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FARNESOIC ACID-STIMULATED RATES OF JUVENILE HORMONE BIOSYNTHESIS DURING THE GONOTROPHIC CYCLE IN *BLATTELLA GERMANICA*

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Abstract—Juvenile hormone III biosynthesis *in vitro* by the corpora allata of adult female *Blattella germanica* is precisely correlated with the gonotrophic cycle. Farnesoic acid-stimulated rates of juvenile hormone synthesis are always higher than the corresponding basal rates, and follow the same pattern of cyclic changes during oöcyte maturation. Both basal and farnesoic acid-stimulated rates decline to undetectable levels while the female carries an oötheca and oöcyte development is inhibited ('pregnancy'). This suggests that, in this species, the main control mechanism of the corpora allata operates through structural/developmental processes, affecting the maximal biochemical capacity of the glands. Morphometric data relating gland volume and the main events during oocyte development and 'pregnancy' support this conclusion. We speculate that the type of corpora allata control which is predominant in different insects might be functionally related to their ovipositional behavior.

Key Word Index: Juvenile hormone; *in vitro* assay; Farnesoic acid; Corpora allata regulation; Ovarian cycle; Pregnancy; *Blattella germanica*

INTRODUCTION

Farnesoic acid biosynthesis in the corpora allata is one of the last two steps in juvenile hormone (juvenile hormone III) biosynthesis, and is considered to be non-rate-limiting (Tobe and Pratt, 1976; Feyereisen, 1985). Isolated corpora allata, incubated *in vitro*, have been shown to utilize exogenous farnesoic acid with high efficiency in a number of insect species (e.g. Tobe and Pratt, 1974; Pratt *et al.*, 1975; Weaver *et al.*, 1980; Feyereisen *et al.*, 1981; Gadot and Applebaum, 1986). The farnesoic acid-stimulated activity of the glands is considered to reflect their maximal biochemical capacity and, in all cases examined, was found to correlate with the size of the glands (Tobe and Pratt, 1976; Feyereisen, 1985). On the basis of their research on the locust, *Schistocerca gregaria*, Tobe and Pratt (1976) proposed a dual control mechanism of corpora allata activity: rapid modulation of rate-limiting step(s) provides an independent control of the basal activity of the glands, while structural and ultrastructural changes are responsible for the slower responses related to the maximal capacity of the glands. Experimental evidence on the control of corpora allata activity in *Locusta migratoria* was recently presented in support of this hypothesis (Gadot and Applebaum, 1986; Baehr *et al.*, 1986; Gadot *et al.*, 1987; Dale and Tobe, 1988; Couillaud *et al.*, 1988).

Independent control of basal activity and maximal capacity of the corpora allata was also demonstrated in the cockroach *Periplaneta americana* (Weaver and Pratt, 1981). However, in the cockroach *Diploptera punctata*, a close relationship between these parameters suggests that the relative importance of the two control modes of the corpora allata (i.e. through the rapid and slow responses) may not be the same in different species (Feyereisen, 1985).

Blattella germanica exhibits an ovipositional behavior that is functionally intermediate between oviparity and ovoviviparity (review: Roth, 1970): unlike most oviparous cockroaches, such as *P. americana*, which form and deposit oöthecae in rapid succession, *B. germanica* forms an oötheca which is extruded but not deposited. Rather, the oötheca is carried externally attached to the genital atrium and basal oöcyte growth is arrested until the nymphs hatch (Roth and Stay, 1962). This incubation period is, therefore, functionally similar to pregnancy in ovoviviparous (e.g. *Nauphoeta cinerea*, *Leucophaea maderae*) and viviparous (*D. punctata*) cockroaches which retract the oötheca into a brood sac and incubate the embryos internally. We refer to this period in *B. germanica* as 'pregnancy'.

In this paper we show that the basal rate of juvenile hormone synthesis in the adult female *B. germanica* is dictated to a large extent by the total synthetic capacity of the corpora allata, as measured by the farnesoic acid-stimulated rate of juvenile hormone synthesis *in vitro*. Morphometric evidence supports the conclusion that the main control mechanism of corpora allata activity in this species operates through structural changes in the glands. By comparing several species of cockroaches and locusts, we propose a hypothesis which relates the type of corpora allata control and ovipositional behavior in the female adult insect.

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MATERIALS AND METHODS

German cockroach (*B. germanica*) nymphs were reared at 27°C under 12 h light–12 h dark, with dog food and water provided *ad lib*. Newly emerged adult females (day 0) were isolated daily and maintained under the same environmental conditions either individually or in groups, as indicated.

Farnesoic acid (about 70% pure) was a generous gift from Dr Staal (Zoecon Corp., Palo Alto, Calif.). L-[methyl-^{3}H]methionine (93% pure, specific activity of 200 mCi/mmol) was obtained from New England Nuclear, Wilmington, Del.

The radiochemical assay for juvenile hormone biosynthesis was adapted from Pratt and Tobe (1974) with modifications after Feyereisen and Tobe (1981) and Gadot and Applebaum (1985): corpora allata–corpora cardiaca complexes were dissected from carbon dioxide-anaesthetized females in modified methionine-free TC-199 medium (GIBCO, Grand Island, N.Y.; special formulation after Kikukawa *et al.*, 1987) and transferred to 100 μl of the same medium containing 100 μM of L-[methyl-^{3}H]methionine (2 μCi) in tissue-culture-treated disposable Cell Wells multidishes (Corning, N.Y.). The glands were incubated with gentle shaking in the dark, at 28°C for 2 h, and then were transferred to fresh medium containing 100 μM farnesoic acid for an additional incubation period of 2 h. The medium from each incubation was collected into 1.5 ml Eppendorf tubes and extracted with 200 μl of isooctane. The glands were removed and extracted separately.

De novo synthesis of juvenile hormone, from either endogenous or exogenous farnesoic acid, was assayed from an aliquot of the isooctane phase by liquid scintillation spectrometry, and corrected by a blank incubation. Thin-layer chromatography confirmed that more than 85% of the radioactivity in the isooctane, which was not attributed to methionine (i.e. after blank substraction), corresponded to juvenile hormone III. The remaining radioactivity (about 15%) found in the isooctane phase consisted of a tailing of more polar unidentified substances. It is unknown whether this radioactivity corresponds to other metabolic byproducts or to impurities from methionine which are more soluble in isooctane in the presence of gland extracts than in the isooctane phase of extract of blank incubations.

Corpus allatum volume was determined by the formula $v = 4/3\ \pi abc$, where a, b and c are the radii of the three principal axes measured with an ocular filar micrometer under a dissecting microscope.

RESULTS

Parameters of juvenile hormone synthesis in vitro

Juvenile hormone release rate is an accurate measure for juvenile hormone biosynthesis rate *in vitro* for both basal and farnesoic acid-stimulated synthesis ($r = 0.975$, $n = 28$; Fig. 1), and the two terms will be used interchangeably throughout this paper. Juvenile hormone synthesis rate is unaffected by the presence of the corpora cardiaca (t-test, $P > 0.05$; Table 1) and since corpora allata-corpora cardiaca complexes are easier to dissect, they were used in all physiological studies which follow.

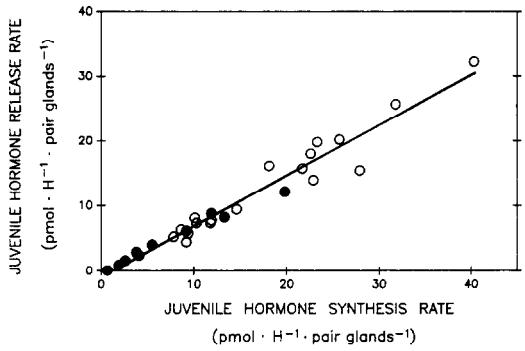

Fig. 1. Relationship between juvenile hormone synthesis and release by corpora allata incubated with (○) and without (●) farnesoic acid (100 μM). Each point represents an individual assay.

Linear rates of juvenile hormone synthesis were demonstrated in individual corpora allata–corpora cardiaca complexes through at least 8 h *in vitro* (Fig. 2). Dose–response effects of methionine and farnesoic acid concentrations on juvenile hormone synthesis were demonstrated with high-activity corpora allata (Figs 3 and 4).

In vitro rates of juvenile hormone synthesis during the gonotrophic cycle

The farnesoic acid-stimulated rate of juvenile hormone synthesis is highly correlated to the basal rate of synthesis [$r = 0.93$, $n = 127$, and $y = 2.56x + 3.35$ is the regression of farnesoic acid-stimulated rate (y) on the basal rate (x)]. Both rates exhibit similar cyclic patterns during at least the first two ovarian cycles (Fig. 5). Both basal rates and farnesoic acid-stimulated rates of synthesis are undetectable in mid-'pregnancy' (day 25). The farnesoic acid-stimulated activity of the corpora allata increases before the termination of 'pregnancy' (Fig. 5).

Using the length of the basal oöcytes as a correlate of juvenile hormone synthesis *in vitro*, no significant differences in the rates of juvenile hormone synthesis are evident between the first two gonotrophic cycles (t-test for each oöcyte length, $P < 0.05$, Fig. 6). The rate of juvenile hormone synthesis peaks in females with basal oöcyte lengths in the range of 1.4–2.2 mm, and declines just before ovulation, when oöcytes are larger than 2.3 mm (Fig. 6). During 'pregnancy' (about 22 days in our colony), both juvenile hormone synthesis and maturation of basal oöcytes are inhibited.

Table 1. The effect of coincubation of corpora allata with corpora cardiaca on juvenile hormone biosynthesis *in vitro*

	Juvenile hormone synthesis (pmol h^{-1} pair glands^{-1})*	
	Pair of corpora allata	Corpora allata–corpora cardiaca
Low-activity glands†	1.4 ± 0.2 (5)	1.5 ± 0.3 (6)
High-activity glands‡	10.5 ± 1.4 (9)	7.8 ± 0.9 (8)

*Mean ± SEM from pairs of corpora allata incubated for 2 h either alone or as intact complexes with corpora cardiaca.
Number of replicates indicated in parentheses.
†Glands obtained from 3-day isolated females.
‡Glands obtained from 5-day grouped females.

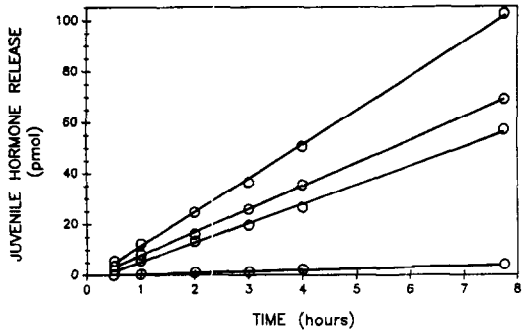

Fig. 2. Time-course of juvenile hormone release *in vitro*. Each point represents the cumulative juvenile hormone released by an individual pair of corpora allata. The glands were transferred to fresh media for each successive period.

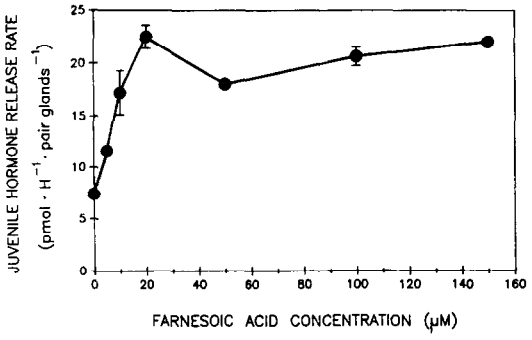

Fig. 4. Dose–response relationship for juvenile hormone release *in vitro* as a function of farnesoic acid concentration. Each point is the mean ± SEM of 3–7 glands from 5–6-day grouped virgin females.

The cyclic changes in the volume of the corpora allata correspond to the cyclic changes in their activity (Table 2). The volume of the glands increases 2-fold during oöcyte development and decreases again at ovulation. The corpora allata are smallest in mid-'pregnancy', when both basal and farnesoic acid-stimulated activity are undetectable (Table 2).

DISCUSSION

Juvenile hormone III was found to be the only juvenile hormone homologue in *B. germanica* adult females, in both *in vivo* and *in vitro* studies (Camps *et al.*, 1987; Belles *et al.*, 1987). Stoichiometry of the *in vitro* incorporation of the radiolabelled methyl group from methionine into juvenile hormone was also demonstrated in this species (Belles *et al.*, 1987). We examined several other parameters of the *in vitro* system in order to verify its adequacy as a tool for physiological investigations on the regulation of the corpora allata in this insect. All of these parameters (Table 1, Figs 1–4) are in agreement with similar studies in other cockroach species (reviews: Tobe and Stay, 1985; Feyereisen, 1985). Although our rates of juvenile hormone synthesis are quite moderate in comparison to rates reported for other insect systems, they are much higher than reported for *B. germanica* by Belles *et al.* (1987), who used somewhat different incubation conditions and employed different methods for the purification and assay of the radiolabelled juvenile hormone. Thus, until a reliable method of determining the *in situ* rates of juvenile hormone synthesis is developed, all comparisons among *in vitro* studies must be made in relative terms, while absolute values are viewed only as approximations.

A precise relation between juvenile hormone synthesis *in vitro* and the gonotrophic cycle was shown: rates of juvenile hormone synthesis increase during vitellogenesis, peak in late vitellogenesis and decline sharply before ovulation (Figs 5, 6). During 'pregnancy' the basal activity of the corpora allata is undetectable, but resumes after hatching of the nymphs. Volumetric changes in the corpora allata follow a similar cyclic pattern (Table 2) as was also shown by Belles *et al.* (1987). In adult females reared in isolation and mated on day 8, the second oöcyte maturation cycle is shorter than the first, but the patterns of corpora allata activity, relative to maturation of the oöcytes, are identical in both cycles (Figs 5, 6). External stimuli, such as from mating and grouping, which modulate the activity pattern of the corpora allata, may confound the relationship

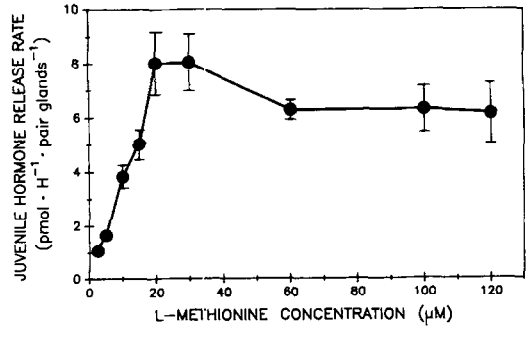

Fig. 3. Dose–response relationship for juvenile hormone release *in vitro* as a function of L-methionine concentration. Each point is the mean ± SEM of 3–4 glands from 5–6-day grouped virgin females.

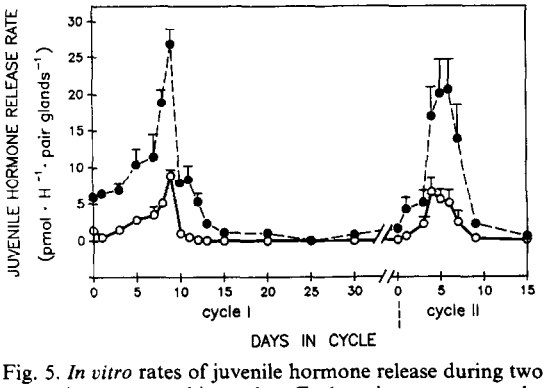

Fig. 5. *In vitro* rates of juvenile hormone release during two successive gonotrophic cycles. Each point represents the mean ± SEM of the basal (○) or farnesoic acid-stimulated (●) rates from 4–20 assays. Day 0 represents either the day of adult emergence, or the day of hatching of the nymphs (females enter second gonotrophic cycle). Isolated females were allowed to mate on day 8 of the first gonotrophic cycle, and ovulation occurred on day 12, on the average.

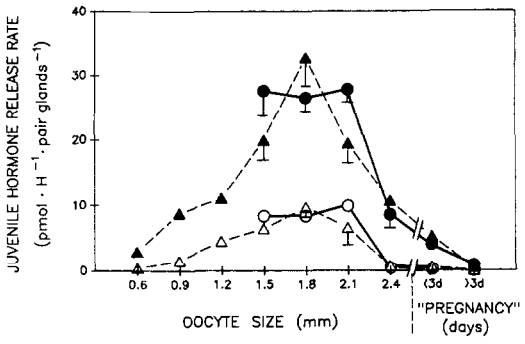

Fig. 6. Rate of juvenile hormone release as a function of oöcyte size (±0.1 mm) during the first two gonotrophic cycles. Each point is the mean ± SEM of 4–17 glands in the first cycle (circles) and in the second cycle (triangles), except for oöcytes of 0.8–1.0 mm which are represented by two glands. Basal rates of release are represented by open symbols and farnesoic acid-stimulated rates by filled symbols. After ovulation (dashed line), glands from females carrying oothecae for <3 days and >3 days were assayed. Isolated females were allowed to mate on day 8 of the first gonotrophic cycle, at which time none of the mated females had oöcytes <1.4 mm long.

between corpora allata activity and age, as will be reported in a separate paper (Gadot *et al.*, unpublished).

Similar patterns of the *in vitro* activity of corpora allata in relation to oöcyte maturation were demonstrated in *D. punctata* (Tobe and Stay, 1977) and in *Supella longipalpa* (Smith, 1988). In other cockroaches, the same relationship was implied by studies relating oöcyte maturation and corpora allata activity *in vitro* with age of the adult female (*N. cinerea*, Lanzrein *et al.*, 1978; *P. americana*, Weaver and Pratt, 1977). In orthopteran species (*L. migratoria*, *S. gregaria* and *Melanoplus sanguinipes*), the relation between the *in vitro* rates of juvenile hormone synthesis and oöcyte development is much less precise (review: Feyereisen, 1985), although a general trend does exist, at least in *L. migratoria* (Gadot and Applebaum, 1985, 1986). Tobe (1980) suggested that the orthopteran species lack a precise regulatory mechanism of juvenile hormone synthesis because they rely on only one mode of regulation (i.e. excitatory mechanism), while the cockroach species have a more complex mechanism, which involves several levels and different modes of regulation (i.e. inhibitory and excitatory mechanisms). Tobe (1980) associates the degree of regulation of the corpora allata with the reproductive mode of the female, predicting that insects which show periods of ovarian 'quiescence' will possess a more precise regulatory mechanism of the corpora allata to insure the inactivation of the glands during these periods.

Table 2. Corpus allatum volume in relation to the gonotrophic cycle

	Gland volume ($10^6\ \mu m^3$)*
Newly emerged	0.87 ± 0.06a (8)
Peak activity†	1.63 ± 0.09b (16)
At ovulation	0.83 ± 0.03a (8)
Mid-'pregnancy'‡	0.63 ± 0.11a (8)

*Means ± SEM followed by the same letter are not significantly different (Duncan's multiple range test, $P < 0.05$). Number of replicates indicated in parentheses.

†Glands from virgin grouped females in the first ovarian cycle with basal oöcytes 1.6–1.8 mm in length.

‡Glands from mated grouped females in the first ovarian cycle 11-days after ovulation.

Farnesoic acid stimulation

Farnesoic acid-stimulated rates of juvenile hormone synthesis in *B. germanica* indicates that the activity of the non-rate limiting enzymes, which dictates the maximal capacity of the glands, is highly correlated to the activity of the rate-limiting enzymes, which dictate the basal rate of juvenile hormone synthesis. The cyclic changes in the farnesoic acid-stimulated rates during the gonotrophic cycle (Fig. 5) and in relation to oöcyte maturation (Fig. 6) are accompanied by a similar pattern of changes in corpora allata volume (Table 2). Scanning electron microscopy shows that the basal lamina of the small inactive glands from females in 'mid-pregnancy' is different from that of the small inactive glands from newly emerged females (data not shown). Large invaginations of the basal lamina in the first case are probably indicative of cell degeneration inside the glands. We suggest that during pregnancy a process of cell death and regrowth takes place, and inhibition of activity at this stage is due to the overall low biochemical capacity of the degenerated cells. Further experiments to test this hypothesis are now in progress.

Similar patterns of changes in farnesoic acid-stimulated rates and in gland volume in relation to the gonotrophic cycle were also demonstrated in *D. punctata* (Feyereisen *et al.*, 1981; Feyereisen, 1985). High correlation between corpora allata morphometrics and ovarian development was demonstrated in other ovoviviparous cockroaches (review: Engelmann, 1970), but no data exist on the total biochemical capacity of the glands (i.e. their farnesoic acid-stimulated rates of juvenile hormone synthesis). In contrast, both gland volume and total biochemical capacity are not correlated with the ovarian cycle in the locusts (review: Feyereisen, 1985). In *L. migratoria*, farnesoic acid-stimulated rates remain relatively constant once the corpora allata have matured (fully active), although the basal rates change during subsequent gonotrophic cycles (Gadot and Applebaum, 1986). Relative stability of the total biochemical capacity of the corpora allata was also found in *P. americana* (Weaver and Pratt, 1981). Thus, regulation of the basal activity of the corpora allata in these insects appears to be dominated by "rapid responses" (Feyereisen, 1985) probably by changes in the activation and/or quantity of a limited number of enzymes, including the rate-limiting enzyme (Feyereisen *et al.*, 1981).

On the other hand, in *B. germanica*, as well as in ovoviviparous and viviparous cockroaches, the 'slow' developmental responses of the corpora allata are not restricted to their maturation period. These 'slow' changes are probably regulated by more than one factor, which may be coordinated (e.g. mitogenic factors, factors which activate RNA synthesis, etc.)

but may also operate independently, as suggested by the hypertrophy of low-activity corpora allata in ovariectomized females of *D. punctata* and *N. cinerea* (Tobe *et al.*, 1984; Lanzrein *et al.*, 1981). Independent activation of rate-limiting steps may be superimposed on these cyclic developmental changes, as indicated by the following examples: during peak activity of the corpora allata in *D. punctata*, basal rates increase relatively more than farnesoic acid-stimulated rates of juvenile hormone synthesis (Feyereisen *et al.*, 1981). Using brain extracts of adult female *D. punctata*, rapid and reversible inhibition of juvenile hormone synthesis was demonstrated *in vitro*; corpora allata with low spontaneous rates of synthesis showed higher sensitivity to the allatostatic factor (Rankin and Stay, 1987). Lastly, following unilateral allatectomy, compensation in juvenile hormone synthesis by the remaining corpus allatum is also indicative of independent activation of specific enzymes, since these elevated rates are not matched by increases in gland volume or cell number (Szibbo and Tobe, 1981).

It is tempting to speculate that in species which require rigorous control of juvenile hormone levels, especially during a protracted pregnancy involving arrest of oöcyte maturation, the preferred mechanism of 'restraining' the corpora allata will rely on structural/developmental processes, which affect the whole biochemical machinery, as well as the synthesis of specific key enzymes. Conversely, in species which develop basal oocytes in rapid succession, or even overlap oöcyte maturation cycles, as in the oviparous *P. americana* and the locusts, the faster and more flexible control through rate-limiting enzymes will be preferred, although not exclusively. Before it is generalized to other insects, the proposed functional relation between the type of control of the corpora allata and the mode of ovipositional behavior can best be evaluated through comparative studies with many insect species.

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REFERENCES

Baehr J. C., Caruelle J. P. and Poras M. (1986) The activity of denervated corpora allata in the diapausing strain of *Locusta migratoria*: *in vivo* and *in vitro* studies. *Int. J. Invert. Reprod. Dev.* **10,** 143–150.

Belles X., Casas J., Messeguer A. and Piulachs M. D. (1987) *In vitro* biosynthesis of JH III by the corpora allata of adult females of *Blattella germanica* (L). *Insect Biochem.* **17,** 1007–1010.

Camps F., Casas J., Sachez F. J. and Messeguer A. (1987) Identification of juvenile hormone III in the hemolymph of *Blattela germanica* adult females by gas chromatography–mass spectrometry. *Archs Insect Biochem. Physiol.* **6,** 181–189.

Couillaud F., Mauchamp B., Girardie A. and De Kort S. (1988) Enhancement by farnesol and farnesoic acid of juvenile-hormone biosynthesis in stimulated low-activity locust corpora allata. *Archs Insect Biochem. Physiol.* **7,** 133–143.

Dale J. F. and Tobe S. S. (1988) Differences in the stimulation by calcium ionophore of juvenile hormone III release from corpora allata of solitarious and gregarious *Locusta migratoria*. *Experientia* **44,** 240–242.

Engelmann F. (1970) *The Physiology of Insect Reproduction*. 1st Edn, p. 152. Pergamon Press, Oxford.

Feyereisen R. (1985) Regulation of juvenile hormone titer: synthesis. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology* (Edited by Kerkut G. A. and Gilbert L. I.), Vol. 7, 1st Edn, pp. 391–429. Pergamon Press, Oxford.

Feyereisen R., Friedel T. and Tobe S. S. (1981) Farnesoic acid stimulation of C_{16} juvenile hormone biosynthesis by corpora allata of adult female *Diploptera punctata*. *Insect Biochem.* **11,** 401–409.

Feyereisen R. and Tobe S. S. (1981) A rapid partition assay for routine analysis of juvenile hormone release by insect corpora allata. *Analyt. Biochem.* **111,** 372–375.

Gadot M. and Applebaum S. W. (1985) Rapid in vitro activation of corpora allata by extracted locust brain allatotropic factor. *Archs Insect Biochem. Physiol.* **2,** 117–129.

Gadot M. and Applebaum S. W. (1986) Farnesoic acid and allatotropin stimulation in relation to locust allatal maturation. *Molec. cell. Endocr.* **48,** 69–76.

Gadot M., Factor O. and Applebaum S. W. (1987) Maturation of locust corpora allata during the reproductive cycle: effect of reserpine on allatotropic activity, juvenile hormone III biosynthesis, and oöcyte development. *Archs Insect Biochem. Physiol.* **4,** 17–27.

Kikukawa S., Tobe S. S., Solowiej S., Rankin S. M. and Stay B. (1987) Calcium as a regulator of juvenile hormone biosynthesis and release in the cockroach *Diploptera punctata*. *Insect Biochem.* **17,** 179–187.

Lanzrein B., Gentinetta V., Fehr R. and Luscher M. (1978) Correlation between haemolymph juvenile hormone titer, corpus allatum volume, and corpus allatum *in vivo* and *in vitro* activity during oöcyte maturation in a cockroach (*Nauphoeta cinerea*). *Gen. comp. Endocr.* **36,** 339–345.

Lanzrein B., Wilhelm R. and Buschor J. (1981) On the regulation of the corpora allata activity in adult females of the ovoviviparous cockroach *Nauphoeta cinerea*. In *Juvenile Hormone Biochemistry, Action, Agonism and Antagonism* (Edited by Pratt G. E. and Brooks G. T.), 1st Edn, pp. 147–160. Elsevier, Amsterdam.

Pratt G. E. and Tobe S. S. (1974) Juvenile hormone radiobiosynthesised by corpora allata of adult female locust *in vitro*. *Life Sci.* **14,** 575–586.

Pratt G. E., Tobe S. S. and Weaver R. J. (1975) Relative oxygenase activities in juvenile hormone biosynthesis of corpora allata of an African locust (*Schistocerca gregaria*) and American cockroach (*Periplaneta americana*). *Experientia* **31,** 120–122.

Rankin S. M. and Stay B. (1987) Distribution of allatostatin in the adult cockroach, *Diploptera punctata* and effects on corpora allata *in vitro*. *J. Insect Physiol.* **33,** 551–558.

Roth L. M. (1970) Evolution and taxonomic significance of reproduction in Blattaria. *A. Rev. Ent.* **15,** 71–96.

Roth L. M. and Stay B. (1962) Oocyte development in *Blattella germanica* and *Blattella vaga* (Blattaria). *A. ent. Soc. Am.* **55,** 633–642.

Smith A. F. (1988) Endogenous and exogenous factors regulating pheromone production and release in the adult female brown-banded cockroach. Ph.D. thesis, Rutgers, the State University of New Jersey.

Szibbo C. M. and Tobe S. S. (1981) The mechanism of compensation in juvenile hormone synthesis following unilateral allatectomy in *Diploptera punctata*. *J. Insect Physiol.* **27,** 609–613.

Tobe S. S. (1980) Regulation of the corpora allata in adult female insects. In *Insect Biology In The Future* (Edited by Locke M. L. and Smith D. S.), 1st Edn, pp. 345–367. Academic Press, New York.

Tobe S. S., Clarke N., Stay B. and Ruegg R. P. (1984) Changes in cell number and activity of the corpora allata of the cockroach *Diploptera punctata*: a role for mating and the ovary. *Can J. Zool.* **62,** 2178–2182.

Tobe S. S. and Pratt G. E. (1974) The influence of substrate concentrations on the rate of insect juvenile hormone biosynthesis by corpora allata of the desert locust *in vitro*. *Biochem. J.* **144,** 107–113.

Tobe S. S. and Pratt G. E. (1976) Farnesenic acid stimulation of juvenile hormone biosynthesis as an experimental probe in corpus allatum physiology. In *The Juvenile Hormones* (Edited by Gilbert L. I.), 1st Edn, pp. 147–163. Plenum Press, New York.

Tobe S. S. and Stay B. (1977) Corpus allatum activity *in vitro* during the reproductive cycle of the viviparous cockroach, *Diploptera punctata* (Eschscholtz). *Gen. comp. Endocr.* **31,** 138–147.

Tobe S. S. and Stay B. (1985) Structure and regulation of the corpus allatum. *Adv. Insect Physiol.* **18,** 305–432.

Weaver R. J. and Pratt G. E. (1977) The effect of enforced virginity and subsequent mating on the activity of the corpus allatum of *Periplaneta americana* measured *in vitro*, as related to changes in the rate of ovarian maturation. *Physiol. Ent.* **2,** 59–76.

Weaver R. J. and Pratt G. E. (1981) Effects of starvation and feeding upon corpus allatum activity and oocyte growth in adult female *Periplaneta americana*. *J. Insect Physiol.* **27,** 75–83.

Weaver R. J., Pratt G. E., Hamnett A. F. and Jennings R. C. (1980) The influence of incubation conditions on the rates of juvenile hormone biosynthesis by corpora allata isolated from adult females of the beetle *Tenebrio molitor*. *Insect Biochem.* **10,** 245–254.