence from hibernation. The influence of these three factors on steroid hormone levels of two groups of females were then compared. In experiment I, females were obtained in the fall, subjected to an artificial dormancy period, and placed on warm, summer-like conditions in the laboratory. In experiment II, females were collected in the spring and sampled in the field. They were held in the field on fluctuating conditions for several weeks and then returned to the laboratory for sampling during early vitellogenesis. Females in experiment I had a shortened but otherwise normal ovarian and gestational cycle, whereas females in experiment II had an ovarian and gestational cycle typical of females in the field. In spite of these differences, the steroid hormone levels in relation to the ovarian cycle were remarkably similar for the two groups of females. We confirmed that mating in the spring induces a surge in E_2; E_2 also was elevated in a single sample obtained from animals collected in the fall. This elevation in plasma levels of E_2 in the fall occurs at a time when the majority of females have recently deposited sperm in their oviducts. Plasma levels of T, P, and B were not significantly influenced by mating. Unlike previous reports of other viviparous snakes, plasma levels of P were low and mostly nondetectable, even during late gestation. Plasma T was significantly elevated around the time of late vitellogenesis and ovulation, and there was a tendency for E_2 levels to be elevated at this time. In the field, plasma B levels were initially high immediately after capture and declined with time. Plasma B was significantly elevated in all females several weeks after emergence, suggesting that levels of B may vary with other annual cycles. © 1987 Academic Press, Inc.

Limited information is available on steroid hormone profiles of female snakes during the reproductive cycle (Garstka *et al.*, 1985; Bona-Gallo *et al.*, 1980; Lance and Callard, 1978; Callard and Lance, 1977; Highfill and Mead, 1975; Chan *et al.*, 1973). Generally, only progesterone and estradiol levels have been measured, and these only in females undergoing ovarian development. Furthermore, with the exception of Highfill and Mead (1975), most investigators have relied on animal suppliers as a source of animals and the history of the animals was unknown. The aim of this study was to measure and examine the functional roles of plasma steroid hormone levels in relation to mating and ovarian development of female red-sided garter snakes. We measured plasma levels of estradiol (E_2), progesterone (P), testosterone (T), and corticosterone (B) in each sample using chromatographic separation and radioimmunoassay techniques. This study was conducted during the period of the garter snake's emergence from hibernation to late gestation. Further, we compared the influence of capture at different times of year and subsequent holding conditions on the ovarian cycle and plasma steroid levels.

Recent evidence of the influence of capture and laboratory handling on plasma

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hormone levels and other reproductive functions makes a comparison of field and laboratory experiments relevant (Wingfield *et al.*, 1982; Licht *et al.*, 1983, 1985; Mendonca and Licht, 1986; Bourne *et al.*, 1986). In this study, all measures of hormone levels were obtained from two groups of females. One group of females ("laboratory-hibernated females") was purchased from animal suppliers and was subjected to a standard laboratory hibernation regime followed by placement on summer-like conditions. A second group of females ("field-hibernated females") was obtained in the field on emergence from hibernacula. These females were held on fluctuating conditions in the field for several weeks and then returned to the laboratory and maintained on summer-like conditions. Since animals are difficult to locate in the field in sufficient numbers during gestation, such field-hibernated females are the best approximation of a field animal available.

The annual reproductive cycle of female red-sided garter snakes has recently been shown to be more complex than originally suspected (Whittier and Crews, 1986a). In autumn (September–November) females return to hibernacula with regressed ovarian follicles. The ovaries remain in this condition throughout hibernation (November–April) and females emerge from hibernacula in the spring (April and May) with completely regressed follicles. Sperm are stored in specialized regions of the oviduct (Halpert *et al.*, 1982) until ovulation, which occurs 6–8 weeks after spring emergence (Gregory, 1977). Offspring are born live in this species approximately 6 weeks following ovulation (in August and September); length of gestation may vary with ambient temperatures (Gregory, 1977).

Females may mate either in late summer before returning to winter hibernacula, in the spring of the year on emergence, or at both times (Whittier and Crews, 1986a). Females that mate in the fall of the year are as likely to undergo vitellogenesis and deliver offspring as are females that mate in the spring of the year (Whittier and Crews, 1986a).

Mating in the spring is associated with a surge in circulating levels of plasma E_2 from 6 to 48 hr after coitus (Garstka *et al.*, 1985; Whittier and Crews, 1986b). This increase in the level of E_2 does not occur in females that are courted by males but do not mate; thus stimuli associated with mating appear to be responsible. While Garstka *et al.* (1985) suggested that mating induces a neuroendocrine reflex that in turn stimulates ovarian steroidogenesis, the function of the E_2 peak has not been clarified. For example, previous studies have not tested whether the mating-induced surge of E_2 is a requirement for subsequent ovarian development. In this study we looked for evidence of a functional association between ovarian development and elevated levels of E_2 induced by mating.

We also measured other steroid hormones after mating in this species. For example, recent reports of high levels of plasma T around the time of ovulation in vertebrates suggest that this may be a common phenomenon that has not received widespread attention (fish: Liley *et al.*, 1986; Fitzpatrick *et al.*, 1986; amphibia: Licht *et al.*, 1983; painted turtle: Callard *et al.*, 1978). In this study we examined the relation between mating, ovarian state, and plasma levels of T. Further, plasma levels of B are related to mating, ovarian state, and capture.

In all previous studies of steroid hormone levels of female snakes, the influences of mating in the spring of the year and that of vitellogenesis were not examined separately. In this study, a separate analysis of the influences of mating in the spring of the year and of vitellogenesis is possible because we have larger sample sizes and these events were independent in this study. The steroid hormone data in this study were also interpreted in light of evi-

dence that females mate in the spring and late summer. Together, these data indicate that a complex set of control mechanisms regulates ovarian responses in red-sided garter snakes.

MATERIALS AND METHODS

Animals and maintenance. Laboratory-hibernated animals were obtained from suppliers in the fall of 1982 (Lemberger–Kons, Oshkosh, WI). Snakes purchased from this supplier were collected in the fall from south-central Manitoba, Canada. On arrival in the laboratory, all snakes were placed in hibernation (OL:24D, 4°) for a total of 17 weeks. Animals were then transferred to summer-like conditions (14L:10D, 28°) for the duration of the experiment.

Field-hibernated animals were collected near Narcisse Pasture, Manitoba, Canada, in the spring of 1983. All females were collected unmated as they emerged from the hibernaculum. Due to limited facilities in the field, these snakes were held in an unheated indoor area in cloth bags and subjected to ambient temperature (4 to 18°) and light conditions for 3–5 weeks in May. Animals were placed in outdoor arenas every other day and offered food and water; during this time they were exposed to sunlight and attained body temperatures characteristic of animals on sunny days (27–32°). Following transport by air to the laboratory, animals were placed on summer-like conditions (14L:10D, 28°).

In both the laboratory and in the field, females were exposed to males on emergence until 50% of the females mated. A detailed protocol of the procedures used during mating trials is available in Whittier and Crews (1986a).

In the laboratory, all animals were housed in 29-gallon glass aquaria with wood chips as a substrate. A Durotest Vitalite bulb provided illumination. Snakes were fed once per week with chopped smelt or mackerel mixed with multiple vitamins (Petco). Water was available at all times.

Collection of plasma. Females were bled from the orbital sinus using heparinized collection tubes. Samples were collected between 0900 and 1400 hr. Blood samples were immediately centrifuged and plasma was collected and stored at −20° until assayed.

Extraction, chromatography, and radioimmunoassay of blood samples. Plasma levels of progesterone, testosterone, estradiol-17β, and corticosterone were measured by radioimmunoassay following chromatographic separation on celite minicolumns according to the methods, with minor modifications, of Moore *et al.* (1985) and Wingfield and Farner (1975). Briefly, plasma samples (100–200 μl) were equilibrated with 2000 cpm of tritiated steroid overnight. Each sample was then extracted with ether (3 ml) twice, and the extracted sample was dried under a stream of nitrogen. Extracts were resuspended in isooctane saturated with ethylene glycol and chromatographed on individual Celite minicolumns (Celite:ethylene glycol:propylene glycol, 6:1.5:1.5, w:v:v) with "glycol traps" (Celite:water, 3:1.5, w:v), rinsed with isooctane. Fractions were eluted as follows: neutral lipids, pure isooctane (4.5 ml); P, 10% ethyl acetate in isooctane (4.5 ml); T, 20% ethyl acetate in isooctane (4.5 ml); E_2, 30% ethyl acetate in isooctane (4 ml); and B, 50% ethyl acetate in isooctane (3.5 ml). There is less than 5% overlap of the hormones in adjacent fractions. Fractions were dried under nitrogen and resuspended in 0.5 ml (1.0 ml for B) phosphate-buffered saline–1% bovine serum albumin (PBS–BSA) for radioimmunoassay. Two-hundred microliters (100 μl of B fractions) was assayed in duplicate for each hormone fraction. A 50-μl aliquot (100 μl of B fractions) was placed in scintillant (Omnifluor) and counted for recovery determination. The values from radioimmunoassays were corrected for individual recovery and are expressed in nanograms per milliliter. Recoveries for each hormone averaged as follows: P, 86.4%; T, 84.2%; E_2, 61.6%; and B, 77.8%. Solvent blanks ranged from 93 to 100% bound. Percentage bound of each sample was considered detectable if it was two standard errors less than the mean of the four solvent blanks included in each assay. The lowest detectable standards for each assay were P, 7.8 pg/tube; T, 1.9 pg/tube; E_2, 0.98 pg/tube; and B, 3.9 pg/tube. Intraassay variation ranged from 7 to 9%. Samples were run in multiple sets; interassay variation averaged 17%. Sample values were not corrected for interassay variation.

Assessment of ovarian condition. Development of ovaries in all females was assessed directly by laparotomy or palpation and indirectly by observation of birth or substantial weight loss and the presence of young in aquaria. Results from all of these methods are combined for each treatment group in a single measure of incidence of ovarian recrudescence or vitellogenesis. Laparotomy was performed under anesthesia (0.0015-[illegible] 1.0% sodium brevital/g body wt) to determine the size of regressed and early vitellogenic (<5–12 mm) follicles.

Statistical methods. Log-transformed plasma hormone levels were analyzed using a multivariate analysis of variance statistical package (BMDP) with mating, pregnancy, and time as factors. Post hoc multiple-means comparisons were used to determine significant differences among the means. Factors resulting in small, nonsignificant F values were pooled for graphical representation.

Experiment 1: Plasma hormone levels of female garter snakes hibernated in the laboratory. Mated and unmated females were randomly assigned to either a 6-hr or a 24-hr sampling schedule. Females were then

randomly assigned to either an odd-week sampling schedule (weeks 1, 3, 5, and 7) or an even-week sampling schedule (weeks 2, 4, 6, and 8). Data from all weeks were analyzed together in a single ANOVA for each hormone.

Experiment II: Plasma steroid hormone levels of female garter snakes hibernated in the field. Females were bled immediately (time 0) on capture after mating in the field. Control females that were courted by males but had not yet mated were bled at the same time. Additional mated females and controls were collected and held segregated in cloth bags for blood sampling at 6 and 24 hr. A portion of these animals were bled again at 2.5 weeks (at the field station); laparotomies were also performed at this time. After return to the laboratory, females were resampled at 5 and 8 weeks and were palpated to determine ovarian condition. A final sample was collected from freshly captured animals at the den site in the field during the postreproductive season (September 1984). All animals bled in the field were sampled within 1 min of capture. In the laboratory all animals were bled within 1 min of retrieval from their enclosure.

RESULTS

Ovarian Responses of Females in Experiments I and II

Vitellogenesis occurred in mated and unmated females in experiments I and II (see Whittier and Crews, 1986a). In experiment I, vitellogenesis commenced immediately after emergence and ovulation occurred within 3–4 weeks. Of all females in experiment I, 61% underwent vitellogenesis. By the end of 9 weeks postemergence, all gravid females delivered offspring. By contrast, the few females in experiment II that underwent vitellogenesis (20%) still had small, nonvitellogenic follicles at 2.5 weeks. Females at 5 weeks had large, vitellogenic, preovulatory follicles; ovulation occurred from 6 to 8 weeks after emergence. Fifteen weeks after emergence, all females in experiment II that were gravid delivered offspring. A high degree of synchrony relative to date of emergence from hibernation were found in the ovarian cycle among females in each experiment.

Experiment I: Plasma Steroid Hormone Levels of Female Garter Snakes Hibernated in the Laboratory

Plasma levels of E_2 were significantly influenced by mating and time of sampling ($F(1,171) = 4.20$ and $F(9,171) = 13.16$; $P < 0.05$ and 0.001, respectively; Fig. 1A), but not by pregnancy ($F(1,171) = 0.47$; $P > 0.5$). There was an interaction between the effects of mating and time of sampling on the level of E_2 ($F(9,171) = 1.85$; $P < 0.06$); peak E_2 levels occurred in mated females 6 hr after coitus (Fig. 1A). Plasma E_2 levels

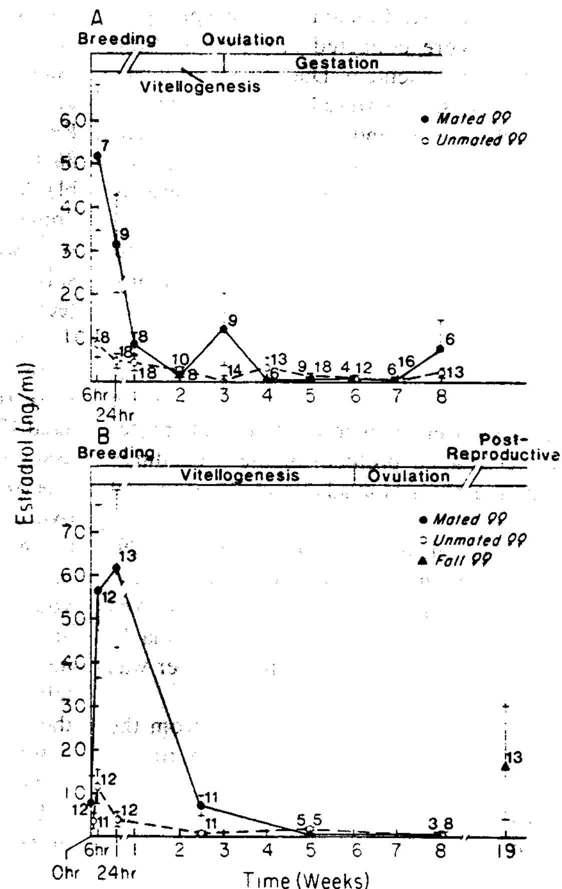

FIG. 1. Plasma levels of estradiol (ng/ml; means ± SD) of mated (solid circles) and unmated (open circles) females hibernated in the laboratory (A) or in the field (B). Sample sizes are indicated by the small numbers near each observation. The reproductive cycle of females appears at the top of each panel. In (B), a final sample from postreproductive females was taken in the field during the fall.

began to decline in mated females at 24 hr and continued during the first week following coitus. Levels then dropped to their lowest point during the second week. During the third week a few females that had mated exhibited high levels of plasma E_2, resulting in a significant increase in the mean. Thereafter levels of E_2 remained basal in mated females. Unmated females exhibited basal levels of plasma E_2 from the first sampling period at 6 hr to the last at 8 weeks.

Plasma P levels remained low, averaging less than 0.50 ng/ml throughout the 8 weeks of sampling. No significant increases were noted with mating status, pregnancy, or time for all females ($F(1,96)$ = 1.33, and $F(1,96)$ = 0.76, and $F(8,96)$ = 1.72; P > 0.20, 0.35, and 0.09, respectively). These unexpected low levels of P, particularly during the latter two-thirds of gestation, were confirmed by having samples reassayed by an independent laboratory (Dr. John Wingfield, Rockefeller Field Research Laboratory, Millbrook, NY).

Plasma T levels were significantly influenced by pregnancy and time ($F(1,150)$ = 7.97 and $F(9,150)$ = 7.44; $P < 0.005$ and 0.001, respectively; Fig. 2A), but not by mating ($F(1,150)$ = 0.92; $P > 0.30$). There was an indication of an interaction between pregnancy and time ($F(9,150)$ = 1.90; P = 0.056): T peaked during the first and second weeks of pregnancy and then decreased to basal levels thereafter (Fig. 2A).

Plasma B levels were significantly influenced by time ($F(9,167)$ = 2.49; P = 0.01; Fig. 3A), but not by mating or pregnancy ($F(1,167)$ = 0.27 and $F(1,167)$ = 0.32; P > 0.55 and 0.55, respectively). Levels of plasma B gradually dropped a total of 12.5 ng/ml from 6 hr to 3 weeks after mating or exposure to males in all females. Between Week 3 and Week 4, plasma B levels increased significantly among all females. Thereafter, plasma B levels declined gradually in both gestating and nongestating animals. It is important to note that these changes in plasma B over time were synchronous with the ovarian cycle but were not dependent on it.

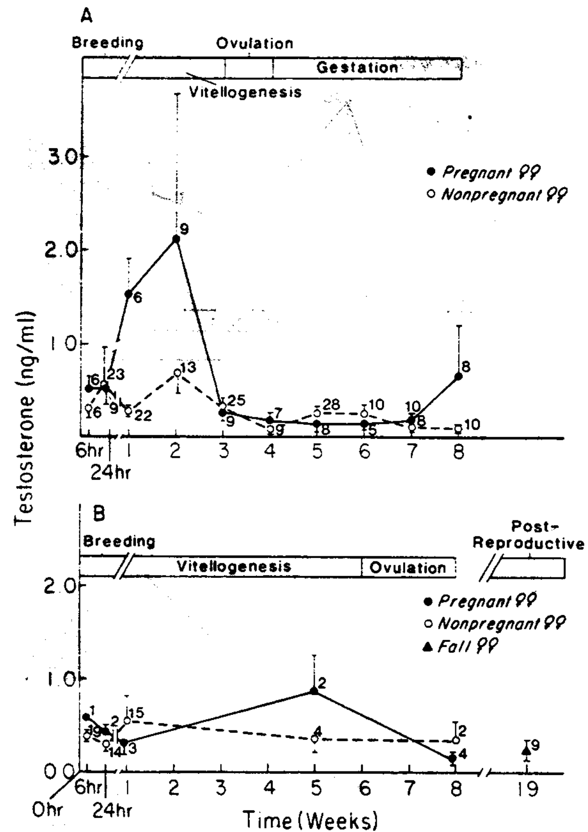

FIG. 2. Plasma levels of testosterone (ng/ml; means ± SD) of pregnant (solid circles) and nonpregnant (open circles) females hibernated in the laboratory (A) or in the field (B). See Fig. 1 for explanation.

Experiment II: Plasma Steroid Hormone Levels of Female Garter Snakes Hibernated in the Field

Because so few animals in this experiment became vitellogenic, the data of levels of E_2, P, and B were analyzed with respect to mating status and time of sampling only. Plasma levels of T were analyzed with respect to pregnancy status and time after sampling only.

The levels of plasma E_2 observed in field-hibernated females (experiment II) were remarkably similar to those in laboratory-hibernated females (experiment I) in

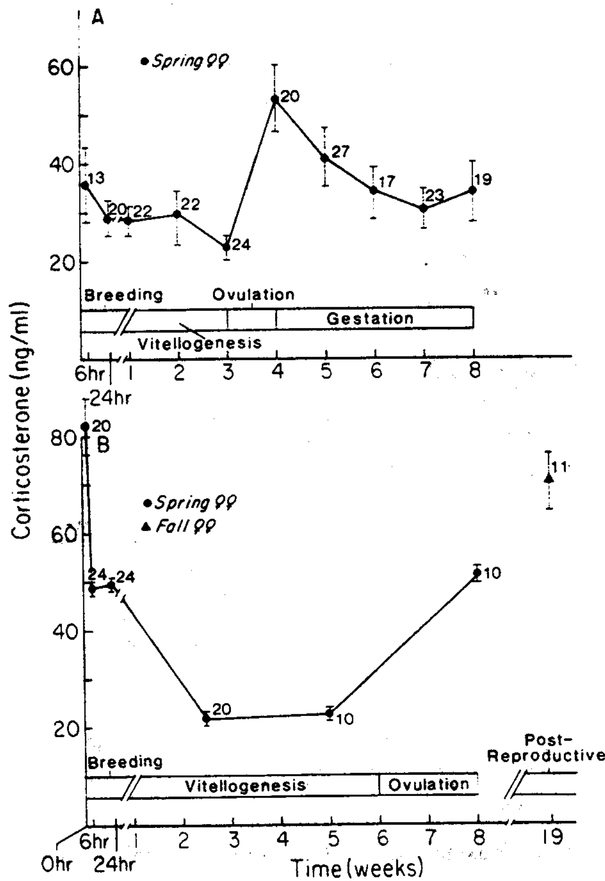

FIG. 3. Plasma levels of corticosterone (ng/ml: means ± SD) of all females hibernated in the laboratory (A) or in the field (B). The reproductive cycle of females appears at the bottom of each panel. See Fig. 1 for further explanation.

relation to mating, time, and absolute level (compare Figs. 1B and 1A, respectively). Plasma levels of E_2 were significantly influenced by mating and time of sampling ($F(1,103) = 9.84$ and $F(5,103) = 7.32$; $P < 0.002$ and 0.001, respectively; Fig. 1B). However, there was no significant interaction between the effects of mating and time of blood sampling on the level of plasma E_2 ($F(5,103) = 1.84$; $P = 0.11$). In addition, the levels of E_2 in the plasma of mated females and unmated females at time 0 were very similar. Estradiol rose precipitously by 6 hr after mating. High levels of E_2 were sustained in mated females through at least 24 hr; by 2.5 weeks plasma E_2 levels dropped but were still significantly different from those of unmated controls ($F = 6.178$, $P = 0.02$). E_2 in mated females remained low thereafter. This contrasts with the near basal levels maintained by unmated females throughout the course of the experiment. Plasma levels of E_2 in females sampled during the postreproductive season were highly variable; some females had levels of plasma E_2 much higher than basal levels.

As in experiment I, plasma P levels were low, with the majority of values nondetectable. No significant differences were noted due to mating or time for all females ($F(1,74) = 2.60$ and $F(5,74) = 1.83$; $P > 0.11$ and 0.12, respectively). As in experiment I, no increase in plasma P was noted at the later sampling times, even among females in early gestation.

In field-hibernated females, plasma T levels did not significantly vary with respect to pregnancy or time ($F(1,73) = 0.21$ and $F(5,73) = 1.10$; $P > 0.65$ and 0.36, respectively).

Plasma B levels, as in experiment I, were significantly influenced by time ($F(5,100) = 4.87$; $P < .001$; Fig. 3A), but not by mating ($F(1,100) = 0.08$; $P > 0.77$). Plasma B levels were initially high at time 0, but then dropped precipitously by 6 hr after collection and remained stable through 24 hr. Another large decline occurred by 2.5 weeks after capture; plasma B remained low through Week 5 and then climbed at Week 8. This pattern is remarkably similar to the timing of the ovarian cycle as seen in females in experiment I. This increase in plasma B was also seen in nonreproductive animals. Females sampled in the field during the postreproductive season had high levels of plasma B that approached the levels found in recently captured females in the spring.

DISCUSSION

We have found remarkable similarity between patterns and magnitude of plasma

steroid hormone levels in female garter snakes exposed to two conditions. Females in experiment I were obtained in the fall, hibernated in the laboratory, and placed on summer-like conditions in the laboratory. In experiment II, females were captured in the spring of the year on emergence from natural hibernation, held on fluctuating conditions, and then transferred to the laboratory during the first 5 weeks after emergence. The similarity in hormone cycles observed under these differing conditions suggests that garter snakes are less susceptible to the disruption of neuroendocrine events induced by handling and captivity (over periods of hours and days) as have been noted in other classes of vertebrates (Wingfield *et al.*, 1982; Licht *et al.*, 1983) and some reptiles (turtles: Licht *et al.*, 1985; Mendonca and Licht, 1986; alligators: Lance, 1985). This lack of suppression of reproductive hormones in the female garter snake is similar to the lack of suppression in androgens observed in the male scincid lizard *Tiliqua* 24 hr after capture (Bourne *et al.*, 1986). In all other studies examining the influence of capture on suppression of reproductive hormones in reptiles, corticoids have not been measured. In amphibians, corticosterone is responsible for directly inhibiting LH-RH (Moore, 1983). Our data support the hypothesis that in the garter snake reproductive suppression does not occur due to captivity and handling because corticosterone levels do not increase under these conditions.

On emergence from hibernation in both experiments, females were exposed to males and approximately half were allowed to mate. Females that did not mate in the spring were as likely to become vitellogenic as females that did mate in the spring. Indeed, females of this species often mate in the fall and store sperm over the winter (see Halpert *et al.*, 1982; Whittier and Crews, 1986a).

Before discussing the hormone data, it is important to note some differences between the incidence of ovarian development in experiments I and II (see Whittier and Crews, 1986a). Overall, 61% of females in experiment I became vitellogenic and produced offspring during this study; in experiment II, only 20% of the females did so. This difference in the incidence of reproduction between females captured in the fall and those captured in the spring may be due to differences in conditions during or immediately after hibernation. Furthermore, there was a significant difference between the length of the ovarian cycle (among those females that ovulated) between the two experiments. Females hibernated on the laboratory (experiment I) had an extremely compressed ovarian cycle compared with females that hibernated in the field (experiment II). The latter had an ovarian cycle much like that described in field animals (Gregory, 1977; Garstka *et al.*, 1982). We have chosen to express our steroid hormone data in terms of time after hibernation; when ovarian state was found to be related to hormone levels, the respective cycles have been indicated on the figures.

In both experiments, plasma levels of E_2 were significantly influenced by mating, as has been shown in previous studies (Garstka *et al.*, 1985; Whittier and Crews, 1986b). Plasma E_2 was low in both mated and unmated females immediately after capture in the field, indicating that the act of mating is not dependent on high levels of E_2. Plasma levels of E_2 rose precipitously only in mated females, and were sustained at high levels for at least 24 hr. Mated females sampled in experiment II maintained high levels of E_2 for a longer period of time (24 hr to 2.5 weeks), perhaps due to lower clearance, than did females that were sampled in experiment I (decline after 6 hr). These results indicate that while captivity does not markedly impair the elevation of

E_2 in response to mating, it may suppress it somewhat. Laboratory-hibernated animals frequently exhibit less sexual behavior as compared with animals observed in the field (Whittier *et al.*, 1985; Whittier and Crews, 1986b).

It is surprising that plasma E_2 levels were not significantly related to subsequent ovarian development. Females that mated but failed to become vitellogenic experienced similar rises in plasma E_2 during the first 24 hr as females that mated and became vitellogenic. Thus, the presence of a peak in E_2 after mating in the spring was not a predictor of subsequent ovarian development. Further, females that did not mate on emergence and became vitellogenic did not experience a peak in E_2 levels at 6 and 24 hr after emergence. A conservative conclusion is that a peak in E_2 during the first 24 hr after emergence is not a requirement for subsequent ovarian development.

While unmated females that became vitellogenic did not experience a peak in E_2 within 24 hr of emergence, this does not imply that vitellogenesis is initiated in the absence of E_2. All evidence in vertebrates that undergo vitellogenesis points to a critical role of estrogen in activating the process (Wallace, 1979). One hypothesis is that in the red-sided garter snake, unmated females in the spring experience a transient peak in E_2 (which was not detected in our sampling) during the first few weeks after emergence, and that this is used to activate vitellogenesis. An alternative hypothesis is that these females have a mating-induced surge in E_2 associated with mating in the previous late summer or fall, before hibernation. The high and variable levels of E_2 detected in females returning to the dens in the fall of the year support this latter alternative. Females mating in late summer would then arrest vitellogenesis in an extremely early stage, as no females with vitellogenic follicles have ever been found entering or leaving the hibernacula (Garstka *et al.*, 1982; J. M. Whittier and R. T. Mason, personal observation). On emergence in the following spring, females would proceed with ovarian development in the absence of an E_2 surge. Thus, the neuroendocrine "signal" to the ovary that occurs in response to mating would be preserved over winter (see Whittier and Crews, 1986a).

Increasing plasma E_2 levels before ovulation have been noted in other species of snakes (see Lance and Callard, 1978). In the present study a highly variable (but nonsignificant) increase in plasma E_2 was observed at Week 3 in females that mated in the laboratory. Only vitellogenic females had very high levels. However, not all vitellogenic females showed such an increase, including both females that had mated and those that had not mated in the spring. It is possible that this rise in E_2 is transient and was not detected due to the infrequency of sampling. A much more detailed analysis of the changes and pattern of plasma levels of E_2 in relation to ovulation needs to be carried out in this species.

The absence of any marked plasma levels of P during the entire 8-week period, and particularly during late gestation, was notable. This was so unexpected that we had several samples reassayed by an independent laboratory to confirm the low levels of P. Values of 6–10 ng/ml of plasma P during gestation have been reported in other snakes (*Thamnophis elegans:* Highfill and Mead, 1975; Nerodia (formerly *Natrix*): Chan *et al.*, 1973; Lance and Callard, 1978; Callard and Lance, 1977; Kleis-San Francisco and Callard, 1986; *Naja naja:* Bona-Gallo *et al.*, 1980). The absence of high levels of P during gestation in the red-sided garter snake indicates there may be more variability in the evolution of viviparity and related dependence on postovulatory levels of P than has been thought previously (see Kleis-San Francisco and Callard, 1986; Guillette, 1987). Alternatively, female red-sided garter snakes may

elaborate a slightly different progestin during gestation which was not detected by our specific radioimmunoassay. The present data only raise questions that must be resolved by future research.

Although levels of T were not particularly high (compared with approximately 10–20 ng/ml in field-captured males and 5–10 ng/ml in laboratory-maintained animals: D. Crews and W. R. Garskta, unpublished data), they were detectable and changed in relation to the ovarian cycle. In experiment I, T increased significantly during the first 2 weeks of vitellogenesis. The few females in experiment II that became vitellogenic also tended to have elevated levels of T during the same phase of vitellogenesis (week 5). Elevated levels of T have been noted in relation to the ovarian cycles of the cobra (*N. naja:* Bona-Gallo *et al.*, 1980) and of some turtles (*Chrysemys picta:* Callard *et al.*, 1978) in concert with E_2. In fact, a correlation between plasma T and preovulatory events has been found in other nonmammalilan species (fish: Liley *et al.*, 1986; Fitzpatrick *et al.*, 1986; amphibia: Licht *et al.*, 1983). However, the functional role of T in relation to ovarian physiology is controversial. Callard *et al.* (1978) suggest that elevated plasma levels of T reflect the role of T in the biosynthetic pathway of E_2. Recent work suggests a more complex role of T in female endocrine physiology (see Fitzpatrick *et al.*, 1986) and is a problem that needs further study.

There was no influence of mating on plasma T levels in either experiment even though T was correlated with vitellogenesis. This is in contrast to the dramatic influence of mating on plasma E_2 levels. Furthermore, there was no overall correlation between plasma levels of T and E_2. This observation demonstrates that circulating levels of T (the biochemical precursor of E_2) do not necessarily reflect aromatase activity nor circulating levels of E_2.

Plasma levels of B decreased dramatically by 6 hr after capture in the field and were elevated in females sampled in the field during the fall. This result contrasts with the large increases in plasma B that occur on capture in some vertebrates (Wingfield *et al.*, 1982; Licht *et al.*, 1983). The present data suggest that the red-sided garter snakes may not experience a classical stress response, characterized by sustained elevations in circulating corticoids, for hours or days after capture. However, short-term elevations in plasma corticoids, such as characterize stress response to capture in some birds (Wingfield *et al.*, 1982), were not examined in this study. The lack of influence of capture on the pattern and levels of sex steroid hormones observed in this and other studies (see Bona-Gallo *et al.*, 1980; Bourne *et al.*, 1986) indicates that reproduction and accompanied hormone secretion are not necessarily constrained by captive conditions in all species. Further, the red-sided garter snake is easily bred in captivity, whereas other species that experience captive-induced stress responses typically do not breed spontaneously in the laboratory (Moore, 1983; J. C. Wingfield and P. Licht, personal communication). Another interesting stress-related correlate, aggression, is completely absent in red-sided garter snakes.

Plasma B changed significantly over time in both experiments I and II but did not vary with respect to mating or ovarian state. The change in plasma B noted in both experiments was synchronous with the time of late vitellogenesis. However, the rise in plasma B occurred at the same time in females that did not yolk follicles. This suggests that the level of plasma B is varying with a nonbreeding physiological cycle. Such changes in plasma B have been noted in other species (see Wingfield and Grimm, 1977) and probably are related to seasonal cycles in fattening or migration. In the garter snake the observed rise in plasma B corresponds roughly to the time when snakes begin feeding (after a prolonged period of aphagia) and also begin to

shed. In this study, however, detailed records of these events were not kept.

This study has yielded interesting data that suggest several avenues of future research. More information is needed on the influence of late summer mating on levels of plasma E_2 and concomitant neuroendocrine changes. Further, how neuroendocrine signals are activated and then arrested after late summer mating, and then reactivated on emergence in the spring, is not known. After emergence in the spring, a more detailed profile of these hormones during the time around ovulation as well as investigations aimed at uncovering their functional role in ovarian events is needed. During gestation we must examine whether there are other ovarian progestins elaborated in this species. Finally the suggestion of the lack of a classical stress response due to handling, as evidenced by no significant elevations in plasma B hours after capture, raises the interesting question as to the generality of this phenomenon.

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