Juvenile Hormone: Effects on a Higher Dipteran

Abstract. *Injection of* dl-*juvenile hormone or* C_{17} *methyl ester into* Sarcophaga bullata *larvae prevents puparium formation or arrests development at about the 3rd day of pupal-adult development. Topical application to the abdomens of young pupae results in the secretion of a second pupal cuticle. This is the first reported morphogenetic effect of juvenile hormone on a fly.*

There have been numerous reports on the morphogenetic effects of the juvenile hormone, or chemical mimics of the juvenile hormone, on several orders of insects (*1*), but none dealing with the higher flies. This has led to informal speculation that the Diptera possess a unique juvenile hormone. The question of juvenile hormone in flies is of more than parochial interest since *Drosophila*, a member of the higher Diptera, is the only experimental animal about which enough genetics is known for a molecular genetic analysis of differentiation. In addition, several dipterans are vectors of disease, and the juvenile hormone may well have practical importance as a "third generation pesticide" (*2*).

With the elucidation of the structure of the natural juvenile hormone from the male *Hyalophora cecropia* moth (*3*) and the preparation of a crude synthetic mixture with potent juvenile hormone activity (*4*), serious attempts could be made to demonstrate effects of juvenile hormone in higher Diptera. For these studies we used larvae and pupae of the large blowfly *Sarcophaga bullata*. The substances tested were: *dl*-juvenile hormone (*dl* mixture of methyl-*trans, trans, cis*-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate) with a biological activity of 5000 Tenebrio Units (TU)/μg; C_{17} methyl ester (methyl-*trans, trans, cis*-7-ethyl-3,11-dimethyl-2,6,10-tridecatrienoate) with a biological activity of 500 TU/μg (*5*); and a crude mixture made by bubbling HCl gas through an alcoholic solution of farnesenic acid (*4*). When tested on lepidopteran pupae the latter material is about 100 times less active than *dl*-juvenile hormone. The *dl*-juvenile hormone and C_{17} methyl ester were diluted in refined olive oil, whereas the crude mixture was diluted in peanut oil. All experiments included equal numbers of control animals receiving the diluent.

Third instar larvae of various ages were injected with 1-μl quantities of olive oil containing either *dl*-juvenile hormone or the C_{17} methyl ester. Less than 1000 TU had no effect, and 91 percent of the larvae formed normal puparia within a few hours of the controls. Injection of 2000 to 2500 TU into 1- or 2-day-old larvae resulted in the death of 70 percent of the animals. Some of these larvae lived up to 14 days, whereas they should have pupated within 3 days. Of the 30 percent that pupated, puparium formation was delayed an average of 3 days. A large percentage of the latter larvae did not assume the typical shortened form before puparium formation, and the shape of the resulting puparia approximated that of the mid-third instar larva. Two weeks later the puparia were opened (after the control flies had emerged), and we found that several of the animals had not passed the 3rd day of pupal-adult development. When 2000 to 3500 TU were injected into late (3rd day) larvae, 72 to 96 percent went on to pupate normally, but most were arrested in their development on about the 3rd day of adult development. Thus, injections into larvae prolonged larval life or interfered with normal puparium formation and in some cases resulted in developmental arrest during pupal-adult development. The latter effect is similar to that described for the action of the crude synthetic mixture (*4*) on mosquitos (*6*). We believe these results with larvae to be equivocal juvenile hormone effects since we did not obtain either superlarvae or larval-pupal intermediates. We therefore concentrated our efforts on the pupal-adult transformation.

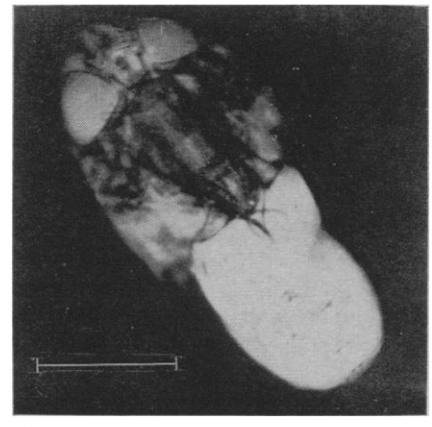

Fig. 1. Pupal-adult intermediate of *Sarcophaga bullata*; 2500 TU of *dl*-juvenile hormone were topically applied to a 52-hour-old pupa. Note normal adult head and thorax and typical pupal abdomen (scale, 3 mm).

Pieces of puparium (2 to 3 mm^2) were excised from the dorsum of the puparium of pupae of various ages, and 1-μl quantities of hormone diluted in olive oil were topically applied to various regions of the pupa within the puparium. After 30 minutes, the opening was covered with a piece from another puparium and sealed with wax. Controls, similarly treated, received only olive oil and never showed morphological abnormalities upon adult emergence. When the controls completed adult development, the puparia of the pupae treated with juvenile hormone were opened, and the organisms within were examined under the dissecting microscope and in several cases, histologically. Table 1 reveals that 1000 or less TU had little or no effect on development. However, the application of 2000 to 2500 TU to the abdomens of young pupae (within 58 hours of the onset of tanning of the puparium) resulted in about 75 percent of the animals having the appearance of pupal-adult intermediates (Fig. 1). The head and thorax appear typically adult, with antennae, mouthparts, legs, and an adult cuticle bearing normally pig-

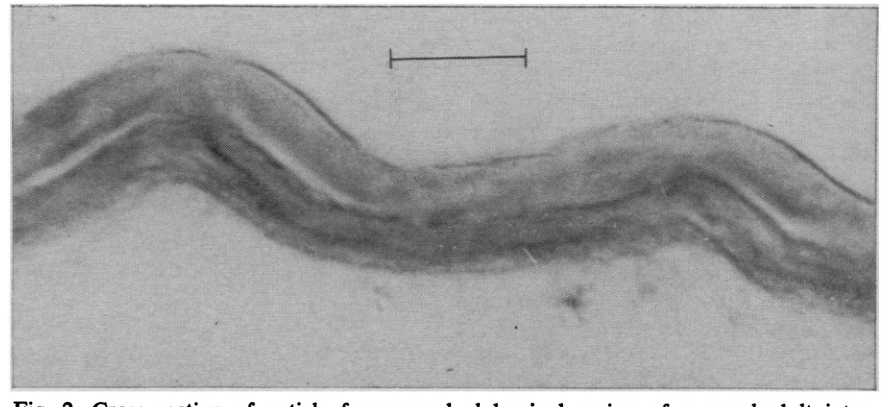

Fig. 2. Cross section of cuticle from pupal abdominal region of a pupal-adult intermediate. Note the two layers of pupal cuticle. The top cuticle is the original pupal cuticle; the lower is the newly secreted second pupal cuticle (scale, 10 μ).

Table 1. Effects of juvenile hormone on *Sarcophaga bullata* pupae. The juvenile hormone and methyl ester were diluted to 1 μl in olive oil and applied topically to the pupal abdomen. Control animals received 1 μl of olive oil, and all emerged as normal adults. The age of the pupa is determined from the onset of tanning of the puparium.

Dose (Tenebrio Units)	Pupal age (hr)	Animals (No.)	Pupal-adult intermediates (No.)
	dl-juvenile hormones		
500	50–55	25	0
1000	50–55	10	0
2000	53–58	20	16
2500	46–52	10	10
2500	70–75	15	0
5000	90–95	10	0
	C_{17} methyl ester		
500	50–55	30	1
1000	48–52	20	0
2500	46–50	10	9
2500	50–55	32	27
2500	65–70	12	6
2500	72–76	12	0

mented hairs and setae. However, the abdomen is typically pupal, with a smooth, transparent cuticle devoid of hairs and pigmentation. The external genitalia are undeveloped or incompletely developed, and the fat body can be seen clearly through the cuticle. Histological sections of eight of these intermediates showed that the head, thorax, and occasionally part of the abdomen were covered by typical adult cuticle. However, in the "pupal" region of the abdomen there are two layers of cuticle (including exo- and endocuticle), one being the original pupal cuticle which had not been digested away and a new pupal cuticle interior to it (Fig. 2). Even the fat body of the pupal region is composed of spherical masses typical of the early pupal stage, whereas in the adult region of the fat body cells have separated normally. Thus, the application of *dl*-juvenile hormone or C_{17} methyl ester induced the epidermal cells of the abdomen to lay down a second pupal cuticle rather than adult cuticle, and this is without doubt a true juvenile hormone effect.

In virtually all corresponding experiments on pupae older than 46 hours in which the hormone was applied to the head or thorax, these parts developed normally, although the abdomen was sometimes affected. In six of ten young pupae (40 to 44 hours after onset of tanning of puparium) receiving 2500 TU of the C_{17} methyl ester, portions of the head and thorax were induced to lay down a second pupal cuticle. It thus appears that the epidermal cells of the head and thorax are determined earlier than those of the abdomen, and, if the technical difficulties involved in applying the hormone at an earlier stage (before the pupal cuticle separates from the puparium) are overcome, we should be able to induce the formation of complete second pupae. It is likely that the imaginal disks of the head and thorax are refractory to the juvenile hormone by 40 hours after initiation of tanning of the puparium.

Using the crude synthetic mixture discussed above (*4*), we conducted two series of experiments. One-microliter samples containing 0.02 μl of the mixture and 0.98 μl of peanut oil were applied to the abdomens of 20 pupae (48 to 56 hours old). Nineteen of the twenty developed into pupal-adult intermediates indistinguishable from those treated with *dl*-juvenile hormone except for a paucity of pigmentation on the head and thoracic hairs. We have not identified the active principle in the mixture, although the major component is the methyl ester of 3,7,11-trimethyl-7,11-dichloro-2-dodecenic acid which exhibits potent juvenile hormone activity when tested on *Pyrrhocoris apterus* (*7*).

Our data demonstrate that the higher Diptera do respond to juvenile hormone when it is applied to the organism at the correct stage and suggest that the natural dipteran juvenile hormone is the same or very similar to that obtained from the *H. cecropia* moth. Further, a unique chemical tool is now available to the developmental geneticist and molecular biologist interested in studying animal differentiation.

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5-Diazo-4-Oxo-L-Norvaline: Reactive Asparagine Analog with Biological Specificity

Abstract. *The* L-*asparagine analog, 5-diazo-4-oxo-*L-*norvaline, specifically inactivates* L-*asparaginase and inhibits the growth of* L-*asparagine-dependent or* L-*asparaginase-sensitive tumor cells in culture. With 5-^{14}C-labeled compound, a biphasic incorporation into sensitive cells occurs, but the inhibition of cell multiplication is manifest much later than the rapid phase of incorporation of the analog.*

The inhibition of the growth of certain transplantable and spontaneous tumors by the enzyme L-asparaginase [L-asparagine amidohydrolase (E.C. 3.5.1.1)] has been briefly reviewed (*1*) and extended to the treatment of human disease with the enzyme from *Escherichia coli* (*2*). An alternate approach in the treatment of these selected neoplasms would be the development of L-asparagine analogs that might act as antagonists of the synthesis or utilization of L-asparagine. Such an analog is 5-diazo-4-oxo-L-norvaline (DONV). An improved preparation and some biological properties of this compound are now reported.

The synthesis of DONV was first reported by Liwschitz *et al.* (*3*). The difficult stage in the synthesis was the removal of the protecting trifluoroacetyl and methyl ester groups from

Table 1. Specificity of action of diazo-oxo-norvaline. Asparaginase (0.5 ml of guinea pig serum in a total volume of 1 ml of solution) and glutaminase (2.5 mg in a total of 0.5 ml solution) were first incubated at *p*H 8.5 with the inhibitors and protecting agents as indicated for 30 minutes. Enzymic activity was then determined after gel filtration to remove small molecules. Activity is expressed as the number of micromoles of NH_3 liberated per hour per milligram of protein.

Prior treatment		Activity (μmole $hr^{-1}mg^{-1}$)
Compound	Concentration	
	Asparaginase	
None		2.3
DONValine	$6 \times 10^{-4}M$	0.6
DONLeucine	$6 \times 10^{-4}M$	2.3
DONValine	$6 \times 10^{-4}M$	
+ Asparagine	$5 \times 10^{-2}M$	1.8
+ Glutamine	$5 \times 10^{-2}M$	0.7
DONValine	$1 \times 10^{-4}M$	
+ aspartic acid (*p*H 4.5)	1×10^{-2}M	2.1
+ aspartic acid (*p*H 8.5)	$1 \times 10^{-2}M$	0.8
	Glutaminase	
None		18.5
DONValine	$6 \times 10^{-4}M$	18.6
DONLeucine	$6 \times 10^{-5}M$	0.4