separation of those biochemical properties of a malignant cell associated with growth from those related to invasion and metastasis.

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Prevention of Metamorphosis by Exposure of Insect Eggs to Juvenile Hormone Analogs

Abstract. *Metamorphosis of the bugs* Pyrrhocoris apterus *and* Oncopeltus fasciatus *is blocked by the application of juvenile hormone analogs to the eggs 4 weeks earlier. One or more supernumerary larval molts occur to form "giant" larvae which routinely die without undergoing metamorphosis. When the corpora allata were excised at the outset of the fifth larval stage, the entire phenomenon vanished and all individuals underwent normal metamorphosis. The inhibition of metamorphosis can therefore be attributed to a continuation of the secretion of endogenous juvenile hormone by the corpora allata of mature larvae derived from eggs treated with juvenile hormone.*

The capacity of juvenile hormone to block the embryonic development of insects was discovered by Sláma and Williams (*1*) in their study of the sensitivity of the eggs of the bug *Pyrrhocoris apterus* to the juvenile hormone analog "juvabione." Topical application of juvabione to mated females or to the freshly oviposited eggs prevented the eggs from hatching. In both cases embryonic development proceeded to a certain stage and then stopped. Similar effects have been observed after application of other juvenile hormone analogs to the eggs of silkworms (*2*) and locusts (*3*).

Studies of the effects of juvenile hormone (JH) analogs on silkworm eggs (*2, 4*) provided the first indication that JH can also have latent effects which are realized days or weeks later during postembryonic development. In *Pyrrhocoris* and *Oncopeltus*, latent effects of JH are also seen. Deaths during larval life, especially at the time of molting, are prevalent among larvae hatching from eggs treated during the first 4 days after oviposition or from the eggs of females treated with JH (*2, 5, 6*). I report here that metamorphosis can be completely blocked by exposure of the late embryo to JH.

I used a mixture of JH analogs that was synthesized as described (*7*). The JH was dissolved in acetone (Mallinckrodt "Nanograde") and dispensed from a 30-gauge needle fitted with a polyethylene tip and sealed onto a 50-μl microsyringe (Hamilton Co.); the latter was operated by an Agla micrometer (Burroughs Wellcome & Co.).

Eggs were collected from mated female *Pyrrhocoris apterus* and *Oncopeltus fasciatus* and incubated at 27°C and 60 percent relative humidity under 17 hours of daily illumination. Specific amounts of JH were topically applied in 0.05 μl of acetone to eggs anchored on Scotch tape. Control eggs received either pure acetone or no treatment. The eggs were carefully removed from the tape and placed in petri dishes at 27°C. Unhatched eggs and egg shells were removed. A maximum of 20 hatched larvae were reared in each dish to which a cotton-plugged vial of water was added along with linden seed for *Pyrrhocoris* and milkweed seed for *Oncopeltus* (*8*). About a month later, adultoids and sixth instar larvae were separated according to type and reared until death.

Application of 2.5 μg of JH to *Pyrrhocoris* eggs 121 to 168 hours after oviposition (about 6 to 30 hours before hatching) was fully effective in preventing the metamorphosis of fifth instar larvae (Table 1). Nearly all molted into perfect sixth stage larvae, and, of these, 16 percent molted to perfect seventh stage larvae. All ultimately died without initiating metamorphosis. This dose is about three times the amount (0.8 μg) which, when applied to a freshly molted fifth instar, produced a supernumerary sixth instar larva (*7, 9*). Treatment of the eggs with a dose of 0.25 μg usually produced adultoids of types I through III (*10, 11*).

The latent effects of JH were studied by applying 1 μg of H^3-juvabione (*12*) to *Pyrrhocoris* eggs at hour 144 of incubation. After the material was homogenized in 15 ml of toluene scintillation fluid containing 10 percent Biosolve (Beckman Instruments, Inc.), the total radioactivity were measured in triplicate samples of eggs and of individual larvae after hatching and after each larval molt. Radioactivity showed a rapid decline and none was detectable after the molt to the third instar.

A conspicuous feature of embryonic development is the onset of eye pigmentation which occurs immediately after blastokinesis. In *Oncopeltus* pigmentation begins after 105 hours of incubation at 27°C, and hatching occurs after 156 hours; the corresponding values for *Pyrrhocoris* are 115 and 174 hours. Prior to blastokinesis the effects of JH were mainly on embryogenesis and larval development (*1, 2, 5*). Thus, when 0.25 μg of JH was applied to 20 *Oncopeltus* eggs at hour 84, four failed to hatch and two failed to complete the molt which occurs immediately after hatching. Five of the remaining larvae subsequenlty died during one of the larval molts.

When the exposure to JH was post-

Table 1. Delayed effects of treatment of hemipteran eggs with synthetic juvenile hormone. The abnormal and normal adult percentages do not sum to 100 percent because of mortality not connected with the experiment.

Time of application (hours)	Hatched eggs (No.)	Abnormal (%)				Normal adults (%)
		Sixth	Pharate sixth	Adultoids	Sterile adults	
Pyrrhocoris apterus						
			Control			
No treatment	50	0	0	0	0	88
			Acetone (0.05 μl)			
121, 168	28	0	0	0	0	93
			Juvenile hormone (0.25 μg)			
109	16	0	0	6	6	81
121	15	0	0	13	44	43
144	27	0	0	85	0	15
157–168	24	0	0	73	0	8
			Juvenile hormone (2.5 μg)			
109	13	23	0	46*	0	0
121	10	90†	0	0	0	0
144	18	100†	0	0	0	0
157–168	23	74†	9	0	0	0
Oncopeltus fasciatus						
			Control			
No treatment	50	0	0	0	0	96
			Acetone (0.05 μl)			
105, 128, 153	28	0	0	0	0	90
			Juvenile hormone (0.25 μg)			
105	8	0	0	25	0	25
128	52	0	0	66	2	2
153	17	0	0	18	59	14
			Juvenile hormone (2.5 μg)			
105	27	22	59‡	0	0	0
128	30	7	40‡	33	0	0
153	15	0	87‡	7	0	0

* Nearly perfect sixth instar larvae except for slight genitalia development. † At least one-half of these sixths molted to seventh stage larvae or became pharate sevenths. ‡ About 20 percent of these died at the end of the fifth instar without definite signs of molting.

poned until after the onset of eye pigmentation, hatching and larval development nearly always occurred in a normal manner, and abnormalities first appeared weeks later at the time of metamorphosis (Table 1). The eggs of both species were maximally sensitive about a day before hatching, that is, at a stage when the pharate first instar is undergoing a rapid terminal phase of growth and differentiation.

These effects on metamorphosis are similar to those encountered after the application of JH to freshly molted fifth instar larvae (*10*). One difference was evident in the sixth stage *Pyrrhocoris* that underwent a further supernumerary molt. Sixth instars produced by JH treatment of fifth instar larvae molted into adultoids (*13*), whereas those produced by JH treatment of the eggs molted into perfect seventh instar larvae. This implies that the effect of JH administered to the larvae requires the persistence of hormone and therefore diminishes as the hormone is metabolized. By contrast, the effects of hormone administered to the eggs are not diminished. Indeed, as seen in the experiments utilizing tritiated juvabione, the effects on metamorphosis are realized long after the administered analog has disappeared.

One possibility is that exposure of the egg to JH interferes with the programming of the corpus allatum for the cessation of JH secretion which must take place before metamorphosis (*15*). The aberrations in metamorphosis would then be directly attributable to the continued presence of high titers of endogenous hormone (*16*). To test this hypothesis, corpora allata were removed from freshly molted fifth instar *Pyrrhocoris* which had been treated with 2.5 μg of JH as late embryos. These allatectomized animals molted to normal adults, thereby confirming the role of the corpus allatum in the production of supernumerary larval instars. The endocrine activity of the excised glands was assayed by implanting them into freshly molted fifth instar larvae (*15*). The loose gland showed little or no endocrine activity when tested in this manner. Apparently, the nervous connections of the corpus allatum are responsible for the abnormal activity of glands of mature larvae derived from eggs treated with JH. In this sense the basic defect provoked by JH treatment of the eggs is a failure of the neurotropic mechanism to inactivate the corpus allatum at the outset of the fifth instar (*17*).

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8. To control for possible contamination of the dishes by excretion of the JH analogs, 100 *Pyrrhocoris* eggs were treated with JH at 144 hours. Fifty were raised in the same dishes (10 to 15 per dish) throughout larval life; the other fifty were transferred to clean dishes after each molt. Sixth instar larvae and adultoids were produced in each group.
9. The relation between the effects produced by application to a freshly molted fifth instar and by application to the embryo varied with the particular hormonal mixture used. When *Pyrrhocoris* eggs were treated at 144 hours with 0.08 μg of a different JH preparation, 33 percent of the hatched larvae became sixth instar larvae. Yet this same dose applied to freshly molted fifth instar larvae produced type III adultoids, just as did the preparation used here (*7*).
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11. During this study *Pyrrhocoris* adultoids were classified as type I through IV, according to the system outlined by Williams and Sláma (*10*); *Oncopeltus* adultoids were classified according to W. S. Bowers [*Science* **161**, 895 (1968)].
12. The juvabione was tritiated by catalytic exchange with H^3-water by the New England Nuclear Corporation. The purified H^3-juvabione had a specific activity of 2.1 c/mmole; 1 μg produced a type III adultoid in the *Pyrrhocoris* assay (*10*). The same results were obtained with synthetically prepared tritiated methyldichlorofarnesoat [M. K. Romanuk, K. Sláma, F. Sorm, *Proc. Nat. Acad. Sci. U.S.* **57**, 349 (1967)] provided by Dr. K. Sláma.
13. Sixth instar *Pyrrhocoris* larvae produced by 1 μg of the synthetic JH mixture topically applied at the beginning of the fifth instar either died or formed type I or II adultoids at the succeeding molt. The sixth instar *Oncopeltus* produced by 1, 5, or 10 μg did not survive to molt again. Similar effects with the production of a few normal adults after a supernumerary larval instar have been reported for *Rhodnius prolixus* (*14*).
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17. The inactivation of the corpora allata by the brain at the outset of the final larval instar has been shown in *Rhodnius* [V. B. Wigglesworth, *J. Exp. Biol.* **25**, 1 (1948); (*14*)].
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