CHAPTER V

Effect of precocene-II on the neuroendocrine regulation of reproduction.
MATERIALS AND METHODS

The source of chemicals and insects used, was already given in Chapter II. Precocene-II was dissolved in acetone (5 mg/ml) and was applied topically to newly emerged females (within 10-15 min of emergence), at a dosage of 50 µg/insect which was found to be the optimal dose to bring about total inhibition of egg-maturation with the least mortality rate. The topical application was made on the dorsal surface of the abdomen below the wings, with the help of a microsyringe (TOP Company, Bombay). The control females were treated with an equal volume of acetone. All these insects were maintained in a glass container on soaked cotton seeds for the required duration together with an equal number of males. The methods used for extraction and estimation of RNA, $H^3$-uridine incorporation and determination of protein content were the same as described in Chapter II.

Precocene as well as acetone-treated 5 days old control insects were injected with $H^3$-leucine (2 µCi/20 mg body wt.) and incubated for 4 h. The ovaries were dissected, fixed at the end of 4 h and were processed for autoradio-
graphic studies. Paraldehyde-fuchsin staining was performed on the whole brains dissected out from 4 and 6 days old experimental and control insects to visualise the distribution of neurosecretory material. For electron microscopic studies, the corpora allata of precocene and acetone treated 6 days old insects were used. To study the recovery effect of exogenous JH-III on precocene treated animals, JH-III dissolved in acetone, was topically applied to precocene-primed 3 days old insects at two different dosages of 5 and 10 μg/insect. The precocene treated insects of the same age group were used as controls. The insects were sacrificed on day 8 and the protein content of the ovary was estimated, according to the method of Lowry et al. (1951).

In order to study the effect of exogenous JH-III on RNA metabolism in the fat body, JH-III was dissolved in acetone and applied topically to the 2 days old female insects in various dosages ranging from 5–15 μg. The insects were sacrificed 24 h after JH III treatment and the RNA of fat body was extracted by the method of Fong and Fuchs (1976) and estimated with the help of orcinol reaction.
Effect of precocene on the body weight and egg maturation:

Table 1 shows the changes in the body weight and ovarian weight in acetone and precocene treated insects. The acetone treated 6 days old insects have a much higher body weight ($105 \pm 12.05$ mg/insect) in comparison to the precocene treated insects ($60 \pm 5.22$ mg/insect) and this is more or less equal to the body weight found in 3 days old normal insects ($62 \pm 3.71$ mg/insect). The ovarian weight of 6 days old acetone treated insects is fairly high and is nearly 15 times greater, as compared to the precocene treated insects of the same age group. The ovarian weight of precocene treated 6 days old insects is nearly equal to that of the 3 days old normal insects. This clearly indicates the deleterious effect of precocene on the ovarian weight.

Plate IV shows the effect of precocene treatment (50 $\mu$g/insect) on the general appearance of the insect. Acetone treated insects (Fig.1), usually possess a bulged abdomen which is largely due to the unhindered normal
ovarian development. On the other hand, precocene treated insects (Fig. 2) show a narrow and flat abdomen whose dimensions and appearance are closely similar to the abdomen of 3 days old normal insects (Fig. 3).

Dissected ovaries of 6 days old precocene treated females remained considerably small and contained undifferentiated eggs, as compared to acetone-treated control insects, wherein the ovarioles showed the presence of a series of well differentiated mature oocytes (cf. Fig. 4a and b). The ovaries of the precocene treated 6 days old insects appear to be about the same as those of 3 days old normal immature insects (cf. Fig. 5a and b). This indicates that precocene has effectively blocked the normal ovarian growth and differentiation. Examination of histological preparations of the ovarioles showed that in the ovaries of control insects, there is a uniformly active vitellogenesis corresponding to the histological pictures of vitellogenic follicles shown in Figs. 8-10 (Plate II). However, in the ovaries of experimental insects, vitellogenesis was inhibited and the oocytes were devoid of yolk platelets and they remained fairly small in size. These gross morphological and histological observations on the ovaries were confirmed by tracer studies also, using
H³-leucine as the precursor for yolk proteins. Control insects showed the appearance of several radioactive yolk spheres at the oocyte cortex in all the vitellogenic follicles. However, with 4 h incubation period, the labelled yolk droplets were mainly confined to the periphery of the oocyte and did not yet move far inwards (Figs. 6 and 7). The follicle epithelium also showed moderate labelling (Fig. 8). With the same incubation time, the incorporation patterns in precocene treated insects, however, presented a strikingly different picture (Figs. 9, 10 and 11). The follicle epithelium showed a moderate labelling but the ooplasm was practically devoid of labelled yolk droplets (Fig. 10) and showed in some of the younger oocytes a diffuse non-specific radioactivity. The terminal follicle of acetone treated insects was fairly large and showed the presence of large number of proteid yolk globules (Fig. 6) suggesting hectic vitellogenic activity, whereas in the precocene treated females even the terminal follicles remained smaller, with homogeneous practically unlabelled ooplasm and with few lipid yolk droplets but no proteid yolk spheres at all (cf. Figs. 6 and 11).
Effect of precocene on the weight and the RNA content of the fat body:

Table 2 shows the effect of precocene treatment on the weight and the RNA content of the fat body, after different durations of treatment. The weight of the fat body was found to be low in precocene treated 1 day old insects. This increased gradually and reached its highest value in precocene treated 6-days old insects. On the other hand, in the control insects, the fat body weight was initially low up to 3 days but increased significantly (2 fold) at 4 days and remained more or less at the same level up to 6 days. The total RNA content of the fat body in precocene treated 1 day old insects was fairly low (50%) when compared with control insects. In precocene treated insects, a gradual increase was found up to 3 days, which remained nearly at the same level thereafter. However, in acetone control insects, it increased from day 2 to 3. Once again, a significant increase (2 fold) in total RNA content was observed in 4 days old control insects. Thereafter, a slight decrease was observed in 5 days old insects which remained nearly at the same level up to 6 days. Acetone control insects showed a gradual increase in the RNA content when expressed per mg tissue.
up to 3 days, which shot up to a significant level (38%) at 4 days, but declined slightly in 5 days old females and remained nearly constant in 6 days old insects. In the case of precocene treated insects, the RNA content, when expressed per mg tissue, increased gradually from 1 to 3 days and declined gradually thereafter till 6 days.

Effect of precocene on H$^3$-uridine incorporation into the fat body RNA:

The effect of precocene (50 µg/insect) on the H$^3$-uridine incorporation into the fat body RNA is presented in Table 3. The rate of RNA synthesis was found to be high in 1 and 2 days old insects but declined gradually up to 6 days and it showed more or less the same pattern in experimental as well as control insects during the first gonotrophic cycle. But the total RNA synthesised in the fat body varied conspicuously in experimental and control insects. The total RNA synthesis was fairly low (50%) in precocene treated 1 day old insects in comparison to controls, but it was found to be more or less the same in 2 and 3 days old experimental and control insects. However, the total RNA synthesis was significantly low
In 4 days old experimental insects in comparison with the controls and this is mainly due to the diminished quantity of fat body. Thereafter, the total RNA synthesis declined gradually up to 6 days in precocene treated insects. Even in controls, the total RNA synthesised, has declined significantly from day 5 to day 6.

Effect of precocene on the protein content of the fat body:

Protein content of the fat body in precocene treated and acetone control insects are set forth in Table 4. The total protein content of the fat body was fairly low in precocene treated insects throughout the first reproductive cycle as compared to the control insects and this difference was more pronounced in the second half of the cycle. When the protein content was expressed in terms of per mg tissue, it was found to be higher in 1 day old control insects than in precocene treated insects. However, the pattern remained more or less the same in 2 to 6 days old experimental and control insects.
Changes in the protein content of haemolymph after precocene treatment:

The data obtained on the effect of precocene treatment on the haemolymph protein content has been presented in Table 5. One day old experimental as well as control insects showed a high haemolymph protein content which declined in 2 days old insects. In precocene treated 3 days old insects, a further decrease was found but it again increased slightly on day 4 and remained nearly constant up to 6 days. On the other hand, 3 days old control insects showed more or less the same protein as in 2 days old insects. However, it shot up significantly (2.5 fold) in 4 days old insects and remained more or less the same in the 5 days old insects but decreased drastically in 6 days old controls.

Effect of precocene on the neurosecretory cells of the brain:

Two groups of median neurosecretory cells occur medio-dorsally in the pars intercerebralis of the protocerebral lobe of the brain. They consist of 9 cells on each side of the pars intercerebralis and they are
prominently stainable with paraaldehyde–fuchsin technique (PF). In precocene treated 4 days (Fig. 14) and 6 days (Fig. 15) old insects, the cells are intensely stainable with purple colour. They have a large amount of cytoplasm filled with abundant neurosecretory granules with a relatively inconspicuous nucleus (Figs. 14 and 15). On comparing with the same kind of preparations of acetone treated control insects of the same age group, it is seen that the cells are faintly stained with PF and the cytoplasm of cells shows the presence of only a small quantity of neurosecretory material (Figs. 12 and 13). They bear conspicuously visible cell nuclei. These illustrations convincingly demonstrate that precocene is interfering with the release mechanism, leading to their massive accumulation in the perikarya of the neurosecretory cells. In the acetone controls there is a rapid turnover of the neurosecretory material.

Effect of precocene-II on the volume of corpus allatum (CA):

In acetone treated insects the CA showed remarkable fluctuations in its volume during the first reproductive cycle (Table 6). The CA volume increased gradually from 1 to 4 days in control insects but declined thereafter,
whereas, the CA volume did not show such marked fluctuation and remained at a low level in precocene-treated insects throughout the first reproductive cycle. In general, the CA of the experimental groups of insects of all ages, revealed lower values of their volume as compared to acetone treated controls. This indicates that precocene has a definite deleterious effect on the allatal growth.

**Electron microscopic observations on the corpus allatum:**

How the volume changes in CA caused by precocene treatment, reflect themselves in the ultrastructural organisation of the allatal cells was investigated with the electron microscope.

**Acetone treated control insect:**

The gland cells rest externally on a basement membrane of moderate thickness (Fig.16). The cell nuclei are branched. In the cytoplasmic space, several inclusions are noticeable. These include rough endoplasmic reticulum, mitochondria, golgi vesicles and numerous free ribosomes (Fig. 17).
The rough endoplasmic reticulum is often present in form of stacks or cisternae (Fig. 17). The ribosomes may sometimes form dense aggregates and exhibit polysomal configuration (Fig. 18). Typical Golgi lamellae found in the vertebrate cells are not present here. But few vesicular type of Golgi bodies are seen scattered in the cytoplasmic space, sometimes very close to the rough endoplasmic reticulum (Fig. 17). The cytoplasm shows the presence of large number of evenly distributed mitochondria of ordinary size with well marked cristae (Fig. 18). The cytoarchitecture reveals the picture of all cell organelles associated with active secretory cells.

Precocene treated insect:

Ultrastructural studies revealed a number of degenerative changes in the allatal cells of precocene treated insects. The basement membrane became loose, disorganised and detached from the cellular layer (Fig. 19). Mitochondria tend to become aggregated but they retain, by and large, their internal organisation (Fig. 20). Cisternae like organisation of rough endoplasmic reticulum is also not visible any more. The most conspicuous feature is the presence of large number of intracellular
vacuoles some of which contain electron dense material (Fig. 21). These may be the autophagic vacuoles and/or the multivesicular bodies associated with primary lysosomes. Such structures are not detectable in the CA cells of acetone treated controls.

Effect of exogenous JH on precocene treated insects:

The precocene primed (50 µg/insect were topically administered two dosages of JH-III at 5 and 10 µg/insect on the 4th day after precocene treatment. As shown in Table 7, when 5 µg JH was applied, there was a 3 fold increase in ovarian weight and a 4 fold increase in total ovarian protein, in comparison with that of precocene control insects. However, 10 µg JH had less pronounced effect than 5 µg treatment.

Effect of exogenous JH on fat body RNA of normal insects:

The effect of JH on the fat body RNA in 2 days old insects, after 24 h treatment, can be seen from the data given in Table 8. The weight of the fat body increased significantly (43%) in 5 µg JH treated insects, as compared to the acetone treated controls. In those
Insects treated with 10 and 15 µg JH, the weight of the fat body remained more or less the same as in acetone controls. The total RNA content of the fat body per insect increased by about 64% in 5 µg JH treated insects when compared to the controls, whereas only a slight increase was observed in case of 10 and 15 µg JH treated insects. The RNA concentration (/mg fat body) also followed the same pattern as that of total RNA content.
Figs.1-3. Macrophotographs of 6 days old females show the bulged abdomen due to ovarian maturation in acetone treated control Fig.2 illustrates the effect of precocene on the general body appearance. Note the presence of a flat abdomen in precocene treated 6 days old insects which resembles closely that of the 3 days old normal immature insects (Fig.3).

x 60

Fig.4. Shows a macrophotograph of the female internal reproductive systems of precocene-treated (a) and acetone treated (b) 6 days old insects as they appear in the dissected condition. Note the presence of well developed mature oocytes in acetone controls, while the ovaries in (b) appear degenerate.

x 75

Fig.5. Illustrates the comparison of internal reproductive system of precocene treated 6 days (a) old insects with 3 days old normal insects (b). They appear roughly to be equal.

x 75
Plate V

Figs. 6-11. Illustrate the pattern of H$^3$-leucine incorporation in acetone (Figs. 6-8) and precocene (Figs. 9-11) treated 5 days old insects with 4 h incubation. Note the large number of labelled yolk droplets (→) at the cortex of the terminal (Fig. 6) and other developing oocytes (Figs. 7 and 8) in acetone treated 5 days old insects. On comparing the acetone autoradiograms with those of precocene (Figs. 9-11), it is seen that there is a total absence of radioactive yolk spheres at the follicle cell/oocyte interface, suggesting the cessation of vitellogenin deposition in the oocyte (OC). PD = pedicel.

<table>
<thead>
<tr>
<th>Figures</th>
<th>Magnification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figs. 6 and 9</td>
<td>x 160</td>
</tr>
<tr>
<td>Figs. 7 and 10</td>
<td>x 320</td>
</tr>
<tr>
<td>Fig. 8</td>
<td>x 480</td>
</tr>
<tr>
<td>Fig. 11</td>
<td>x 375</td>
</tr>
</tbody>
</table>
Plate VI

Figs.12-13. While preparations of brain showing the median neurosecretory cells of acetone treated 4 (Fig.12) and 6 (Fig.13) days old insects, and precocene treated 4 (Fig.14) and 6 (Fig.15) days old insects. Note the small amount of neurosecretory colloids in perikarya of acetone treated insects (Figs. 12 and 13) indicating a rapid turnover while the precocene treated (Figs.14 and 15) insects show the accumulation of large amount of colloid in the perikarya probably due to the inhibition of release. N = Nucleus, CY = Cytoplasm. Bouin/Aldehyde Fuchsina.

x 520
Figs. 16 and 17. Electron micrographs of the corpus allatum of acetone treated 5 days old insects, depicting the gland cells closely adhering to the basement membrane (BM). The cells show intercellular spaces (IS) in Fig. 16, mitochondria (M) in Figs. 16 and 17, nucleus (N) with several nucleoli in Fig. 16. The rough endoplasmic reticulum exists as stacks of cisternae in close association with Golgi vesicles (GV) (Fig. 17).
Fig. 18. Electron micrograph of gland cells of the corpus allatum of acetone treated 5 days old insects. Picture shows the presence of intercellular spaces (IS), mitochondria (M) and numerous polysomes (→).

Fig. 19. Electron micrograph of gland cells of the corpus allatum of precocene treated 5 days old insects, showing the detached basement membrane (BM) which becomes disorganised, clustered mitochondria (M). The cisternae-like organisation of rough endoplasmic reticulum (rER) is no longer in evidence.
Plate IX

Figs. 20 and 21. Electron micrographs showing the presence of large number of intracellular vacuoles (IV) which appear in the corpus allatum gland cells after precocene treatment. Note the presence of clusters of mitochondria (M).
TABLE 1
Effect of precocene on the body and ovary weight of *Dysdercus koenigii*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body wt. mg/Insect</th>
<th>Ovary wt. mg/Insect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 days old (7)</td>
<td>106 ± 9.84</td>
<td>45.15 ± 2.8</td>
</tr>
<tr>
<td>Acetone treated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 days old (8)</td>
<td>105 ± 12.05</td>
<td>38.46 ± 2.61</td>
</tr>
<tr>
<td>Precocene treated (50 µg/insect)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 days old (8)</td>
<td>60 ± 5.22*</td>
<td>3.53 ± 0.51*</td>
</tr>
<tr>
<td>Normal insect</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 days old (6)</td>
<td>62 ± 3.71</td>
<td>3.15 ± 0.44</td>
</tr>
</tbody>
</table>

The values represent the means ± S.D. of the number of determinations given in the parentheses.

* These values are significantly different (P<0.001) from the corresponding control values.
TABLE 2

Effect of precocene on the fat body weight and RNA content of the fat body.

<table>
<thead>
<tr>
<th>Age</th>
<th>Acetone treated</th>
<th>Precocene treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fat body wt. mg/Insect</td>
<td>Fat body RNA (μg)/Insect</td>
</tr>
<tr>
<td>1 day (6)</td>
<td>3.74 ± 0.50</td>
<td>18.41 ± 6.99</td>
</tr>
<tr>
<td>2 days (7)</td>
<td>4.24 ± 0.90</td>
<td>18.74 ± 4.72</td>
</tr>
<tr>
<td>3 days (5)</td>
<td>3.98 ± 0.50</td>
<td>32.16 ± 5.80</td>
</tr>
<tr>
<td>4 days (14)</td>
<td>9.17* ± 2.40</td>
<td>85.16 ± 11.10</td>
</tr>
<tr>
<td>5 days (14)</td>
<td>8.81 ± 2.00</td>
<td>68.63 ± 11.50</td>
</tr>
<tr>
<td>6 days (10)</td>
<td>8.84 ± 1.20</td>
<td>75.20 ± 11.00</td>
</tr>
</tbody>
</table>

The values represent the means ± S.D. of the number of determinations given in parentheses.

* This value is significantly different (P<0.001) from acetone treated 3 days old insect as well as corresponding control value.
<table>
<thead>
<tr>
<th>Age</th>
<th>Acetone treated</th>
<th></th>
<th></th>
<th>Precocene treated</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CPM/mg RNA</td>
<td>CPM/Insect fat body</td>
<td></td>
<td>CPM/mg RNA</td>
<td>CPM/Insect fat body</td>
<td></td>
</tr>
<tr>
<td>1 day (6)</td>
<td>2,59,340</td>
<td>4037</td>
<td></td>
<td>2,34,090</td>
<td>2850</td>
<td></td>
</tr>
<tr>
<td>± 49,650</td>
<td>± 116</td>
<td></td>
<td>± 39,570</td>
<td>± 667</td>
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<tr>
<td>2 days (7)</td>
<td>2,94,780</td>
<td>5907</td>
<td></td>
<td>2,77,090</td>
<td>5705</td>
<td></td>
</tr>
<tr>
<td>± 65,000</td>
<td>± 893</td>
<td></td>
<td>± 10,350</td>
<td>± 237</td>
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<tr>
<td>3 days (5)</td>
<td>1,80,830</td>
<td>5086</td>
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<td>1,56,460</td>
<td>4999</td>
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<tr>
<td>± 24,000</td>
<td>± 136</td>
<td></td>
<td>± 30,130</td>
<td>± 431</td>
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<tr>
<td>4 days (13)</td>
<td>92,950</td>
<td>6310</td>
<td></td>
<td>1,32,420</td>
<td>* 3921</td>
<td></td>
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<tr>
<td>± 24,420</td>
<td>± 1267</td>
<td></td>
<td>± 25,700</td>
<td>± 1172</td>
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<tr>
<td>5 days (13)</td>
<td>84,610</td>
<td>6625</td>
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<td>1,16,990</td>
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<tr>
<td>± 9,660</td>
<td>± 1165</td>
<td></td>
<td>± 25,700</td>
<td>± 693</td>
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<tr>
<td>6 days (10)</td>
<td>71,580</td>
<td>3820</td>
<td></td>
<td>86,433</td>
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<td></td>
</tr>
<tr>
<td>± 18,000</td>
<td>± 1625</td>
<td></td>
<td>± 11,340</td>
<td>± 865</td>
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</table>

The values represent the means ± S.D. of the number of determinations given in parentheses.

* This value is significantly different (P<0.001) from the corresponding control value.
Changes in the protein content of the fat body after precocene treatment.

<table>
<thead>
<tr>
<th>Age</th>
<th>Acetone Treated</th>
<th>Preccocene treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein (µg)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insect fat body</td>
<td>mg tissue</td>
</tr>
<tr>
<td>1 day</td>
<td>134.04 ± 20.20</td>
<td>39.10 ± 0.70</td>
</tr>
<tr>
<td>2 days</td>
<td>204.32 ± 32.50</td>
<td>27.13 ± 3.80</td>
</tr>
<tr>
<td>3 days</td>
<td>152.27 ± 16.60</td>
<td>27.03 ± 2.20</td>
</tr>
<tr>
<td>4 days</td>
<td>393.38 ± 31.10</td>
<td>36.31 ± 3.60</td>
</tr>
<tr>
<td>5 days</td>
<td>378.02 ± 28.40</td>
<td>42.91 ± 3.40</td>
</tr>
<tr>
<td>6 days</td>
<td>399.78 ± 10.04</td>
<td>52.72 ± 4.00</td>
</tr>
</tbody>
</table>

The values are means ± S.D. of 3–5 determinations from 5–7 insects per group.
TABLE 5

Effect of precocene on the protein content of the haemolymph.

<table>
<thead>
<tr>
<th>Age</th>
<th>Acetone treated</th>
<th></th>
<th>Preecene treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein (μg)/μl haemolymph</td>
<td></td>
<td>Protein (μg)/μl haemolymph</td>
</tr>
<tr>
<td>1 day</td>
<td>26.42 ± 4.65</td>
<td></td>
<td>30.94 ± 1.05</td>
</tr>
<tr>
<td>2 days</td>
<td>18.19 ± 3.35</td>
<td></td>
<td>18.30 ± 5.37</td>
</tr>
<tr>
<td>3 days</td>
<td>17.07 ± 1.91</td>
<td></td>
<td>10.45 ± 2.00</td>
</tr>
<tr>
<td>4 days</td>
<td>45.16 ± 7.60</td>
<td></td>
<td>14.89 ± 2.63</td>
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<tr>
<td>5 days</td>
<td>39.22 ± 4.06</td>
<td></td>
<td>16.50 ± 1.88</td>
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<td>6 days</td>
<td>22.34 ± 0.42</td>
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<td>15.75 ± 2.86</td>
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Results are mean ± S.D. of 3-5 determinations from 5-7 insects per group.
<table>
<thead>
<tr>
<th>Age</th>
<th>Volume (μm$^3 \times 10^5$)</th>
<th>Age</th>
<th>Volume (μm$^3 \times 10^5$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>±</td>
<td></td>
<td>±</td>
</tr>
<tr>
<td>1 day (8)</td>
<td>15.27 ± 5.50</td>
<td>1 day (8)</td>
<td>11.14 ± 2.79</td>
</tr>
<tr>
<td>2 days (8)</td>
<td>17.95 ± 2.34</td>
<td>2 days (8)</td>
<td>7.90 ± 1.04</td>
</tr>
<tr>
<td>3 days (10)</td>
<td>22.34 ± 7.91</td>
<td>3 days (15)</td>
<td>13.09 ± 4.03</td>
</tr>
<tr>
<td>4 days (9)</td>
<td>28.04 ± 7.64</td>
<td>4 days (15)</td>
<td>11.29 ± 3.44</td>
</tr>
<tr>
<td>5 days (8)</td>
<td>21.46 ± 3.43</td>
<td>5 days (10)</td>
<td>8.06 ± 2.50</td>
</tr>
<tr>
<td>6 days (6)</td>
<td>10.81 ± 3.08</td>
<td>6 days (17)</td>
<td>9.67 ± 2.64</td>
</tr>
</tbody>
</table>

Results are means ± SD of the number of determinations given in parentheses.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ovary wt. (mg)/Insect</th>
<th>Protein (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>/Insect ovary /mg tissue</td>
</tr>
<tr>
<td>Precocene treated</td>
<td>3.95 ± 0.32</td>
<td>198.47 ± 40.35 / 41.38 ± 6.26</td>
</tr>
<tr>
<td>5 µg JH on 4th day</td>
<td>12.04 ± 1.40</td>
<td>802.50 ± 27.18 / 67.29 ± 6.02</td>
</tr>
<tr>
<td>10 µg JH on 4th day</td>
<td>8.67 ± 0.35</td>
<td>437.32 ± 95.47 / 63.85 ± 7.11</td>
</tr>
<tr>
<td>Acetone treated</td>
<td>50.75 ± 4.50</td>
<td>4827.00 ± 385.50 / 121.46 ± 22.24</td>
</tr>
</tbody>
</table>

These values represent means ± S.D. of 4-5 determinations. For each determination, 5-7 insects were used.
TABLE 8

Effect of JH-III on fat body RNA content in 2 days old insects.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wt. of fat body</th>
<th>RNA(μg)/Insect</th>
<th>RNA(μg)/mg tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-Acetone</td>
<td>5.234 ± 0.649</td>
<td>59.22 ± 8.17</td>
<td>15.25 ± 1.70</td>
</tr>
<tr>
<td>treated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JH-5μg/Insect</td>
<td>7.501 ± 0.646</td>
<td>97.88 ± 20.52</td>
<td>25.31 ± 3.51</td>
</tr>
<tr>
<td>JH-10μg/Insect</td>
<td>5.612 ± 0.990</td>
<td>64.97 ± 10.92</td>
<td>19.18 ± 2.77</td>
</tr>
<tr>
<td>JH-15μg/Insect</td>
<td>5.438 ± 0.872</td>
<td>73.72 ± 15.41</td>
<td>19.59 ± 4.52</td>
</tr>
</tbody>
</table>

The values represent means ± S.D. of the 5-7 determinations, for each determination 4-5 insects were used.