

## Delayed Effects of Juvenile Hormone on Insect Metamorphosis are mediated by the Corpus Allatum

EXPOSURE of insect embryos to juvenile hormone or to any of its analogues can result in delayed effects which are realized later at metamorphosis<sup>1-5</sup>. These have been attributed to: (1) persistence of the applied hormone through larval life<sup>2</sup>; (2) interference with the overall programming of the embryo for postembryonic development<sup>1,3</sup>; and (3) the selective interference with the embryonic programming of the corpus allatum<sup>3,5,6</sup>. This study presents evidence that juvenile hormone disrupts the embryonic programming of the corpus allatum such that it does not cease secretion of the hormone as a prelude to metamorphosis.

Eggs of the linden bug, *Pyrrhocoris apterus*, were exposed to a mixture of juvenile hormone analogues<sup>7</sup> 1.5 days before hatching. A solution of 2.5 µg of the analogues in 0.1 µl. of acetone (Mallinkrodt 'Nanograde') was applied to each egg<sup>8</sup>. Eggs were placed in 100 mm plastic Petri dishes lined with Whatman No. 1 filter paper (20 eggs per dish) and supplied with linden seeds and water. Unhatched eggs and shells were removed the day after hatching, and shed skins were removed after each moult. Larvae (hereafter referred to as treated larvae) were moved to new dishes after the moult to the third and to the fifth instar.

Larvae destined for surgical manipulations were deprived of food within 6 h of the moult to the fifth (last) instar. Twenty-four hours later, the corpus allatum was removed with fine forceps through a transverse slit in the neck membrane. The gland was then implanted through a slit in the metathoracic pleuron of a recipient of similar age.

As seen in Table 1, 72% of the treated *Pyrrhocoris* larvae formed adultoids which showed effects ranging from a marked juvenilization of the wings to the formation of supernumerary sixth instar larvae. By contrast, untreated larvae in similar conditions consistently formed normal adults.

Allatectomy of the treated fifth instar larvae reversed the effects of the prior treatment with juvenile hormone (Table 1, Fig. 1). Of thirty-two animals which were allatectomized, twenty-seven formed externally normal adults, although the females in this group failed to mature eggs. Thus, the complete removal of the gland was confirmed, since *Pyrrhocoris* requires the corpus allatum for vitellogenesis<sup>9</sup>. The remaining five animals showed only minor juvenilization of the wings, although autopsy revealed no trace of the corpus allatum.

The endocrine activity of the corpus allatum was determined by gland implantation. As seen in Table 1, glands from larvae at the outset of the fifth instar were inactive<sup>10</sup>. By contrast, corpus allatum from treated larvae produced 51% adultoids.

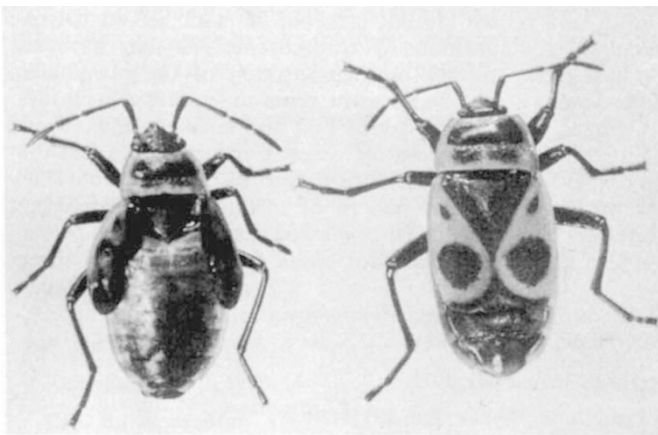


Fig. 1 Left, an untreated individual which had received the corpus allatum from the treated animal on the right at the outset of the fifth instar. The larval type wings and the red patches of larval cuticle on the ventral abdomen classify it as a +2 adultoid. Right, an externally normal *Pyrrhocoris* adult which was treated with the mixture of juvenile hormone analogues as a late embryo. It was allatectomized after the moult to the fifth instar.

The recipients showed effects which ranged up to the reformation of large patches of larval cuticle on the abdomen (Fig. 1). But these effects were not as marked as those in the donors. Similarly, removal and reimplantation of the corpus allatum of five treated larvae caused decreased juvenilization (Table 1). To test the effectiveness of transplanted glands, active corpora allata of fourth instar larvae were used; the recipients showed effects ranging from supernumerary larvae to normal adults, thus indicating the problems inherent in transplantation.

The results reported here are consistent with the experiments of Willis and Lawrence which involved grafts of integument between treated and untreated *Oncopeltus*<sup>2</sup>. Contamination due to rearing treated larvae in the same cage throughout larval life may account for their "bristle-oriented patches" of larval cuticle, which was the evidence for the conclusion that the delayed effects arise from persistence of applied hormone<sup>2</sup>. When treated *Pyrrhocoris* are left in the same dish throughout larval life, there is a slight enhancement of juvenilizing effects which we presume arises from juvenile hormone analogues or active metabolites in the excreta and cast skins.

These experiments, therefore, show that juvenile hormone can somehow interfere with the programming of the embryonic corpus allatum. This gland then fails to cease secretion of the hormone at the outset of the last larval instar.

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<sup>1</sup> Riddiford, L. M., and Williams, C. M., *Proc. US Nat. Acad. Sci.*, **57**, 595 (1967).

<sup>2</sup> Willis, J. H., and Lawrence, P., *Nature*, **225**, 81 (1970).

<sup>3</sup> Riddiford, L. M., *Science*, **167**, 287 (1970).

<sup>4</sup> Riddiford, L. M., *Develop. Biol.*, **22**, 249 (1970).

<sup>5</sup> Riddiford, L. M., in *Chemistry and Action of Insect Juvenile Hormones* (edit. by Beroza, M., and Menn, J. J.) (Academic Press, New York, in the press).

<sup>6</sup> Willis, J. H., *J. Exp. Embryol. Morphol.*, **22**, 27 (1969).

<sup>7</sup> Vinson, J. W., and Williams, C. M., *Proc. US Nat. Acad. Sci.*, **58**, 294 (1967).

<sup>8</sup> Williams, C. M., and Sláma, K., *Biol. Bull.*, **130**, 235 (1966).

<sup>9</sup> Sláma, K., and Hrubesová, H., *Zool. Jb. Physiol.*, **70**, 291 (1963).

<sup>10</sup> Sláma, K., *Zool. Jb. Physiol.*, **70**, 427 (1964).

**Table 1** Application of Juvenile Hormone Analogues to Late *Pyrrhocoris* Embryos: Effects on the Function of the Corpus Allatum at the Outset of the Fifth Instar

Treatment of embryo	Treatment of fifth instar larva	No.	Normal adults (%)	Adultoids (%) <sup>*</sup>				
				+1	+2	+3	+4	+5
None	None	713	100	0	0	0	0	0
JHA	None	54	28	11	20	13	24	4
JHA	Excise and reimplant CA	5	40	20	20	20	0	0
JHA	Excise CA	32	84	16	0	0	0	0
None	Implant CA from fifth instar larvae treated with JHA as embryo	59	49	29	22	0	0	0
None	Implant CA from untreated fifth instar larva	67	100	0	0	0	0	0

JHA, juvenile hormone analogues; CA, corpus allatum.

<sup>\*</sup> The classification of adultoids was based on the scoring system of Williams and Sláma<sup>8</sup>.

0, Normal adult; +5, supernumerary sixth instar larva.