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0014-4754/85/050683-02$1.50 + 0.20/0

Brain stimulation of juvenile hormone production in insect larvae[1]

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Summary. The titer of juvenile hormone (JH II) in *Galleria mellonella* decreases from 3 pmol/g b.wt in the penultimate to less than 0.1 pmol/g in the last larval instar. Hormone production is resumed when newly ecdysed last instar larvae either receive brain implants or are chilled on ice. The implanted brains as well as the chilled in situ brains stimulate JH production by a blood-borne allatotropin.
Key words. Wax moth; *Galleria mellonella;* juvenile hormone production; corpora allata; allatotropin, cerebral.

It has been known for fifty years that in insects a decrease or cessation of juvenile hormone (JH) secretion from the corpora allata is necessary for the termination of larval development and the initiation of metamorphosis[2]. How this change in JH production is effected, however, has never been fully elucidated. Experiments with the wax moth, *Galleria mellonella,* indicated that the larval corpora allata are stimulated by an allatotropin from the median neurosecretory cells of the brain[3]. Stimulation by the brain appears to cease at the beginning of the last larval instar. The corpora allata subsequently become insensitive to allatotropin as well as nervously inhibited[4]. This model of stimulatory and inhibitory control of corpora allata has been confirmed in experiments with another moth, *Manduca sexta*[5].

Evidence for the stimulation of the corpora allata by a cerebral allatotropin derives from the observation that brain implantations induce an extra larval molt in intact, but not in allatectomized, last instar larvae. According to an alternative explanation of these results, however, the implanted brains stimulate a precocious secretion of ecdysteroids which in turn elicit a prompt molt[6]. The nature of the molt is determined by the presence or absence of corpora allata in the larval host, because in intact larvae these glands are producing enough JH at the beginning of the last instar to support a larval molt. By contrast, allatectomized larvae lack JH and therefore undergo a pupal molt. The present study provides proof that the original interpretation of the results of the implantation studies was correct: implanted brains stimulate an increase in the body concentration of JH. It is also shown that chilling of freshly ecdysed last instar *Galleria* larvae, a treatment which causes an extra larval molt[6,7], elicits this effect by increasing the JH titer.

Materials and methods. This study utilized penultimate and last instar larvae of *Galleria mellonella* (Lepidoptera, Pyralidae) reared under standard conditions[8] and staged within ± 6 h. Last (VIIth) instar larvae 12 h after ecdysis were taken for brain implantations and chilling. Those selected for the implantation each received three brains dissected from 1-day-old last instar larvae[3,4]. Chilling involved placing larvae on melting ice for 30 min.

Duplicate or triplicate samples for JH determination each weighed about 2 g and contained 15–100 larvae, depending on their developmental stage. The larvae were frozen on dry ice, lyophilized, and homogenized in 3 ml methanol (p.a. grade, Lachema). The homogenate was left overnight at 0 °C, then centrifuged and the pellet washed three times with methanol. The pooled supernatants (30 ml) were reduced to 3 ml under vacuum at 50 °C, sealed in glass ampoules and stored for 1–6 months at −20 °C before further processing.

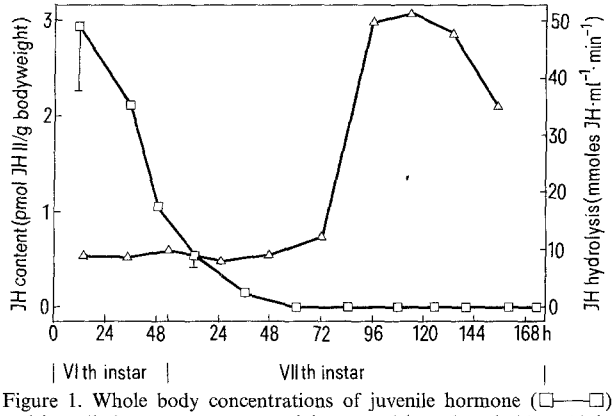

Figure 1. Whole body concentrations of juvenile hormone (□—□) and juvenile hormone esterase activity per ml hemolymph (△—△) in penultimate and last instar larvae of *G. mellonella.* Data on JH-esterase activity is reproduced from Hwang-Hsu et al.[15].

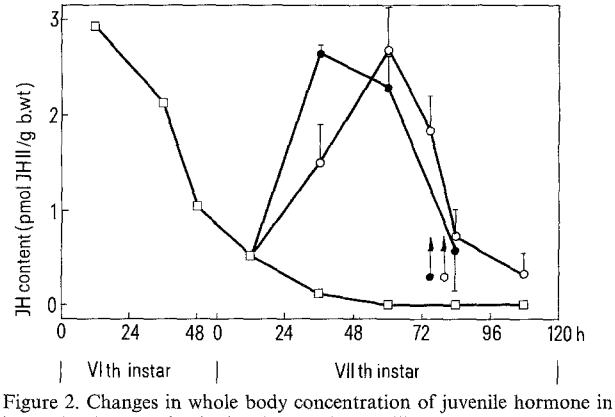

Figure 2. Changes in whole body concentration of juvenile hormone in larvae implanted with brains (●—●) or chilled (○—○) 12 h after the seventh (normally last) larval ecdysis. Hormone content of control larvae is also shown (□—□). Vertical bars indicate standard deviations and the arrows (e) the times of the supernumerary larval ecdysis.

After the addition of a defined mixture of ethyl ester homologues of JH I, II, and III as an internal standard, the extracts were purified by adsorption chromatography on silica gel and by high pressure liquid chromatography. The JHs and the internal standards were then converted to their 10-hydroxy-11-nonafluorohexoxy derivatives[9]. Following purification by high pressure liquid chromatography the samples were subjected to gas chromatography combined with selected-ion-monitoring mass spectrometry by which the individual JHs were identified and quantified[10].

Results and discussion. Only JH II was found in measurable amounts; this was consistent with the report that the corpora allata of *Galleria* produce this homologue exclusively when cultured in vitro[11]. Penultimate instar larvae contained 1–3 pmol JH II/g b.wt but this concentration rapidly decreased to about 0.1 pmol/g by 36 h after the last larval ecdysis (fig. 1). No hormone was detected later in the last instar. This developmental profile of the whole body JH titer is similar to that established with biological assays[12,13], except that the biological tests revealed a small peak of JH at the time of cocoon spinning, at about 140 h of the last instar. According to Peferoen and De Loof[13] the hormone should then rise to about 20 pg/g (i.e. less than 0.1 pmol/g). This quantity could not be detected by our physico-chemical method.

The sharp decline in JH titer at the end of the penultimate and beginning of the last larval instars is obviously caused by a reduction of hormone production and not by an increase in hormone breakdown. This is indicated by the fact that the activity of hemolymph esterases, which play the major role in JH inactivation in Lepidoptera[14], remains at a steady level during this period[15,16] (see fig. 1).

Figure 2 clearly shows that JH production resumes when freshly ecdysed last instar larvae are implanted with brains or are chilled on ice. Within 24 h after either treatment, the JH concentration rose to a value comparable to that found in early penultimate instar larvae. A decrease then occurred around the supernumerary larval molt, similar to the decrease at the time of molt from the penultimate to the last instar. The supernumerary larvae one day after ecdysis contained in average 0.5 pmol JH/g, a concentration virtually identical to that in newly ecdysed last instar larvae. Since neither brain implantation nor chilling have a significant effect on JH esterase activity[17], the increase in JH titer after these treatments must be due to stimulation of the corpora allata rather than a decrease in the rate of hormone degradation. These data provide compelling evidence that the corpora allata of *Galleria* larvae are stimulated by a cerebral allatotropin. The secretion of this factor from the brain in situ apparently ceases after the last larval ecdysis, except when the larvae are exposed to temperatures around 0 °C.

1 We are grateful to Mr B. Lackner for his expert technical assistance in the JH measurements, and to Dr. N.A. Granger, University of North Carolina, Chapel Hill, for her help with the manuscript.

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0014-4754/85/050684-02$1.50 + 0.20/0

Morphological differentiation of the growing oocyte of *Ctenomys torquatus* (Rodentia, Octodontidae)

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Summary. The ultrastructural changes observed during the growth phase of oocytes of *Ctenomys torquatus* (Rodentia, Octodontidae) are reported. Interest was particularly centered on the transformation and/or distribution of the components of the endoplasmic reticulum. According to the observations made it is suggested that the endoplasmic reticulum stores some kind of material which may support early stages of development.
Key words. Ctenomys torquatus; oocyte; endoplasmic reticulum; ultrastructure; storage materials.

Mammalian oocytes grow and differentiate after their meiotic prophase has been arrested at the dyctiate stage[1,2]. During this stage oocytes increase their volume by the synthesis of macromolecules and accumulation of organelles[3–7], some of which probably support early embryogenesis, as is the case in lower species[8].

The morphological aspects of oocyte differentiation have been the subject of several light[9,10] and electron-microscopic[11–14] studies. These studies show that oocytes from different mammalian species present similar changes during their growth phase; however, details of these changes may be quite different.

In the present paper the most conspicuous ultrastructural aspects of differentiation of oocytes of *Ctenomys torquatus* are reported. Some of these results have been published as an abstract[15].

Materials and methods. Seven adult female *Ctenomys torquatus* (Rodentia, Octodontidae) were collected in the field at Carrasco, Uruguay. The diploid chromosome number of this population is 2n = 56, as determined by Kiblisky et al.[16]. Ovaries were fixed in 2.5% buffered glutaraldehyde (pH 7.2) at 4 °C; postfixed in 1% buffered OsO_4 and embedded in Durcupan ACM (Fluka). Sections were cut in a Sorval MT2 ultrami-