of the secondary recipients to survive (Table 1), it is possible to estimate that the GVL reaction, as employed in these experiments, reduced the number of leukemia cells from about 10^7 to close to 10^2.

Because of the many assumptions made in attempting to analyze the cellular events which took place in these experiments, it is not possible to state the precise number of leukemia cells killed by the GVL reaction. Nonetheless, some of the capacities and limitations of adoptive immunotherapy are pointed up when the data are reviewed within the framework of current cytokinetic theory. The results reported here suggest that adoptive immunotherapy, under carefully controlled conditions, may prove useful as an adjunct to conventional antileukemic therapy.

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Juvenile Hormone Mimics: Effect on Cirriped Crustacean Metamorphosis

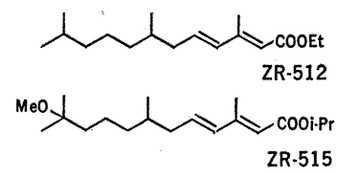
Abstract. *A synthetic juvenile hormone mimic has been shown to cause premature metamorphosis of the cyprid larva of an acorn barnacle in concentrations as low as 10 parts per billion in filtered seawater. The effect of a juvenile hormone mimic on a crustacean has not previously been demonstrated.*

In 1956 Williams (*1*) recognized the potentialities of the cecropia juvenile hormone as an insecticide. Shortly after the structure of the juvenile hormone became known (*2*), and synthetic material had been tested as an insecticide, it became apparent that the cecropia juvenile hormone lacked the necessary chemical stability and species specificity for widespread agricultural use. Meanwhile, juvenile hormone activity was demonstrated for several natural and synthetic compounds. Moreover, some of these compounds exhibited the desired chemical stability and some specificity (*3*).

Two reports led us to believe that juvenile hormone mimics might affect the metamorphosis of crustaceans. Schneiderman and Gilbert (*4*) found that the richest source of juvenile hormone outside of insects was the eyestalk of Crustacea and suggested that juvenile hormone plays a role in crustacean physiology. Furthermore, closely related steroidal molting hormones have been isolated from both insects and crustaceans (*5*).

For the test organism we decided on the acorn barnacle, *Balanus galeatus* (L.) because one of us (E.D.G.) was thoroughly familiar with its rearing and development from larval to adult stages (*6*). The barnacles, members of the subclass Cirripedia, possess a developmental pattern that is analogous to that of the holometabolous insects. Acorn barnacles generally pass through six free-swimming, feeding larval stages (nauplii), followed by a distinct motile but nonfeeding cyprid stage whose function is to find a suitable substrate for settlement where it will attach and metamorphose into the sessile adult form.

We chose to test the juvenile hormone mimics ZR-512 (ethyl 3,7,11-trimethyldodeca-2,4-dienoate) and ZR-515 (isopropyl 11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate)



since both compounds were undergoing field trials as insecticides. The two insect hormone mimics were dissolved in ethanol to make stock solutions (25 mg/ml) which were stored at − 10°C. Aliquots were diluted such that addition of 0.5-ml portions of the ethanolic solutions to 200 ml of filtered seawater yielded final concentrations ranging from 0.1 to 500 ppb (parts per billion). Ethanol (0.5 ml) was added to the filtered seawater (200 ml) in the control experiments.

Adult *B. galeatus* collected from the nearshore waters off the coast at La Jolla provided the larvae, which were reared through naupliar and cyprid larval stages according to established methods (*6*). Preliminary attempts to test the juvenile hormone mimics on the naupliar stages proved unsuccessful and were abandoned. Tentative results showed no apparent effects on nauplii through stage 4, although it was determined that hormone mimic concentrations greater than 500 ppb were lethal to larvae.

Cyprids which were kept at room temperature (25° ± 2°C) in beakers containing various concentrations of ZR-512 in seawater metamorphosed prematurely without attaching to a substrate (*7*). While cyprids normally continue to swim or "walk" at least 30

Table 1. Terminal state of cyprids in ZR-512 experiments. The average duration of each experiment was 6 days. The total number of cyprids was 1129; M, metamorphosed; U, unmetamorphosed; *N*, number of replicate experiments.

ZR-512 (ppb)	Number of cyprids			*N*
	M	U	Dead	
Control	0	131	27	6
0.1	0	71	8	4
1	0	92	12	5
10	140	14	29	9
50	158	0	11	6
100	141	0	17	7
250	76	0	6	4
500	180	2	14	8

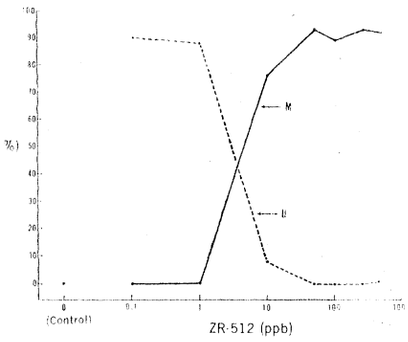


Fig. 1. Percentage of metamorphosed (*M*) and unmetamorphosed (*U*) cyprids at each concentration of ZR-512, showing a threshold value between 1 and 10 ppb. The average mortality for each concentration was 11 percent.

days, the cyprids in the experimental vessels ceased their searching behavior within several hours and lay on their sides with their attachment appendages free of the bottom. This was taken as an indication of the onset of metamorphosis. Complete adults with beating cirri appeared as early as 24 hours after the initial exposure. Some cyprids initiated metamorphosis but failed to attain the normal adult form.

After an average duration of 6 days, the numbers of metamorphosed, unmetamorphosed, and dead cyprids were recorded (Table 1). These results are expressed graphically in Fig. 1, which indicates a threshold concentration between 1 and 10 ppb.

Time effect tests on a small sample ($N = 100$) indicated that 3 hours of exposure to 100 ppb of ZR-512 was sufficient to produce a total effect. One hour of exposure to the same concentration caused only 50 percent of the cyprids to metamorphose.

Experiments with ZR-515 showed that it did not cause premature metamorphosis, nor did this hormone mimic prevent settling and metamorphosis of cyprids that were provided with the proper substrate.

The preceding results are confined to the effects of two synthetic hormone mimics on a single crustacean species. We cannot come to the conclusion that these potential insecticides will disrupt the marine crustacean life cycles without first testing their action on other important groups, such as decapods, copepods, and amphipods.

It appears that the compound ZR-512 may be a useful tool in studies of barnacle development (*8*). There is a further possibility that a synthetic juvenile hormone mimic might be developed as an antifouling agent, since there can be no doubt that an unattached barnacle is a dead barnacle.

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Evoked Potential Correlates of Expected Stimulus Intensity

Abstract. *The electrophysiological responses to a flash of medium intensity have different wave shapes in trials in which the occurrence of bright stimuli or dim stimuli is expected. When a bright or dim stimulus is signaled, the potentials evoked by the medium stimulus resemble the responses evoked by a real bright or dim flash.*

The wave shape of a visual evoked potential (VEP) is primarily determined by two factors: (i) the physical parameters of the stimulus that can be changed by the experimenter, and (ii) the significance or meaning of the stimulus that is dependent on subjective experience. With regard to the first factor, it has been demonstrated that stimulus intensity (*1*), spectral composition (*2*), and frequency and duration (*3*) influence the VEP. Modification of the VEP by the second factor has been reported with regard to uncertainty about the nature of the forthcoming stimulus (*4*). It has been shown that affective meaning can significantly alter the response evoked by a visual stimulus (*5*). We have reported on changes in the VEP by classical conditioning. We found that during acquisition, changes in the significance of the stimulus were accompanied by modifications of the later activity of the evoked potentials (*6*).

In the experiment reported here, different wave shapes were obtained for potentials evoked by visual stimuli of constant intensity, whose perceived intensities were modified by psychological set. All data were derived from monopolar scalp recordings of 20 college students. One active electrode was located on the midline, 2.5 cm above the inion (Oz); the other was at the vertex (Cz). The electrode at the left earlobe was used as reference, and that at the right earlobe as ground. Evoked potentials were recorded by means of a Grass model 78 wide-band a-c electroencephalograph amplifier, whose low-frequency cutoff filter was set at 0.3 hertz. The high-frequency cutoff filter of the driver amplifier was set at 100 hertz, and the gain at 5 μv/mm. The evoked potentials were summed by a Mnemotron computer (CAT 1000) and written out on a Moseley XY plotter.

The subject was seated in an acoustically shielded enclosure, so that he was looking directly into a viewing hood which was flush against the one-way mirror of the enclosure. On the other side of the glass window a Grass PS-2 photostimulator was mounted and set at No. 2 intensity. The stimuli were presented in front of the photostimulator located 50 cm from the subject's eyes and subtended the central 20° of the visual field.

The visual stimuli were flashes transmitted through three different neutral density filters, which were used to reduce the light intensity of the photostimulator by a definite ratio. The dim stimulus corresponded to 20 percent transmittance and the bright stimulus to 80 percent transmittance. For the stimulus of medium intensity, we used a filter that allowed 50 percent transmittance. All stimuli were 2-inch ($\sim$ 5-cm) squares which were placed in a random access projector.