at a rate not exceeding 0.5 ml/mn. Then the columns were washed with 10 ml 0.02 *M* phosphate buffer (pH 6.5) containing 0.2% EDTA. Elution was performed with 1 *N* HCl. The first 6 ml of the elution contained 5-HT. Serotonin was determined fluorimetrically[7].

The results were expressed in $\gamma$/g of pineal tissue, and the standard error is measured for each 3 determinations.

*Results.* 4 days after the operation, no statistically significant differences in the quantity of autofluorescent cells (Figure 1 and Table I) and the quantity of serotonin (Figure 2 and Table II) between the castrated and sham-operated rat pineal glands were observed.

One week after the castration, the quantity of autofluorescent cells in the pineal gland of the operated rats was about 37% increased in respect to the sham-operated rat group (Figure 1, Table I). No statistically significant differences have been observed in the serotonin concentration in the castrated and sham-operated rat pineal glands (Figure 2, Table II).

Three weeks after the operation, the quantity of autofluorescent cells in the pineal gland of the castrated rats was increased, this increase being larger than after 1 week (about 66% in respect to the sham-operated group) (Figure 1, Table I). However, no statistically significant differences were observed between the serotonin concentration of the castrated and sham-operated rat pineal gland (Figure 2, Table II).

*Discussion.* Pineal response to castration appeared to be very slow. 4 days after the operation we have not observed any effect on the number of autofluorescent cells and the 5-HT levels. After 1 week, a small increase of the quantity of autofluorescent cells was observed, while after 3 weeks this increase is larger. This slowness of the pineal response is possibly due to the parameters chosen. Indeed, in other experiments using different parameters (metabolic compounds[8]), we have observed a quick response of the pineal gland to castration.

The fact that there was no modification in serotonin levels in the pineal gland of orchidectomized adult rats is interesting but difficult to interprete. It is possible that the castration does not effect the production of pineal indole derivatives but it is also possible that, after castration, the 5-HT turnover changes without any change in the 5HT levels in tissue.

The increase of quantity of cells containing autofluorescent material demonstrated that orchidectomy has an effect on the pineal gland. Just after castration, an abrupt drop of androgen plasma levels occurs. This causes a rapid increase in plasma gonadotropin[9–11]. At that moment it is not possible to determine whether the effect of castration, described in the present paper, is due to the decreasing plasma androgen level or to the increase of FSH and LH levels. Perhaps both may have an effect on the pineal gland. It is interesting that, some time after castration, a notable increase was observed of pineal protein synthesis, i.e., increase of the quantity of autofluorescent material. Intensified pineal protein synthesis is also known to occur in estrogen-treated immature female rats. This is caused by increased LH secretion by the adenohypophysis[12]. As castration also provokes an increase of LH secretion[9–11], it may therefore be that the increase of autofluorescent pinealocytes after castration is caused by increased LH secretion.

It is possible that the yellow autofluorescent material represents an active protein compound (pineal hormone ?) different to pineal indole derivatives. Under these experimental conditions, castration may be restricted to the synthesis of these active protein compounds[13].

*Summary.* The orchidectomy of the adult rat induces an increased quantity of cells containing autofluorescent material (proteinaceous material[1,2]).

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## Suppression of Pupal Esterase Activity in *Aedes aegypti* (Diptera: Culicidae) by an Insect Growth Regulator

Considerable interest has been generated by the suggestion that insect populations may be controlled by use of analogues and mimics of natural juvenile hormone[1,2]. These insect growth regulators (IGR) are believed to alter the normal hormonal balance and thus interfere with post-embryonic development; however, the precise mode of action has not yet been resolved. Slade and Wilkinson[3] have indicated that, because many IGR's are structurally dissimilar to the natural hormone, it is unlikely that their effect is mediated directly through an interaction with the natural hormone receptor. They propose that the IGR's stabilize the natural hormone by inhibiting the normal degradation pathways. One enzyme responsible for catabolism of endogenous juvenile hormone is a carboxyesterase which is induced within 30 min of the appearance of juvenile hormone[4]. The present study was undertaken to determine the effect of one IGR (isopropyl 11-methoxy-3, 7, 11-trimethyl-dodeca-2, 4- dienoate, Altosid ®) (ZR 515) on the esterases of the mosquito, *Aedes aegypti* (L.), an insect species which is particularly sensitive to the effects of IGR's[5].

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*Materials and Methods.* Experimental animals were raised from eggs obtained from a stock colony of the yellow-fever mosquito, *A. aegypti* (from a colony originating from the former Canada Agriculture laboratory at Belleville, Ontario) maintained under standard conditions of 28 °C and a photoperiod of LD: 12:12. Eggs were hatched at 1 h following the initiation of the photophase; to insure synchrony of hatch, eggs were subjected to a reduced pressure of 350 mm Hg as a hatching stimulus. 1000 newly hatched larvae were aded to 2 l of distilled water in enamel pans (9 × 14 × 2 inches), containing $^1/_4$ tsp of liver powder as a larval diet. The diet was replenished at intervals of 48 h and, during the 4th instar, at intervals of 24 h.

Fourth-instar larvae appeared at 96 h following hatching and at this time a single application of ZR 515 was added to the culture at a concentration of 0.1 ppm. This dose has previously been shown to cause 100% mortality under laboratory conditions[6]. Control and experimental animals were maintained in separate incubators to avoid contamination of control cultures due to cold distillation of the analogue. At given times during subsequent larval and pupal development, 50 individuals were removed from treated and control cultures and stored at −20 °C.

Prior to electrophoretic separation of soluble proteins, a sample of 40–60 mg (wet weight) was homogenized at 0 °C in 0.2 ml of a 25% sucrose solution containing a small amount of phenylthiourea. The homogenate was centrifuged at 3,000 *g* for 30 min and 50 µl infranatant applied to a pre-run 5% acrylamide gel for conventional disc electrophoresis[7]. The run was performed at 4 °C with *Tris*-glycine buffer (pH 8.6) and using bromophenol blue as tracker dye.

Esterase activity was detected by the method of NACHLAS and SELIGMAN[8] using α-naphthylacetate as substrate and Fast Blue BB as a diazo stain. The relative concentration of each band was estimated using a recording densitometer (Clifford Instruments Ltd.).

*Results.* Fourth-instar larvae of *A. aegypti* which have been treated with a lethal dose of ZR 515 do not display obvious adverse effects until pupation. Preliminary experiments in the present study failed to reveal any marked differences in non-specific esterases between treated and non-treated larvae.

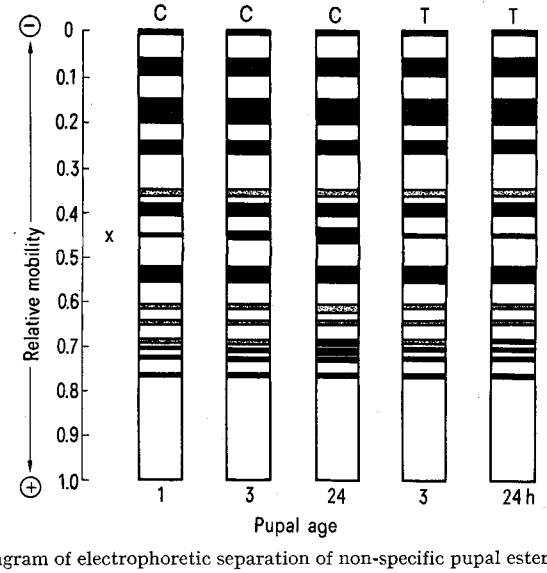


Diagram of electrophoretic separation of non-specific pupal esterases of *Aedes aegypti*, with (T) and without (C) prior larval treatment with a lethal dose of ZR 515.

Developmental changes occurring in pupal esterases have not previously been described for this species; there is a marked increase in activity of 1 fraction ('X', relative mobility 0.45) which occurs during pupal development (Figure). 13 bands were present throughout the pupal stage with only minor quantitative differences apparent at different pupal ages. The Figure also demonstrates the suppression of band 'X' due to larval treatment with a lethal dose of ZR 515. The possibility of direct inhibition of the enzyme by the analogue was tested by incubating homogenized control pupae with ZR 515 and determining enzyme activity. No loss of activity was detected when this experiment was performed[9].

*Discussion.* The ontogenesis of non-specific esterases in *A. aegypti* has been described previously[10], although no attempt was made to distinguish between the various pupal ages. The present study fills this gap and reveals a marked increase in activity associated with one esterase fraction, with increasing pupal age.

Juvenile hormone functions to favour the expression of larval characteristics and is not normally present in the pupa[11]. The absence of the hormone may result from decreased production by the corpora allata and (or) an increased rate of degradation of the hormone. One of the enzymes responsible for degradation of the natural hormone is a carboxyesterase[4], and it is possible that this enzyme may play a role in maintaining a low endogenous level of juvenile hormone during the pupal stage. Thus the increased esterase activity observed in one fraction during normal pupal development may reflect increased activity of the carboxyesterase responsible for hydrolysis of natural juvenile hormone.

Suppression of this activity by larval treatment with ZR 515 supports the proposal of SLADE and WILKINSON[3] that many IGR's function to stabilize endogenous levels of natural juvenile hormone, rather than operating antagonistically at the site of the hormone receptor. The study does not, however, explain the mechanism by which larval treatment induces the suppression of pupal enzyme.

*Summary.* Changes in non-specific esterases of *Aedes aegypti* were noted during pupal development. One esterase band was found to increase markedly within 3 h of pupation and this increase in activity was suppressed by prior treatment of larvae with an insect growth regulator, ZR 515. It is suggested that the esterase activity may help to reduce endogenous levels of juvenile hormone during metamorphosis and that the growth regulator may prevent this normal regulation.

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