The Cecropia juvenile hormone (JH) has been shown to have a number of morphogenetic effects on Drosophila (1-4). These include on the abdominal tergites and sternites a reduction in bristle size, abnormal bristle morphology, a reduction in bristle number, regions without any hairs (trichomes), regions without any pigment, failure of male genitalia to rotate and decrease in eclosion frequency. In order to provide a useful JH assay, these parameters have here been quantified.

Oregon RC animals were cultured as usual and individual animals were collected as white prepupae, either before or after JH treatment, to provide animals of known age at the time of hormone application. JH was dissolved in acetone and delivered topically to animals in 0.3 μl drops. Eclosed or uneclosed animals were fixed in 70% ethanol 7 days after pupariation.

The abdominal cuticle was mounted between two cover glasses and examined under 400X magnification. The 5th tergite of the male and the 6th tergite of the female were scored, as were the three posterior most sternites. A total of 1071 JH treated animals provide the data for Figures 1 and 2. An additional 359 animals provide the data in Table 1.

Table 1. Relative abilities of 24 JH analogues to block metamorphosis.

<table>
<thead>
<tr>
<th>Analogue</th>
<th>% aberrant bristles per tergite</th>
<th>Cecropia JH equivalent*</th>
<th>Analogues</th>
<th>% aberrant bristles per tergite</th>
<th>Cecropia JH equivalent (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epoxygeranyl-sesamole</td>
<td>95</td>
<td>3,400</td>
<td>Bishomofarnesoate</td>
<td>1</td>
<td>2</td>
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<tr>
<td>Iso C17JH</td>
<td>55</td>
<td>120</td>
<td>Williams-Law</td>
<td>0.4</td>
<td>2</td>
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<tr>
<td>C16JH</td>
<td>39</td>
<td>56</td>
<td>Ethyl dichloro farnesoate</td>
<td>0.2</td>
<td>1</td>
</tr>
<tr>
<td>C18JH</td>
<td>36</td>
<td>34</td>
<td>Farnesenic acid</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td>C18JH</td>
<td>19</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18JH + C16JH</td>
<td>19</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18JH + sesamex</td>
<td>13</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C17JH</td>
<td>18</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epoxygeranyl-p-ethylbenzene</td>
<td>19</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Epoxygeranyl-p-propylbenzene</td>
<td>19</td>
<td>19</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Epoxygeranyl-p-propylbenzene</td>
<td>8</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Geranyl sesamole</td>
<td>5</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloromethyl C16JH</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16episulfide</td>
<td>4</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

*Amount of cercropia JH required to cause the same degree of abnormality.
To insure a sensitive assay the most sensitive developmental stage must be found. Figure 1 shows how four parameters - eclosion frequency, angle of rotation of male genitalia, fre-
quency of abnormal tergite bristles, and bristle number - vary with age. Each point repre-
sents 10 - 20 animals. The most sensitive stage includes the first 10 hours after eclosion.

Figure 2 shows the dose-
response curve for sternite and
tergite bristle number, and eclo-
sion frequency. The threshold
response for the posterior stern-
ites is 0.002 µg/individual.
Figure 3 shows the sternites from a
graded series of JH doses, as
well as normal adult cuticle and
normal pupal cuticle.

Several JH analogues were
tested using this assay, and the
results are given in Table 1. All
analogues were delivered at 34 µg/
gm animal, and the results are re-
corded as the dose of cecropia JH
required to give quantitatively
similar results.

The dose resulting in 50%
morphological inhibition of meta-
morphosis (I.D.50 Morph.,5) is 5 µg
/g live weight. The I.D.50 Morph.
for topically applied Cecropia JH
is about 8 µg/g for Tenebrio and
25 µg/g for Pyrrhocoris (5,6). So
Drosophila is about as sensitive
as some other insects. The JH
sensitive phase is during the
period the abdominal histoblasts
are dividing most rapidly (7-10).

Figure 2. Dose-response curve
for JH applied to white pre-
pupae. Filled circles: failure
to eclose; Squares: % aberrant
bristles on tergite; Stars: %
aberrant bristles on sternite.

Figure 3. Response of stern-
ites to graded dosages of JH.
A. Acetone treated control
sternite. B. .002 µg JH/
animal. C. .016 µg JH/animal.
D. .05 µg JF/animal. E. .16
µg JH/animal. F. Pupal
cuticle from an acetone treated
control. ab: abnormal bristle;
ac: aberrant cuticle.

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References: see next page.


Entomol. 12:229, 1967). Since metabolic activation of indirect carcinogens such as aryldialkyltriazenes and azoxyalkanes is also performed by mixed-function oxidases (R. Preussmann et al., Ann. Acad. Sci. 163:697-716, 1960), the question that presented itself was whether a similar effect might occur with respect to chemical mutagens.

The strains selected to study this question were our wild strain Berlin K and a resistant one, Hikone R. Dosage-mortality effects and the induction of X-chromosome recessive lethals were analyzed by treating adult males of the two strains. 1-2 day old males were exposed to test solutions of 1.0 mM/1 2,4,6-trichloro-phenyldimethyltriazene or 1.3 mM/1 azoxymethane for three days and recessive lethals tested for. To recover stage-dependent sensitivity differences, three broods of three days duration each were set up (Table 1).

Table 1. Frequencies of X-chromosome recessive lethals induced by 2,4,6-trichloro-phenyldimethyltriazene (a) and azoxymethane (b).

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Strain</th>
<th>Brood I lethal/chrom.</th>
<th>%</th>
<th>Brood II 1/chr.</th>
<th>%</th>
<th>Brood III 1/chr.</th>
<th>%</th>
<th>I-III (II) 1/chr.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Berlin K</td>
<td>131/553</td>
<td>23.7</td>
<td>102/364</td>
<td>28.0</td>
<td>sterile</td>
<td></td>
<td>233/917</td>
<td>25.4 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Hikone R</td>
<td>29/617</td>
<td>4.7</td>
<td>39/603</td>
<td>6.5</td>
<td>20/753</td>
<td>2.7</td>
<td>88/1973</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td>b</td>
<td>Berlin K</td>
<td>6/617</td>
<td>0.97</td>
<td>27/582</td>
<td>4.6</td>
<td>2/202</td>
<td>0.99</td>
<td>35/1401</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Hikone R</td>
<td>5/608</td>
<td>0.82</td>
<td>49/603</td>
<td>8.1</td>
<td>sterile</td>
<td></td>
<td>54/1211</td>
<td>4.5 ± 0.6</td>
</tr>
</tbody>
</table>

The experiments revealed pronounced differences in mutation frequencies between both strains. Recessive lethals were induced to a much greater extent in Berlin K males by the triazene, while more lethals were produced by azoxymethane in Hikone R males. Analyses of the data from the different brood pattern experiments (I - III) using the $\chi^2$ test revealed highly significant differences between the samples.

With the compounds so far tested, there was a positive correlation between toxicity and genetic activity for triazenes and azoxyalkanes. Triazenes were more toxic to Berlin K males, while Hikone R males showed higher sensitivity to azoxymethane (as well as the structural isomer of azoxyethane-diethylnitrosamine).

The data are interpreted to be due to genotype-dependent differences in activation of these indirect mutagens resulting in differing concentrations of mutagenic products in various parts of the body including the gonads. This assumption is supported by:

1) the positive correlation between mutation frequency and the observed sterilizing effects.

2) the inhibitory action of proper enzyme inhibitors on mutation induction by indirect mutagens (Vogel, unpublished), and

3) our finding that the mutation frequency in Berlin K - Hikone R hybrids (Berlin K qq x Hikone R dd) treated with the triazenes is almost exactly half that in the wild strain.

Whatever the correct explanation of the result is, the data show that group-specific cross-resistance to certain chemical mutagens seems to exist in Drosophila.