find a clear explanation for the distribution of crustacean porphyropsin (3-dehydroretinal).
There is another possible function for crayfish porphyropsin; if the porphyropsin and rhodopsin were present in different photoreceptor cells, wavelength discrimination could be possible using the two visual pigments. Intra-ommatidial distribution of 3-dehydroretinal should be investigated to evaluate this possibility.

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In vitro biosynthesis of juvenile hormone III by the corpora allata of *Calliphora vomitoria* and its role in ovarian maturation and sexual receptivity

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Summary. JH III is the only JH detected by GLC-MS in medium from in vitro incubations of corpora allata of adult females of *Calliphora vomitoria*. When corpora allata were removed from females at various times during the reproductive cycle and the JH III produced by the glands in vitro measured by a JH III radioimmunoassay, an increase in the level of synthesis was found to occur before previtellogenesis (0–24 h). A second increase appeared at the onset of vitellogenesis (72–83 h) and continued until the end of vitellogenesis (96 h) and the occurrence of chorionation (120 h). Since sexual receptivity develops with vitellogenesis, the significantly higher levels of JH III biosynthesis in vitro at this time supports a possible role for JH in the acquisitive of receptivity.
Key words. Juvenile hormone III biosynthesis; radioimmunoassay; ovarian maturation; sexual receptivity; *Calliphora vomitoria*.

In higher flies, vitellogenesis and oocyte growth both appear to be controlled by juvenile hormone (JH) and ecdysteroids[1,2]. In *Calliphora vomitoria*, parallel fluctuations occur in the ecdysteroid titers in the hemolymph and ovaries during oocyte growth, with a simultaneous drop during chorionation[3]. In this same species, destruction of the corpora allata (CA) before oocyte growth begins prevents ovarian development and sexual receptivity. Both processes are restored by the application of a JH analogue, S-methoprene, to the allatectomized females[4]. These same results have been obtained with *Musca domestica*[5], *Drosophila melanogaster*[6], and *Lucilia cuprina*[7], thus suggesting that JH plays a major role in ovarian development and receptivity in flies.
Knowledge of the JH titers in adult females is critical to an understanding of the function of JH in these processes. However, little information is available on the identification and titers of the JHs in Diptera, except for *Aedes aegypti*[8] and *Drosophila hydei*[9–11], in which only JH III is present in whole body extracts of larvae and adults.
We confirmed by gas-liquid chromatography and mass spectrometry (GLC-MS) that JH III is the only JH in *C. vomitoria*. They also document that specific fluctuations in JH biosynthesis in vitro by CA taken during the first gonadotropic cycle correlate with the development of female receptivity, indicating that JH titers may fluctuate in the same way.

Materials and methods. Adults of *C. vomitoria* were reared at 25 °C under a L:D 12:12 photoperiod and were fed daily with a diet of water, sugar and minced meat. Under these conditions, the gonadotropic cycle lasts 5 days. Experimental animals were isolated at emergence. Behavioral observations were carried out at a time of day determined to be optimal for sexual activity, between 09.00 and 11.00 h. Females of known imaginal age (3, 6, 12, 24, 48, 72, 83, 92, 96 or 120 h) were kept together with 5-day-old mature and sexually motivated males for a period up to 1 h. Female receptivity was scored by the claspering of male and female genitalia, stopped after 4 min to prevent insemination. Ovarian development in both unreceptive and receptive females was evaluated by the stage of oocyte development based on the observation that the polytrophic ovary contains 1, 2, 3 and 4 oocytes in succession and by the degree of completion of vitellogenesis in the terminal follicle. Vitellogenesis began at 72 h and finished at 96 h. Because of the rapidity of this phenomenon, JH production levels were measured at 83 h and 92 h. For JH identification, ten CA taken from females 48 h after emergence and thus well before the onset of vitellogenesis (72 h) were incubated in triplicate for 4 h at 25 °C in 100 µl of medium TC 199 (with Hanks' salts, L-glutamine, and 25 mM HEPES buffer – Gibco). Non-polar products of the glands were extracted with 1 ml of pentane. The efficiency of this

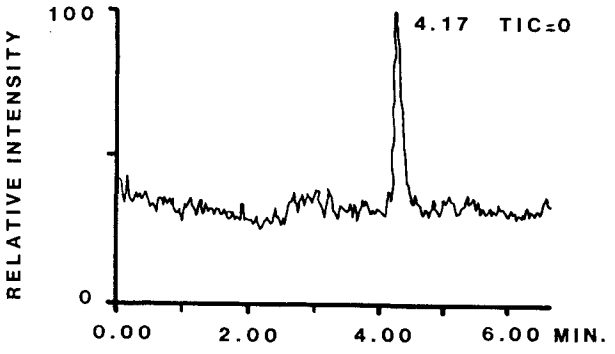

Figure 1. Identification of C16-JH III released by corpora allata of *Calliphora vomitoria* (Diptera) females. Total Ion Current (TIC) GC chromatogram of C16-JH III extracted from the medium. The retention time (RT = 4 min 17) corresponds to the C16-JH III standard analyzed in separate run. Same chromatograms were obtained by Selected Ion Monitoring (SIM = 235, 252, 267).

extraction is better than 95%. The extract was dried under a gentle stream of nitrogen in a silicone-coated glass tube, resuspended in 50 µl of pentane, and directly injected into a GLC-MS apparatus for JH identification[12]. For quantification of JH biosynthesis in vitro, individual CA were incubated for 4 h at 25 °C in 500 µl of TC 199 and the incubation medium was extracted with 4 ml of pentane. The extracted fraction was dried under nitrogen and resuspended by sonication in 500 µl of 0.02 M phosphate buffer containing 1% BSA before analysis by radioimmunoassay (RIA). The RIA was performed as previously described[13]. The antiserum used in the RIA is highly specific for JH III; its cross-reactivity with JH I and JH II is 0.3% and 0.9%, respectively. The lower limit of detection of the assay was 10 pg. For HPLC analysis of the products of the CA in vitro, dried pentane extracts of incubation medium were resuspended in 200 µl of methanol: water (45:55) and applied to a reverse phase radial compression column (Radial Pack CN, Waters) with methanol: water (45:55) as the solvent. At a flow rate of 1 ml min^{-1}, the retention times of JH I, JH II and JH III standards were 10, 9 and 7 min, respectively. Fractions of 0.5 ml were collected and the immunoreactive material in fractions 1–30 quantified with RIAs specific for JH I, II or III[13].

Results. JH identification. GLC-MS analysis of the non-polar products in the medium of incubation of *Calliphora vomitoria* CA revealed that only JH III was present (fig. 1). Mass spectrum of compounds detected at this time confirms that it is JH III. Thus JH biosynthesis in vitro by the CA of this species was demonstrated for the first time.

Verification of JH RIA identification of CA products. To verify that the JH III RIA used for subsequent quantification of gland activity in vitro accurately identified the product(s) of the gland, medium from CA incubations was extracted and chromatographed by reverse phase HPLC, and the resulting fractions assayed by RIAs with specific affinity for JH I, II, or III (see 'Materials and methods'[13]). Immunoreactive material was detected solely in those fractions with a retention time identical to the JH III standard. A dose response was obtained with a serial dilution of these combined fractions. No RIA activity was detected in extracts of medium incubated without CA; this control proved that no immunoreactive material appeared because of medium alteration during the incubation.

The same amount of JH III RIA active material was measured in extracts of medium before and after HPLC separation. Thus since JH III was the only JH detected by GLC-MS in medium from the CA incubations, and since only the JH III RIA detected immunoreactive material in non-polar extracts of the incubation medium, it was concluded that the JH III RIA activity measured in the incubation medium reflected JH III biosynthesis/release by the CA and thus CA activity. Moreover no JH III immunoreactive material related to JH III synthesis or degradation was detected in the medium after CA incubation.

JH synthesis during oocyte development. Changes in the in in vitro rate of JH III biosynthesis/release by the CA during oocyte development are shown in figure 2. The first significant increase was noted just before previtellogenesis approximately between 12 and 24 h. Previtellogenesis (24–72 h)

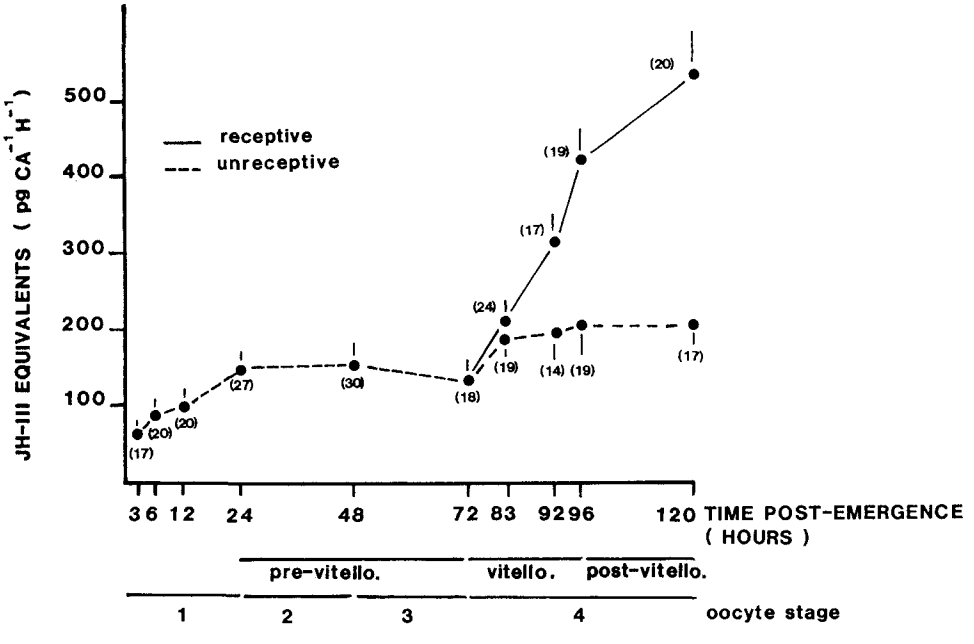

Figure 2. Variation of the in vitro C16-JH III biosynthesis rate by individual corpora allata (CA) during the first gonadotropic cycle (120 h) in female *Calliphora vomitoria*. The number of flies per sample is given in brackets. Comparison between receptive (——) and unreceptive (-----) female. Ordinate: juvenile hormone III equivalents (pg corpora allata^{-1} h^{-1}) (with their SEM). Abscissa: time post-emergence (h).

itself was characterized by a steady rate of synthesis of 143 pg gland^{-1} h^{-1}.

When the onset of receptivity appeared at the beginning of vitellogenesis (72–83 h), rates of JH synthesis/release in receptive and unreceptive females were not significantly different (approximately 200 pg gland^{-1}h^{-1}). However, at 92 h, the rate of JH III synthesis/release by CA from receptive females increased significantly, reaching a rate of 533 pg gland^{-1} h^{-1} at chorionation. By contrast, synthesis/release by CA from unreceptive females did not increase significantly during this same period of time.

Discussion. The results of the present study show that JH III is the sole JH in *C. vomitoria,* in agreement with previous identifications of the JH in Diptera[8–11]. The measurement of the biosynthesis/release of JH III by *Calliphora* CA in vitro is the first such demonstration for dipteran CA, and the developmental study of gland activity during the gonadotropic cycle indicated a coincidence of increasing gland activity with the onset of receptivity. The nature of the relationship between receptivity, JH and ovarian development has yet to be resolved, although some information is available from a previous study[4]. On the basis of the results of that study, it was concluded that the occurrence of receptivity coincided with the beginning of vitellogenesis and that JH was necessary for receptivity. This latter conclusion was derived from the fact that vitellogenesis and receptivity were abolished in allatectomized females and that the application of JH analogue to the allatectomized animals restored both processes. Furthermore, the ovary itself appeared to have a role in the maintenance of receptivity, since less than 24% of ovariectomized females remained receptive[4]. It could be postulated from this information that the JH titer stimulates vitellogenesis in *C. vomitoria,* as it does in other fly species[5–7]. The occurrence of receptivity may then be affected indirectly by JH, through the ovarian development resulting from vitellogenesis. However, it is also possible that JH affects receptivity directly. Supporting this possibility it was shown, in this work, that ovarian development is the same in both receptive and unreceptive females and that receptive females possess a higher JH titer than unreceptive females. The function of the ovary may thus be to maintain receptivity once established, rather than to affect its initiation.

There also appears to be a link between receptivity and ecdysteroid levels. In *C. vomitoria,* it has been found that ecdysteroid levels are highest just at the onset of vitellogenesis and receptivity[3,4]; the same situation appears to occur in *Musca domestica*[14,15]. Thus the *Calliphora* system may prove to be ideal for studying the interendocrine relationships of JH and ecdysteroids in the regulation of a reproductive process. In this species, critical questions remain to be answered concerning the role of JH and ecdysteroids in the regulation of vitellogenesis, ovarian development and receptivity. For example, does JH directly or indirectly affect receptivity? What is the specific role of the ovary in this process? With the in vitro systems currently available, solutions to these problems should be forthcoming.

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Ecdysteroid-dependent larval-adult oviduct transformation in the milkweed bug *Oncopeltus fasciatus* requires absence of juvenile hormone[1]

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Summary. It has been tested whether juvenile hormone plays a role in the larval-adult transformation of lateral oviducts in the milkweed bug. The transformation is ecdysteroid-dependent, as was reported previously[2]. Application of precocene or juvenile hormone III proved that the absence of juvenile hormone is required.

Key words. Insect oviduct; larval-adult transformation; juvenile hormone; *Oncopeltus.*

The lateral oviducts of the milkweed bug *Oncopeltus fasciatus* undergo a drastic larval-adult transformation during the last larval instar. Between day 3 and day 5 the long and thin larval oviducts shorten and become very wide. Previous studies have shown that this process is ecdysteroid-dependent in a dose-related manner[2]. Juvenile hormone is expected to be absent in the stage preceding adult molt[3]. We therefore examined whether the oviductal transformation requires the absence of juvenile hormone or not.

Materials and methods. Oncopeltus fasciatus Dallas (Heteroptera, Lygaeidae) was reared on peeled sunflower seeds at 27.5 °C, in a relative humidity between 60% and 70% and without photoperiod. The animals were staged at 4th, 5th (= last larval) or adult molt. Those which ecdyzed during the night were separated the following morning and defined as day-0 adults, or as day-0 larvae of the ultimate (5th) or penultimate (4th) stage.

The treatment with precocene II (Aldrich, Steinheim) was