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Title: Activity of the Corpora Allata of Adult Female Leucophaea maderae

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In the cockroach Leucophaea maderae, a centre in the brain controls the female's acceptance of courting males. This centre is affected by starvation. A brief exposure to food can induce mating but is inadequate for oocyte development. In fed L. maderae there appears to be a synergistic action of nutrition and mating in controlling the rate of oocyte development. Mating (mechanical) and feeding (chemical) stimuli are both required for activating the corpora allata (CA) to their fullest extent so that the oocytes mature at their maximum rate. A rate of 0.4 mm/day of oocyte growth was recorded.

Activity of CA in vitro was quantified using a radiochemical assay. In starved virgin females, no detectable activity was recorded by our assay conditions. The CA were virtually inactive and yolk was not deposited in the oocytes; mating had no effect on CA and oocyte

development in starved females. Experiments with fed females showed that feeding increased CA activity to about 2 pmol JH-III/pair/hr of glands until the roaches were mated (11-12 days after eclosion). After mating, the activity of the CA increased over a period of 9 days to a peak of 52.7± 1.5 pmol JH-III/hr/pair of glands.

Farnesol stimulation experiments showed that the inhibition of juvenile hormone synthesis in virgin females was exerted early in the biosynthetic pathway.

Activity of the Corpora Allata of Adult Female *Leucophaea maderae*

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Activity of the Corpora Allata of Adult Female *Leucophaea maderae*.

INTRODUCTION

The corpus allatum (CA) hormone, juvenile hormone (JH), and its effect on metamorphosis was first demonstrated by Wigglesworth (1936) in his classical parabiosis experiments with the south American blood-sucking bug *Rhodnius prolixus*. In the same paper it was shown that the presence of active CA is also necessary in the activity of both the ovarian follicle cells in adult females and the accessory glands in adult males. Since then a large number of papers have appeared demonstrating similar effects of JH in other groups of insects, together with a number of other physiological effects following the implantation or extirpation of the CA. The CA are said to influence polymorphism in various groups of insects, to affect regeneration and tumorous growth, as well as a number of other physiological functions of the body (proteosynthesis, respiration, molting, secretory activity of the silk gland, pheromones production, and in some cases instinctive behavior, etc.) (Scharrer, 1958, 1959; Staal, 1975; Sehnal, 1976; Edwards and Menn, 1981). For example, insect eggs are sensitive to JH, especially in the early stages of embryogenesis, and the application of JH prevents the development of viable larvae. Also, in some species the treatment of adult females with JH

results in the deposition of eggs that fail to hatch. The growth of immature larvae requires the presence of JH, but in order for mature larvae to undergo metamorphosis into adults the production of the hormone must decline. Contact with JH at this stage can cause the death of the insect due to the derangement of development.

The interest of insect physiologists in JH has been further stimulated by the discovery of similar morphogenetical effects produced by a large number of substances, such as farnesol and its derivatives (Wigglesworth, 1963), some saturated and insaturated fatty acids (Slama, 1961, 1962), the so-called "paper factor" of Slama and Williams (1965, 1966), and a number of other substances, some of them being active in surprisingly low amounts.

The search for safer and more selective insecticides has prompted extensive research during the past 15 years in the area of compounds showing insect JH activity (juvenoids). The alkyl and alkynyl (2E,4E)-3,7,11-Trimethyl-2,4-dodecadienoates (Henrick, 1977; Henrick and Siddall, 1975; Henrick et al., 1973) are the most commercially important group of known juvenoids. Structure - Biological activity relationships for this class of compounds have been studied in considerable detail (Henrick et al., 1973. 1975a, 1976 a,b, 1978b), and the juvenoids registered (1980) with the U.S.

Environmental Protection Agency (EPA) for use in insect control, methoprene and kinoprene, belong to this group.

In addition to these sources of interest the activity of the CA under various physiological conditions have been investigated using classical experimental methods of extirpation, implantation, and the status of an oocyte growth have been investigated by authors using several species; the viviparous cockroach Diploptera punctata (Roth and Stay; 1961), Nauphoeta cinerea and Pycnoscelus surinamensis (Roth; 1962, 1964), Leucophaea maderae (Roth; 1964, Engelmann and Rau; 1965) and Byrsotria fumigata (Barth; 1962). The aim of this research was to quantitatively investigate the activity of the CA of adult female L. maderae and to show the effects of feeding and mating. For this purpose, the activity of the CA from starved, fed, and fed and mated females was assayed in vitro using either the partition assay or thin layer chromatography (TLC) assay. Egg length was determined in order to correlate this parameter with the activity observed.

Routine use of the radiochemical assay is dependent on the establishment of several parameters (Pratt and Weaver 1978; Tobe and Feyereisen 1983) such as:

- The JH(s) produced in vitro must be identified.
- Optimal incubation conditions must be found and the CA must not be damaged during dissection or

incubation.

- Synthesis and/or release of JH during the incubation period must be linear for glands of high and low activity.

- A fixed molar incorporation ratio of label from methionine should be demontrated.

I have investigated the first three requirements. I was unable to demontrate the last point because the technical materials needed were not available in our laboratory at this time.

The CA from starved females synthesize little if any JH. Experiments were performed to determine whether the lack of synthesis was the result of inhibition or of a deficiency of enzymes brought on by nutritional deprivation.

REVIEW OF LITERATURE

Definition of the Corpora Allata

The CA are paired endocrine glands situated behind the brain. The CA are the site of synthesis and release of juvenile hormones which control a variety of developmental and reproductive processes in insects.

Structure and Biosynthesis of Juvenile Hormones

Purification of extracts from lepidopterous insects led to the identification of JH-I as methyl 10,11-epoxy-7-ethyl-3,11-dimethyl-trans-2,6-tridecadienoate (fig. 1). JH-II (a 17-carbon homologue) was identified shortly thereafter. JH-III was identified directly from material released by CA cultured in vitro. Numerous investigators have now demonstrated that the isolated CA of a variety of insects will produce JH-I, JH-II, and/or JH-III (King,1983).

JH-III is the only hormone found in most species of insects examined. To date, the higher homologs of JH-III have been found only in lepidoptera.

The carbon skeleton of JH-III is synthesized from acetate precursors that are converted to mevalonate and various intermediates. JH-I and JH-II utilize propionate

as source of the "extra" carbon that they possess (fig. 2).

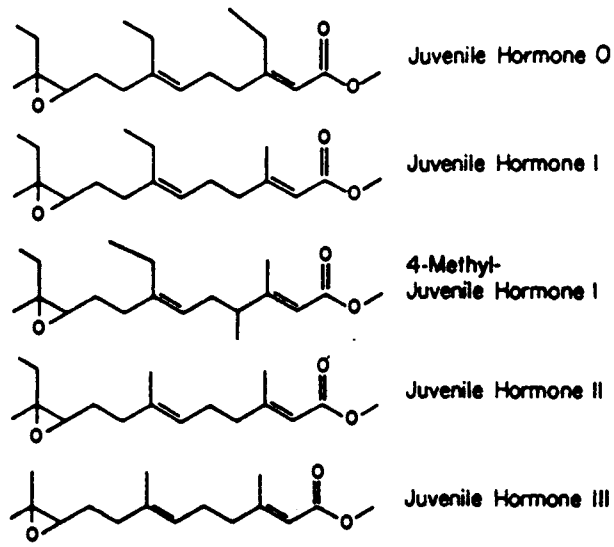

Figure 1. Structure of juvenile hormones

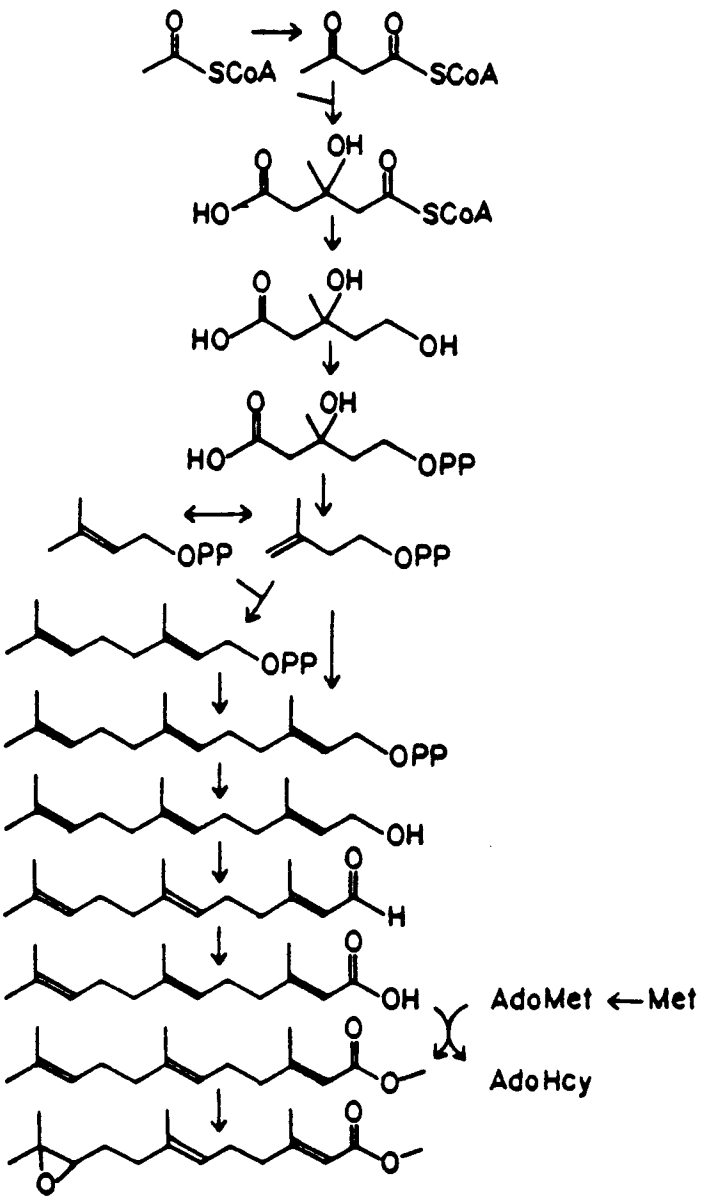

Figure 2. Biosynthesis of juvenile hormones

Histology and Morphometry of the Corpora Allata

Early work of Scharrer (1948; 1961) on L. maderae demontrated that the secretion of the CA produces juvenile characteristics in the larva and is responsible for oocyte development and the secretion of secondary sexual organs in the adult.

The CA of L. maderae undergo marked histological changes in conjunction with various phases of the reproductive cycle (Engelmann, 1957; Scharrer and Von Harnack, 1958). Activation of the CA is characterized by an increase in organ volume, in number of nuclei, and in absolute and relative cytoplasmic content.

Luscher and Engelmann (1959;1960) investigated the changes in the CA which are associated with its declining output of JH, thus allowing the final molt to be a metamorphic one. They used estimates of glandular volume and the density of nuclei to determine whether the fall in activity was simply a case of negative allometry or involved active inhibition. Nuclear and volume measurement were combined to give a value known as the "activity volume" (difference between the volume of the gland and the minimal volume of a gland with the same number of nuclei). These investigations found that the "activity volume" decreased during early larval life, but increased rapidly after the third instar, and dropped to

zero during the last instar. The authors also found that if nerves to the CA are sectioned soon after the last larval molt, the "activity volume" of the CA isolated fail to drop although mitotic activity is reduced. The CA retain their activity and this results in supernumerary moults. It is evident therefore that the CA are being inactivated in normal development by nervous stimuli from the protocerebrum. The slightly negative allometric growth of the CA does not account for the functional insufficiency in the last instar.

Assay for Juvenile Hormone Biosynthesis: The Radiochemical Assay.

Prior to 1974, there were no reliable methods for accurately determining the activity of the CA; transplantation bioassay, hormone replacement therapy, and CA volume were used as indicators of CA activity.

In 1973 and 1974, work at the university of Sussex led to the development of a rapid radiochemical assay for JH synthesis in vitro (Pratt and Tobe, 1974; Tobe and Pratt, 1974a). This assay utilized the fact that in the presence of radiolabelled methionine, the molar incorporation ratio for methionine and farnesoic acid into JH was approximately 1:1. In other words, equimolar quantities of the methyl moiety of methionine and farnesoic acid were incorporated into JH-III. Methionine

is the major source of the methyl group in the esterification of farnesoic acid (Tobe and Pratt, 1974a; 1976). Accordingly, by following the incorporation of the radiolabelled methyl moiety of methionine into JH-III, the precise rate of synthesis of JH-III can be measured. Under normal circumstances, using (methyl-^{14}C) methionine, the sensitivity of this assay is approximately 10^{-13} mol (0.1 pmol). This can be extended by almost two orders of magnitude 10^{-15} mol with the use of high specific radioactivity (methyl-^{3}H) methionine (Hamnett and Pratt, 1978). Using this method, identification of the higher homologues in the presence of JH-III can be performed, to a ratio of $1:10^{-5}$. At these levels of sensitivity, measurement of JH synthesis by individual pairs of CA over short-term incubation periods can be performed routinely.

Feeding and the Sexual Cycle

Females of *L. maderae* eat large amount of food shortly after eclosion and after parturition. This increased food consumption is apparently necessary for the activation of the CA, because in starved animals no eggs mature (Engelmann, 1957; Von Harnack, 1958). Mating is required as an additional stimulus for egg maturation at a normal rate.

Two opposing factors influence feeding in *L. maderae*. Egg maturation stimulates feeding when not enough reserves are

available for the completion of yolk deposition; feeding is reduced by the brood sac. Removal of the egg case at parturition or during pregnancy stops the restriction of feeding (Engelmann and Rau, 1964).

It has been reported that a factor released by the egg case or the brood sac inhibits the CA during pregnancy. This factor seems to affect the ganglia of the ventral nerve cord and the brain as well; the brain in turn inhibits the CA (Engelmann, 1964).

Mating Stimuli

In L. maderae, mating normally takes place between 11^{th} and 12^{th} day after adult emergence (Engelmann, 1957). Stimuli received in the genital apparatus of the female during mating are transmitted to the brain via the last abdominal ganglion and ventral nerve cord, and the brain then ceases to inhibit the CA. The CA then produce and release a gonadotropic hormone (JH-III), which induces vitellogenesis and activation of the accessory sex glands. After ovulation of the mature eggs and their deposition in the brood sac, the CA are again inhibited by the brain, as described above.

Vitellogenesis

The growth of the terminal oocytes to their final size before ovulation, is achieved by the massive incorporation of protein and lipid yolk and to a lesser extent of carbohydrates.

a) Vitellogenin induction. It has been documented for several species of insects belonging to different orders that the predominant yolk protein precursor, or vitellogenin, is synthesized by the fat body of the female. The fat body produces this female specific protein and exports it into the hemolymph, from which it is taken by the growing oocytes via pinocytosis. It is generally acknowledged that JH regulates vitellogenin mRNA synthesis and several additional aspects associated with the development of a fully grown egg (Engelmann; 1979, 1983). Among these are the accelerated proliferation of the endoplasmic reticulum in fat bodies, vitellogenin export to the hemolymph, juvenile hormone esterase induction, DNA replication, and vitelogenin uptake by the growing oocytes.

b) Characteristics of the yolk protein. The technique of sucrose density gradient centrifugation identifies the vitellogenin of the hemolymph as a 14S

unit. Several of these units appear to aggregate in the oocytes to 28S units (Dejmal and Brookes, 1972). The molecular weight of the vitellogenin was reported to be equal to $5.59x10^5$ daltons (Dejmal and Brookes, 1972) using sedimentation analysis and equal to $5.35x10^5$ daltons (Engelmann et al., 1976) using polyacrylamide gels. The egg protein of L. maderae is virtually unsoluble in water but soluble in strong saline solutions (Dejmal and Brookes, 1968, 1972; Brookes, 1969).

Reinvestigation of the vitellogenin complex by Della-Cioppa and Engelmann (1987), shows that the vitellogenin molecule consists of at least five polypeptides which had apparent molecular masses of 155, 112, 95, 92 and 54 kD. Determination of molecular weight were performed by these authors using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) autoradiography.

Comparison of some Gonotrophic Cycles

Oviparous insects lay their eggs after fertilization and before substantial embryonic development takes place. In some groups of insects, such as viviparous and ovoviviparous insects, eggs are retained by female after they are fertilized. In a few species the embryos are partially nourished by secretions of the female.

In the oviparous insect, Periplaneta americana, the CA

undergo cyclic changes in JH synthesis, with the number of peaks corresponding to the number of batches of oocytes formed (Weaver et al.,1975; Weaver and Pratt, 1977). In this species both basal and penultimate oocytes are vitellogenic at the same time. Therefore, a functional correspondence between CA activity and maturation of oocytes is difficult to show even though discrete cycles of JH can be observed (Weaver and Pratt, 1977).

In the ovoviviparous cockroach Nauphoeta cinerea, basal oocytes grow synchronously. Developing embryos are retained in the brood sac until gestation is completed and during this period, no additional oocytes are matured. The CA of Nauphoeta cinerea show a cycle of JH synthesis associated with the gonotrophic cycle, with maximal rate of JH synthesis occuring during the period of rapid ovarian growth (Lanzrein et al., 1978). The correspondence between CA activity and oocyte maturation is more obvious in this species because only one batch of oocytes is matured for each pregnancy cycle.

In the viviparous cockroach Diploptera punctata, oocytes mature synchronously. The developing embryos are incubated in the brood sac and each wave of oocytes become vitellogenic just before parturition (Roth and Stay, 1961). The CA show a cycle of activity precisely correlated with oocyte growth and maturation (Feyereisen et al.,1981).

In L. maderae, eggs mature synchronously and the CA activity is correlated with oocyte growth as in D. punctata.

All four species have synchronous oocyte growth. The principal difference between their reproductive modes is that P. americana produces batches of oocytes continously whereas in the other three, each wave of oocyte maturation is followed by a prolonged period of ovarian "quiescence".

In Tenebrio molitor, oocytes develop asynchronously and continously. The regulation of the CA is less precise (Weaver et al., 1980). The CA probably remain active for the duration of the reproductive cycle.

Origin, Distribution, Biology, and Ecology.

The Madeira cockroach, Leucophaea maderae, is probably from Africa, but was first reported from Madeira by Heevin in 1864.

Leucophaea maderae is a large cockroach. The adult male is between 40 and 44 mm length; the adult female 42-50 mm. The insect is whitish-yellow or straw-coloured with greyish-brown speckles on the wings and thorax. The underparts and legs are black or blackish-brown. The wings are fully developed.

Leucophaea maderae has been observed in fruit stores and houses throughout the West Indies, and also in Central and South America, and Tropical Africa from which it

probably originated. No serious economic and medical problems have been assessed with this species.

MATERIALS AND METHODS

Rearing of the Insects

The animals were taken from a laboratory colony raised in a walk-in incubator at 30°C and 12:12 light-dark cycle. Newly emerged females were collected from the colonies and housed in hard-paper boxes, fed dog chow and water. The first period of egg maturation was synchronized by pairing 11-12 day old females with an equal number of males. Mating occured within a few hours after pairing, and mated females were segregated after 24 hours. Successful mating indicated by the presence of a spermatophore in the bursa copulatrix. The mated females used in the experiments are identified by the number of days after mating, i.e. 8-day mated female, a female mated for 8 days. Day 0 is the day of pairing.

Dissection and Incubation of the CA

The CA were dissected in medium 199 with Hank's salts, pH 7.2. The heads of the animals were cut with a piece of razor blade and the complex brain-CA pulled out. The CA were cleaned as much as possible, then separated from the corpora cardiaca (CC). To avoid wounding the CA, a small piece of CC was left with it. The CA were then

transfered into vials containing Gibco TC 199 medium with 2% Ficoll (50 ul/gland). (methyl-^{3}H) Methionine was added to the buffer at concentration of 50 uM. The CA were incubated at 30^{o}C.

Juvenile Hormone Extraction

After incubation, the reaction was stopped by removal of the CA on a steel loop. The CA were washed and their hormonal content extracted separately. One hundred ul of methanol (HPLC grade), 1000 ul chloroform (spectra) and 20 ul unlabelled carrier JH and methyl farnesoate (MF) were added to each tube. The tubes were vortexed and spun for 5 minutes. The lower chloroform layer was carefully removed by using drawn out Pasteur pipettes and put onto a "pencil" drying tube of sodium sulfate on ether-washed cotton wool. The extraction was repeated with 1 ml of chloroform (no methanol, no marker).

High Pressure Liquid Chromatography (HPLC)

Chloroform/methanol extracts of CA incubated media containing 70-100,000 d.p.m. of ^{3}H-labelled products were analyzed by reversed phase chromatography on a 100 x 2.1 mm octadecyl column (Pierce) at 0.3 mls/min, using a series 400 Liquid Chromatograph and LC-90 Detector (Perkin Elmer) fitted with a 100 ul loop injector (Rheodyne 7125); the gradient elapsed volume of the system was 1.95 ml. Elution was achieved by two linear gradients of increasing acetonitrile in water (both containing 0.2% 1,2-dihydroxypropane):(1) 10-60% over 5 min, (2) 60% isochratic for 10 min, (3) 60-100% over 5 min. Authentic compounds were monitored at 250 nm, and fractions collected automatically every 12 seconds into 4.5 ml minivials preloaded with scintillation cocktail.

Thin Layer Chromatography (TLC)

The extracted JH-III plus chloroform was evaporated to 50 ul with nitrogen. Two hundred ul of dry ether were added to each tube, vortexed, and spotted on plastic backed silica TLC plates. The tubes were rinsed with 200 ul dry ether and added to TLC plates. The plates were then chromatographed with 300ml of solvent (75:25 hexane/ethyl-acetate) for 45 minutes. The JH and MF bands were visualized under UV light, cut with dissecting scissors, and put in 10 ml of liquid scintillation cocktail (F-963, New England Nuclear) and counted for 10 min.

Partition Assay

After incubation of the CA, a partition assay, described by Feyereisen and Tobe (1981), was used to quantitatively separate the biosynthesized JH from the precursor L-methionine. The JH in the incubation medium was extracted into an isooctane phase and determined by liquid scintillation counting .

RESULTS

I: THE RADIOCHEMICAL ASSAY

Relationship between incubation time and JH synthesis

The CA of females 6 days after mating were assayed over a 4 hour period for JH synthesis. The incubation medium was changed and measured each hour using the partition assay. The graphic representation (figure 3) shows a linear synthesis of JH over the incubation time. The lag period of 30 to 35 min. may represent the time needed for the radiolabelled methionine to enter the glands and for the synthesis of the hormone to start.

Methionine dose-response

The CA of females 6 days after mating were investigated. After 30 min. preincubation of the glands, the medium was changed each hour and the concentrations of methionine measured from 25uM to 200uM. The JH released was measured using the partition assay. There was no statistical difference between high and low methionine concentrations (table 1). For subsequent assays, a concentration of 50uM was chosen.

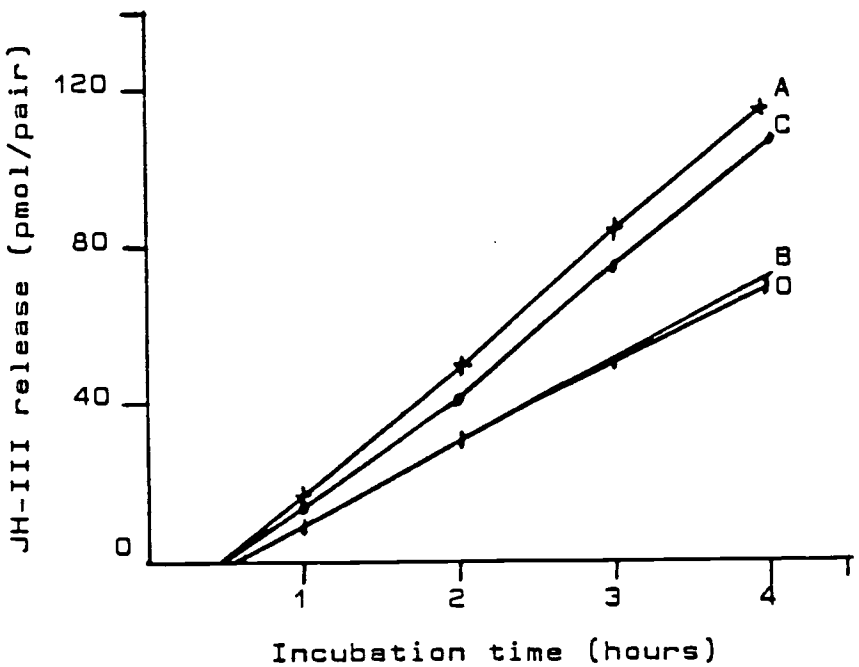

Figure 3. Relationship of JH-III release with incubation time. Four individual pairs of glands were followed over a period of 4 hours.

Table 1. Methionine dose-response. Each point is mean±SE of 7 pairs of glands.

methionine (uM)	day 6 JH-III release (pmol/hr)	
25	11.4±2.8	a
50	18.5±4.6	ab
100	21.9±5.9	ab
200	25.4±4.7	b

JH identification

Eight-day mated insects were used. Their CA were incubated for 4 hours, the hormone(s) extracted , and analyzed by HPLC (fig. 4). The JH synthesized and released was identified as JH-III. Neither JH-I nor JH-II was found in the extract. The identification of the hormone was performed by Dr. G. E. Pratt.

Thin layer chromatography

An example of TLC is shown on figure 5. Six day mated female CA were incubated for 3 hours, the hormone extracted and the TLC performed as described previously in the materials and methods section.

II: RELEASE OF JUVENILE HORMONE

JH release/JH synthesis

There was a simple linear ($y = 0.7565x + 0.4097$) relationship between the amount of JH synthesized and the amount released (figure 6). Statistical test showed that the r value ($r= 0.9625$) was highly significant. High levels of JH synthesized were associated with high rate of JH released. Therefore the hormone produced was released in the incubation medium and did not accumulate within the glands.

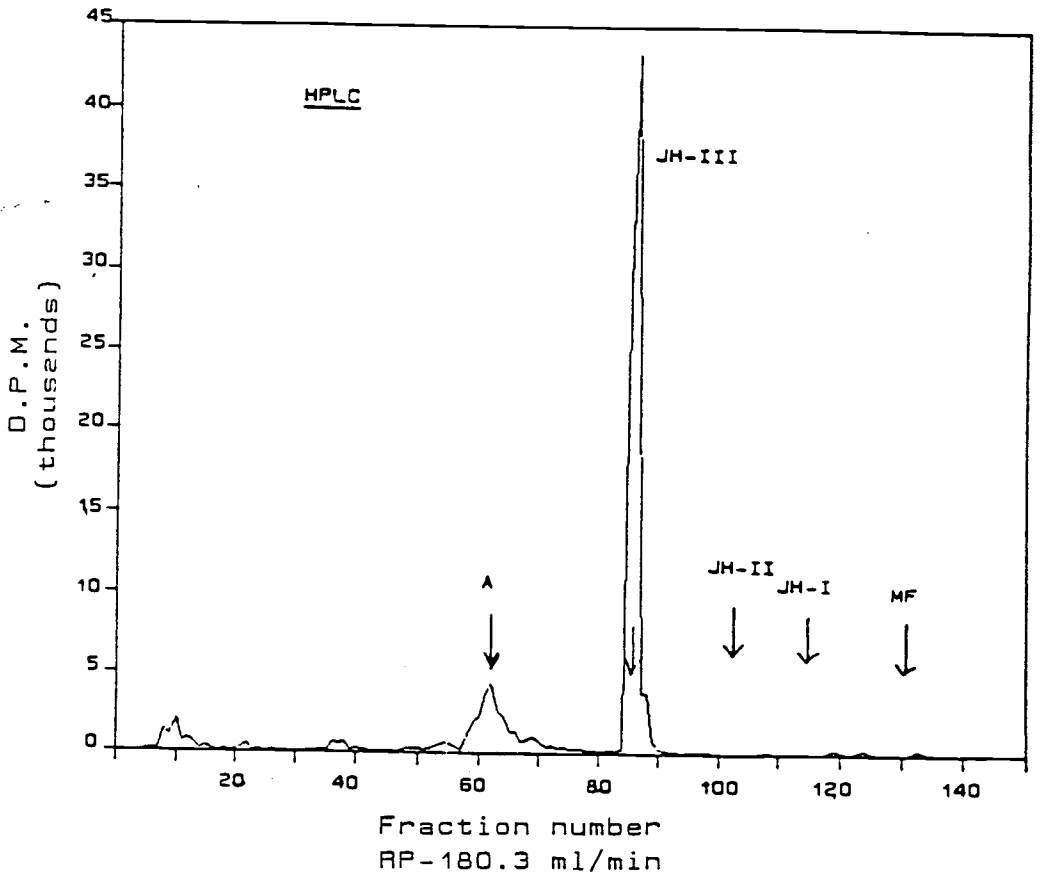

Figure 4. Identification of juvenile hormones. Only JH-III was found in the extract. A = unidentified materials.

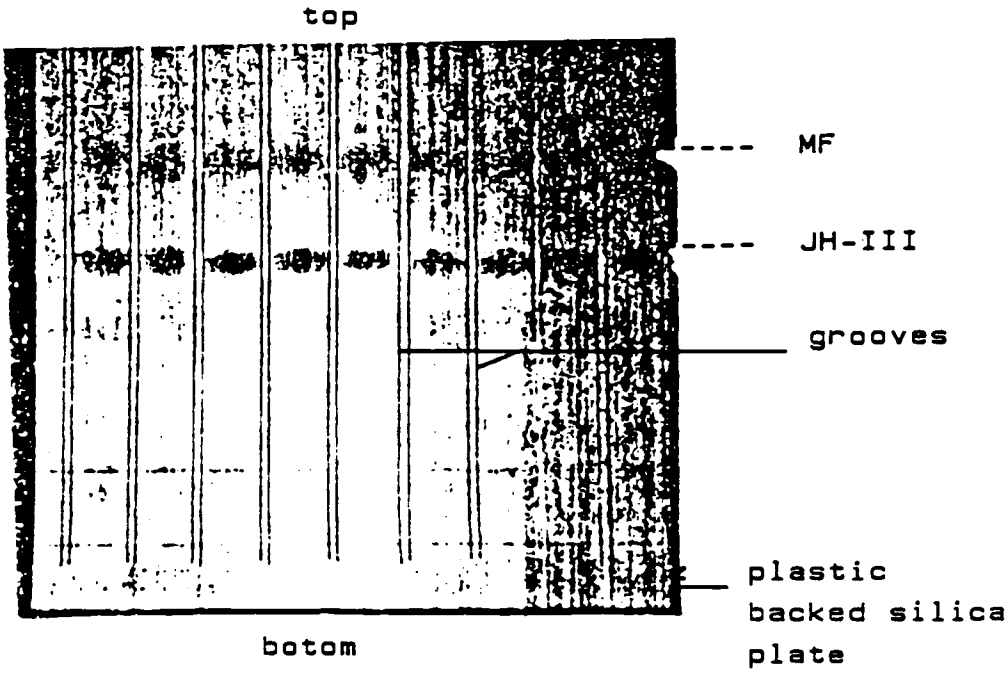

Figure 5. Thin layer chromatography (TLC) plate. The two spots in the same row are the marker (carrier) MF and JH identified under UV light.

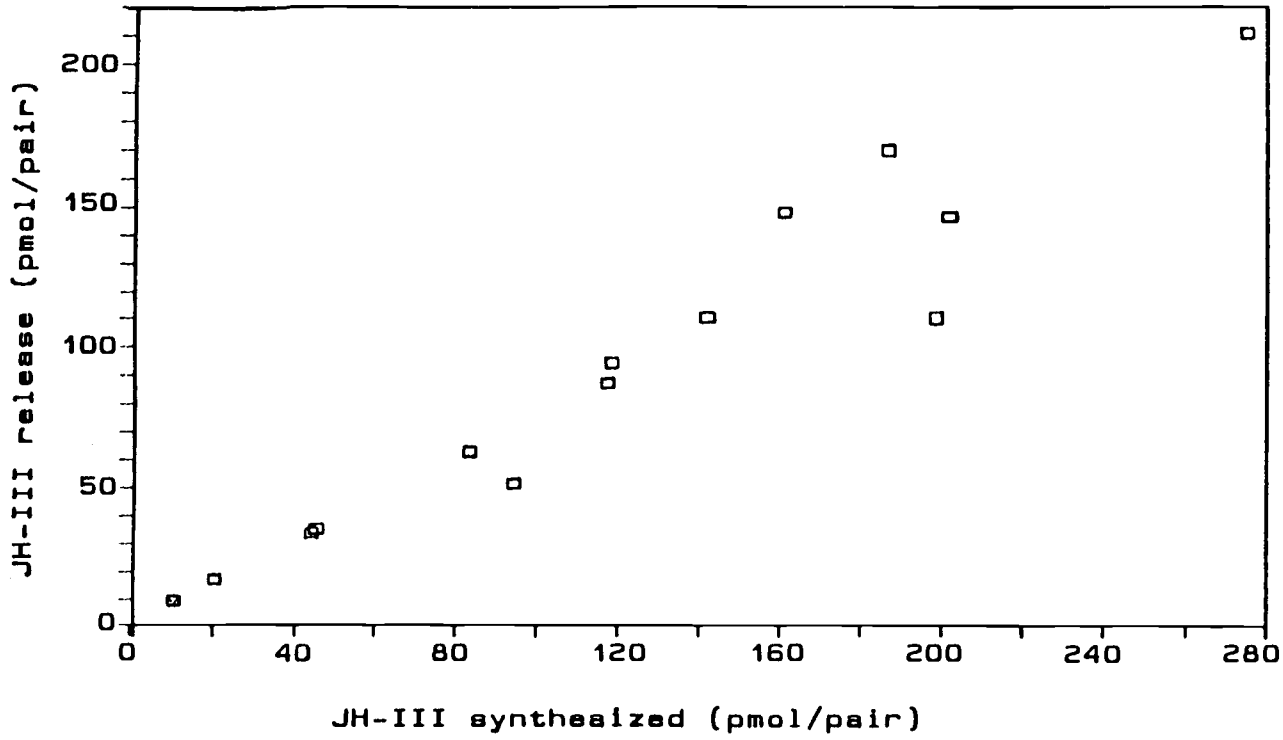

Figure 6. Relationship between JH-III synthesis and release. Each point represents a single assay for one pair of glands.

Y = 0.7565X + 0.4097; r = 0.9625, where Y is JH-III released, X JH-III synthesized, and r the coefficient of correlation between Y and X.

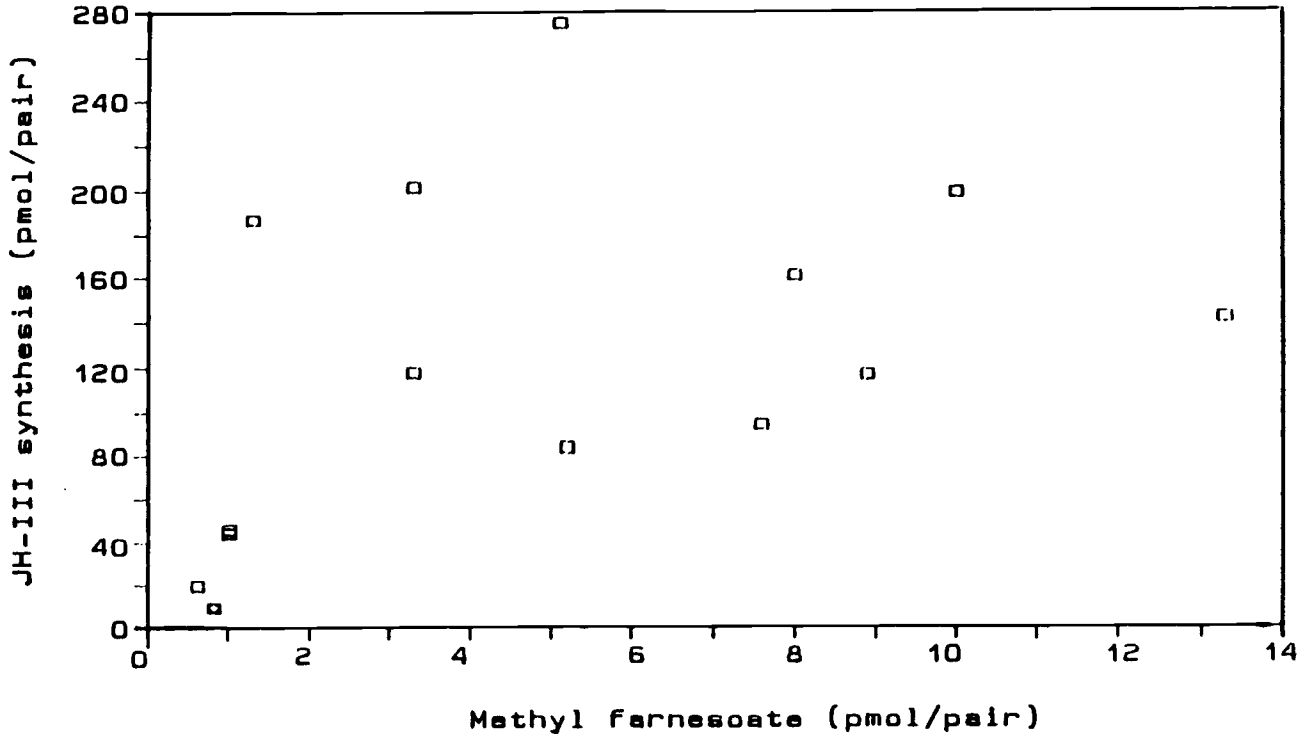

Figure 7. Relationship of JH-III synthesis with intraglandular methyl farnesoate. Each point is one assay for one pair of glands.

JH synthesis/methyl farnesoate (MF)

The relationship between JH synthesis and intraglandular MF was not quite an enzymatic, Michaelis Menten, type saturation (figure 7). To obtain a Michaelis Menten relationship, the direct enzymatic epoxidation of methyl farnesoate to JH-III should have been studied, as was done for Locusta migratoria CA by Feyereisen, Pratt and Hamnett (1981).

III : ACTIVITY OF THE CORPORA ALLATA

Relationship age/oocyte length

Measurement of oocyte length showed that the oocyte was about 1.0-1.3 mm before mating. There was a rapid oocyte growth after mating with an average increase in the length of .4 mm/24 hours (fig 9) until day 12. After day 12, the growth dropped and stopped (day 13,14). In L. maderae 20 eggs are matured by each ovary and 40 will go into the egg case.

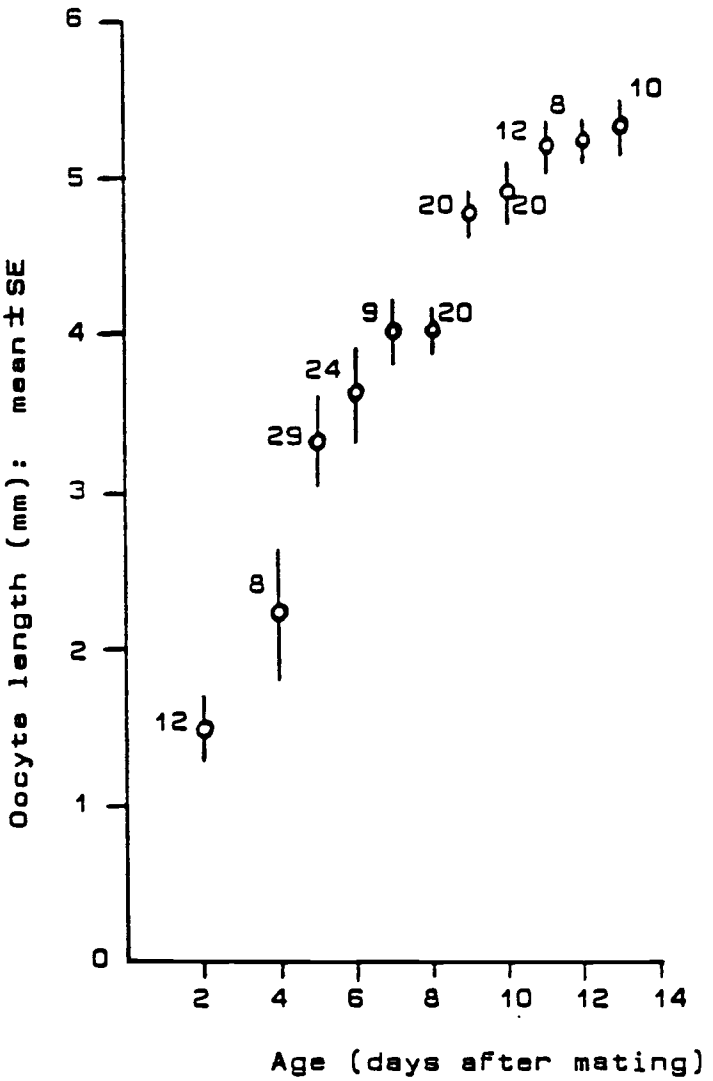

Figure 8. Oocyte growth rate. Each point is the mean±SE of n individual insects. In each insect, 3 ovariols were measured.

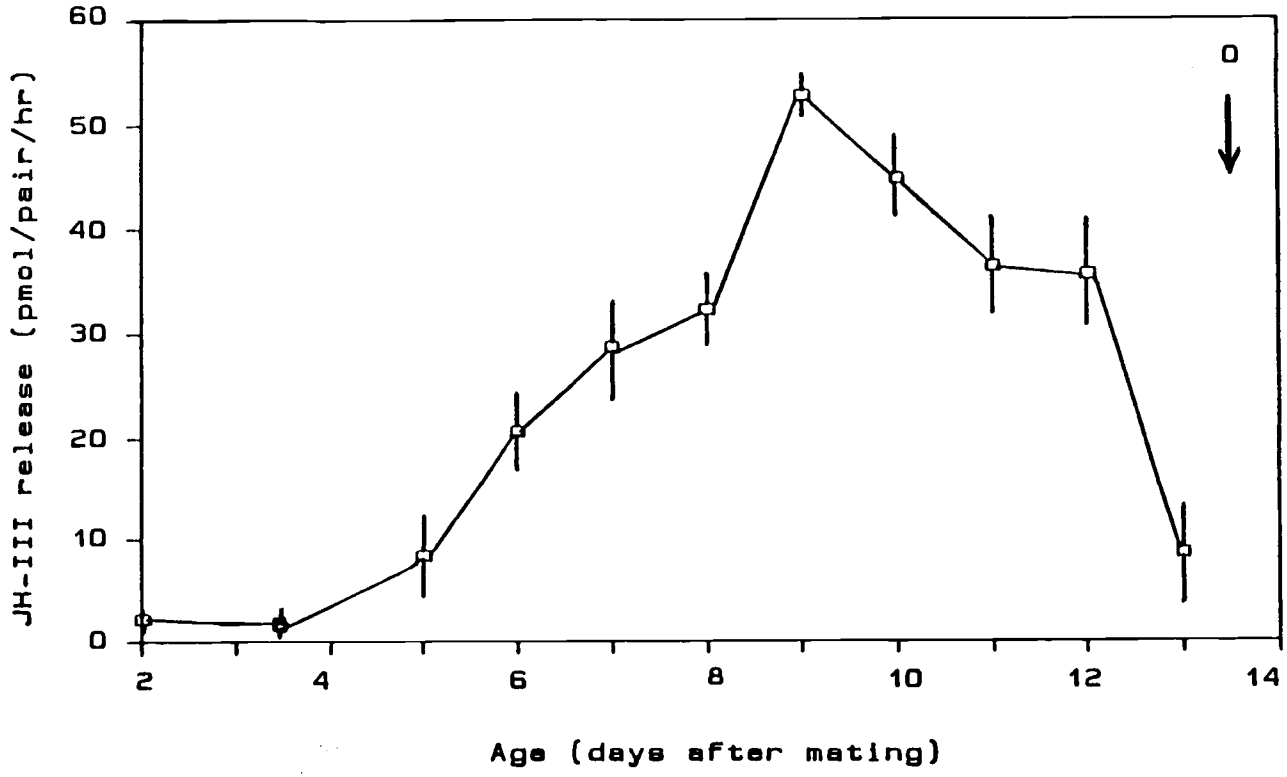

Figure 9. Relationship of CA activity with insect age. Each point is mean ± SE of 10 to 20 pairs of glands. O = oviposition

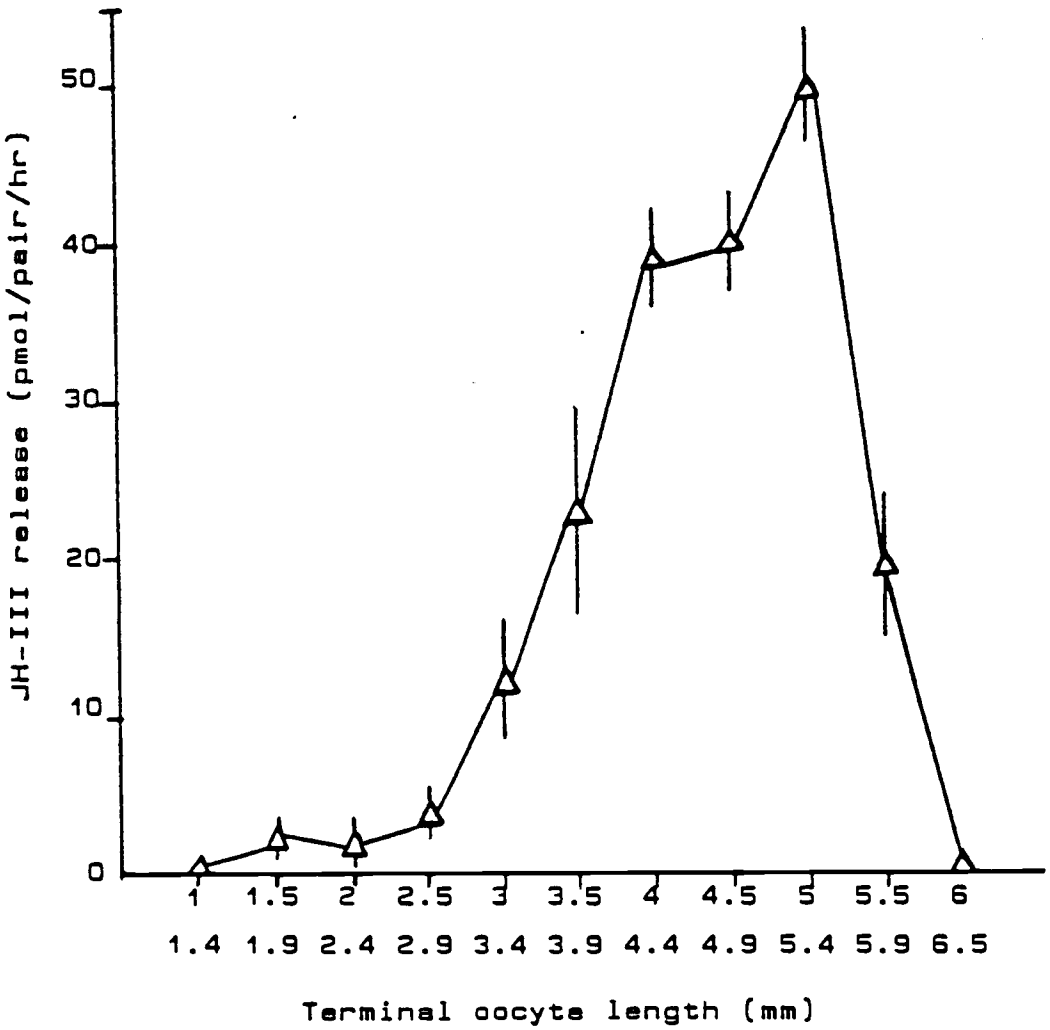

Figure 10. Relationship of CA activity with oocyte length. Each point is mean±SE of 10 to 20 pair of CA.

Relationship age/ JH release/oocyte length

Oocyte growth and CA activity were investigated during the first gonotrophic cycle. Two to 13-day mated females were used. Their oocytes were removed, measured, and their CA assayed for JH synthesis (fig. 9,10). The CA underwent increases of up to 9-fold in rate of JH, this increase was closely correlated with oocyte growth.

IV. EFFECTS OF FEEDING AND MATING UPON CA ACTIVITY

Effect of mating on JH release and oocyte growth

A study of starved and mated females was conducted in order to determine the effect of mating on JH release and oocyte growth.

Adult female L. maderae were starved until the 11th day after eclosion, fed once for 4 hours, and mated. Their CA were assayed using the partition assay. Oocyte length and CA volume were also measured. The data are shown in table 2. Fifteen hours, two days, four days, and six day starved and mated female CA showed no activity; oocyte growth was poor. CA activity of 9.2 ± 2.0pmol JH/pair/hr (20 % less than fed mated female CA activity) was recorded on 9-day starved mated females. When compared with data on fed mated females, the results suggested that mating alone had no effect on CA activity and oocyte growth. Both feeding and mating were necessary for activation of the CA to their fullest extent so that the oocytes could mature at their maximum rate. Figure 11 shows the effect of mating on JH release when the animals were fed.

Effect of starvation on JH release

Fourty two virgin and starved females ranging from day 3 to day 23 after eclosion were investigated. No activity was recorded using the partition assay. Day 14 and day 19 starved virgins were assayed using TLC. The entire incubation medium as well as the glands were extracted in order to measure the JH biosynthesized (JH released + JH intraglandular). The values recorded were still below opmol (19 day female CA gave 0.1pmol/pair/hr). The CA of starved virgin females appear to be inactive.

Effect of feeding on JH release

CA activity of fed virgin females was assayed in vitro (table 3). Feeding alone did permit some oocyte growth, but at a lower rate when compared with fed and mated females.

The activity of the CA was also lower than in mated females of comparable age.

Table 2. Effect of mating on JH release of starved females

Age	15 hrs	2 days	4 days	6 days	9 days
JH-III/pair/ hour	0	0	0	0	9.2±2.0
oocyte length (mm): mean±SD	1.0±0.1	1.0±0.3	2.0±0.5	2.0±0.6	4.0±0.4
CA volume ($\times 10^{-3}$ mm 3) mean±SD	15.0±5.0	9.4±2.1	9.9±7.2	9.9±7.2	19.0±10.0
n	6	5	5	5	7

Table 3. Effect of feeding upon JH release of virgin females

Age (days)	JH-III/pair/hr mean±SE	oocyte length (mm) mean±SE	n
1	0		10
2	0		8
4	0	0.5±0.2	5
5	0		8
6	0.3±0.0		18
7	0.1±0.1	1.0±0.3	9
8	2.6±1.7		7
12	2.6±1.5	1.2±0.5	8
15	2.5±1.5	1.2±0.5	10
20	3.1±1.6	1.8±0.4	8
22	6.4±3.9	2.0±0.5	10
23	6.5±2.0	2.0±0.5	10

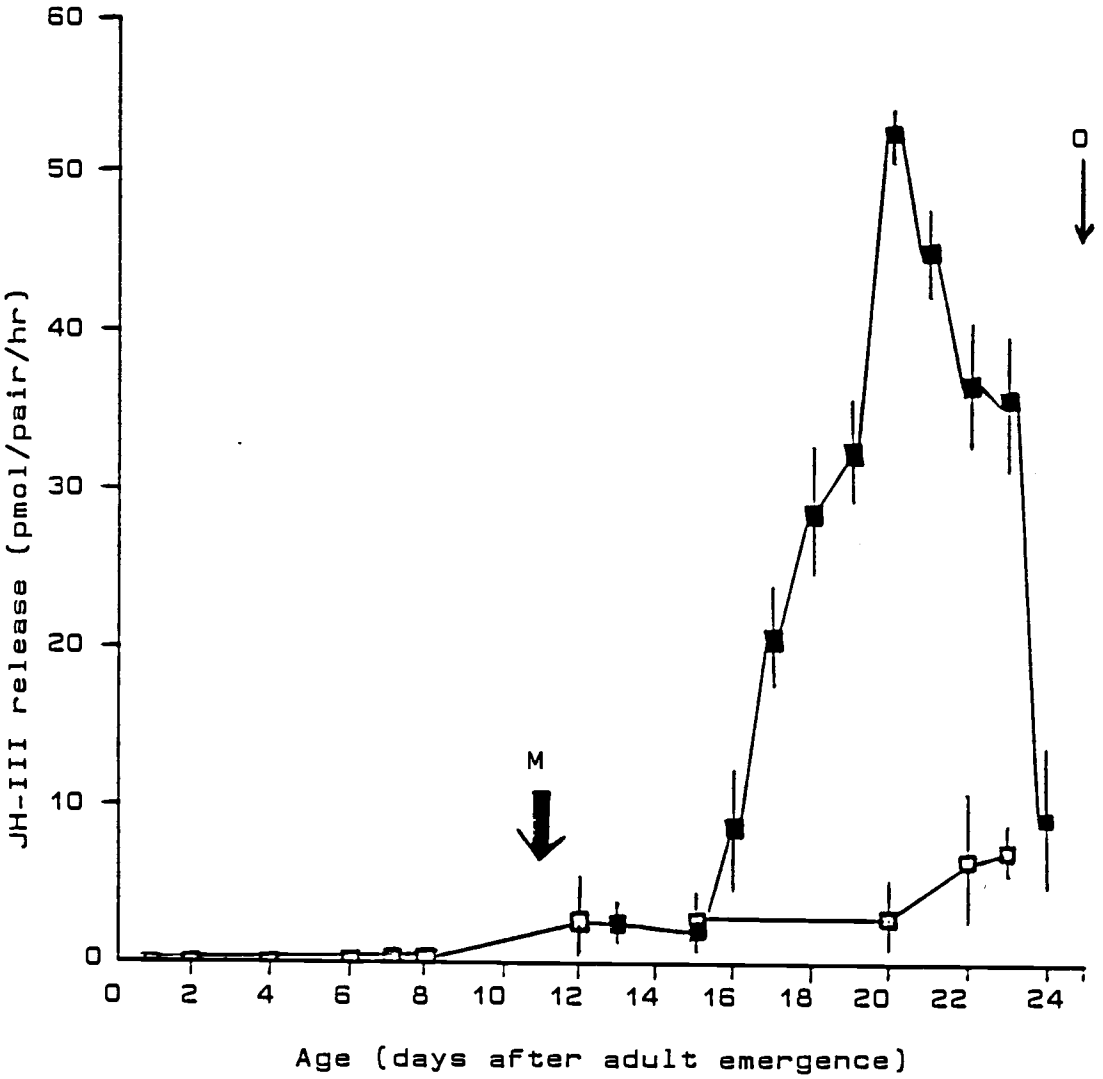

Figure 11. Effect of mating upon JH release of fed females. Each point is mean±SE of 10 to 20 pairs of CA. M = mating; O = oviposition

activity after mating

activity of virgin female CA

V. FARNESOL STIMULATION OF THE CA

The low activity in glands from starving females may be the result of inhibition of existing enzymes in the synthetic pathway or to the absence of enzymes resulting from nutritional deprivation. To test which of these may be the basis for the low activity, the glands from starving females were incubated in the presence of farnesol, a precursor of the synthesis of JH-III.

Farnesol dose-response

The CA from 5 and 6-day, fed, virgins were assayed in the presence of different amounts of 2E,6E-farnesol ranging between 25uM and 250uM. The JH released was measured using the TLC assay. The optimum concentration of farnesol for synthesis of JH and MF was established at 150uM (fig. 12).

Stimulation of CA from starved females

Three groups of 4-day mated female CA were investigated. The synthesis of JH by the glands is shown in table 4. The difference between farnesol stimulated glands from fed females was not statistically significant. The results indicated that CA from starved females contained appreciable levels of enzymes, farnesol and

farnesal deshydrogenases, O-methyl transferase and epoxidase, and a sufficient supply of ATP and reducing equivalents. This indicated that the rate of JH-III synthesis was suppressed at a control point earlier in the pathway.

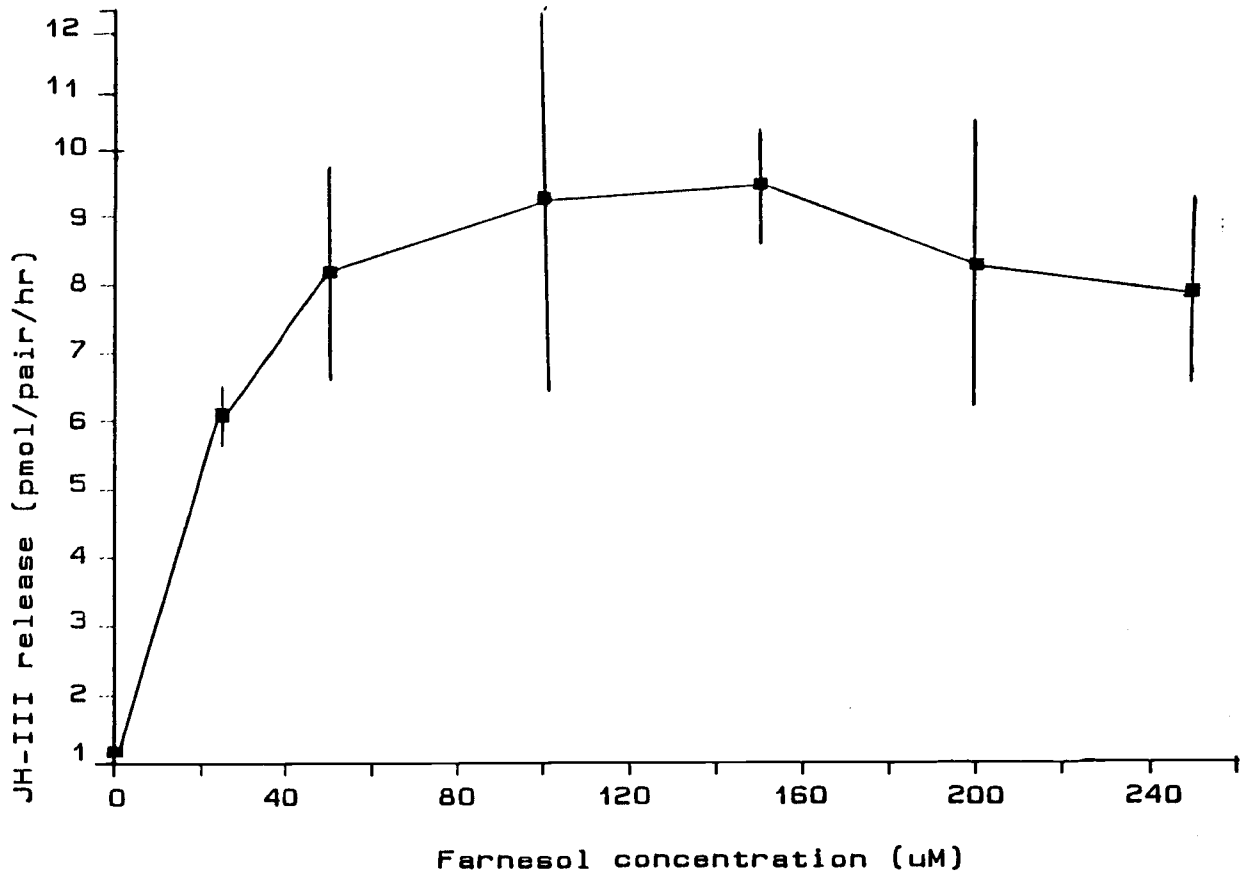

Figure 12. Farnesol dose-response. Each point represents the mean ± SE of 3 pairs of glands. The optimum concentration of farnesol was established at 150uM.

Table 4. Effect of farnesol on JH synthesis. JH was measured on day 15 (day 4 after mating). Values in pmol/pair /hour are means± SE of 8 to 15 pairs of CA. Farnesol-treated glands were incubated in the presence of 0.15 mM 2E,6E-farnesol.

	control	+farnesol
fed virgins	2.5±1.5	17.9±2.9
fed mated	2.0±1.0	29.9±4.9
starved mated	n.d (n=12)	24.7±5.8

DISCUSSION

The activity of the CA has been estimated either indirectly, by the size and the histological appearance of the gland, or directly, by its effects on the recipient after transplantation. Each of these methods has both advantages and disadvantages. The reliability of estimating the activity of the CA by an increase in the volume of the gland has been criticized because of various instances of increase in volume, both normal and pathological, not connected with a corresponding increase in hormone production. For example, the gland increases in size from one instar to the next and this increase is not connected with an increase in activity per volume unit (Scharrer, 1946a; 1952). Similarly, the increase in volume observed following castration in adult females is probably due not to an increase in the secretory activity of the gland, but rather, to a restriction of the removal of the secretion by the haemolymph (Scharrer and Von Harnack, 1976). However, in D. punctata and L. maderae, characteristic histological changes in the CA do occur in association with different phases of ovarian activity (Engelmann,1957,1959; Scharrer and Von Harnack,1958). When eggs are maturing, the volume of the CA increases,

primarily because of an increase in the cytoplasmic volume of its cells; during the following quiescent stage of the ovaries the amount of cytoplasm in the CA is reduced. From these finding it seemed feasible to grade the activity of the CA by calculating the volume of the gland per number of nuclei (Engelmann,1957).

Because of its simplicity and high sensitivity, the radiochemical assay is a very good test for CA activity. De Kort and Granger (1981), and Tobe and Feyereisen (1983), showed that the rate of JH synthesis in vitro shortly after extirpation of the glands is probably a good estimator of the rate of synthesis of those glands in situ (Pratt et al.,1976). Glands which are dependent on intact nervous connections for sustained activity in vitro (Tobe et al.,1977), still maintain a constant rate of activity for 3 hours in vitro. This is sufficient time to allow precise quantification of JH produced (Pratt et al.,1975). I showed that the release of JH in L. maderae was linear over a period of 4 hours; suggesting that the activity in vitro was probably close to the activity in situ.

Only JH-III was identified from the CA extract. The same result was previously obtained by Koeppe et al (1980). These authors showed that 50% 0f the CA extracted label was JH-III. JH-I and JH-II were not detected. In the same paper, the curve that represent the haemolymph JH titre during the first reproductive cycle of L. maderae

shows similar pattern as the activity of the CA I have investigated.

The regulation of JH may involve either the release or the synthesis of the hormone. In several species, including P. americana and D. punctata, it is synthesis rather than release which ultimately regulates the amount of JH that enters the hemolymph (Tobe and Pratt, 1974b; Pratt et al., 1975b; Tobe and Stay, 1977; Weaver and Pratt, 1977). JH is released as soon as it is synthesized and does not accumulate within the glands. The rate of release of JH is directly proportional to intraglandular content; thus high intraglandular levels of JH can be associated with high rates of release (Pratt et al., 1975b; Tobe and Stay, 1977). I have investigated this relationship in L. maderae. The results showed that this relationship is also true in L. maderae.

According to Feyereisen (1985), advantages of the radiochemical assay are its extreme simplicity and its high sensitivity, allowing the activity of single pairs of glands or single glands to be monitored. Disadvantages are that it cannot be used to determine the activity of glands which do not esterify their secretory products, such as the CA from adult male Hyalophora cecropia (Peter et al.,1981). Also, it cannot be used in those species where dissection is so difficult that the CA cannot be obtained free of tissue (including hemolymph)

contaminating the medium with JH esterase activity (Feyereisen, 1981).

Mating in D. punctata usually occurs immediately following adult eclosion, and is essential for a normal rate of oocyte growth (Engelmann, 1959a; Roth and Stay, 1961). CA activity of virgin D. punctata is very low compared to the activity of mated female CA, and vitellogenic oocytes are observed in only very few insects (Stay and Tobe, 1977). Mating in female Nauphoeta cinerea occurs 3-4 days after adult emergence. Whereas ovulation first occurs on day 12 in mated females, it is delayed until day 24 in virgin females, and only fragile egg cases are expelled (Lanzrein et al., 1981a). JH synthesis by virgin females shows a peak on day 9 as in mated females, but it is much smaller (5-fold). In any case, within those two species, feeding is not a major constraint on oocyte development. L. maderae is different in that both mating and feeding are necessary for optimal growth of the oocytes, and the CA appear to be inactive in starved individuals even after they are mated. The activity recorded on day 9 (table 4) is probably due to factors other than the mating stimulus which should have activated the CA on day 3 or 4 (at most).

The ability of starved P. americana CA to utilize farnesoic acid to promote rates of JH biosynthesis testifies to the continuing presence of appreciable levels

of O-methyl transferase and epoxidase, and to a metabolic supply of ATP and reducing equivalent (Weaver and Pratt, 1981). These authors concluded that the spontaneous rate of JH biosynthesis was suppressed at a control point earlier in the pathway. In L. maderae, the synthesis of JH is inhibited in starved female CA. Farnesol stimulation of the glands showed that the starved female CA were able to utilize the exogenous metabolite to manufacture JH. Therefore, like in P. americana, the inhibition of JH biosynthesis in starved L. maderae CA occured early in the pathway.

CONCLUSIONS

1. Only juvenile hormone III (JH-III) was identified using our assay conditions.

2. The radiochemical assay gave a good estimation of the activity of the corpus allatum in vitro. However, the conditions of the assay can be improved.

3. Feeding and mating were both required for a normal oocyte development and JH synthesis.

4. Farnesol stimulation of starved female CA showed that the inhibition of JH synthesis was produced early in the pathway.

REFERENCES

Barth, R. H. (1962). The endocrine control of mating behaviour in the cokroach Byrsotria fumigata (Guerin). Gen. Comp. Endocr. 2, 53-69.

Bodenstein, D. and Sprague, E. (1959). The developmental capacities of the accessory sex glands in Pleriplaneta americana. J. exp. Zool., 142, 177 -202.

Brookes, V. J. (1969). The induction of yolk protein synthesis in the fat body of an insect, Leucophaea maderae, by an analog of juvenile hormone. Devel. biol. 20, 459-471.

Dejmal, R.K. and Brookes, V.J. (1968). Solubility and electrophoretic properties of ovarial protein of the cockroach, Leucophaea maderae. J. Insect Physiol. 14, 371-381.

Dejmal,R.K. and Brookes, V.J. (1972). Chemical and physical caracteristics of a yolk protein from the ovaries of Leucophaea maderae. J. Biol. Chem. 247, 869-874.

De Kort, C. A. D. and Granger, N. A. (1981). Regulation of the juvenile hormone titer. Ann. Rev. Ent. 26, 1 -28.

Della-Cioppa, G. and Engelmann, F. (1987). The vitellogenin of Leucophaea maderae synthesis as a large phosphorylated precursor. Insect Biochem. 17, 401-415.

Edwards, J. P., and Menn, J. J. (1981). The use of juvenoids in insect pest management. In "Chemie der Pflanzenschutz-und Schadlingsbekampfungsmittel" (R. Wegler, ed.), Vol. 6, pp. 185-214. Springer-Verlag, Berlin and New York.

Engelmann, F. (1957). Die steuerung der ovarfunktion ben derovoviviparen schabe Leucophaea maderae. J. Ins. physiol. 1, 257-278.

Engelmann, F. (1959a). The control of reproduction in Diploptera punctata (Blattaria). Biol. Bull. 116, 406-419.

Engelmann, F. (1959b). Uber die Wirkung Implantierter

Prothoraxdrusen im Adulten Weibchen von Leucophaea maderae (Blattaria). Z. Vergl. Physiol. 41, 456 -470.

Engelmann, F. (1962). Further experiments on the regulation of the sexual cycle in females of Leucophaea maderae. Gen. and comparative endocrinology 2, 183-192.

Engelmann, F. (1964). The inhibition of egg maturation in a pregnant cokroach. Nature, Lond. 202, 724-725.

Engelmann, F. (1965). The mode of regulation of the corpus allatum in adult insects. Arch. Ana. Micr. 54, 387-404.

Engelmann, F. (1970). The physiology of insect Reproduction. Pergamon Press, Oxford.

Engelmann, F. (1971). Juvenile hormone-controled synthesis of female-specific protein in the cockroach Leucophae maderae. Arch. Biochem. and Biophys. 145, 439-447.

Engelmann, F. (1978). Synthesis of vitellogenin after ovariectomy in a cockroach. Insect Biochem. 8, 149 -154.

Engelmann, F. Fridel, T. and Ladduwahetty, M. (1976). Insec biochem. 6, 211-220.

Engelmann, F. and Ladduwahetty, A. M. (1974). The female specific protein, an extraovarian protein essential for egg maturation in insects. Zool. Jb. Physiol. Bd. 78, S. 289-300.

Engelmann, F. and Luscher, M. (1956). Die hemmende wirkung des gehirus auf die corpora allata bei Leucophaea maderae (orthoptera). Verh. dt. Zool., Ges. Hamburgh., 215-20.

Engelmann, F. and Rau, I. (1965). A correlation between feeding and the sexual cycle in Leucophaea maderae. J. Ins. physiol. 11, 53-64.

Feyereisen, R. (1985a). Regulation of juvenile hormone titer: Synthesis. In: Comprehensive Insect Physiology, Biochemistry and Pharmacology (Edited by Kerkut, G. A. and Gilbert, L. I.), pp. 391-429.

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.

Feyereisen, R., Pratt, G. E. and Hamnett, A. F. (1981b). Enzymic synthesis of juvenile hormone in locust corpora allata: evidence for a microsomal cytochrome P-450 linked methyl farnesoate epoxidase. Eur. J. Biochem. 118, 231-238.

Hamnett, A. F. and Pratt, G. E. (1978) Use of automated capillary column radio gas chromatography in the identification of insect juvenile hormones. J. Chromatogr. 158, 387-399.

Henrick, C. A. (1977). Aliphatic hydrocarbon 2,4-dienoic acids, esters and derivatives thereof. U.S. Patient 4,021,461

Henrick, C. A., and Anderson, R. J. (1973). Zoecon Corporation, Palo Alto, California (unpublished results).

Henrick, C. A., and Siddall, J. B. (1975). 2,4-Dienoic Acids, Esters, and derivatives. U.S. Patient 3,904,622.

Henrick, C. A., and Staal, G. B. (1974). Zoecon Corporation, Palo Alto, California (unpublished results).

Henrick, C. A., Staal, G. B., and Siddall, J. B. (1973). Alkyl 3,7,11-trimethyl-2,4-dodecadienoates, a new class of potent insect growth regulators with juvenile hormone activity. J. Agric. Food Chem. 21, 354-359.

Henrick, C. A., Willy, W. E., Garcia, B. A., and Staal, G. B. (1975a). Insect juvenile hormone activity of the stereoisomeres of ethyl 3,7,11-trimethyl-2,4 -dodecadienoate. J. Agri. Food Chem. 23, 396-400.

Henrick, C. A., Staal, G. B., and Siddall, J. B. (1976a). Structure activity relationships in some juvenile hormone analogs, In "The Juvenile Hormones" (L. I. Gilbert, ed.), pp. 48-60. Plenum, New York.

Henrick, C. A., Willy, W. E., and Staal, G. B. (1976b). Insect juvenile hormone activity of alkyl(2E,4E) -3,7,11-trimethyl-2,4-dodecadienoates. Variations in the ester function and in the carbon chain. J. Agric. Food Chem. 24, 207-218.

Henrick, C. A., Labovitz, J. N., Graves, V. L., and Staal, G. B. (1978). Ethyl (E)-3,5-ethano-7,11-dimethyl -2,4-dodecadienoate, a new insect growth regulator with potent juvenile hormone activity. Bioorg. Chem. 7, 235-250.

Koeppe, J. K., Brantley, S. G. and Nijhout, M. M. (1980). Structure, haemolymph titre, and regulatory effects of juvenile hormone during ovarian maturation in Leucophaea maderae. J. Insect physiol. 26, 749-753.

Lanzrein, B., Gentinetta, V., Fehr, R. and Luscher, M. (1978) Correlation between hemolymph juvenile hormone titer, corpus allatum volume, and corpus allatum in vivo and in vitro activity during oocyte maturation in the cokroach (Nauphoeta cinerea). Gen. Comp. Endocr. 36, 339-345.

Lanzrein, B., Wilhelm, R. and Buschor, J. (1981a). On the regulation of the corpora allata activity in adult females of the ovoviparous cockroach Nauphoeta cinerea. In juvenile hormone biochemistry. Edited by G. E. Pratt and G. T. Brooks. Pages 147-160. Elsevier/North Holland,Amsterdam.

Lanzrein, B., Wilhelm, R. and Gentinneta, V. (1981b). On relations between corpus allatum activity and oocyte maturation in the cockroach Nauphoeta cinerea. In regulation of insect Development and Behaviour. Edited by F.Sehnal,A.Zabza,J.J.Menn and B.Cymborowski, Part II, pages 523-534. Wroclaw Technical University Press, Wroclaw.

Peter, M. G., Shirk, P. D., Dahm, K. H. and Roller, H. (1981). On the specificity of juvenile hormone biosynthesis in the male Cecropia moth. Z. Naturf. 36c, 579-585.

Pratt, G. E. and Tobe, S. S. (1874) Juvenile hormones biosynthesised by corpora allata of adult female locusts in vitro. Life Sci. 14, 575-586.

Pratt, G. E., Tobe S. S., Weaver, R. J. and Finney, J. R. (1975a). Spontaneous synthesis and release of C16 juvenile hormone by isolated corpora allata of female locust Schistocerca gregaria and female cockroach Periplaneta americana. Gen. Comp. Endocr. 26, 478 -484.

Pratt, G. E., Tobe, S. S. and Weaver, R. J. (1975b). Relative oxygenase activities in juvenile hormone

biosynthesis of the corpora allata of an african locust (Schistocerca gregaria) and american cockroach (Periplaneta americana). Experientia 31, 120-122.

Pratt, G. E., Weaver, R. J. and Hamnett, A. F. (1976). Continuous monitoring of juvenile hormone release by superfused corpora allata of Periplaneta americana. In The Juvenile Hormones. Edited by L. I. Gilbert. Pages 164- 178. Plenum Press, New York.

Roth, L. (1964) Control of reproduction in female cokroaches with special reference to Nauphoeta cinerea - I. First pre-oviposition period. J. Insect Physiol. 10, 915-945.

Roth, L. M. and Stay, B. (1961). Oocyte development in Diploptera punctata (Eschscholtz) (Blattaria). J. Insect Physiol. 7, 186-202.

Scharrer,B.(1946). The relationship between corpora allata and reproductive organs in adult Leucophaea maderae (Orthoptera). Endocrinology 38, 46-55.

Scharrer, B. (1978). Histophysiological studies on the corpus allatum of Leucophaea maderae. VI. Ultrastructure characteristics in gonadectomized females. Cell. Tiss. Res. 194, 533-545.

Scharrer, B. and Von Harnack, M. (1958). Histophysiological studies on the corpus allatum of Leucophaea maderae. I. Normal life cycle in male and female adults. Biol. Bull. 115, 508-520.

Scharrer, B. and Von Harnack, M. (1961). Histophysiological studies on the corpus allatum of Leucophaea maderae. III. The effect of castration. Biol. Bull. 121, 193 -208.

Sehnal, F. (1976). Action of juvenoids on different groups of insects. In "The Juvenile Hormones" (L. I. Gilbert, ed.), pp. 301-322. Plenum, New York.

Slama, K. (1961). Physiology of sawfly metamorphosis. I. Continuous respiration in diapausing prepupae and pupae. J. Insect physiol. 5(3/4): 341-348.

Slama, K. (1962). The juvenile hormone like effect of fatty acids, fatty alcohols, and some other compounds in insect metamorphosis. Acta Soc. Ent. Cechoslov. 59, 323-340.

Slama, K., and Williams, C. M. (1966). "Paper factor" as an inhibitory of the embryonic development of the European bug, Pyrrhocoris apterus. Nature 210, 329 -330.

Slama, K. (1965). The effect of hormone mimetic substances on the ovarian development and oxygen consumption in allatectomized adult females of Pyrrhocoris apterus L.(Hemiptera).. J. Insect Physiol. 11(8): 1121-1129.

Slama, K., Romanuk, M., and Sorm, F. (1974). "Insect Hormones and Bioanalogues." Springer-Verlag, Berlin and New York.

Staal, G. B. (1975). Insect regulators with juvenile hormone activity. Annu. Rev. Entomol. 20, 417-460.

Stay, B. and Tobe, S. S. (1977). Control of juvenile hormone biosynthesis during the reproductive cycle of a viviparous cockroach. I. Activation and inhibition of corpora allata. Gen. Comp. Endocr. 33, 531-540.

Tobe, S. S. and Feyereisen, R. (1983). Juvenile hormone biosynthesis: regulation and assay. In Invertebrate Endocrinology. Endocrinology of insects. Edited by R. G. H. Downer and H. Laufer. Vol 1, pages 161 -178. Alan R. Liss, New York.

Tobe, S. S. and Pratt, G. E. (1974b). Dependence of juvenile hormone release from corpus allatum on intraglandular content. Nature 252, 474-476.

Tobe, S. S. and Pratt, G. E. (1975). The synthetic activity and glandular volume of the corpus allatum during ovarian maturation in the desert locust Schistocerca gregaria. Life Sciences 17, 417-422.

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., Stay, B., Friedel, T., Feyereisen, R. and Paulson, C. (1981). The role of the brain in regulation of the corpora allata in female Diploptera punctata. In Juvenile hormone biochemistry. Edited by G. E. Pratt, and G. T. Brooks. Pages 161-174. Elsevier/North Holland, Amsterdam.

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. and Finny, J. R. (1975) Cyclic activity of the corpus allatum related to gonotrophic cycles in adult female Periplaneta americana. Experientia 31, 597-598.

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.

Wigglesworth, V. B. (1936) The function of the corpus allatum in the growth and reproduction of Rhodnius prolixus (Hemiptera). Quart. J. Mic. Sci. 79, 91 -121.

Wigglesworth, V. B. (1963). The juvenile hormone effect of farnesol and some related compounds: quantitative experiments. J. Insect Physiol. 9, 105-119.