GENERAL AND COMPARATIVE ENDOCRINOLOGY 82, 163–171 (1991)

# Cyclic Juvenile Hormone Biosynthesis in the Cockroach, *Blattella germanica:* Effects of Ovariectomy and Corpus Allatum Denervation

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Accepted June 18, 1990

The corpora allata (CA) of ovariectomized adult *Blattella germanica* females exhibited delayed but high rates of juvenile hormone biosynthesis *in vitro*. Using the onset of sexual receptivity as a probe of the degree of CA activation in females, we demonstrated at least one cycle of CA activity in the experimentally synchronized ovariectomized females. Following their activation, the CA exhibited a partial and transient decline in activity, but in contrast to the CA of intact females, this decline was not accompanied by a regression in CA volume. CA of intact and ovariectomized females that were denervated from the brain were activated, but the subsequent decline in CA activity at the end of the cycle was prevented in ovariectomized females. The presence of an egg-case suppressed the reactivation of the inactive CA in intact females but not in CA-denervated females. We conclude that activation of the CA in *B. germanica* is not dependent upon either the presence of the ovary or intact nervous connections between the CA and the brain. The brain exerts a partial inhibition on CA activity through intact nerves which is relieved (by disinhibition) in the presence of a young ovary but is enhanced and sustained in the presence of the egg-case. Inhibition of the CA also occurs independently of nervous connections with the brain through factors that originate in the mature ovary and affect both CA activity and morphology. 

The brain–corpora allata (CA)–ovary axis in insects has been compared to the hypothalamus–hypophysis–ovary axis in vertebrates (Tobe, 1980; Scharrer, 1987). Of special interest are insects with gonadotrophic cycles as in vertebrates, i.e., ovoviviparous and viviparous cockroach species. In both vertebrate and insect systems, ovarian development and pregnancy depend on accurate timing of gonadotropin cycles in the blood. Ovarian hormones are known to control gonadotrophic cycles in mammals, acting at both the hypophysis and the hypothalamus and generating inhibitory as well as stimulatory signals through alternating negative and positive feedback loops (Knobil, 1980; Ojeda, 1988). In insects, the precise interactions among the ovary, the CA, and the brain are less clearly understood, although considerable evidence from several species supports the hypothesis that both stimulatory and inhibitory ovarian factors (hormones ?) modulate the cyclic production of juvenile hormone (JH) during the reproductive cycle (Scharrer and von Harnack, 1961; Stay *et al.*, 1980, 1983; Lanzrein *et al.*, 1981; Weaver, 1981; Rankin and Stay, 1983, 1985).

In cockroaches, the brain exerts mainly inhibitory effects on the activity of the CA, which can be relieved by transection of the nervous connections between the brain and the CA or by appropriate stimuli (e.g., mating and feeding in *Diploptera punctata* (Stay and Tobe, 1977; Woodhead and Stay, 1989) and in *Periplaneta americana* (Pipa, 1986), and grouping in *Blattella germanica* (Gadot *et al.*, 1989a). Decapitation of *D. punctata* and *Nauphoeta cinerea* young adult females does not prevent the normal

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0016-6480/91 $1.50

activation and cyclic JH synthesis by implanted CA, but the ovary is required for allatal activation in both species (Lanzrein *et al.*, 1981; Rankin and Stay, 1983). It is interesting, however, that the brain of male *D. punctata* stimulates transplanted female CA in the absence of the ovary (Tobe *et al.*, 1981).

In this paper we report on the effects of ovariectomy and denervation of the CA on the cyclic production of juvenile hormone (JH) III in the oviparous cockroach, *B. germanica*. The adult female produces an egg-case and carries it externally, in her genital atrium, for the duration of embryonic development, thus exhibiting functional "pregnancy" (Roth and Stay, 1962). We previously demonstrated that in this species, as in ovoviviparous *N. cinerea* and viviparous *D. punctata*, JH III synthesis *in vitro* is precisely regulated in relation to the gonadotrophic cycle: CA activity and oocyte development are inhibited in the gravid female and both resume immediately after the nymphs hatch and the egg-case is dropped (Roth and Stay, 1962; Gadot *et al.*, 1989b). We report that the CA of ovariectomized females attain the same high rates of JH synthesis as do CA of intact *B. germanica*, but the cyclic pattern of hormone synthesis depends upon the presence of the ovary and the egg-case. The brain mediates the disinhibition of the CA by the ovary. The interactions among the ovary, the brain, and the CA are discussed, and a model of ovarian regulation of the cyclic activity of the CA is presented.

## MATERIALS AND METHODS

*Blattella germanica* was reared at 27° as described previously (Gadot *et al.*, 1989a); adult females were housed in groups of 2–10. Ovariectomy was performed on early last instar nymphs. Transection of the nervi corpori cardiaci (NCC) I and II was performed on newly emerged adult females as described by Pipa (1986). Juvenile hormone synthesis was determined *in vitro* by the radiochemical assay of Pratt and Tobe (1974) as modified and described by Gadot *et al.* (1989a,b). The volume of freshly dissected CA was determined by the formula $V = \frac{4}{3}\pi abc$, where $a$, $b$, and $c$ are the radii of the three principal axes measured with an ocular filar micrometer under a dissecting microscope.

Newly emerged (Day 0) adult females (sham as well as treated) were placed with mature males for at least 8 hr daily until mating was observed. Thereafter each mated female was housed with two males. Females that did not mate by Day 7 were discarded, except where indicated.

## RESULTS

### *Juvenile Hormone Synthesis in Ovariectomized Females and the Effect of Mating*

Previous studies have shown that in their first gonadotrophic cycle normal *B. germanica* females become sexually receptive when their basal oocytes attain a minimal length of 1.2–1.5 mm, suggesting a relation between receptivity and JH levels (Roth and Stay, 1962; Gadot *et al.*, 1989a). Also, ovariectomized females exhibit a delayed and variable first mating, while allatectomized females do not mate during 30 days of continuous observations (Schal, unpublished observations). Because the CA of ovariectomized females of different chronological ages exhibited similar levels of activity when assayed the same number of days after mating (Table 1), it suggests that the onset of sexual receptivity is a reliable

TABLE 1
JUVENILE HORMONE SYNTHESIS (pmol/hr/PAIR CA) IN OVARIECTOMIZED *B. germanica* FEMALES IN RELATION TO THE ONSET OF SEXUAL RECEPTIVITY AND COPULATION

| Age mated | Days after mating | |
|---|---|---|
| | 7 | 14 |
| 6–7 days | 7.5 ± 1.6 (6) | 3.1 ± 0.9 (9) |
| 8–19 days | 9.1 ± 1.6 (7) | 3.7 ± 0.2 (5) |

*Note.* All operations were performed on last stadium nymphs. Values are means ± SEM ($N$). No significant differences were observed between different aged females the same number of days after mating ($P > 0.05$, Student's $t$ test).

probe of the physiological stage (and level of CA activity) of ovariectomized females.

However, mating may also directly affect CA activity. To test this hypothesis, we compared ovariectomized females that mated normally on Day 6 with females that were separated from the males within 1 min of the start of copulation; no spermatophore or accessory male secretions are transferred during the first minute of copulation (Khalifa, 1950; Schal, personal observations). On Day 14 the CA from females that were separated from males had slightly lower rates of JH synthesis than the CA from control females that completed copulation (3.4 ± 0.5, $N = 10$ and 5.8 ± 1.6 pmol/hr/pair CA, $N = 7$, respectively; $P = 0.18$, Student's $t$ test). However, in both groups the rates of JH biosynthesis were significantly higher than in unreceptive females of the same age (0.5 ± 0.2 pmol/hr/pair CA, $N = 4$; $P < 0.05$, Duncan's multiple range test (MRT)).

Only 53% of ovariectomized females that were presented with males daily mated by Day 7 ($N = 172$), compared with 98% of intact females ($N = 179$). A group of ovariectomized females was monitored continuously by time-lapse video recording for 28 days: 93% of the females mated by this age, indicating that in contrast to intact females, the onset of sexual receptivity in ovariectomized females is significantly delayed and presumably reflects variable rates of CA activation. Therefore, we selected ovariectomized females that mated by Day 7 in order to obtain a population with relatively synchronous activation of the CA. These females exhibited at least one cycle of CA activity with comparable average peak rates of JH biosynthesis to intact females (Fig. 1). However, their CA activity cycle differed from that of intact mated females by the delayed activation of the CA and the moderate but significant decline ($P < 0.05$; Duncan's MRT) in JH synthesis on Days 17 and 21. These low rates never fell below the limits of detection in any individual determination, as they do in intact mated females during and after ovulation (Fig. 1, Table 2; see Gadot *et al.*, 1989b). The CA of ovariectomized females increased their JH biosynthetic activity after Day 21 to a peak on Day 35 of similar magnitude to that on Day 14 ($P > 0.05$; Duncan's MRT). It is unclear whether the decline on Day 42 represents a dampened cycle due to the greater variability among CA of older females.

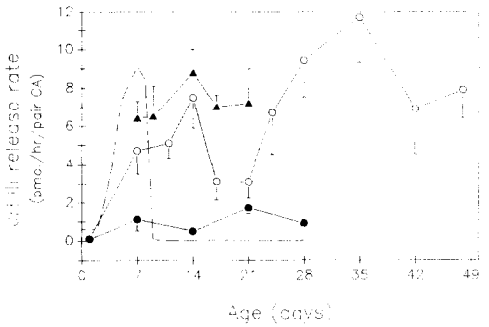


FIG. 1. Rates of juvenile hormone biosynthesis by CA from adult females ovariectomized as last stadium nymphs. Females either mated by Day 7 after adult ecdysis (open circles) or refused to mate by the day of the assay (solid circles). The nervous connections between the brain and the CA were transected in some newly ecdysed ovariectomized females and they mated by Day 7 (solid triangles). The dashed line represents the values of JH III biosynthesis by CA from intact-mated females, extrapolated from data relating oocyte size and JH III release rates (Gadot *et al.*, 1989a). Each point is the mean of 4–17 females and the vertical bars represent the SEM.

In contrast, the CA of ovariectomized females that did not mate (have not become sexually receptive) in daily exposure to males exhibited low and relatively invariable rates of JH synthesis *in vitro* (Fig. 1). Thus, if all the JH biosynthesis data are averaged chronologically without regard to whether and when females become receptive, then no clear cycle of JH synthesis is evident, and the average daily rate of JH synthesis is significantly lower than in intact females.

### *Effect of CA-Denervation*

Intact females with denervated CA that

TABLE 2
JUVENILE HORMONE SYNTHESIS AND OOCYTE LENGTH IN CA-DENERVATED AND SHAM-TREATED *B. germanica* FEMALES

| Age (days) | Sham-treated | | CA-denervated | |
|---|---|---|---|---|
| | JH release rate (pmol/hr/pair CA) | Basal oocyte length (mm) | JH release rate (pmol/hr/pair CA) | Basal oocyte length (mm) |
| 7[a] | 8.5 ± 0.9 (5) | 1.81 ± 0.2 (6) | 7.9 ± 0.7 (10) | 1.3 to 2.0 |
| | | | 0.4 ± 0.2 (14) | 2.3 to ovulated |
| 17 | ND[b] | egg case (0.5)[c] | 0.8 ± 0.3 (5) | egg case (0.6 to 1.2)[c] |
| 21 | ND[b] | egg case (0.5)[c] | 6.9 ± 1.9 (4) | egg case (1.5 to 2.0)[c] |

*Note.* All values are means ± SEM (*N*).

[a] Seven-day-old females were separated according to their physiological stage into two groups: those with maturing oocytes (1.3 to 2.0 mm in length) and those with oocytes undergoing chorionation and ovulation with basal oocytes at least 2.3 mm in length.

[b] ND, not detectable.

[c] The values in parenthesis denote the length of the penultimate oocytes which developed while the first egg case was still carried by the female.

mated by Day 7 (i.e., selected as before, 84%, $N = 32$) exhibited a clear cycle of CA activity (Table 2). Their CA became active earlier than in sham-denervated females, as evidenced by the decline in JH synthesis on Day 7 in association with early ovulation: 10 of 24 CA-denervated females had vitellogenic basal oocytes (1.3–2.0 mm long) and their CA exhibited high rates of JH synthesis, while the other 14 females had chorionated basal oocytes or they had just ovulated and their CA exhibited low rates of JH synthesis (Table 2). Sham-operated females that mate by Day 7 normally ovulate on days 8–9. Thus, CA-denervation not only accelerated the activation of the CA, but also did not abolish or delay the decline in CA activity during ovulation, suggesting that inhibitory factors that probably originate in the mature ovary are transmitted via the hemolymph.

On Day 17, while the first egg-case was still attached and at least 10 days before it would normally hatch, JH biosynthesis increased in the CA-denervated females, and some oocyte development was evident (Table 2). By Day 21 the denervated CA resumed their high activity, and the new basal oocytes had nearly completed their development, indicating that the inhibiting effect of the egg-case on CA activity is mediated by the brain and transmitted to the CA via the NCC.

Transection of the nerves between the brain and the CA in ovariectomized females also accelerated the activation of the CA as evidenced by the higher rates on Day 7 (Fig. 1). However, CA denervation in these females prevented the decline in JH biosynthesis which remained high at least until Day 21, with no apparent cycle (Fig. 1).

To further elucidate the roles of the ovary and the brain in the activation of the CA during the first gonotrophic cycle, females were either ovariectomized, CA-denervated, or both, and compared with sham-operated females. Mating was not used to select females in this experiment because CA activity was assayed only on Day 7. Figure 2 shows that while CA-denervation alone did not affect the activity of the CA (compared with sham-operated controls), ovariectomy alone significantly reduced the average rate of JH synthesis. The double operation resulted in intermediate rates of JH synthesis in both sham-operated and experimentally operated females, but they did not differ significantly from each other. The rates of JH synthesis by denervated CA in both ovariectomized

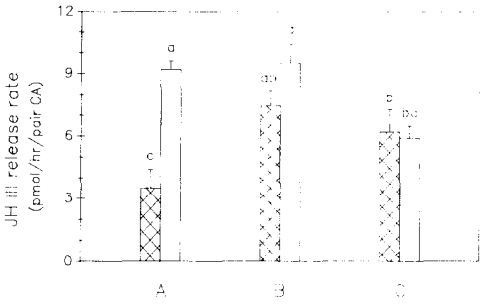


FIG. 2. Rates of juvenile hormone biosynthesis by CA from 7-day-old virgin females ovariectomized as last stadium nymphs (A), or CA-denervated on Day 0, the day of adult ecdysis (B), or both ovariectomized and CA-denervated (C). The hatched bars represent the means of treated females and the blank bars represent the means of sham-operated females. The vertical lines represent the SEM of 5–21 females. Means with different letters differ significantly from each other ($P < 0.05$, Duncan's multiple range test).

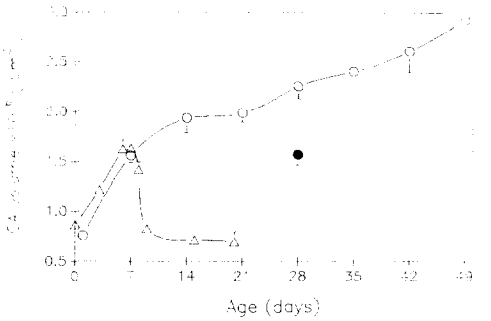


FIG. 3. Corpus allatum volume in intact (open triangles) and ovariectomized (open circles) adult *B. germanica* females that mated by Day 7. The CA volume of ovariectomized females that did not mate by Day 28 is also shown (solid circle).

and in intact (sham-ovariectomized) females were as high as in the corresponding sham-operated females, indicating that the presence or absence of the ovary does not appear to affect the rate of activation of denervated CA (Fig. 2). This suggests that stimulation of CA activity by the young ovary occurs through brain disinhibition, which is mediated through intact nerves to the CA.

### *Developmental Changes in CA of Ovariectomized Females*

The volume of the CA in intact females changes in relation to JH synthesis, with a sharp decline before and during ovulation (Fig. 3). In ovariectomized females, however, CA volume increased gradually with age. The lower CA activity in 21-day-old ovariectomized females (Fig. 1) was not accompanied by a decline in gland volume (Fig. 3). The CA of females that did not mate also increased in volume although they exhibited low rates of JH synthesis; on Day 28 they were as large as fully active CA of intact females (Fig. 3). Farnesoic acid (FA) is a late precursor of JH III biosynthesis and it is utilized efficiently by CA *in vitro*, including in *B. germanica* (Gadot *et al.*, 1989b). The pattern of FA-stimulated JH synthesis resembled the pattern of spontaneous CA activity (Figs. 1 and 4) with significantly lower rates on Day 21 than on Days 14 and 28 ($P < 0.05$, Duncan's MRT). The FA-stimulated rates of JH synthesis in unmated females were invariably low at all ages (Fig. 4).

## DISCUSSION

The onset of sexual receptivity in the first gonotrophic cycle of intact *B. germanica* females is coincident with a relatively low level of circulating JH. Sexual receptivity is expressed only after the oocytes reach a critical size (Roth and Stay, 1962; Gadot *et*

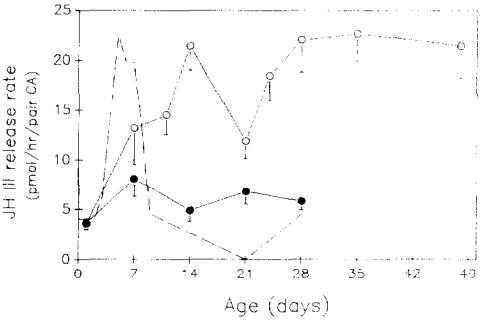


FIG. 4. Rates of juvenile hormone biosynthesis by CA incubated in the presence of farnesoic acid. Symbols as in Fig. 1.

*al.*, 1989a) and allatectomized females do not mate (Schal, unpublished observations). The ovaries do not appear to influence sexual receptivity directly since both ovariectomized females and allatectomized females exposed to JH mate (Schal, unpublished observations). Therefore, in the absence of the ovaries, we used readiness of females to copulate as a probe for their physiological stage (i.e., moderate CA activity). Females that become sexually receptive at different chronological ages exhibit similar rates of JH synthesis when assayed the same number of days after copulation (Table 1), while females that are offered males daily and are not sexually responsive exhibit low rates of JH synthesis (Fig. 1). Copulation itself may accelerate CA activity because females that are sexually receptive but are not inseminated exhibit slightly lower rates of JH synthesis than do inseminated females (Table 2).

### *Activation of the CA*

Ovariectomy of last instar *B. germanica* nymphs does not prevent the activation of the CA in the adult female. The pattern of CA activation is significantly delayed and more variable than in either intact or sham-operated females, but in experimentally synchronized ovariectomized females the average peak rates of JH synthesis are as high as in intact females (Fig. 1). This is in contrast to ovariectomized females of the cockroaches *D. punctata* and *N. cinerea* in which the CA are not activated and low rates of JH synthesis are sustained (Stay and Tobe, 1978; Lanzrein *et al.*, 1981; Rankin and Stay, 1983; Stay *et al.*, 1983). In *P. americana* the CA from ovariectomized females that mated by Day 6 became active in the absence of the ovaries, but their activity was noncyclic, highly variable, and significantly lower than in intact females (Weaver, 1981). Since the gonotrophic cycles in intact *P. americana* are very rapid and are markedly accelerated by mating, a large variability among females might stem from differences in the onset of sexual receptivity in ovariectomized females.

The pattern of CA activation in grouped ovariectomized *B. germanica* females resembles to some extent the asynchronous and delayed pattern of CA activation in intact isolated females (Gadot *et al.*, 1989a). In both cases, denervation of the CA removes a partial brain inhibition of their activity and accelerates their activation (Fig. 2; Gadot *et al.*, 1989a). These results are consistent with experimental evidence from several cockroach species in which a general inhibition (restraining) by the brain is transmitted through nerves to the CA. This inhibition can be overriden (disinhibited) by various external and internal stimuli that presumably affect the brain. It is important to note that in contrast to *B. germanica*, ovariectomized *D. punctata* females with denervated CA synthesize JH at only slightly higher rates than do ovariectomized females with innervated CA, and the peak rate of JH III synthesis in both groups is less than ⅓ that of intact females (Stay *et al.*, 1983). The role of the ovary in CA activation thus appears to be species-specific in cockroaches.

### *Activity Cycle of CA in Ovariectomized Females*

The use of sexual readiness to select synchronous ovariectomized females enabled us to identify a cycle of CA activity in *B. germanica* females (Fig. 1). The lower JH synthesis rates on Days 17 and 21 represent a true and relatively synchronous decline in all females, as evident from other JH-mediated reproductive events. In intact and ovariectomized *B. germanica* females, the size of the left colleterial gland and protein accumulation within the gland increase in response to JH induction and are arrested upon allatectomy (Burns *et al.*, 1991). In ovariectomized females that mated by Day 7, the colleterial gland is larger on Day 21 than on Day 14, indicating that each female

with a low rate of JH synthesis on Day 21 had experienced higher rates earlier. If lower CA activity on Days 17 and 21 was caused by slower activation of the CA in some females, these females would have also exhibited a slower accumulation of colleterial gland proteins. Together with the synchronous decline in CA activity in ovariectomized females 14 days after mating at different ages (Table 1), these data support the conclusion that each ovariectomized female experiences a cycle of CA activity.

However, it is unclear whether more than one cycle is sustained by each CA. No significant decreases were detected after Day 21 either in the basal CA activity or in their FA-stimulated activity, and the CA of older ovariectomized females exhibited greater heterogeneity in their levels of activity (Figs. 1 and 4). These results may reflect a loss of cyclicity in each gland, followed by a highly variable slow decline in CA activity that may result from exhaustion of the CA in older females (see also Scharrer and von Harnack, 1961). Alternatively, as the synchronization of females that mated by Day 7 diminishes gradually, temporal asynchrony among older females will also result in intermediate and highly variable rates of JH synthesis.

### *Factors Involved in CA Inhibition following Their Activation*

In the absence of both the ovary and intact nervous connections between the CA and the brain, CA activity did not decline through at least 21 days (Fig. 1). In the presence of either the ovary or the intact CA–brain nervous connections the CA exhibited a regression in activity (Fig. 1, Table 2). These results suggest that in their first activity cycle at least two independent mechanisms can inhibit the CA.

One mechanism of CA inhibition involves ovarian factors that can operate on innervated as well as on denervated CA (Table 2). These factors may have long-lasting effects, since the denervated CA of otherwise intact *B. germanica* females do not resume their full activity for several days after ovulation (Table 2). Alternatively, the decline in CA activity may be initiated by ovarian factors, but because they also cause dramatic changes in CA morphology and cytology (see below), the CA may be partially or completely refractory to stimulatory signals; the decline in activity may continue for several days after ovulation until the process is reversed either by endogenous events within the CA or through new stimulatory signals from other sources. Ecdysteroids have been suggested to be the active ovarian factors that inhibit CA activity before ovulation in cockroaches (Stay *et al.*, 1980; Lanzrein *et al.*, 1981; Tobe and Stay, 1985) and we speculate that these factors may also operate in *B. germanica*.

The other mechanism of CA inhibition in *B. germanica* is mediated by the brain, and it can operate in the absence of the ovary (Fig. 1). However, this mechanism does not cause a regression in CA volume (Fig. 3). This nonovarian brain-mediated inhibition of CA activity is only partially effective as evidenced by the smaller decline in CA activity in ovariectomized females than in either intact or CA-denervated females at ovulation. These factors may also be ecdysteroids, originating in the prothoracic gland which slowly degenerates after the imaginal molt. This is consistent with the observation that these factors are present only in the first cycle, since the CA resume high activity thereafter with no apparent declines. They may act by programming the brain to effect a CA cycle. We are currently investigating the role of 20-hydroxyecdysone in the CA cycle in *B. germanica* in an effort to elucidate the precise role(s) of this hormone in the control of JH III synthesis.

### *CA Morphology*

In ovariectomized *B. germanica* females CA volume increased gradually with age. Hypertrophy of the CA in ovariectomized

females has been observed also in *Leucophaea maderae* (Scharrer and von Harnack, 1961), suggesting that the ovary has an important role in the volumetric regression of the CA at each ovulation. Moreover, our results with *B. germanica* indicate that in ovariectomized females CA volume does not necessarily reflect the biochemical capacity of the gland to synthesize JH. Hypertrophy of the CA is thus a direct consequence of the absence of the ovaries, while CA activity is clearly affected by nonovarian as well as by ovarian factors.

In the presence of exogenous FA, JH biosynthesis bypasses earlier rate-limiting steps in the sesquiterpenoid pathway and it reflects the maximal competence of the CA to synthesize JH (Tobe and Pratt, 1976; Feyereisen, 1985). In all cockroaches examined to date, the FA-stimulated rates of JH synthesis correlate well with CA volume in intact adult females (Feyereisen, 1985; Gadot *et al.*, 1989b; Chiang, Gadot, and Schal, unpublished observations). However, in ovariectomized *B. germanica* females FA-stimulated rates are more closely related to the spontaneous JH biosynthetic rates than to CA volume (Figs. 1, 3, and 4).

These results suggest that the developmental processes associated with changes in CA volume are controlled independently of the processes associated with JH biosynthesis. Chiang *et al.* (1989, 1990) showed that in intact *B. germanica* adult females, activation of the CA is associated with a synchronous increase in the size of CA cells without significant changes in total cell number. Recently, we extended these observations to ovariectomized females: in contrast to *D. punctata* (Tobe *et al.*, 1984), total CA cell number does not change during the 49 days we examined in ovariectomized *B. germanica* and it does not differ significantly from CA cell number in intact females (Chiang *et al.*, 1991). Our preliminary data indicate that, as in intact females, both the spontaneous and FA-stimulated JH biosynthetic rates in ovariectomized females are closely related to changes in the size of CA cells. Changes in cell size that are independent of changes in gland volume suggest that a morphometric rearrangement occurs within the CA in ovariectomized females. Our preliminary results suggest that in ovariectomized females older than 49 days, intercellular spaces and central cavities may be formed, accounting for the increased volume of the CA without further changes in its activity and total cell number.

## ACKNOWLEDGMENTS

We thank Dr. M. P. Pener for valuable remarks on the manuscript. This study was supported in part by a grant from NIH (HD-21891) to C. Schal and by the Thomas J. Headlee Fellowship to A-S. Chiang. New Jersey Agricultural Experiment Station publication D-08170-03-90 was supported by State Funds and by the U.S. Hatch Act.

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