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Adrenal Activity in the Female Lizard *Lacerta vivipara* Jacquin during Artificial Hibernation

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The variations of interrenal activity were investigated in captive female *Lacerta vivipara* submitted to artificial hibernation (4 months at 6°) and compared to data obtained in nonhibernating females. Plasma corticosterone levels reached 25 ng/ml during the prehibernal period. During the first day following the transfer to cold conditions, an initial significant peak of plasma corticosterone was observed (up to 63 ng/ml). A second, more gradual, but also significant increase was observed thereafter and levels remained maximum during the two first months of artificial hibernation (75 ng/ml). The circulating levels of corticosterone then decreased gradually. At the time of transfer to warm conditions, a third significant peak of corticosterone was observed (up to 82 ng/ml). The minimal values (15 ng/ml) previously described during vitellogenesis were reached within 1 week. High corticosterone levels appeared to be actually related to the "hibernation state" since they were also observed in hibernating males and not in nonhibernating females. In order to explain the pattern of plasma corticosterone, variations of adrenal sensitivity to synthetic ACTH 1–39 were examined *in vitro*, using a perifusion system technique. Surprisingly, ACTH-induced stimulation of corticosterone and aldosterone release was significantly reduced during hibernation, whatever the temperature of the perifusion bath (30 or 6°). Nevertheless, a fourfold increase in the half-life of injected tritiated corticosterone was observed during hibernation which likely contributes to maintain high levels of corticosterone despite a low production rate of the hormone.

As in other ectotherms, the biology of reptiles remains strictly dependent upon environmental conditions of temperature and most reptiles living in temperate zones survive to severe winter conditions by continuous or discontinuous winter dormancy (reviewed by Gregory, 1982; Gavaud and Xavier, 1986). This phenomenon is generally called hibernation by analogy with mammals since it implies more than a mere cold-induced torpor and encompasses temperature-independent physiological changes. Behavioral anticipation of winter arrival and seasonal metabolic changes have been observed in many reptile species (reviewed in Bennett and Dawson, 1976; Gregory, 1982; Gavaud and Xavier, 1986).

Adrenals have been implicated in the endocrine control of hibernation in mammals (reviewed in Musacchia, 1984; Wang, 1982, 1986). In contrast, involution of adrenals and minimal activity during winter has been observed in amphibians (reviewed in Leboulenger, 1986). In reptiles, little is known about the activity and function of adrenal glands during hibernation, although corticosteroids may be important for energetic metabolism (Callard and Chan, 1972; Gist, 1972; reviewed in Callard and Callard, 1978) as in other vertebrates (reviewed in Kraus-Friedman, 1984). Previous histological studies performed in *Lacerta vivipara*

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(Panigel, 1956) and in other reptiles (reviewed in Lofts, 1978; Callard and Callard, 1978) suggested a decrease of adrenal activity during hibernation. However, *in vitro* results obtained in female *L. vivipara* indicated that the steroidogenic capacities at optimal temperature (30°) did not decrease significantly during hibernation as compared to other phases of the annual cycle (Dauphin-Villemant and Xavier, 1985).

L. vivipara, a small European lizard, presents a clear-cut ecological cycle characterized by a phase of activity during which reproduction takes place and a phase of continuous hibernation (Bauwens, 1981) required to obtain reproduction (Hubert and Xavier, 1979; Gavaud, 1983). An extended study of annual adrenal activity has been undertaken in the female *L. vivipara* (Dauphin-Villemant and Xavier, 1985, 1986, 1987; Dauphin-Villemant *et al.*, 1988, 1990). The importance of interrenal activity was investigated in relation to breeding activities (Dauphin-Villemant *et al.*, 1990). The purpose of the present study was to further investigate the changes of interrenal activity during hibernation and the perihibernal period as compared to data obtained in nonhibernating females. The changes in plasma corticosterone were characterized. In order to determine the origin of the observed fluctuations, the control of adrenal activity by adrenocorticotropin was studied using an *in vitro* perifusion technique. In addition, the possible variations of corticosterone metabolic clearance were researched.

MATERIALS AND METHODS

Animals and Maintenance in the Laboratory

Adult *L. vivipara* Jacquin (2–4 g body weight) were collected over 2 years (1986 and 1988) from May to July in natural populations of the Massif Central (1000–1200 m above sea level, France). Animals were first kept in terraria under a summer-like environmental regime as previously described (Dauphin-Villemant and Xavier, 1987). Females were able to complete their annual reproductive cycle under laboratory conditions and gave birth to healthy offspring. At the end of the natural period of active life (September–October), most animals were grouped (four to six animals) and transferred to 6° (between 07.00 and 08.00 AM) in cotton bags placed in soil-filled small cages. Particular care was taken to reduce the duration of handling in order to minimize stress effects (see Dauphin-Villemant and Xavier, 1987). These animals were submitted to a 4-month (October 15th to February 15th) artificial hibernation at a constant temperature of 6°, in complete darkness, without food and water supply, as previously described by Gavaud (1983). Thereafter, animals were switched again to a summer-like environmental regime (between 07.00 and 08.00 AM). For comparison, other animals were similarly grouped and transferred to new terraria but were maintained all the time under the summer-like environmental regime (nonhibernating animals).

Blood Collection

In the different experiments, blood was sampled from the infraorbital sinus and collected into ice-cold heparinized tubes. Blood collection usually lasted less than 3 min and never exceeded 5 min in order to minimize stress effects (Dauphin-Villemant and Xavier, 1987). Plasma (20 to 60 μl) was obtained by centrifugation at 4° (1000*g* for 10 min) of the blood samples and was stored at −20° until assay. Blood sampling always took place between 07.00 and 09.00 AM; it was performed at room temperature for the nonhibernating animals and at 6° for the hibernating animals.

Experimental Procedure

Changes in plasma steroids. Plasma corticosterone and progesterone levels were measured by radioimmunoassay in intact animals (mainly females and some males for comparison). Blood sampling was performed during the prehibernal period (15 and 2 days before the exposure to cold conditions), during artificial hibernation (1, 2, 4, 8, 14 hr, 1, 2, 4, 8, 15, 30, 45, 60, 75, 90, 105, and 120 days after the beginning of artificial hibernation), and during the first month following arousal from hibernation (1, 2, 4, 8, hr, 1, 2, 4, 7, 11, 14, 17, 21, and 24 days after the transfer to summer-like environmental conditions). Animals (100 females and 40 males) were used over 2 years (1986–1988); they were divided into several experimental groups so that each individual was only sampled twice during hibernation and twice after emergence. For comparison, plasma corticosterone was also measured in one experimental group of nonhibernating females (in December).

In vitro response to adrenal tissue to ACTH: Perifusion experiments. The perifusion system employed for this study was previously described in detail (Leb-

oulenger *et al.*, 1978). For each perifusion experiment, two female lizards were killed by decapitation between 19.00 and 20.00 PM. Adrenals were quickly placed in a drop of Dulbecco's modified Earle's medium (DMEM; Eurobio, Paris) and sliced into 1-mm^3 fragments. The adrenal tissue was then mixed with Bio-Gel P2 (200–400 mesh wet, Bio-Rad, Richmond, CA), transferred into the perifusion chamber, and continuously perifused with DMEM (pH 7.3) at a constant flow rate (12 ml/hr). After the equilibration period (8 hr), graded concentrations of synthetic human ACTH 1–39 (from 0.32 to 32 n*M*, 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. Three series of perifusion experiments were conducted during the hibernation period: (i) 6 independent perifusions (12 females) were performed at 30° with adrenals from nonhibernating females; (ii) 4 independent perifusions (8 females) were performed at 30° with adrenals from hibernating females; and (iii) 1 perifusion (2 females) was performed at 6° (temperature of hibernation) with adrenals from hibernating females.

Kinetics of tritiated corticosterone disappearance from plasma after a single injection. A single intraperitoneal injection of 1 μCi tritiated corticosterone/animal dissolved in 10 μl ethanol was given to females hibernating for 2 months (2 females) and also to nonhibernating females (4 females). Blood (10 to 50 μl of plasma) was collected at 10 min, 30 min, 1 hr, 3 hr, 6 hr, 10 hr, 24 hr, and 72 hr after the injection. At each time, plasma radioactivity was immediately counted in 5-μl aliquots of plasma, using a Kontron automatic liquid scintillation spectrometer (Model beta V). Steroids were extracted from the remaining part of the plasma (twice with 5 ml of methylene chloride) and the proportion of corticosterone was determined by high-performance liquid chromatography analysis (HPLC) as previously detailed (Dauphin-Villemant *et al.*, 1990). After the elimination of corticosteroid metabolism products, tritiated corticosterone 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 ($T_{1/2}$).

Reagents and Solvents

All organic solvents were from Merck (Darmstadt, West Germany) or Carlo Erba (Milan, Italy). Nonradioactive steroids were supplied by Sigma Chemical Co. (St. Louis, MO). [7(*n*)-^{3}H]progesterone (101 Ci/mmol), [1,2,6,7-^{3}H]corticosterone (98 Ci/mmol), and [1,2,6,7-^{3}H]aldosterone (75 Ci/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) and a progesterone antiserum supplied by Dr. G. Niswender (U.S.A.). Synthetic human ACTH 1–39 was a generous gift from Drs. Scheibli and Andreatta (Ciba-Geigy, Basel, Switzerland).

Steroid Assays

Steroids in plasma samples. Plasma samples were diluted in 1 ml water after addition of 1000 cpm tritiated progesterone and corticosterone for recovery. Progesterone was first extracted by 5 ml isooctane. Polar steroids (corticosteroids) were further extracted by methylene chloride (2 × 5 ml). Progesterone and corticosterone fractions were evaporated to dryness and redissolved in 1 ml phosphate buffer (0.05 *M*, pH 7.8) containing 0.01% gelatin (PBG). Aliquots were taken for recovery (250 μl) and for assays (1 × 500 μl for progesterone; 2 × 100 μl for corticosterone). Corticosterone and progesterone samples were incubated overnight at 6° with 5000 cpm of tritiated hormone and antibody (final dilution required to bind 35 to 50% of labeled hormone) in a final volume of 600 μl PBG. Separation of free and bound fractions was achieved by adding 500 μl of dextran-coated charcoal (0.5% charcoal and 0.05% dextran in phosphate buffer). Recovery after extraction was approximately 100% for progesterone and corticosterone. The usable ranges of the standard curves were 10 to 400 pg/tube for progesterone and 25 to 500 pg/tube for corticosterone. The blank values run with each assay were undetectable. The intra- and interassay reproducibilities were, respectively, 12.1 and 11.0% for progesterone and 9.4 and 6.8% for corticosterone.

Corticosterone and aldosterone concentrations in perifusion experiments were measured without prior extraction 75–300 μl of perifusion effluent. The radioimmunoassay procedures were 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 (Dauphin-Villemant *et al.*, 1990). Corticosterone and aldosterone productions at any time were either expressed as picograms per minute per adrenal or as a percentage of the basal level. The basal levels were calculated as the mean of six samples (30 min) taken just before the infusion of the first secretagogue.

Statistical analysis.

Results were expressed as means ± SE. In the per-

ifusion experiments, correlations between the steroid output and the doses of ACTH were calculated using linear regression analysis. Data were analyzed using ANOVA and some means were compared using Student's t test for nonpaired data.

RESULTS

Changes in Plasma Steroids during Hibernation

Plasma corticosterone. The hibernation to which animals were submitted was artificial but occurred during the natural phase of lizard hibernation and after the premonitory signs (anorexia, more frequent burying) were noticed. The body temperature of the animals decreased rapidly after the transfer to cold conditions, roughly equilibrated with the environmental temperature of hibernation (5–6°) within 4 hr and remained stable thereafter (Fig. 1B). The circulating levels of corticosterone measured during the prehibernal period in adult female *L. vivipara* remained moderate (25 ng/ml) as compared to the results obtained through the entire active life period (Dauphin-Villemant *et al.*, 1990). Plasma corticosterone levels significantly varied with time during hibernation ($F(16,96) = 1.90$; $P < 0.05$; Fig. 1A). A sharp peak of plasma corticosterone (up to 63 ng/ml) was observed during the first day of artificial hibernation (Fig. 1B), followed by a more gradual three-fold increase during the first month of hibernation (maximum values 75 ng/ml). After 3 months of hibernation, the levels began to decrease (51 ng/ml after 4 months hibernation) but remained significantly higher throughout all hibernation than during the prehibernal period ($F(1,141) = 23.16$; $P < 0.001$); Fig. 1A). Plasma corticosterone levels were significantly influenced by time during the emergence period ($F(12,53) = 5.10$; $P < 0.001$; Fig. 2). At the arousal, a significant sharp peak of plasma corticosterone (up to 82 ng/ml) was observed during the first day following the transfer to the summer-like environmental

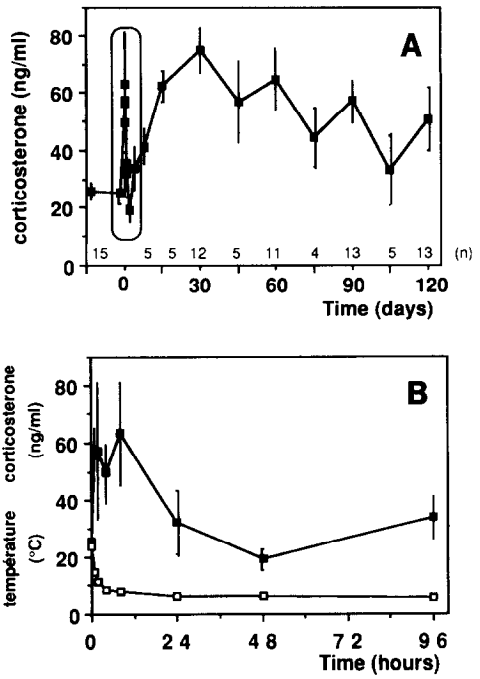

FIG. 1. (A) Changes in plasma corticosterone levels during hibernation, in female *L. vivipara*. Results are expressed as means ± SE; (*n*), number of animals. (B) Changes in body temperature (light symbols) and in plasma corticosterone levels (dark symbols) during the first hours of hibernation (circled region from (A)), in female *L. vivipara*. Results are expressed as means ± SE ($n = 5$ animals for each point).

regime (Fig. 2). The levels gradually decreased thereafter to reach the lowest values previously measured during vitellogenesis (15 ng/ml; Fig. 2 and Dauphin-

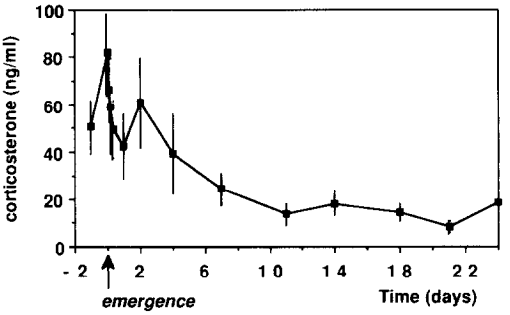

FIG. 2. Changes in plasma corticosterone levels at the emergence period, in female *L. vivipara*. Results are expressed as means ± SE ($n = 5$ animals for each point).

Villemant *et al.*, 1990) from 1 week after emergence.

Except for the rapid changes observed at the beginning and at the end of hibernation, the plasma corticosterone levels remained stable. As these levels did not vary significantly from 1 to 3 months of hibernation ($F(4,40) = 1.23$; $P > 0.05$) and from 7 to 24 days after the arousal from hibernation ($F(5,27) = 0.84$; $P > 0.05$), the results were pooled according to three main phases (prehibernal period, hibernation, and emergence period). Sex-related variations were investigated (Table 1). No significant differences in the concentrations of plasma corticosterone were observed between males and females ($P > 0.05$) over the time of the experiment. Levels of plasma corticosterone were significantly increased during hibernation in both females and males ($P < 0.001$; Table 1). Conversely, the peak of corticosterone appeared to be related to the physiological state of hibernation since, at the same time, significantly lower levels ($P < 0.001$) were measured in the plasma of nonhibernating females (Table 1).

The circulating levels of corticosterone during hibernation were surprisingly high when compared to the results obtained in other ectotherms. Although corticosterone radioimmunoassay was performed after a purification step, we wanted to confirm its identity. Extracts were chromatographed by HPLC before radioimmunoassay. As shown in Fig. 3, the immunoreactivity found in plasma of hibernating females only corresponded to standard corticosterone.

Plasma progesterone. This was also measured during hibernation in order to examine whether the rise in plasma corticosterone was specific to this hormone or not. Progesterone plasma levels were determined simultaneously with corticosterone. Titers remained low without significant variations ($P > 0.05$; Table 1) during the prehibernal period, the artificial hibernation, and the postemergence phase.

In Vitro Response of Adrenal Tissue to ACTH

The ACTH-induced stimulation of corticosterone and aldosterone releases was studied at 30°, in females which were hibernating for 2 months and simultaneously compared to nonhibernating females. The

TABLE 1

CIRCULATING LEVELS OF CORTICOSTERONE AND PROGESTERONE IN ADULT *L. vivipara* FROM THE PREHIBERNAL PERIOD TO THE EMERGENCE (EXPRESSED AS ng/ml; MEAN ± SE; (*n*), NUMBER OF ANIMALS)

Period of the annual cycle	Corticosterone		Progesterone
	Females	Males	Females
Prehibernal period (September)	25.7 ± 2.5[a] (30)	28.0 ± 6.6[a] (10)	2.0 ± 0.4[d] (16)
Hibernation			
Hibernating animals (November to January)	62.6 ± 4.2[b] (45)	61.5 ± 7.1[b] (19)	1.1 ± 0.3[d] (25)
Active animals (December)	29.0 ± 4.5[a] (14)	—	—
Emergence period* (February)	17.0 ± 2.2[c] (33)	22.5 ± 3.7[a,c] (13)	1.7 ± 0.3[d] (23)

Note. Means with different letters in exponent are statistically different according to student *t* test for nonpaired data.

* Seven to 24 days after emergence for corticosterone plasma levels; only 7–11 days after emergence for progesterone plasma levels: thereafter, plasma progesterone levels begin to increase.

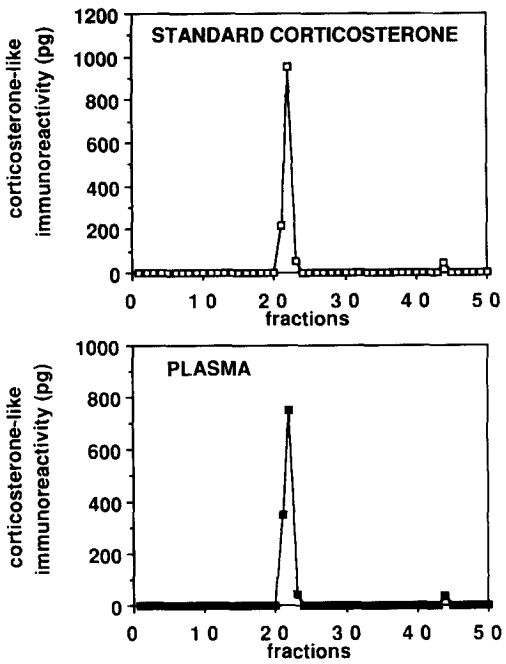

FIG. 3. HPLC analysis of methylene chloride extracts of plasma samples from hibernating female as compared to standard corticosterone. The gradient of solvent used consisted of 50 to 83% methanol in water (v:v) for 40 min, as previously described (Dauphin-Villemant and Xavier, 1985). One hundred fractions (0.4 ml each) were collected and corticosterone-like material was radioimmunoassayed as described in text.

release of corticosterone and aldosterone was increased in a dose-dependent manner, both in hibernating and nonhibernating females (Fig. 4A), but the slope of the dose–response curve, for corticosterone and for aldosterone, was much steeper in active than in hibernating females (Fig. 4B). However, the basal levels of corticosterone and aldosterone measured after the equilibration period of 8 hr did not vary significantly between hibernating and active females ($P > 0.05$; Table 2) and stabilized at a rate of 147 to 162 pg/min per adrenal for corticosterone and 52 to 55 pg/min per adrenal for aldosterone.

In order to investigate the effect of temperature on the secretory response of adrenal tissue to ACTH, a perifusion experiment was conducted at 6°. The basal levels of corticosterone and aldosterone production were strongly reduced (Table 2). The response to ACTH was also depressed as well as delayed in comparison with that obtained with adrenals from hibernating females, perifused at 30° (Fig. 5).

These results clearly indicate that the sensitivity of adrenal tissue to ACTH is significantly decreased during hibernation. In addition, the results obtained at 6° suggest that the spontaneous production rate of corticosteroids is also strongly reduced during hibernation, as a direct effect of temperature.

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, in nonhibernating females and in females hibernating for 2 months. The disappearance rate of tritiated corticosterone was much more rapid in active than in hibernating females (Fig. 6; $T_{1/2}$ respectively about 2 and 8 hr). The difference was due to both slower elimination of radioactivity from plasma and to slower metabolization of corticosterone in hibernating than in active females.

DISCUSSION

In reptiles, as in mammals, hibernation appears to be a complex adaptive behavior and not only a cold-induced torpor (Joy and Crews, 1987; reviewed in Gregory 1982), suggesting the existence of complex endocrine control mechanisms of hibernation. The present study focused on the relations between adrenal activity and hibernation in captive females *L. vivipara*.

In the viviparous lizard *L. vivipara*, behavioral events anticipate the winter arrival. Anorexia or a decline in appetite has been observed prior to hibernation, regard-

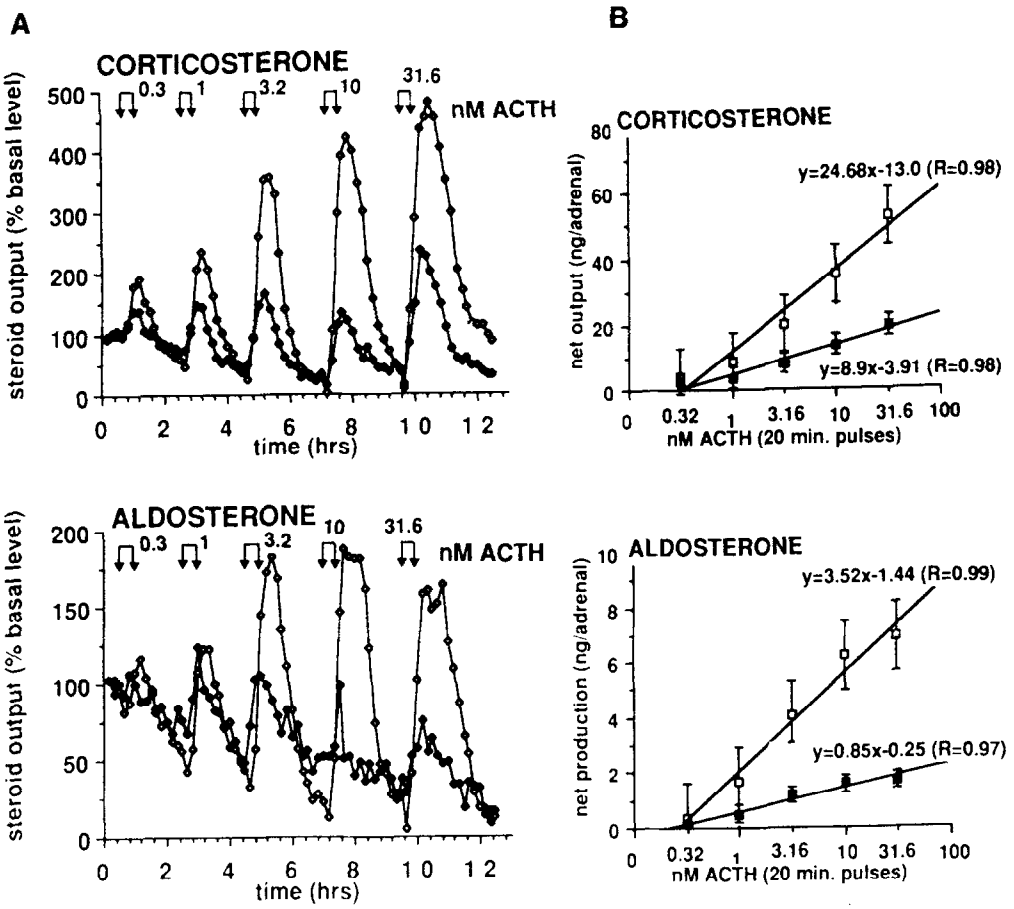

FIG. 4. (A) Effect of graded doses of 1–39 ACTH on corticosterone and aldosterone release by perifused adrenal slices from hibernating (dark symbols) and nonhibernating (light symbols) females. Each dose of ACTH was infused for 20 min. The profiles represent the mean secretion pattern of four and six independent perifusion experiments for hibernating and nonhibernating animals, respectively. The mean secretion rates of corticosterone and aldosterone in basal conditions are reported in Table 2. (B) Relationship 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.

less of the temperature and photoperiod (Patterson and Davies, 1978a; reviewed in Gavaud and Xavier, 1986). A spontaneous reluctance to become active in fall has been noticed, even under constant laboratory conditions (Gavaud and Xavier, 1986). Similar behaviors have been observed in many reptilian species (Hernandez and Coulson, 1963; Mayhew, 1965; Case, 1976; Naulleau, 1986). Moreover, in *L. vivipara,* as in several snake and lizard species (reviewed in Gregory, 1982; Naulleau, 1986; Gavaud and Xavier, 1986), hibernation is obligatory for the future fitness of the species since the resumption of reproductive activity in the spring depends upon hibernation (Hubert and Xavier, 1979; Gavaud, 1983). In the present study, captive females *L. vivipara* were submitted to a cold environmental regime during 4 months (6°, complete darkness, no food and water supply). This treatment seemed to be more than a simple induced hypothermia as defined by Musacchia (1984). It was considered an ar-

TABLE 2

BASAL CORTICOSTEROID PRODUCTION[a] BY ADRENAL SLICES FROM HIBERNATING AND NONHIBERNATING FEMALES *L. vivipara* PERIFUSED AT 30 OR 6° (EXPRESSED AS pg/min PER ADRENAL; MEAN ± SE OVER (*n*) INDEPENDENT PERIFUSIONS

	Conditions of perifusion		Hormone	
State of animals	Temp.	No.	Corticosterone	Aldosterone
Active females	30°	6	147 ± 9	55 ± 13
Hibernating females	30°	4	162 ± 36	52 ± 29
	6°	1	29	ND[b]

[a] For each perifusion experiment, the basal level of corticosteroid production was calculated as the mean of six samples (30 min) taken just before the infusion of the first secretagogue.

[b] Nondetectable in our assay conditions, that is <0.8 pg/min/adr.

tificial hibernation since (i) the exposure to cold conditions was applied just after the premonitory signs of hibernation were noticed, and (ii) such an artificial hibernation enabled the animals to reproduce (Gavaud, 1983).

In the first part of this paper, the changes in plasma corticosteroids were studied during hibernation and the perihibernal period. Corticosterone is the major circulating corticosteroid in reptiles (reviewed in Licht, 1974; Sandor *et al.*, 1976; Callard and Callard, 1978; Duggan, 1981), including the viviparous lizard *L. vivipara* (Dauphin-Villemant and Xavier, 1986, 1987), but to our knowledge, *in vivo* changes in adrenal activity have never been serially measured along the hibernation period.

Rapid changes were observed in plasma corticosterone levels as the animals entered and emerged from hibernation. A sharp peak of circulating corticosterone was observed during the first day of artificial hibernation which might be related to the sudden temperature change imposed to the animals and corresponding to the rapid decrease in the body temperature of lizards (6° within about 4 hr). Corticosteroids are known to have thermogenic effects and to regulate carbohydrate metabolism in mammals submitted to acute cold exposures leading to short-term hypothermia (Tang *et al.*, 1984; Werner and Vens-Cappel, 1985; reviewed in Musacchia, 1988). A similar

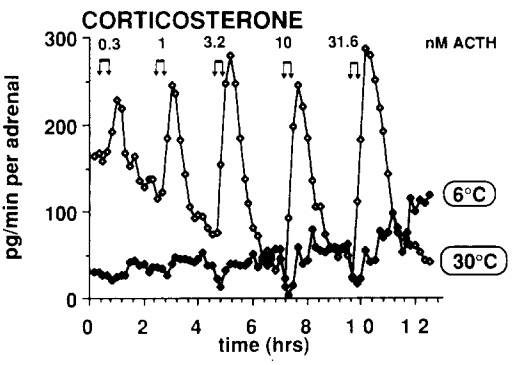

FIG. 5. Effect of graded doses of 1–39 ACTH on corticosterone release by adrenal slices from hibernating females perifused at 6° (dark symbols) or 30° (light symbols). Each dose of ACTH was infused for 20 min.

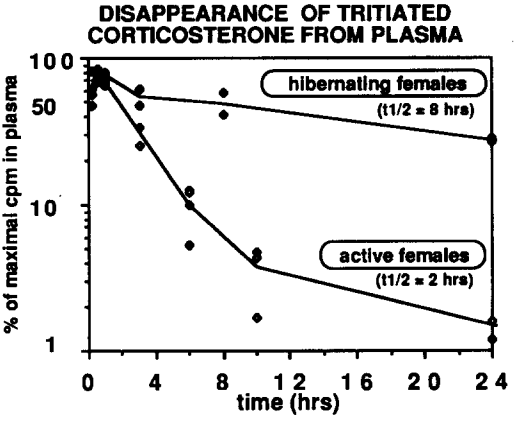

FIG. 6. Changes in corticosterone half-life after a single injection of tritiated corticosterone in adult female *L. vivipara* during hibernation (n = 2 animals) and the prehibernal period (n = 4 animals).

function could be hypothesized in reptiles which are known to present a relative physiological thermoregulation (reviewed in Gregory, 1982). Another peak of plasma corticosterone was observed in female *L. vivipara* during the first day following transfer to warm conditions. Corticosterone plasma levels then stabilized at minimal values within 6 days. A similar rise in plasma corticosteroids has always been described in mammals, in association with the arousals observed during a discontinuous hibernation or at the end of any type of hibernation (reviewed in Wang, 1982, 1986; Petrovic *et al.*, 1985; Shivatcheva *et al.*, 1988). It is nevertheless questionable whether these rapid changes in circulating corticosterone would occur during natural hibernation. In fact, various stressful factors were previously investigated in *L. vivipara*. Particular care was taken in the present study to restrain handling and bleeding of the animals, but confinement or transfer to new conditions was also demonstrated to activate the pituitary–adrenal axis in several reptilian species, including *L. vivipara* (Bradshaw, 1975; Lance and Elsey, 1986; Dauphin-Villemant and Xavier, 1987). Thus, the rapid rises in corticosterone observed immediately after placing the animals under hibernation or at the arousal may well represent transient stress effects.

Nevertheless, a prolonged increase in plasma corticosterone was also observed in female *L. vivipara* during the major part of hibernation. After a return to the prehibernal level, plasma corticosterone levels increased gradually during the first month of hibernation. Titers then began to decrease slowly but remained significantly higher throughout hibernation than during the perihibernal period. High corticosterone levels appeared to be actually related to the "hibernation state" since they were not observed in nonhibernating females and there was no sex-related difference. Moreover, corticosterone increase did not seem to be only a passive phenomenon since other steroids, like progesterone in females *L. vivipara* (Xavier, 1982, and the present study) or testosterone in males *V. aspis* (Naulleau *et al.*, 1987; Fleury and Naulleau, 1987), remained at very low levels. Again, these results are more similar to what is observed in hibernating mammals than in other ectotherms. High corticosteroid levels were measured in several hibernating mammals (Saboureau *et al.*, 1980; Gustafson and Belt, 1981), whereas in amphibians, the activity of interrenals *in vivo* generally presents a marked decline during hibernation (Leboulenger *et al.*, 1979; Licht *et al.*, 1983; Jolivet-Jaudet *et al.*, 1984a, b). It must, however, be noticed that the pattern of adrenal activity in hibernating mammals greatly depends on the species and involution of adrenals has been reported in several species during a major part of hibernation (reviewed Wang, 1982).

To our knowledge, no studies have been reported on the function of adrenal steroids during hibernation in reptiles. In active animals, corticosteroids appear to be involved in the regulation of energetic and hydric metabolism (reviewed in Callard and Callard, 1978; Minnick, 1979). Corticosterone would improve glycogenesis and gluconeogenesis (Callard and Chan, 1972; Gist, 1972; reviewed in Callard and Callard, 1978), possibly in synergy with prolactin (Callard and Chan, 1972). Conflicting results were obtained concerning a possible lipolytic effect of corticosteroids (reviewed in Callard and Callard, 1978). Important metabolic changes characterize the hibernation period in reptiles as in mammals. Even if a higher depression of metabolic rates has been observed during hibernation than in response to short-term cold exposure (Patterson and Davies, 1980 for *L. vivipara;* reviewed in Bennett and Dawson, 1976; Gregory, 1982 for other reptiles), there are seasonal cycles of energy-yielding substances. Depending on the reptile species, the major energy source stored prior

to and utilized during hibernation is either carbohydrates (mainly glycogen) or lipids (reviewed in Gregory, 1982; Costanzo, 1985; Gonzalez *et al.*, 1988). In our viviparous lizard, a considerable amount of lipid is stored in abdominal and caudal fat bodies and a major part of it is used during hibernation (Avery, 1970, 1974; Avery *et al.*, 1974). An important part of liver glycogen is also consumed during hibernation but it represents only a small proportion of the energy stores (only 3% of the stored energy, Patterson and Davies, 1978b; Patterson *et al.*, 1978). In hibernating mammals, corticosteroids are known to promote carbohydrate homeostasis (Petrovic *et al.*, 1985; reviewed in Musacchia, 1988) and the adrenals also seemed necessary to establish lethargy (reviewed in Wang, 1982). Thus, on the basis of the temporal correlations observed, corticosteroids might also be involved in the regulation of energetic metabolism in hibernating reptiles.

The important increase in plasma corticosterone during hibernation was surprising as compared to the results obtained in other ectotherms and to *in vitro* results. As expected for a Q_{10} coefficient higher than 1, *in vitro* incubations of adrenals with exogenous precursors or production of corticosteroids in perifusion experiments performed at low temperature indicated a low spontaneous production of corticosteroids in the female *L. vivipara* (Dauphin-Villemant, 1987 and the present study) even if the enzymatic machinery remained functional (Dauphin-Villemant and Xavier, 1985 and the present study). Similar results were obtained in temperate zone amphibians (reviewed in Leboulenger *et al.*, 1979). Histological studies previously performed in reptiles (reviewed in Gabe, 1970; Gregory, 1982), including *L. vivipara* (Panigel, 1956), also indicated an involution of interrenal tissue during hibernation (reviewed in Gabe *et al.*, 1964; Lofts, 1978; Callard and Callard, 1978). Nevertheless, signs of histological activity were noticed before the end of hibernation in vipers (Gabe, 1970).

In the second part of this paper, we have therefore investigated factors which might explain the elevated corticosterone levels in plasma, despite a probable low production rate. First, the validity of our corticosterone radioimmunoassay was ascertained by several purification steps. No unknown factor appeared to interfere with corticosterone in our radioimmunoassay procedure.

Second, the sensitivity of the interrenal tissue to ACTH during hibernation was investigated. It has previously been shown that adrenocorticotropin plays a major role in the control of steroid secretion *in vivo* (Bradshaw, 1978; Lance and Lauren, 1984; Vallarino *et al.*, 1985; Dauphin-Villemant *et al.*, 1990) and *in vitro* (Leloup-Hatey, 1968; reviewed in Callard and Callard, 1978; Dauphin-Villemant *et al.*, 1988, 1990). The perifusion system technique allowed us to determine whether the plasma changes of corticosterone could be ascribed to an increase of adrenal sensitivity to adrenocorticotropin. The stimulation of corticosteroid output by synthetic ACTH 1–39 was significantly decreased in hibernating females as compared to nonhibernating animals, even under optimal conditions of perifusion (30°). These results indicate that the sensitivity of the adrenal tissue to ACTH is significantly reduced during hibernation. In addition, a direct effect of low temperature on the secretion rate of corticosteroids was superimposed to the reduction of the responsiveness of interrenal tissue to ACTH in hibernating animals. Similar results have been obtained in the turtle *Chrysemys picta* (Callard, 1975; Callard *et al.*, 1975) and in amphibians (Leboulenger *et al.*, 1978, 1981; Delarue *et al.*, 1979). In the frog *Rana ridibunda*, the sensitivity of the adrenal gland to adrenocorticotropin is significantly reduced during hibernation (Leboulenger *et al.*, 1979) and the effect of ACTH is highly temperature dependent (Leboulenger *et al.*, 1978, 1981; Delarue *et al.*, 1979). Although our results clearly indicate that the production rate of corticosterone is markedly reduced during hibernation, it cannot be pres-

ently excluded that high circulating levels of ACTH (or other corticotropic factors) may stimulate corticosterone production during the hibernation period.

In order to investigate factors which may contribute to the high circulating levels of corticosterone (while the production rate of the hormone was low), we have examined the possible modifications of the corticosterone metabolic clearance. After a single injection of tritiated corticosterone, the hormone disappeared four times more rapidly from the plasma of nonhibernating than hibernating females. This fourfold increase of $T_{1/2}$ resulted from both a slower elimination of radioactivity from plasma and from a reduced metabolism of corticosterone (evaluated by the proportion of corticosterone compared to other tritiated metabolites found in plasma after a single injection of [^{3}H]corticosterone, data not shown). The occurrence of a transcortin-like protein has previously been evidenced in the viviparous lizard. Although this binding protein exhibits a lower affinity for corticosterone than progesterone, it may significantly contribute to the decrease of corticosterone metabolic clearance during hibernation, when the corticosterone/progesterone ratio is very high (Martin and Xavier, 1981). In addition, an important decrease in plasma volume has been observed in hibernating reptiles (reviewed in Minnick, 1979) and should lead to a decrease of the distribution volume of corticosterone. Taken together, these results may account for an accumulation of corticosterone in plasma during hibernation, even if a production rate of this hormone appeared to be low. It must, however, be noted that these findings do not explain why there was a specific increase of the corticosterone level while the concentrations of other steroids were not modified.

In conclusion, the results of the present studies demonstrate temporal correlations between adrenal activity and hibernation in the female of the viviparous lizard. Reptiles appear to hold a key position with respect to hibernation, sharing characteristics of adrenal activity with both lower and higher vertebrates. Further investigations are needed to determine if the metabolic changes observed during the same period are actually regulated by endocrine factors and if corticosterone is actually involved in such a regulation.

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REFERENCES

Avery, R. A. (1970). Utilization of caudal fat by hibernating common lizards, *Lacerta vivipara*. *Comp. Biochem. Physiol.* **37**, 119–121.

Avery, R. A. (1974). Storage of lipids in the lizard *Lacerta vivipara:* A quantitative study. *J. Zool. (Lond.)* **173**, 419–425.

Avery, R. A., Shewry, P. R., and Stobart, A. K. (1974). A comparison of lipids from the fat body and tail of the common lizard, *Lacerta vivipara*. *Brit. J. Herpetol.* **5**, 410–412.

Bauwens, D. (1981). Survivorship during hibernation in the European common lizard *Lacerta vivipara*. *Copeia* 741–744.

Bennett, D. A., and Dawson, W. R. (1976). Metabolism. *In* "Biology of the Reptilia" (C. Gans and W. R. Dawson, Eds.), Vol. 5, pp. 127–223. Academic Press, New York/London.

Bradshaw, S. D. (1975). Osmoregulation and pituitary-adrenal function in desert reptiles. *Gen. Comp. Endocrinol.* **25**, 230–248.

Bradshaw, S. D. (1978). Volume regulation in desert reptiles and its control by pituitary and adrenal hormones. *In* "Osmotic and Volume Regulation," pp. 38–53. The Alfred Benzon Symposium XI, Copenhagen.

Callard, G. V. (1975). Control of the interrenal gland of the freshwater turtle *Chrysemys picta in vivo* and *in vitro*. *Gen. Comp. Endocrinol.* **25**, 323–331.

Callard, I. P., and Callard, G. V. (1978). The adrenal gland of Reptilia. *In* "General, Comparative and Clinical Endocrinology of the Adrenal Cortex" (I. Chester-Jones and I. W. Henderson, Eds.), Vol.

2, pp. 370–414. Academic Press, London/New York.

Callard, I. P., and Chan, D. K. O. (1972). Hormonal effects on liver glycogen and blood sugar level in the iguanid lizard *Dipsosaurus dorsalis*. *Gen. Comp. Endocrinol.* **18,** 552–556.

Callard, G. V., Chan, S. W. C., and Callard, I. P. (1975). Temperature effects on ACTH-stimulating adrenocortical secretion and carbohydrate metabolism in the lizard *Dipsosaurus dorsalis*. *J. Comp. Physiol.* **99,** 271–277.

Case, T. J. (1976). Seasonal aspects of thermoregulatory behavior in the chuckwalla *Sauromalus obesus* (Reptilia, Lacertilia: Iguanidae). *J. Herpetol.* **10,** 85–95.

Costanzo, J. P. (1985). The bioenergetics of hibernation in the eastern garter snake *Thamnophis sirtalis sirtalis*. *Physiol. Zool.* **58,** 682–692.

Dauphin-Villemant, C. (1987). "Etude du fonetionnement de l'interrénale (corticosurrénale) chez la femelle du lézard vivipare *Lacerta vivipara* Jacquin 8 Evolution au cours du cycle annuel d'activité et de reproduction,"; p. 171. Thèse dc Doctorat es Sciences, Univ. Paris VI, Paris.

Dauphin-Villemant, C., and Xavier F. (1985). *In vitro* steroid biosynthesis by the adrenal gland of the female *Lacerta vivipara* Jacquin: The metabolism of exogenous precursors. *Gen. Comp. Endocrinol.* **58,** 1–9.

Dauphin-Villemant, C., and Xavier, F. (1986). Adrenal activity in the female *Lacerta vivipara* Jacquin: Possible involvement in the success of gestation. *In* "Endocrine Regulations as Adaptive Mechanisms to the Environment" (I. Assenmacher and J. Boissin, Eds.), pp. 241–250. Editions de CNRS, Paris.

Dauphin-Villemant, C., and Xavier, F. (1987). Nychthemeral variations of plasma corticosteroids in captive female *Lacerta vivipara* Jacquin: Influence of stress and reproductive state. *Gen. Comp. Endocrinol.* **67,** 292–302.

Dauphin-Villemant, C., Leboulenger, F., Xavier, F., and Vaudry, H. (1988). Interrenal activity in the female lizard *Lacerta vivipara* J.: *In vitro* response to ACTH 1-39 and to [Sar^1, Var^5] Angiotensin II (ANG II). *J. Steroid Biochem.* **29,** 431–434.

Dauphin-Villemant, C., Leboulenger, F., Xavier, F., and Vaudry, H. (1990). Interrenal activity in the female lizard *Lacerta vivipara* Jacquin associated with breeding activities. *Gen. Comp. Endocrinol.* **78,**

Delarue, C., Tonon, M. C., Leboulenger, F., Jegou, S., Leroux, P., and Vaudry, H. (1979). *In vitro* study of frog (*Rana ridibunda* Pallas) interrenal function by use of a simplified perifusion system. II. Influence of adrenocorticotropin upon aldosterone production. *Gen. Comp. Endocrinol.* **38,** 399–409.

Duggan (1981). Plasma corticosteroids in marine, terrestrial and freshwater snakes. *Comp. Biochem. Physiol.* **68,** 115–118.

Fleury, F., and Naulleau, G. (1987). Relations entre l'hivernage et la reprise des activités endocrines, testiculaire et thyroïdienne, chez *Vipera aspis* L. (Reptilia, Viperidae). *Gen. Comp. Endocrinol.* **68,** 271–277.

Gabe, M. (1970). The adrenal. *In* "Biology of the Reptilia" (C. Gans and T. S. Parsons, Eds.), Vol. 3, pp. 263–318. Academic Press, New York/London.

Gabe, M., Martoja, M., and Saint-Girons, M. (1964). Etat actuel des connaissances sur la glande surrénale des Reptiles. *Ann. Biol.* **3,** 303–376.

Gavaud, J. (1983). Obligatory hibernation for completion of vitellogenesis in the lizard, *Lacerta vivipara* J. *J. Exp. Zool.* **225,** 397–406.

Gavaud, J., and Xavier, F. (1986). The possible significance of hibernation in the determinism of the reptilian reproductive cycle. *In* "Endocrine Regulations as Adaptive Mechanisms to the Environment" (I. Assenmacher and J. Boissin, Eds.), pp. 251–261. Editions de CNRS, Paris.

Gist, D. H. (1972). Effects of mammalian ACTH on liver and muscle glycogen levels in the South American caiman (*Caiman sclerops*). *Gen. Comp. Endocrinol.* **19,** 1–6.

Gonzalez, C., Rodriguez-Fernandez, J. L., Cabrera, I., Poch, H., Frias, I., Perez, J. A., and Siverio, J. M. (1988). Fructose-2-6-biphosphate and other metabolites and enzymes in the process of cold-induced lethargy and starvation in lizard liver. *Comp. Biochem. Physiol. B.* **89,** 131–135.

Gregory, P. T. (1982). Reptilian hibernation. *In* "Biology of the Reptilia" (C. Gans and F. H. Pough, Eds.), Vol. 13, pp. 53–154. Academic Press, New York/London.

Gustafson, A. W., and Belt, W. D. (1981). The adrenal cortex during activity and hibernation in the male little brown bat, *Myotis lucifugus lucifugus:* Annual rhythm of plasma cortisol levels. *Gen. Comp. Endocrinol.* **44,** 269–278.

Hernandez, T., and Coulson, R. A. (1963). Hibernation in the alligator. *Proc. Soc. Exp. Biol. Med.* **79,** 145–149.

Hubert, J., and Xavier, F. (1979). Cristallisation ribosomique et hibernation chez le Lézard vivipare *Lacerta vivipara* J. *C.R. Acad. Sci. Paris Sér. D* **228,** 635–636.

Jolivet-Jaudet, G., Inoue, M., Takada, K., and Ishii, S. (1984a). Circannual changes in plasma aldosterone levels in *Bufo japonicus formosus*. *Gen. Comp. Endocrinol.* **53,** 163–167.

Jolivet-Jaudet, G., Inoue, M., Takada, K., and Ishii,

S. (1984b). Circannual changes in corticosterone plasma levels and binding of corticosterone to plasma in *Bufo japonicus formosus*. *Zool. Sci.* **1**, 317–325.

Joy, J. E., and Crews, D. (1987). Hibernation in garter snakes (*Thamnophis sirtalis parietalis*): Seasonal cycles of cold tolerance. *Comp. Biochem. Physiol. A* **87**, 1097–1101.

Kraus-Friedmann, N. (1984). Hormonal regulation of hepatic gluconeogenesis. *Physiol. Rev.* **64**, 170–259.

Lance, V. A., and Elsey, R. M. (1986). Stress-induced suppression of testosterone secretion in male alligators. *J. Exp. Zool.* **239**, 241–246.

Lance, V. A., and Lauren, D. (1984). Circadian variation in plasma corticosterone in the American alligator *Alligator mississippiensis*, and the effects of ACTH injections. *Gen. Comp. Endocrinol.* **54**, 1–7.

Leboulenger, F. (1986). "Contribution à l'étude de l'activité de la corticosurrénale des Amphibiens Anoures, une glande endocrine sous contrôle multifactoriel," p. 318. Thèse Doct. Sc. Univ. Rouen.

Leboulenger, F., Delarue, C., Belanger, A., Perroteau, I., Netchitailo, P., Leroux, P., Jegou, S., Tonon, M. C., and Vaudry, H. (1982). Direct radioimmunoassays for plasma corticosterone and aldosterone in frog. I. Validation of the methods and evidence for daily rhythms in a natural environment. *Gen. Comp. Endocrinol.* **46**, 521–532.

Leboulenger, F., Delarue, C., Tonon, M. C., Jegou, S., and Vaudry, H. (1978). *In vitro* study of frog (*Rana ridibunda* Pallas) interrenal function by use of a simplified perifusion system. I. Influence of adrenocorticotropin upon corticosterone release. *Gen. Comp. Endocrinol.* **36**, 327–338.

Leboulenger, F., Delarue, C., Tonon, M. C., Jegou, S., Leroux, P., and Vaudry, H. (1979). Seasonal study of the interrenal function of the European green frog *in vivo* and *in vitro*. *Gen. Comp. Endocrinol.* **39**, 388–396.

Leloup-Hatey, J. (1968). Controle corticotrope de la corticostéroïdogenèse interrénalienne chez les Vertébrés inférieurs (Reptiles, Téléostéens). *Comp. Biochem. Physiol.* **26**, 997–1013.

Leroux, P., Delarue, C., Leboulenger, F., Jegou, S., Tonon, M. C., Vaillant, R., Corvol, P., and Vaudry, H. (1980). Development and characterization of a radioimmunoassay technique for aldosterone: Application to the study of aldosterone output from perifused frog interrenal tissue. *J. Steroid Biochem.* **12**, 473–478.

Licht, P. (1974). Endocrinology of Reptilia: The pituitary system. *In* "Chemical Zoology: Amphibia and Reptilia" (M. Florkin and B. T. Scheer, Eds.), Vol. 9, pp. 428–437. Academic Press, New York/London.

Licht, P., Mc Geery, B. R., Barnes, R., and Pang, R. (1983). Seasonal and stress related changes in plasma gonadotropins, sex steroids, and corticosterone in the bullfrog. *Rana catesbeiana*. *Gen. Comp. Endocrinol.* **50**, 124–145.

Lofts (1978). The adrenal gland in Reptilia. Part 1. Structure. *In* "General, Comparative and Clinical Endocrinology of the Adrenal Cortex" (I. Chester-Jones and I. W. Henderson, Eds.), Vol. 2, 292–360. Academic Press, New York.

Martin, B., and Xavier, F. (1981). High-affinity binding of progesterone, estradiol-17β, and testosterone by plasma proteins of the reptile *Lacerta vivipara* J. *Gen. Comp. Endocrinol.* **43**, 299–307.

Mayhew, W. W. (1965). Hibernation in the lizard *Phrysonoma M'Calli*. *Comp. Biochem. Physiol.* **16**, 103–119.

Minnich, J. E. (1979). Reptiles. *In* "Comparative Physiology of Osmoregulation in Animals" (G. M. O. Maloy, Ed.), pp. 391–641. Academic Press, New York/London.

Musacchia, X. J. (1984). Comparative and biochemical aspects of hypothermia as a model for hibernation. *Cryobiology* **21**, 583–592.

Musacchia, X. J. (1988). Endocrine regulation of carbohydrate metabolism in hypometabolic animals. *Canad. J. Zool.* **66**, 167–172.

Naulleau, G. (1986). Importance of hibernation on sexual and endocrine cycles in male snakes from temperate regions. *In* "Endocrine Regulations as Adaptive Mechanisms to the Environment" (I. Assenmacher and J. Boissin, Eds.), pp. 213–226. Editions de CNRS, Paris.

Naulleau, G., Fleury, F., and Boissin, J. (1987). Annual cycles in plasma testosterone and thyroxine in the male aspic viper *Vipera aspis* L. (Reptilia, Viperidae), in relation to the sexual cycle and hibernation. *Gen. Comp. Endocrinol.* **65**, 254–263.

Panigel, M. (1956). Contribution à l'étude de l'ovoviviparité chez les Reptiles: Gestation et parturition chez le Lézard *Zootoca vivipara*. *Ann. Sci. Nat. Zool.* **18**, 569–668.

Patterson, J. W., and Davies, P. M. C. (1978a). Preferred body temperature: Seasonal and sexual differences in the lizard *Lacerta vivipara* J. *J. Therm. Biol.* **3**, 39–41.

Patterson, J. W., and Davies, P. M. C. (1978b). Energy expenditure and metabolic adaptation during winter dormancy in the lizard *Lacerta vivipara* Jacquin. *J. Therm. Biol.* **3**, 183–186.

Patterson, J. W., and Davies, P. M. C. (1980). Thermal acclimation in temperature lizards. *Nature (London)* **275**, 640–647.

Patterson, J. W., Davies, P. M. C., Veasey, D. A., and Griffiths, J. R. (1978). The influence of sea-

son on glycogen levels in the lizard *Lacerta vivipara* Jacquin. *Comp. Biochem. Physiol.* **608,** 491–493.

Petrovic, V. M., Rajcic, O., and Janic-Sibalic, V. (1985). Accelerated gluconeogenic processes in ground squirrel (*Citellus citellus*) during the arousal from hibernation. *Comp. Biochem. Physiol. A* **80,** 477–480.

Saboureau, M., Bobet, J.-P., and Boissin, J. (1980). Activité cyclique de la fonction corticosurrénalienne et variations saisonnières du métabolisme périphérique du cortisol chez un Mammifère hibernant, le hérisson (*Erinaceus europaeus* L.). *J. Physiol. Paris* **76,** 617–629.

Sandor, T., Fazekas, A. G., and Robinson, B. H. (1976). The biosynthesis of corticosteroids throughout the vertebrates. *In* "General, Comparative and Clinical Endocrinology of the Adrenal Cortex" (I. Chester Jones and I. W. Henderson, Eds.), Vol. 1, pp. 25–142. Academic Press, New York/London.

Shivatcheva, T. M., Ankov, V. K., and Hadjioloff, A. I. (1988). Circannual fluctuations of the serum cortisol in the european ground squirrel, *Citellus citellus* L. *Comp. Biochem. Physiol. A* **90,** 515–518.

Tang, F., Hsieh, A. C. L., Lee, C. P., and Baconshone, J. (1984). Interaction of cold and starvation in the regulation of plasma corticosterone levels in the male rat. *Horm. Metab. Res.* **16,** 445–450.

Vallarino, M., Uva, B., and Isola, G. (1985). Effect of hypophysectomy on plasma electrolytes and adrenal mineralocorticoid secretion in the terrestrial chelonian *Testudo hermanni. Comp. Biochem. Physiol. A* **81,** 283–286.

Wang, L. C. H. (1982). Hibernation and the endocrines. *In* "Hibernation and Torpor in Mammals and Birds" (C. P. Lyman, J. S. Willis, A. Malan, and L. C. H. Wang, Eds), pp. 206–236. Academic Press, New York/London.

Wang, L. C. H. (1986). Some neuroendocrine aspects of mammalian hibernation. *In* "Endocrine Regulations as Adaptive Mechanisms to the Environment" (I. Assenmacher and J. Boissin, Eds.), pp. 341–349. Editions de CNRS, Paris.

Werner, R., and Vens-Cappel, F. (1985). Changes in plasma cortisol during acute cold exposure in euthermic european hedgehogs: Thermoregulatory role of the hypothalamo-pituitary-adrenal axis. *J. Comp. Physiol. B* **155,** 219–226.

Xavier, F. (1982). Progesterone in the viviparous lizard, *Lacerta vivipara* J.: Ovarian biosynthesis, plasma levels and binding to transcortin-type protein during the sexual cycle. *Herpetologica* **38,** 62–70.