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# QUANTITATIVE DETERMINATION OF THE JUVENILE HORMONES IN THE HAEMOLYMPH OF *LOCUSTA MIGRATORIA* DURING NORMAL DEVELOPMENT AND AFTER IMPLANTATION OF CORPORA ALLATA

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**Abstract**—Simultaneous quantitative determination of the three naturally occurring juvenile hormones in insects (JH-I, JH-II and JH-III) was performed on haemolymph samples of both normally developing locusts and locusts implanted with active corpora allata, using capillary gas chromatography with electron capture detection.

In fourth instar female larvae, 24–48 hr after the third ecdysis, as well as in adult females, 18 days after the imaginal ecdysis, only JH-III was detected. In fifth instar female larvae JH-III was present in very low concentrations, if at all.

After implantation of four pairs of corpora allata taken from young fourth instar female larvae or one pair or corpora allata taken from adult females into fifth instar female larvae 0–24 hr after ecdysis, an elevation of the JH-III titre was observed. Neither JH-I nor JH-II could be detected. The amount of JH-III, already elevated 2 hr after implantation, remained high for several days in comparison to that of control insects. On the third day after the subsequent moult the JH-III level was comparable to that of normally developing fifth instar larvae. Factors involved in the achievement of the haemolymph JH-titre are discussed.

*Key Word Index*: *Locusta migratoria*, juvenile hormones, capillary gas chromatography, electron capture detection, corpora allata, flight muscles.

## INTRODUCTION

IMPLANTATION of active corpora allata (CA) (JOHNSON and HILL, 1975) early in the fifth larval instar of *Locusta migratoria* results in an increase in heart-beat frequency (ROUSSEL, 1969), a change in pigmentation and a disturbance of metamorphosis (JOLY, 1960; STAAL, 1961; POELS and BEENAKKERS, 1969; BEENAKKERS and VAN DEN BROEK, 1974). Similar effects have also been found upon injection of any of the three naturally occurring insect juvenile hormones (JH's); the activity of JH-I being the most potent (ROUSSEL, 1977). Also, in adult female locusts JH is involved in maturation of the eggs (ROUSSEL, 1976).

The JH secreted by the CA reaches its target tissues via the haemolymph, its concentration being controlled by regulatory factors, such as degrading enzymes. In the haemolymph of *Locusta* a high molecular weight JH-transporting lipoprotein has been identified which is probably capable of protecting the hormone from esterase activity (EMMERICH and HARTMANN, 1973).

Up till now, JH in the haemolymph of *Locusta* has only been demonstrated by a *Galleria* bioassay. In fourth instar female larvae JH-activity appeared to reach a maximum on the first days of the instar, whereas activity was virtually absent in the fifth larval instar. In the adult stage an increasing activity during the gonotrophic cycle was observed (JOHNSON and HILL, 1973a, 1975; JOLY *et al*., 1977). The chemical nature of the actual homologue (or homologues) involved, however, is unknown.

Qualitative and quantitative determination of the three naturally occurring juvenile hormones at physiological levels in insects by capillary gas chromatography with electron capture detection (GC-ECD) (HUIBREGTSE-MINDERHOUD, 1979a) now facilitates a definitive analysis of the JH-titre in *Locusta*.

The present paper is concerned with such an analysis of the JH-titre in the haemolymph of young fourth and fifth instar larvae and of adult females of *Locusta*. Furthermore, this study focusses on the question which JH homologue(s) is (are) produced in fifth instar larvae upon implantation of active CA and whether the titre fluctuates in the period following implantation. The latter aspect is studied in view of the fact that implantation of CA in young fifth instar larvae influences the development of the flight muscles, normally taking place during the fifth larval instar and early adult stage. Thus, after implantation of CA, POELS and BEENAKKERS (1969) observed an inhibition of the normal increase in dry weight and protein content of these muscles. This inhibition is accompanied by lower specific activities of enzymes representative of the main metabolic pathways in the

flight muscles (VAN DEN HONDEL-FRANKEN *et al.*, 1980) and with a delay in invagination of tracheoblasts into the muscle fibres (VAN DEN HONDEL-FRANKEN and BEENAKKERS, unpublished), irrespective of the fact whether the CA were taken from young fourth instar larvae (larval CA) or from 18 day adult females (adult CA). The nature and the amount of the JH's in the CA-implanted locusts responsible for the retardation of the flight muscle development, however, is as yet unknown.

## MATERIALS AND METHODS

Locusts (*Locusta migratoria*) were reared under crowded conditions at 30°C, 40% r.h. and a photoperiod of 12 hr light per day. The insects were fed with reed, supplemented with rolled oats.

Haemolymph JH-titre determinations were carried out in normally developing female locusts, 24–48 hr after the third larval ecdysis, 0–24 hr after the fourth larval ecdysis and 18 days after the imaginal ecdysis, as well as in fifth larval instar females implanted with active CA or injected with a Ringer solution. Implantation experiments were performed as previously described (VAN DEN HONDEL-FRANKEN *et al.*, 1980). The CA were taken from fourth instar female larvae, 24–48 hr after the third ecdysis, or from female adults, 18 days after the imaginal ecdysis. Fifth instar female larvae (0–24 hr old) were used as recipient insects, receiving four pairs of larval CA or one pair of adult CA taken up in 25 $\mu$l of Ringer solution (CA-implanted locusts); controls were injected with 25 $\mu$l of the Ringer solution. The concentrations of the JH's in the haemolymph were determined at different times after treatment.

Samples of haemolymph were taken by a puncture in the ventral membrane between the head and thorax using a Hamilton micro-syringe and kept on ice for at most 10 min. Some crystals of reduced glutathione were added to inhibit tyrosinase activity. Unless otherwise stated haemolymph samples obtained from 10 animals (0.25–0.70 g) were pooled for each determination. After sampling, the haemolymph was kept frozen until extraction with ether-ethanol (6:1, v/v) followed by ether extraction. Also, JH-determinations were performed directly on CA taken from 18 day adult females. After dissection, the CA were sonified for 10 min at 0°C in a Ringer solution to which ether was added and subsequently extracted as described above.

After combining the ether–ethanol and the ether phases, two internal standards were added to the JH-extracts, *viz.* the 10-trans-isomer of JH-II and the ethylester of the 2Z-isomer of JH-III. After intensive purification of the JH-extract and micro-derivatization to the 10-heptafluorobutyrate-11-methoxy-JH's (HUIBREGTSE-MINDERHOUD *et al.*, 1979b) JH-I, JH-II and JH-III were determined simultaneously, using a gas chromatographic system consisting of an all-glass solvent-free solid injector, a set of capillary columns—two liquid phases were used separately—and an electron capture detector. In general, the detection limits for JH-I, JH-II and JH-III were as low as 0.5, 0.3 and 0.2 ng/sample, respectively.

The accuracy of the measurements, defined as the deviation from the true value, was found to be +2% at the level of 5 ng/sample ($n = 10$). The precision, expressed as the standard deviation was 8% ($n = 10$). These analyses were performed with a 1:1 ratio of hormone to internal standard. In separate experiments it was demonstrated that the range between ratios 5:1 and 1:4 does not affect the mentioned values. The amount of internal standard was adapted to each individual sample.

## RESULTS

In the haemolymph of normally developing young fourth instar female larvae and of adult females only JH-III was detected (Table 1). Due to an overlapping peak in the gas chromatogram which coincided in part with the peak of JH-III, the presence of JH-III in the fifth larval instar cannot be excluded. The concentration was, however, less than 2.3 ng/g haemolymph. From 24 to 48-hr-old fourth instar female larvae haemolymph was sampled until over 1 g had been collected (about 100 larvae). In these larvae the presence of JH-III could be demonstrated, reaching a level of only 1.1 ng/g haemolymph. The concentration of JH-III was high in the (reproducing) adult females.

The concentrations of both JH-I and of JH-II in the haemolymph samples from the fourth and fifth instar female larvae and from the adult females were below the detection limits.

Table 2 shows the JH-titres in the haemolymph of

Table 1. JH-titres (ng/g haemolymph) in female locusts of the (a) fourth larval, (b) fifth larval, and (c) adult stage

| Age | Sample number | Weight (g) | JH-I* | JH-II* | JH-III |
|---|---|---|---|---|---|
| (a) 24–48 hr old fourth instar larvae | 1 | 1.10 | <0.3 | <0.1 | 0.9 |
| | 2 | 1.20 | <0.3 | <0.1 | 1.3 |
| (b) 0–24 hr old fifth instar larvae | 1 | 0.30 | <1.7 | <0.6 | ≤1.3 |
| | 2 | 0.29 | <1.8 | <1.0 | ≤2.3 |
| (c) 18 day adults | 1 | 0.60 | <1.6 | <1.4 | 44.0 |
| | 2 | 0.64 | <1.6 | <1.5 | 36.0 |
| | 3 | 0.40 | <2.0 | <2.5 | 38.0 |
| | 4 | 0.44 | <1.9 | <2.5 | 74.0 |

* In some cases the detection limits for JH-I and JH-II were higher than 0.5 and 0.3 ng/sample, respectively.

Table 2. JH titres (ng/g haemolymph) in female locusts determined 24 hr after (a) implantation of larval CA, and (b) injection of Ringer solution

| Experiment | Serial number* | JH-I† | JH-II† | JH-III |
|---|---|---|---|---|
| (a) Four pairs of larval CA | a1 | b.d. | b.d. | 15.0 |
| | a2 | b.d. | b.d. | 16.0 |
| (b) Ringer solution | b1 | b.d. | b.d. | ≤2.2 |
| | b2 | b.d. | b.d. | 1.7 |

*b1 and b2 are the determinations of the controls belonging to a1 and a2, respectively.

† b.d.: value below detection limit; JH-I: less than 0.8–1.5 ng/g haemolymph; JH-II: less than 0.5–0.9 ng/g haemolymph.

Each value is obtained from one group of 10 animals.

fifth instar female larvae, 24 hr after implantation of CA taken from 24 to 48-hr-old fourth instar larvae. JH-I and JH-II were below the detection limits (less than 0.8–1.5 ng/g and 0.5–0.9 ng/g haemolymph, respectively). The JH-III titre was strongly elevated as compared with the control insects. This is most interesting, since the level of JH-III in the haemolymph of the donor insects appeared to be very low (approx. 1.1 ng/g haemolymph; Table 1).

The JH-titres, determined in the haemolymph of fifth instar female larvae at different times in the period following implantation of CA taken from 18 day adult females are presented in Table 3.

JH-I and JH-II were again below the detection limits (less than 0.7–2.4 ng/g and 0.4–1.4 ng/g haemolymph, respectively), whereas within 2 hr after implantation of adult CA a considerable elevation of the JH-III titre was observed. Since the CA contained only 0.3 ng JH-III/pair (Table 4), obviously the JH-III is not stored within the CA. It seems clear that a high titre was maintained for at least 3 days after implantation. On the fifth day the concentration was reduced. On the third day after the subsequent moult the JH-III titre was further reduced to the level of 3.0 ng/g haemolymph.

In the control insects the JH-III level was very low, except in one group of locusts 24 hr after injection of Ringer solution in which a titre of 8.5 ng JH-III/g haemolymph was found; for this exception no explanation can be given.

## DISCUSSION

At any moment in an insect's development the JH-titre in the haemolymph is the balance between synthesis and release of JH by the CA, its enzymic degradation and its protection by JH-carriers (Akamatsu *et al.*, 1975). The activity of the CA is regulated by cerebral nervous and/or neurosecretory centres as well as by blood-borne factors such as substrate precursors of JH, other specific hormones and neurosecretions (Williams, 1976).

In the haemolymph of 18 day adult females of *Locusta* only JH-III was detected definitively, which is in agreement with the *in vivo* findings in reproducing adults of *Schistocerca gregaria* (Trautmann *et al.*, 1974; Blight and Wenham, 1976) and observations on *in vitro* incubation experiments with CA from *Schistocerca vaga* (Judy *et al.*, 1973). CA from *Schistocerca gregaria* produce *in vitro* both JH-I and JH-III (Pratt and Tobe, 1974). JH-synthesis in *in vitro* incubations, however, might differ significantly from the *in vivo* synthetic activity of CA (Pratt *et al.*, 1976). Also the haemolymph of young fourth instar female larvae contains detectable amounts of JH-III, which is in agreement with the findings in *Schistocerca gregaria* (Blight and Wenham, 1976). These results indicate that in *Locusta* JH-III has both morphogenetic and gonadotrophic functions, although as demonstrated with exogenous JH the biological activities of JH-I and JH-II surpass that of JH-III (Roussel, 1977). In young fifth instar female larvae JH-III, if at all, is present in very low concentrations.* This may clarify the bioassay results of Johnson and Hill (1973a). However, in experiments in which CA from fifth instar larvae were implanted into *Galleria* they observed that the CA were capable of releasing JH. A conceivable explanation for this phenomenon might be that isolated CA do not behave in the same way as those connected with the brain complex. On the other hand, it is also possible that in the fifth larval instar of *Locusta* JH-specific esterases play an important role in lowering of the hormone titre by hydrolysis of free as well as protein bound JH, as demonstrated for *Manduca sexta* (Sanburg *et al.*, 1975a). The latter possibility seems to be ruled out by the results of our implantation experiments. After implantation of active CA in fifth instar larvae the JH-titre is strongly elevated, indicating that there is no substantial inactivation of the hormone.

The elevated JH-III titres in the haemolymph of the CA-implanted locusts clearly show that the implanted CA synthesize and release JH-III since the isolated CA of adult female locusts contain only small amounts of JH-III; the presence of any JH within the CA confirms the bioassay results of Johnson and Hill (1973b). The JH-III titre, already raised 2 hr after implantation, remained high for several days. Apparently, the implanted CA were capable of prolonged activity which may be explained by the presence of an activating factor in the haemolymph of the fifth instar larvae. On the other hand, this might also imply that the virtual absence of JH in the haemolymph of normally developing fifth instar larvae is caused by an inhibition of secretion by the CA by way of nervous means. Regulation of CA-activity by nervous inhibition and humoral stimulation has already been suggested in adult females of *Diploptera punctata* (Stay and Tobe, 1977) and in larval and adult *Locusta* (Joly, 1967; Moulins *et al.*, 1974). Although determination of JH-titres in the haemolymph samples by GC-ECD reflects both free and lipoprotein bound JH, the relative high JH-titre suggests that JH-transporting carriers in the haemolymph of the CA-implanted locusts are involved in protecting the JH

* Using radioimmunoassay, Baehr *et al.* (1979) recently identified the presence of JH-I immunoreactive substances in the haemolymph of *Locusta* during the last larval instar, with a maximum concentration during the first hours after the fourth ecdysis.

Table 3. JH-titres (ng/g haemolymph) in female locusts during the period following (a) implantation of adult CA, and (b) injection of Ringer solution

| Experiment | Serial number* | Time after implantation of CA | | | | | | | | | | | | Time after ecdysis following implantation of CA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 hr JH† | | | 24 hr JH† | | | 3 days JH† | | | 5 days JH† | | | 3 days JH† | | |
| | | I | II | III | I | II | III | I | II | III | I | II | III | I | II | III |
| (a) One pair of adult CA | a1 | b.d. | b.d. | 31.0 | b.d. | b.d. | 45.0 | b.d. | b.d. | 19.0 | b.d. | b.d. | 8.0 | b.d. | b.d. | 3.0 |
| | a2 | b.d. | b.d. | 19.0 | b.d. | b.d. | 43.0 | b.d. | b.d. | 38.0 | b.d. | b.d. | 9.0 | b.d. | b.d. | ≤5.0 |
| | a3 | | | | b.d. | b.d. | 30.0 | | | | | | | | | |
| | a3 | | | | b.d. | b.d. | 38.0 | | | | | | | | | |
| | a4 | | | | b.d. | b.d. | 16.0 | | | | | | | | | |
| (b) Ringer solution | b1 | b.d. | b.d. | <1.6 | b.d. | b.d. | 1.7 | b.d. | b.d. | ≤0.7 | b.d. | b.d. | ≤2.0‡ | | | |
| | b2 | b.d. | b.d. | ≤5.0 | | | | | | | b.d. | b.d. | ≤3.0‡ | | | |
| | b3 | | | | b.d. | b.d. | 8.5 | | | | | | | | | |
| | b4 | | | | o.d. | b.d. | ≤1.9 | | | | | | | | | |

* b1, b2, b3 and b4 are the determinations of the controls belonging to a1, a2, a3 and a4, respectively.
† b.d.: value below detection limit; JH-1: less than 0.7–2.4 ng/g haemolymph; JH-II: less than 0.4–1.4 ng/g haemolymph.
‡ Injection of Ringer solution was performed one day before collecting haemolymph.
Each value is obtained from one group of 10 animals.

Table 4. JH-concentrations (ng) within CA taken from adult females

| Sample | Number | JH-I | JH-II | JH-III |
|---|---|---|---|---|
| CA taken from 18 day adult females | 7 pairs | <0.5 | <0.3 | 2.3 |

from esterase activity. Results of studies on larval haemolymph lipoproteins that are in progress suggest an induction of JH-transporting lipoprotein synthesis after implantating active CA in fifth instar larvae (VAN DER HORST *et al.*, unpublished). Although it is not clear whether the JH-binding protein carrier exerts a synergistic action with JH at target tissues (SANBURG *et al.*, 1975b) or not (FERKOVICH *et al.*, 1976) the implantation of larval CA as well as of adult CA inhibits normal development, including developmental processes in the flight muscles (VAN DEN HONDEL-FRANKEN *et al.*, 1980).

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