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EFFECTS OF RECOMBINANT JUVENILE HORMONE ESTERASE ON *AEDES AEGYPTI*

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ABSTRACT

Recombinant juvenile hormone esterase (JHE), cloned from *Heliothis virescens*, was injected into *Aedes aegypti* larvae resulting in a dose-dependent decrease in survival. In addition, ovariole maturation in mosquitoes was impaired by injection of JHE into larvae or pupae.

Introduction.

One of the most promising strategies for mosquito control entails the use of recombinant DNA technology to disrupt the insect endocrine system. Juvenile hormone (JH) is a promising target since it regulates a variety of physiological processes in mosquitoes, including ovariole maturation (Masler et al. 1980) and blood feeding (Meola and Petralia 1980) in females. Juvenile hormone titer may also play a role in controlling initiation of mosquito metamorphosis, and reduction of JH titer at the larval stage has the potential to cause premature pupation or block development. There are a number of evolving molecular technologies which may allow us to exploit such effects for mosquito control.

Juvenile hormone titer in insects is controlled by a balance between biosynthesis and degradation, the latter primarily by two classes of hydrolytic enzymes, juvenile hormone esterase (JHE) and juvenile hormone epoxide hydrolase (Hammock 1985). Juvenile hormone esterase was cloned from *Heliothis virescens* (Lepidoptera: Noctuidae) (Hanzlik et al. 1989) and expressed in a baculovirus vector (Hammock et al. 1990). The expressed recombinant juvenile hormone esterase, acting as an anti-JH enzyme, is potentially useful for mosquito control. The goal of this study was to evaluate the effect of recombinant JHE on *Aedes aegypti* (L.) by injecting larvae or pupae with varying amounts of JHE and monitoring larval and pupal survival as well as adult ovariole maturation.

Methods.

Juvenile hormone esterase, for microinjection of larvae, was produced by a previously developed protocol for baculovirus expression in insect cell culture (Hammock et al. 1990). Triton X-100 (0.05%) was added to the cell culture medium to kill baculoviruses after JHE expression. To concentrate and purify the enzyme, baculovirus cell culture medium containing secreted JHE was loaded onto a DEAE ion exchange column and eluted with a salt gradient, 50 mM to 200 mM NaCl in 10 mM Tris-HCl at a pH of 8.5 (Ichinose et al. 1991). Before the DEAE column, the protein concentration was 0.16 mg/ml and the activity of JHE was 80 nmol JH III/min/ml. After the DEAE column, the protein concentration was 4.1 mg/ml and the activity of JHE was 10,000 nmol JH III/min/ml (approximately 75% pure JHE). The dose of JHE administered to mosquitoes will be presented in JHE activity units, defined here as 40 pmol of JH III hydrolyzed per minute. Before injection, JHE was stored at -80° C for 1-4 months in sodium azide. Control injections employed the same concentration of sodium azide that was present in the JHE treatments.

The Rock strain of *Aedes aegypti* was used for all injection experiments. Juvenile hormone esterase was introduced using a stereo dissecting microscope and micromanipulator to carefully control the site of injection. A finely drawn microcapillary tube was coupled to a Narishige microinjector to dispense small volumes of JHE into the side of the thorax. A cold light source was used for illumination during the injection process. For each insect, 1 μl of JHE was added to a finely-drawn calibrated microcapillary tube and the volume completely discharged into the body. This procedure was employed to ensure that a consistent volume was administered to each insect. Extensive methods development and practice was required to avoid excessive mortality resulting from injection of larvae or pupae. Tris-HCl buffer (pH 8.5) or tissue culture media (EX-CELL 400, J.R. Scientific, Woodland CA) were used for control injections.

Determination of ovariole development in treated and control female mosquitoes was done four days after adult eclosion, by placing them on clean microscope slides and dissecting ovarioles in drops of physiological saline under a stereoscopic microscope. After disrupting ovarioles with dissecting needles and covering them with a cover slip, individual follicles were measured under a compound microscope at 40X magnification by means of a squared reticle. Ovariole development was also scored using the scheme of Kawai (1969).

Results and Discussion.

When examined at two days after the injection of 4th stage larvae with JHE, the survival of treated larvae decreased in a dose-dependent manner (logistic regression, $p < 0.0001$)(Fig. 1). Survival to the imago stage was significantly lower for treated larvae than for control larvae (Fisher's exact test, $p = 0.0018$). Another consequence of exposure to recombinant JHE was partial eclosion, which was only observed among treated larvae and pupae (11% incomplete eclosion). A hypothesis to explain the JHE effects observed in this study would include the supposition that recombinant JHE activity has

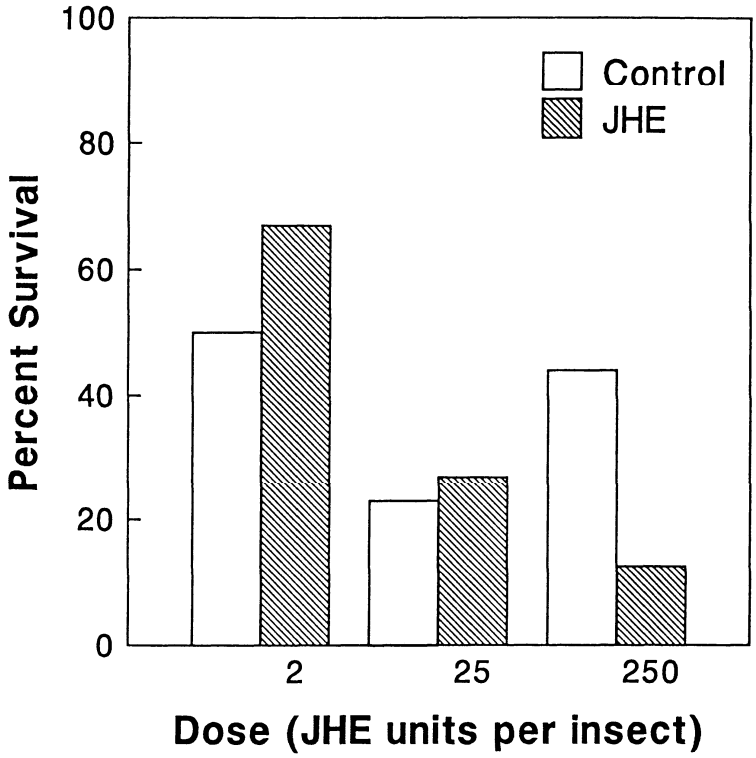

Figure 1. The effect of JHE dose on survival of *Aedes aegypti* larvae. Forty control and forty treatment last instar larvae were injected to evaluate each JHE dose.

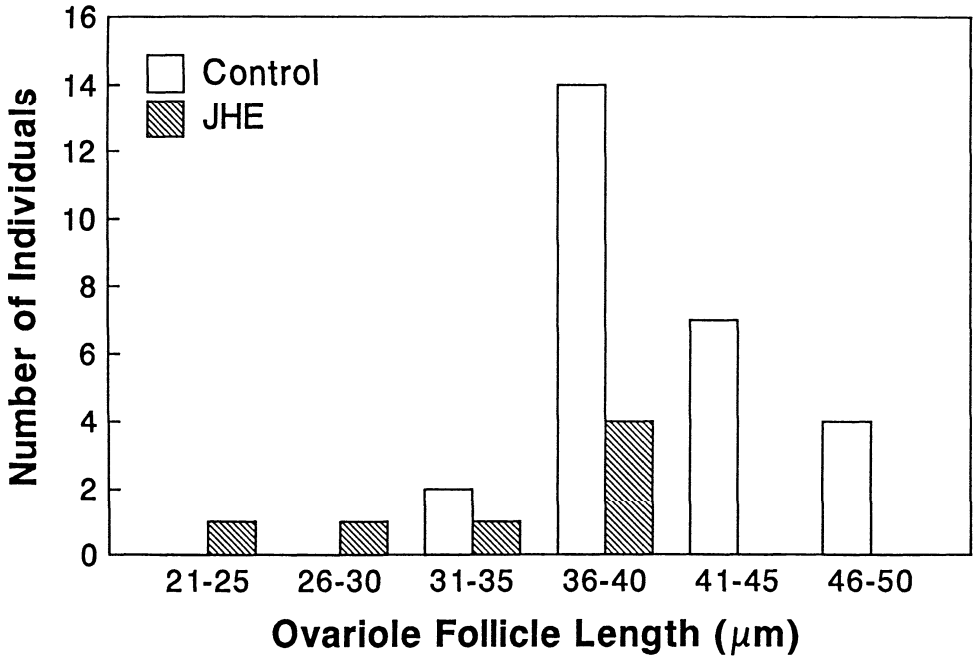

Figure 2. Distribution of mean ovariole follicle size (μm) measured in 4-day old *Aedes aegypti* females after injection of last instar larvae or pupae with JHE (pooled results from injection of 25 or 250 units of JHE per insect).

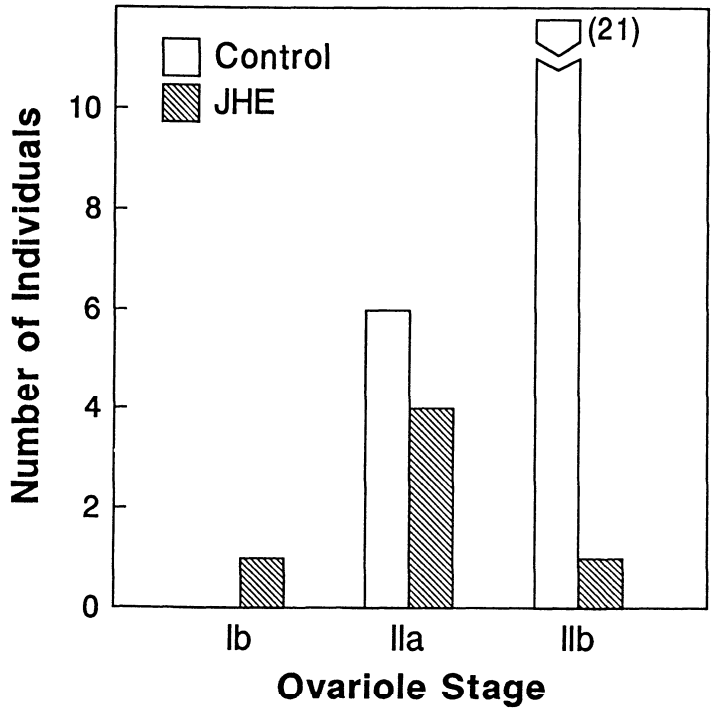

Figure 3. Number of individuals per ovariole stage after injection of last instar larvae or pupae with JHE (pooled results from injection of 25 or 250 units of JHE per insect).

been introduced in excess of analogous endogenous enzymatic activity on JH in the mosquito hemolymph and the assumption that JH titer drops accordingly. In the future, these studies can be extended to evaluate life-stage dependent physiological responses (Eldridge 1968) after perturbation of the endocrine system.

Evidence for female reproductive dysfunction following injection of larvae and pupae is presented in Figures 2 and 3. As shown in Figure 2, a significant reduction in ovariole size was observed in treated animals (Mann Whitney test, $p < 0.01$). All the treated larvae had a mean ovariole follicle length of 40 μm or less, whereas 93% of the control larvae had a mean follicle length of 36 μm or more (Fig. 2). In addition, the reproductive effect of introduced JHE was indicated by ovariole stage classification (Fig. 3). The less developed ovariole stages (Ib, IIa) were relatively prevalent among individuals exposed to JHE as larvae or pupae (Fisher's exact test, $p = 0.0046$). A noteworthy observation from this study is that perturbation at the larval stage can effect adult reproduction. In summary, JHE expressed by an appropriate mosquito vector would be useful for control, both as a mosquitocide and through deleterious effects on reproduction.

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