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Adrenal Activity in the Female Lizard *Lacerta vivipara* Jacquin Associated with Breeding Activities

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Variations of adrenal activity were studied in captive viviparous females *Lacerta vivipara*, in relation to breeding activities. The study was restricted to the period of active life which includes both the phase of annual reproduction and a phase of sexual inactivity. Significant seasonal changes in plasma corticosterone levels were measured with a peak during the second half of gestation followed by an abrupt fall at parturition. No significant variations in plasma aldosterone levels were observed. A limited extraovarian production of progesterone was detected which might be of adrenal origin. The half-life of injected tritiated corticosterone was not longer in pregnant than in nonreproductive females, suggesting that the peak of circulating corticosterone in pregnant females corresponds to an increase in the production rate of the hormone. The functional importance of the pituitary–adrenal axis was demonstrated *in vivo:* plasma corticosteroid levels dropped to the detection limit after adenohypophysectomy. Seasonal variations of adrenal sensitivity to synthetic ACTH 1–39 were examined *in vitro*, using a perifusion system. No significant variations were observed throughout the period of active life. These results suggest that the peak of plasma corticosterone during gestation can be ascribed to activation of the pituitary–adrenal axis. Experimental modifications of circulating corticosterone level during late gestation altered the timing of parturition, thus indicating that the fall of corticosterone just before term may be involved in the process of parturition in the female *L. vivipara*.

Squamate reptiles hold a key position in the vertebrate evolution of viviparity, but most studies concerning the endocrine control mechanisms of their reproduction have focused on gonadal and gonadotropic activities (reviewed in Guillette, 1987; Xavier, 1989). A few morphological and biochemical studies (reviewed in Callard and Callard, 1978) suggested that the adrenal function is influenced by sex-related changes. An extended study of adrenal function was therefore undertaken in the female of the viviparous lizard *Lacerta vivipara* Jacquin (Dauphin-Villemant and Xavier, 1985, 1986, 1987; Dauphin-Villemant *et al.*, 1988). The first histological studies performed in reptiles, including *L. vivipara* (Panigel, 1956), suggested annual variations of adrenal activity (reviewed in Gabe *et al.*, 1964). Our previous results (Dauphin-Villemant and Xavier, 1985) confirmed the existence of seasonal changes in the *in vitro* steroid biosynthesis from exogenous precursors by adrenals. As in other reptiles (reviewed in Licht, 1974; Sandor *et al.*, 1976; Callard and Callard, 1978; Duggan, 1981), corticosterone appears to be the major circulating corticosteroid (Dauphin-Villemant and Xavier, 1986, 1987). In the female viviparous lizard, a nychthemeral rhythm of plasma corticosteroids was recorded with a peak in late morning. Under laboratory conditions, no shift of the peak

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appeared during the period of active life (Dauphin-Villemant and Xavier, 1987), allowing a single daily blood sampling at fixed time to study the seasonal changes in plasma corticosteroids.

The purpose of the present study was to investigate changes in adrenal activity in relation to breeding activities in the female *L. vivipara*. This study was therefore conducted throughout the period of active life which includes a phase of reproduction and a phase of sexual inactivity. The seasonal changes in plasma corticosteroids were characterized. In order to determine the origin of the observed fluctuations, the possible variations of corticosterone metabolic clearance were researched. In addition, the control of adrenal activity by adrenocorticotropin and the possible variations of the sensitivity of the interrenal tissue to ACTH were studied using an *in vitro* perifusion technique. The functional importance of corticosterone during gestation was investigated by studying the effects of *in vivo* modifications of corticosterone levels during late gestation.

MATERIALS AND METHODS

Animals and Maintenance in the Laboratory

Adult *L. vivipara* Jacquin (2–6 g body weight) were collected over a 4-year period (1985–1988) from May to July in natural populations of the Massif Central (1000–1200 m above sea level, France). During the natural period of active life (April–October), the animals were housed in large soil-filled terraria containing heather, in an air-conditioned room with additional heating from 8 AM to 2 PM (temperature in the terraria ranging from 20 to 30° as previously described by Dauphin-Villemant and Xavier, 1987) and exposed to natural photoperiod. Water dishes were present in the terraria and animals were fed *ad libitum* with crickets and mealworms. Captivity lasted from several days to several months. Particular care was taken to reduce handling and confinement of the animals, thus avoiding stress effects (see Dauphin-Villemant and Xavier, 1987). Under these conditions, females completed their annual reproductive cycle (Fig. 1) and gave birth to healthy offspring.

Experimental Procedure

Measurements of plasma steroids. In a first set of experiments, plasma corticosteroids were measured in intact animals throughout the period of active life (about 300 animals, mainly females and some males for comparison, were used over 4 years). Blood was sampled from the infraorbital sinus and collected into ice-cold heparinized tubes. Blood collection usually lasted less than 3 min, never exceeded 5 min, and took place between 07.00 and 09.00 AM. Plasma (10 to 65 μl) was obtained by centrifugation at 4° (1000*g* for 10 min) of the blood samples and was stored at −20° until assay. Individual values were pooled according to the physiological state of the adult females as defined in Fig. 1 (vitellogenesis, gestation, time after parturition). The physiological stage of reproductive females was determined either by laparotomy of control females or at autopsy following the blood collection.

In a second set of experiments, female lizards anesthetized by hypothermia were ovariectomized, dorsally during gestation (n = 24) and ventrally during the

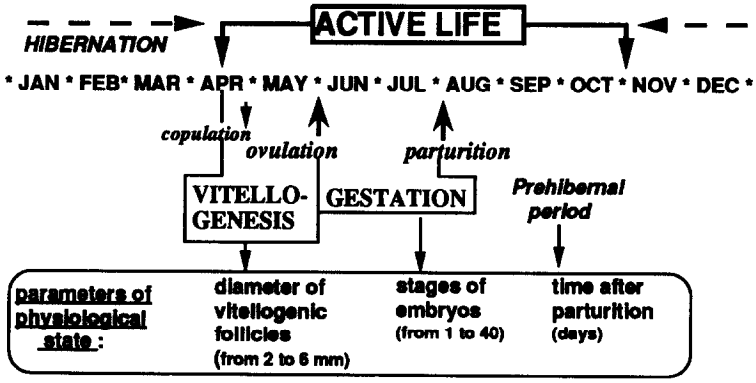

FIG. 1. Reproductive cycle of viviparous female *L. vivipara* and parameters used as reference system for the determination of the physiological state of the females.

prehibernal period (n = 8). Blood was sampled as described above, before and 7 days after ovariectomy. Plasma progesterone levels were measured.

In a third set of experiments, female lizards anesthetized by hypothermia were adenohypophysectomized during gestation (n = 6) or the prehibernal period (n = 5). Blood was sampled as described above, before and 10–13 days afer operation. Plasma corticosteroid concentrations were measured.

Peripheral metabolism of corticosterone: Kinetics of tritiated corticosterone disappearance from plasma after a single injection. Adult lizards received one intraperitoneal injection of 37 kBq (1 μCi) tritiated corticosterone/animal dissolved in 10 μl ethanol. As determined from preliminary experiments, blood (10 to 50 μl of plasma) was sampled at times 10 min, 30 min, 1 hr, 3 hr, 8 hr, 24 hr, and 72 hr after the injection. This experiment was performed once during the gestation period (n = 5♀ + 4♂) and once during the prehibernal period (n = 6♀ + 6♂). At each time, plasma radioactivity was immediately counted in 5-μl aliquots of plasma, using a Kontron automatic liquid scintillation spectrometer (Model beta V). The remaining part of the plasma samples was diluted in 1 ml water. Steroids were extracted twice with 5 ml of methylene chloride. The organic phase was evaporated to dryness under air stream and dissolved in 1 ml of ethyl acetate before chromatography. Corticosterone was separated from other metabolites by high-performance liquid chromatography (HPLC) as previously described (Dauphin-Villemant and Xavier, 1985). Briefly, a 250 × 4.6-mm Ultrasphere ODS column was used with a linear solvent gradient from 50 to 83% methanol in water (v:v), at a flow rate of 1 ml · min^{-1} for 40 min. Fractions were collected every 0.4 min and counted in the scintillation spectrometer. Standard tritiated corticosterone was run before each series. Tritiated corticosterone, after elimination of corticosteroid metabolism products, was expressed at each time as a percentage of the maximal radioactivity recovered in plasma. The time at which tritiated corticosterone in plasma represented 50% of the maximal radioactivity recovered in plasma was called half-life ($T_{1/2}$).

In vitro response of adrenal tissue to ACTH: Perifusion experiments. The perifusion system employed for this study has been previously described in detail (Leboulenger *et al.*, 1978). Three to four independent experiments were performed at each of the main phases during the active life period of the lizards (vitellogenesis, gestation, prehibernal period). For each perifusion experiment, two female lizards were killed by decapitation between 19.00 and 20.00 PM. Adrenals were quickly removed, placed in a drop of Dulbecco's modified Earle's medium (DMEM; Eurobio, Paris) and sliced into 1-mm^3 pieces. The adrenal pieces were transferred into the perifusion chamber (mixed to Bio-Gel P2, 200–400 mesh wet, Bio-Rad) and continuously perifused with DMEM at a constant flow rate (12 ml/hr), temperature (30°), and pH (7.3). After an 8-hr equilibration period, graded concentrations of synthetic human ACTH 1–39 (from 0.32 to 32 nM dissolved in DMEM just before use) were administered as 20-min pulses every 120–150 min. The effluent perifusate was collected in polystyrene tubes at 5-min intervals for 12–13 hr and stored at −20° until steroid assays.

Modifications of plasma corticosterone levels: In vivo experiment during late gestation. This experiment was performed in adult pregnant females near term. A first group of animals (n = 18) received daily intraperitoneal injections of corticosterone in sesame oil (2 to 6 μg · g^{-1} · day^{-1}). A second group of animals (n = 8) was adrenalectomized dorsally with a thermocautery, after anesthesia by hypothermia. Control females (n = 24) were not treated, received sham injections of sesame oil only, or were sham operated. The day the experiment started was called D0. Parturitions were noted from this day. Injections of corticosterone stopped only when the females gave birth to their offspring.

Reagents and Solvents

All organic solvents were from Merck (Darmstadt, West Germany). Nonradioactive steroids were supplied by Sigma Chemical Co. (St. Louis, MO). [7(n)−^{3}H]Progesterone (3737 GBq/mmol), [1,2,6,7−^{3}H]corticosterone (3626 GBq/mmol), and [1,2,6,7−^{3}H]aldosterone (2775 GBq/mmol) were purchased from the Radiochemical Centre, Amersham (France). The radiochemical purity of each steroid was regularly controlled by HPLC. Measurements of plasma steroids were performed with a corticosterone antiserum supplied by Steranti Research Ltd. (UK), an aldosterone antiserum generously given by Dr. Corvol (U36-INSERM, Paris, France), and the progesterone antiserum: anti-progesterone 11-succinyl-BSA FURR (UK). Synthetic human ACTH 1–39 was a generous gift from Drs. Scheibli and Andreatta (Ciba-Geigy, Basel, Switzerland).

Steroid Assays

Corticosteroids in plasma samples. These were extracted by methylene chloride (2 × 5 ml), separated by chromatography on celite microcolumns (Celite 535 from Koch-Light Laboratories Ltd., UK) using a method modified from Magyar *et al.* (1981). Steroid extracts were measured according to the radioimmunoassay procedure previously described (Dauphin-Villemant and Xavier, 1987). When only corticosterone was to be measured, a simplified method of separation was used: plasma samples were first washed by 5 ml isooctane in order to eliminate progesterone. Polar steroids (corticosteroids) were then extracted by methylene chloride and radioimmunoassayed using

the same procedure as mentioned above. Recovery after extraction and chromatography was 75 to 100% and 65 to 85% for corticosterone and aldosterone, respectively. The sensitivities of the standard curves were 25 and 10 pg/tube for corticosterone and aldosterone, respectively. The blank values run with each assay were below the sensitivity of the standard curves. The intra- and interassay reproducibilities were, respectively, 8.6 and 17.2% for corticosterone and 7.8 and 11.6% for aldosterone.

Progesterone in plasma samples. Progesterone was extracted by ether and radioimmunoassayed after chromatography on Sephadex LH-20 microcolumns as described by Thibier and Saumande (1975). Recovery after extraction was 85 to 95%. The sensitivity of the standard curve was 5 pg/tube with nondetectable blank values. The intra- and interassay reproducibilities were, respectively, 17 and 20%.

Corticosterone and aldosterone concentrations in perifusion experiments. These concentrations were measured without prior extraction in 75–200 μl of perifusion effluent. The radioimmunoassay procedure was previously described in detail (Leroux *et al*, 1980; Leboulenger *et al.*, 1982). The sensitivities of the standard curves were 25 and 5 pg/tube for corticosterone and aldosterone, respectively. The intra- and interassay reproducibilities were, respectively, 8.6 and 16.8% for corticosterone and 7.0 and 14.3% for aldosterone. The validity of the assay techniques has been controlled by HPLC analysis of an aliquot of effluent perifusate (Fig. 2). Corticosteroid productions at any time were expressed as pg/min per adrenal. The basal levels were calculated as the mean of six samples (30 min) taken just before the infusion of the first secretagogue.

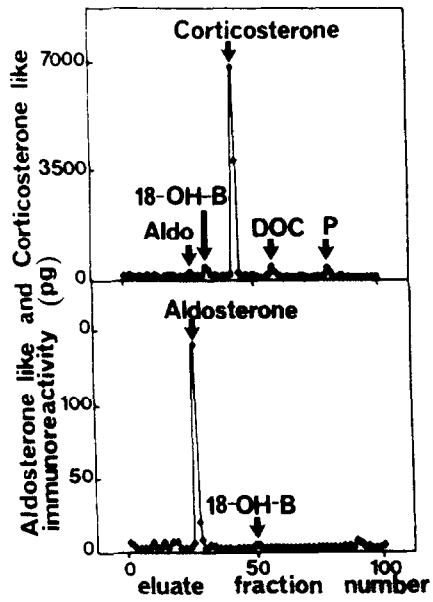

FIG. 2. HPLC analysis of methylene chloride extracts of effluent perifusate. Corticosterone (upper)- and aldosterone- (lower)-like materials were radioimmunoassayed as described in text. The gradient of solvent used consisted of 50 to 83% methanol in water (v:v) for 40 min, as previously described (Dauphin-Villemant and Xavier, 1985). One hundred fractions (0.4 ml each) were collected and radioimmunoassayed for corticosterone and aldosterone.

Statistical Analysis

Results were expressed as mean ± SE. Linear regressions were calculated in several experiments. Data were analyzed using ANOVA and some means were compared using Student's *t* test for nonpaired data. Differences in parturition frequencies were analyzed by χ^2.

RESULTS

Variations of Interrenal Activity

Seasonal changes in plasma corticosteroids. The results collected during the period of active life were first pooled according to the main phases of the reproductive cycle (Table 1). Plasma corticosterone levels were significantly influenced by the physiological state of adult females *L. vivipara* ($F(4,124) = 8.63$; $P < 0.001$). The lowest levels of plasma corticosterone were measured during vitellogenesis (13 ng/ml). Plasma levels gradually increased during the first half of gestation (27 ng/ml, $P < 0.05$) and a significant peak in plasma corticosterone was observed during the second half of gestation (45 ng/ml, $P < 0.01$). Moderate levels were measured during the postpartum and the prehibernal period (24 to 32 ng/ml). Short-term variations of plasma corticosterone were recorded during gestation and postpartum. Blood was regularly sampled in pregnant females. Results were pooled according to the dates of parturition (Fig. 3). During the second half of gestation, two successive peaks of plasma corticosterone were observed: the first, from Days 19 to 15 before parturition (up to 50 ng/ml), was correlated with stages 28 to 32 of the embryos; the second took place dur-

TABLE 1
ANNUAL VARIATIONS OF PLASMA CORTICOSTEROIDS IN ADULT *L. vivipara* (EXPRESSED AS ng/ml; MEAN ± SE; (*n*), NUMBER OF ANIMALS)

Period of the reproductive cycle	Corticosterone	Aldosterone
I. Adult females over 4 years		
Vitellogenesis	14.7 ± 2.9 (18)	3.2 ± 0.8 (10)
Early gestation (stages 1–25[a])	27.0 ± 4.4 (28)	2.7 ± 0.2 (16)
Late gestation (stages 26–40[a])	45.1 ± 4.1 (38)	2.8 ± 0.2 (33)
Postpartum	24.1 ± 3.5 (14)	1.8 ± 0.3 (5)
Prehibernal period	32.1 ± 2.6 (31)	2.4 ± 0.2 (19)
ANOVA	$F(4,124) = 8.63$ ($P < 0.001$)	$F(4,78) = 1.30$ (NS)

II. Adult males and females (May to September 1988)

	Corticosterone	
	Females	Males
Vitellogenesis	15.2 ± 3.5 (7)	17.3 ± 5.2 (6)
Early gestation (stages 23–29[a])	20.6 ± 1.2 (6)	12.8 ± 4.0 (7)
Midgestation (stages 30–36[a])	35.8 ± 7.5 (5)	18.4 ± 2.8 (7) $F(1,10) = 7.67$ ($P < 0.02$)
Prehibernal period	20.5 ± 4.2 (7)	23.6 ± 6.7 (6)
ANOVA	$F(3,21) = 4.53$ ($P < 0.02$)	$F(3,22) = 1.05$ (NS)

[a] Developmental stages of the embryos according to Dufaure and Hubert (1961).

ing late gestation, from Days 8 to 1 before parturition and coincided to the last embryonic stages 39–40 (up to 80 ng/ml). Parturition was characterized by an abrupt fall of plasma corticosterone levels (from 70–80 to 20–30 ng/ml).

From May to September 1988, sex-related variations were investigated (Table 1). No significant differences in the concentrations of plasma corticosterone were observed between males and females, except during the period of gestation ($F(1,10) = 7.67$; $P < 0.02$). Among males, plasma corticosterone levels did not vary significantly throughout the period of active life ($F(3,22) = 1.05$; $P > 0.05$; Table 1).

Aldosterone was only measured in females. No significant variations of plasma aldosterone levels were observed during the period of active life ($F(4,78) = 1.30$; $P > 0.05$; Table 1 and Fig. 3).

Possible production of progesterone by the adrenal glands of the female L. vivipara. In pregnant females, plasma levels of progesterone dropped significantly after ovariectomy ($P < 0.001$; Table 2), but levels remained significantly higher in ovariectomized pregnant females than in intact females during the prehibernal period ($P < 0.05$). During the prehibernal period, plasma progesterone levels did not change significantly before and after ovariectomy ($P > 0.05$; Table 2). These results suggest the existence of an extraovarian source of progesterone which may be the adrenals.

Seasonal Changes in the Kinetics of Tritiated Corticosterone Disappearance from Plasma after a Single Injection

The variations of corticosterone metabolic clearance were evaluated by comparing the disappearance of [^{3}H]corticosterone from plasma after a single injection (Fig. 4), during the gestation period (July) and during the prehibernal period (September) in adult animals. Tritiated corticosterone disappeared more rapidly from plasma in pregnant than in nonreproductive females, although the variation was not statistically

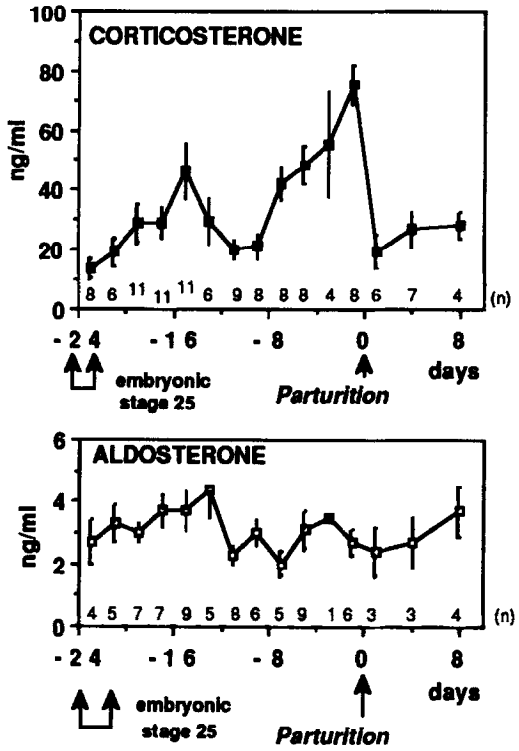

FIG. 3. Changes in plasma corticosterone (upper) and aldosterone (lower) during gestation, in female *L. vivipara*, according to the number of days before or after parturition. Results are expressed as mean ± SE; (*n*), number of animals.

significant (Fig. 4A; $T_{1/2}$, respectively, about 25 and 40 min). For comparison, the kinetics of tritiated corticosterone disappearance were similar in males tested during the same periods (Fig. 4B; $T_{1/2}$ about 40 min) and in nonreproductive females.

ACTH Control of Corticosteroid Production

In vivo effect of adenohypophysectomy. Plasma corticosterone and aldosterone levels were strongly reduced after adenohypophysectomy performed during gestation or during the prehibernal period. Levels became nondetectable or just reached the detection limit (Table 3), illustrating the existence and importance of a functional pituitary–adrenal axis.

In vitro production of corticosteroids by adrenals and effect of ACTH 1–39: Perifusion experiments. After the equilibration period of 8 hr, during which the steroid output gradually decreased, the basal levels of corticosterone and aldosterone stabilized at a rate of 129 to 165 and 44 to 60 pg/min per adrenal, respectively. This rate did not significantly vary according to the period of the reproductive cycle ($F(2,7) = 0.28$ and 0.45 for corticosterone and aldosterone, respectively; $P > 0.05$; Table 4). The release of corticosterone and aldosterone increased in a dose-dependent manner in response to graded doses of ACTH 1–39 as illustrated in a representative experiment in Fig. 5 and previously detailed (Dauphin-Villemant *et al.*, 1988). The relation between the doses of ACTH and the net production of corticosterone and aldosterone could be expressed as a linear regression between the areas under the peaks and the logarithm of the ACTH concentrations (Fig. 5B). Only a slight change appeared during the period of gestation: the maximal stimulatory effect was reached for 10 n*M* ACTH and was maintained for 32 n*M* ACTH. However, when this last concentration was excluded from the linear regression during the gestation period, the slope of the linear response to ACTH did not vary significantly during the period of active life (Table 4), indicating that the sensitivity of adrenal cells to corticotropin remained unchanged.

Physiological Importance of Corticosterone: Effect on Parturition

An abrupt fall in corticosterone plasma levels was observed in the few hours preceeding parturition. As controlled by measurements of plasma corticosterone in treated animals, daily injections of corticosterone maintained high levels of circulating corticosterone (50 to 100 ng/ml) while in adrenalectomized females, an earlier fall in plasma corticosterone was induced (levels dropped to the detection limit). In each experimental group, females gave birth to

TABLE 2
EFFECT OF OVARIECTOMY ON PLASMA PROGESTERONE LEVELS IN FEMALE *L. vivipara* (EXPRESSED AS ng/ml; MEAN ± SE; (*n*), NUMBER OF ANIMALS)

Physiological state of females	Treatment: Before ovariectomy		Treatment: 7 days after ovariectomy		
Early gestation (stage 20[a])	146.8 ± 8.4 (7)	***	5.3 ± 1.3 (7)	***	*
Midgestation (stage 32[a])	275.9 ± 20.6 (17)	***	7.1 ± 0.8 (17)		
Prehibernal period	2.0 ± 0.4 (16)	NS	2.0 ± 0.3 (8)		

Note. Statistical significance: $*P < 0.05$; $***P < 0.001$; NS, nonsignificant.
[a] Developmental stages of the embryos according to Dufaure and Hubert (1961).

healthy offspring. Parturition was significantly advanced in adrenalectomized females: 62.5% of the females gave birth between Day 0 and Day +2 of the experiment as compared to 20.8 and 16.6% for control and females injected with corticosterone, respectively ($\chi^2 = 6.69$; $P < 0.02$; Table 5). On the other hand, parturition was significantly delayed in the case of females receiving daily injections of corticosterone: 55.6% of the females gave birth only between Days +7 and +14 after the start of the experiment as compared to 20.8 and 0% for control and adrenalectomized females, respectively ($\chi^2 = 9.99$; $P < 0.01$; Table 5).

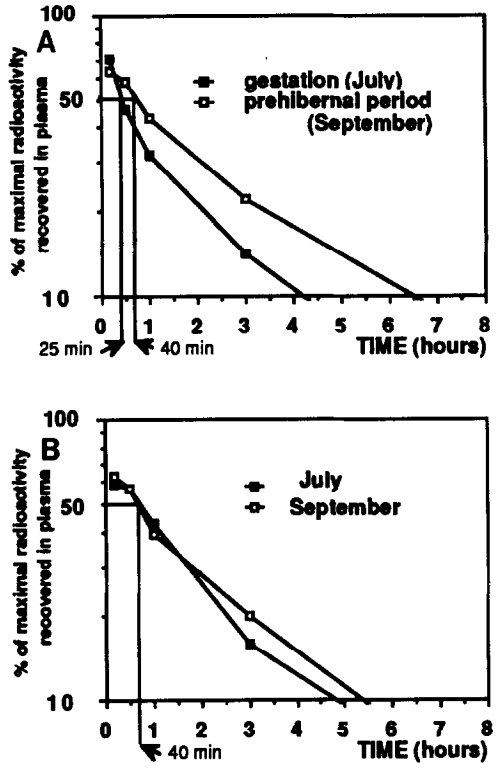

FIG. 4. Changes in corticosterone half-life after a single injection of tritiated corticosterone in adult females (A) and males (B) *L. vivipara*, during the gestation period of the females (July) and during the prehibernal period (September). Results are expressed as means from each tested group (four to six animals).

DISCUSSION

The present paper was aimed at investigating the relations between adrenal and breeding activities in the female *L. vivipara*. We restricted our study to the period of active life which includes both the period of annual reproduction (vitellogenesis, gestation) and a period of sexual inactivity (prehibernal period). Previous *in vitro* study have shown a major production of corticosterone and a notable biosynthesis of aldosterone by adrenals of female *L. vivipara* (Dauphin-Villemant and Xavier, 1985; Dauphin-Villemant *et al.*, 1988). Consequently, in the first part of this paper, the seasonal changes in the circulating levels of these two steroid hormones were studied. Although aldosterone was demonstrated to vary in response to hemorrhage and stress (Dauphin-Villemant and Xavier, 1987), no significant changes in plasma aldosterone were observed under laboratory conditions

TABLE 3
EFFECT OF ADENOHYPOPHYSECTOMY ON PLASMA CORTICOSTERONE AND ALDOSTERONE LEVELS (EXPRESSED AS ng/ml; MEAN ± SE)

Physiological state	Hormone	No. animals	Before hypophysectomy	10–13 days after hypophysectomy
Gestation	Corticosterone	6	44.9 ± 9.1	6.2 (1[a])
	Aldosterone	6	4.0 ± 1.2 (4[a])	2.5 (2[a])
Prehibernal period	Corticosterone	5	19.9 ± 5.8	3.7 ± 1.3 (4[a])
	Aldosterone	3	4.5 ± 2.9	2.0 (2[a])

[a] Number of animals with detectable levels.

during the active life period of lizards. On the other hand, significant seasonal changes in plasma corticosterone were measured with low levels during vitellogenesis, a progressive four-fold increase during the second half of gestation, an abrupt fall at parturition, and a slight increase during the prehibernal period. No adult nonreproductive females could be found in natural populations. Plasma corticosterone was therefore compared between males and females in order to distinguish sex-related changes in adrenal activity from changes induced by environmental factors. The peak of plasma corticosterone seemed to be related to the physiological state of gestation since it was only observed in adult females and not in males compared during the same period. These results agree with our previous studies concerning *in vitro* steroid bio-

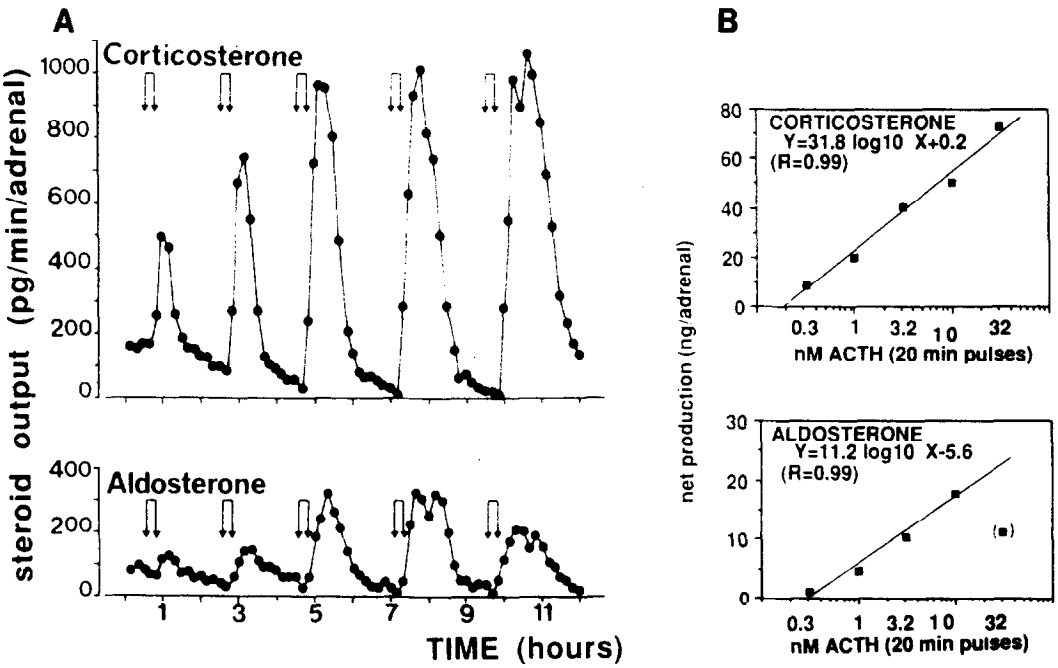

FIG. 5. (A) Effect of graded doses of ACTH 1–39 on corticosterone and aldosterone release by perifused adrenal fragments. Each dose of ACTH was infused for 20 min. This profile was obtained in one representative experiment. (B) Relation between the doses of ACTH and the net production of corticosteroids expressed as a correlation (linear regression) between the areas under the peaks and the logarithm of the ACTH concentrations.

TABLE 4

ANNUAL VARIATIONS OF BASAL CORTICOSTEROID PRODUCTION AND RESPONSE TO ACTH BY PERIFUSED ADRENAL SLICES (EXPRESSED AS VALUES ± SE OVER THREE TO FOUR INDEPENDENT EXPERIMENTS)

Period of the reproductive cycle	Vitellogenesis	Gestation	Prehibernal period
I. Basal levels of corticosteroid production (pg/min/adrenal)			
Corticosterone	129.0 ± 60.2	149.5 ± 27.1	165.3 ± 31.9
Aldosterone	58.0 ± 10.5	59.8 ± 19.0	43.7 ± 7.1
II. Slope of linear response to ACTH[a]			
Corticosterone	22.4 ± 7.0	21.4 ± 2.7[b]	26.4 ± 8.2
Aldosterone	2.33 ± 0.78	3.28 ± 0.76	2.21 ± 0.19

[a] For each experiment, a linear regression was calculated by correlating the net productions of either corticosterone or aldosterone (areas under the peaks; ng/adrenal) with the logarithm of the ACTH concentrations.
[b] The fifth concentration of ACTH (32 n*M*) was excluded as explained in the text.

synthesis by the adrenals: the metabolism of exogenous-tritiated pregnenolone was maximal during gestation (Dauphin-Villemant and Xavier, 1985). Previous histological studies (Panigel, 1956) also indicated an increased adrenal volume and an increased vascularity of adrenals during the gestation period in the female *L. vivipara*. The peak of adrenal activity during the reproduction period appears as a general finding among fishes (Lamba *et al.*, 1983), amphibians (Jolivet-Jaudet *et al.*, 1984; Kühn *et al.*, 1987; Leboulenger *et al.*, 1979; Licht *et al.*, 1983, Mendoça *et al.*, 1985), and the few studies concerning reptiles (Lofts *et al.*, 1971; Tam *et al.*, 1972; Callard and Callard, 1978; Whittier *et al.*, 1987).

A second point investigated in this study was the possible secretion of progesterone by the adrenals. Adrenal production of progesterone was suggested in several turtle and snake species (Chan *et al.*, 1973; Highfill and Mead, 1975, Lance and Callard, 1978) and would be predominant during the nonreproductive period. In the female *L. vivipara*, the corpora lutea represent the main source of progesterone during reproduction (Yvorra, 1986; Xavier, 1987), but our *in vivo* results also indicated an extraovarian source of progesterone, particularly during the nonreproductive period. However, the perifusion experiments gave contradictary results: the *B/P* ratio reached approximately 100 at the end of the equilibration period (according to a radioimmunoassay performed after extraction of

TABLE 5

EFFECTS ON TIME OF PARTURITION OF CORTICOSTERONE INJECTIONS OR ADRENALECTOMY AT LATE GESTATION

Treatment	No. animals	Frequences of parturition (%)		
		J0[a] to J2	J3 to J6	J7 to J14
Control				
No treatment	8	25.0	62.5	12.5
Sham injected	8	12.5	62.5	25.0
Sham operated	8	25.0	50.0	25.0
Adrenalectomy at J0[a]	8	62.5	37.5	0
Injections of corticosterone[b]	18	16.6	27.8	55.6

[a] J0, first day of treatment.
[b] One injection of 2 to 6 μg/g per day.

the steroids and separation by HPLC analysis, data not shown). Such a result indicates that while synthesis of progesterone by the adrenals is possible (Dauphin-Villemant and Xavier, 1985), its actual secretion remains doubtful and its physiological importance was not determined.

In the second part of this study, we attempted to determine the factors which might explain the seasonal rhythm of plasma corticosterone observed in female *L. vivipara*. First, the increase of circulating corticosterone levels might be due to (i) a decrease of the metabolic clearance rate of corticosterone and/or (ii) an increase in the production rate of corticosterone. In females, tritiated corticosterone disappeared more rapidly from plasma after a single injection during gestation than during the prehibernal period, while in males, no differences were observed between the periods tested. These results indicate an increase rather than a decrease in the metabolic clearance rate of corticosterone in females during gestation and cannot account for an accumulation of corticosterone in plasma during this period. Thus, the peak of plasma corticosterone in pregnant females suggest an increased production rate of the hormone.

Second, we investigated factors which might lead to this increased production rate of corticosterone. The integrity of the pituitary–adrenal axis is essential for adrenal activity: as in other reptiles (reviewed in Bradshaw, 1975; Callard and Callard, 1978), hypophysectomy almost suppressed the circulating levels of corticosteroids. Moreover, in several reptile species, including the viviparous lizard, ACTH proved to play a major role *in vivo* (Bradshaw, 1978; Lance and Lauren, 1984; Vallarino *et al.*, 1985) or *in vitro* (Leloup-Hatey, 1968; reviewed in Callard and Callard, 1978; Dauphin-Villemant *et al.*, 1988). Thus, the effect of adrenocorticotropin should be involved to explain the seasonal rhythm of plasma corticosterone. Two hypotheses could be considered: (i) variations of adrenal cell sensitivity to ACTH as was observed in a few studies performed in fishes (Ilan and Yaron, 1976) or amphibians (Leboulenger *et al.*, 1979) or (ii) variations of ACTH levels. The use of the perifusion technique allowed a dynamic study of spontaneous or stimulated corticosteroid production by the adrenals. The spontaneous secretory capacity of the adrenals (basal corticosteroid output at the end of the equilibration period) did not significantly vary during the period of active life, confirming the stimulatory effect of an external factor. Similarly, the stimulation of corticosteroid output by synthetic ACTH 1–39 did not significantly vary during the period of active life. It is then probable that variations of plasma corticosterone are mainly ascribed to variations of adrenocorticotropin levels. In fact, structural variations of the pituitary throughout the annual cycle support this hypothesis. A pituitary hypertrophy has been observed during reproduction in several reptile species (Herlant and Grignon, 1961; Grignon and Grignon, 1965), including the viviparous lizard (Panigel, 1956). These variations were first correlated with gonadotrophs but have since been identified as corticotrophs (Licht and Bradshaw, 1969; Licht, 1974). However, other hypotheses deserve further investigation. First, other corticotropic factors like angiotensin have been shown to play a role in the regulation of adrenal cell activity in reptiles (Dauphin-Villemant *et al.*, 1988; Sanford and Stephens, 1988) as in other vertebrates (reviewed in Wilson, 1984; Delarue *et al.*, 1984) and may interfere with the effect of ACTH. Second, the stimulatory effect of ovarian steroids has been suggested in various reptile species (review in Callard and Callard, 1978). Finally, the hypothesis that fetal corticosteroid production could participate to the peak of maternal circulating corticosterone cannot be ex-

cluded. Morat (1969) observed that a Δ^5-3β dehydrogenase activity appears in fetal adrenals from stage 31 and increases until final stage 40. In addition, when fetal adrenals were incubated *in vitro* without exogenous precursors, corticosterone and aldosterone could be detected in the incubation media (Dauphin-Villemant, 1987). However, this hypothesis should not be overestimated since an *in vivo* experiment of fetectomy performed in female *L. vivipara* at midgestation did not lead to a significant decrease in plasma corticosterone as compared to control pregnant females (Dauphin-Villemant, 1987).

The results of the aforementioned studies demonstrate the existence of temporal correlations between adrenal and breeding activities in *L. vivipara*. In the last part of this study, the functional link between reproduction and the adrenals was studied. First, the significance of high corticosterone levels during gestation was investigated. In amphibians, the increased adrenal activity is observed in males as well as in females and several authors (Leboulenger *et al.*, 1982; Mendoça *et al.*, 1985, reviewed in Leboulenger, 1986) do not relate this increase directly to reproductive events but interpret it as a consequence of a stress effect sustained during all this period of the cycle in relation to behavioral events like increased locomotor activity or social interactions. It should also be noted that in the recent study performed on the female snake *Thamnophis sirtalis parietalis*, Whittier *et al.* (1987) suggest that the levels of plasma corticosterone vary with a nonbreeding physiological cycle in this species since the peak observed during reproduction also occurs in nonreproductive females. In the female viviparous lizard, the change of the disappearance rate of tritiated corticosterone appeared to be mainly due to an increase of peripheral utilization of the hormone in pregnant females since the metabolism of corticosterone (evaluated by the proportion of corticosterone compared with the other tritiated metabolites in plasma) did not significantly vary among all tested groups (data not shown). This result is in favor of a physiological importance of corticosterone during gestation as is also the observed difference in circulating corticosterone levels between males and females. A temporal correlation could be noted between the peak of circulating corticosterone and the increase of transplacental water flows which are observed in the female *L. vivipara* (Dauphin-Villemant and Xavier, 1986) as in other viviparous reptiles (Clark *et al.*, 1955; Thompson, 1977, 1981). The control mechanisms of this process are still unknown, but corticosterone has been involved in the regulation of body fluids in several reptile species (reviewed in Minnich, 1979; Duggan and Lofts, 1978; Brewer and Ensor, 1980a, b; Bradshaw *et al.*, 1984). However, *in vivo* experiments at midgestation (corticosterone supplement or adrenalectomy) did not allow us to implicate corticosterone in the regulation of egg water intake (Dauphin-Villemant, 1987) and further experiments would be needed to clarify the physiological importance of the high circulating levels of corticosterone during the second half of gestation.

By contrast, the last point investigated in the present study shows that the fall of corticosterone just before term seems directly involved in the process of parturition in the female viviparous lizard. The injection of additional corticosterone in late gestation delayed parturition while adrenalectomy provoked premature parturition. These results suggest that high corticosterone levels near the term contribute to the maintenance of gestation whereas the abrupt fall just before term must be considered as one of the events involved in the process of parturition. Such results appear completely opposite to what is known of the effect of adrenal steroids in pregnant mammals. In ovine species, it is well established that cortico-

steroids increase in fetal and maternal plasma during the last few days of gestation and bring about changes in placental steroid production that result in labor (reviewed in Nathanielsz, 1978; Thorburn and Challis, 1979; Magyar *et al.*, 1981). However, in another viviparous lizard (*Sceloporus jarrovi*), Shine and Guillette (1988) evoke similar delayed parturitions after corticosterone or progesterone injections. Studies performed in other oviparous and viviparous reptile species suggest the following hypotheses. The increased corticosterone levels would be due to the activation of the pituitary–adrenal axis. They would increase catecholamine production by adrenal chromaffin tissue and thus potentiate the β-adrenergic effects of relaxation at the uterine level, thus promoting egg retention as proposed in oviparous reptiles by Guillette (1985). In fact, Jones *et al.* (1983) demonstrated that in *Anolis carolinensis*, arginine vasotocin (AVT), which stimulates uterine contractions in reptiles (reviewed in Jones and Guillette, 1982), induced oviposition only if females were pretreated with a β-adrenergic antagonist. Parturition would start in response to the following factors (reviewed in Guillette, 1985; Xavier, 1989). The fall in luteal progesterone would allow the release of AVT from the neurohypophysis. AVT would be effective in inducing uterine contractions only after the fall in circulating corticosterone and the subsequent reduction of β-adrenergic relaxing effects at the uterine level. Further studies are obviously required to confirm this hypothetical scheme and to precise the fine mechanisms involved.

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