The Role of Ecdysteroids and Juvenoids in Vitellogenin Levels and Follicle Development in the Housefly, *Musca domestica*

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The dose dependent effects of ecdysterone on follicle development and vitellogenin concentration were observed in houseflies decapitated 24 hr after hormone treatment. Vitellogenin level induced by ecdysterone was maximally 5.7-fold greater than control flies. Vitellogenin was detectable in decapitated male flies treated with ecdysterone, but not in intact males. Juvenile hormone analog (methoprene) treatment did not induce any vitellogenin in male flies, whereas it induced approximately 4.4-fold the vitellogenin and follicle development in decapitated females over that of control flies. A large amount of vitellogenin was detectable in 70% of the ovarioiectomized females, but only a trace amount was found in male flies holding a vitellogenic ovary. Vitellogenin and ecdysteroid level were coincident and made a similarly shaped parabolic curve during the first gonotrophic cycle. The maximal ecdysteroid level (18 ng/ml) appeared in the middle of the vitellogenic phase. Meanwhile, no ecdysteroid surge was found in the hemolymph of ovarioiectomized females or in that of males holding a vitellogenic ovary. A moderate level of ecdysteroids was detectable in the intact ovary during the vitellogenic and post-vitellogenic stage. The inability to stimulate oogenesis and vitellogenin synthesis in both sexes of houseflies by ecdysteroids and juvenoids is discussed.

INTRODUCTION

It has been demonstrated that oogenesis of insects is entirely controlled by the endocrine system and is dependent on nutrition. The role of corpora allata in egg maturation has been widely reported in many insect species (Engelman, 1970), since it was first reported in *Rhodnius prolixus* as the source of the gonotrophic hormone (juvenile hormone) (Wigglesworth, 1936). After publication of the theory that vitellogenin synthesis might be controlled directly by ecdysterone in the mosquito, *Aedes aegypti* (Hagedorn, 1974; Fallen et al., 1974), the capacity of ecdysteroid for the induction of vitellogenin synthesis was also investigated in both sexes of the cyclorrhaphous flies, Sarcophaga bullata, *Drosophila melanogaster*, Calliphora vicina, Phormia terra-novae and in *Lucilia caesar* (Huybrechts and De Loof 1981, 1982; Postlethwait et al., 1980). In the housefly, *Musca domestica*, few studies have been made on the direct effect of ecdysteroids and juvenoids on either oogenesis or vitellogenesis in either sex, although corpora allata and juvenile hormone itself have been known to play a principal role in controlling the housefly oogenesis (Adams, 1970, 1974; Sakurai, 1977).
In the present paper, we investigated in vivo effects of ecdysterone, juvenile hormone analog (methoprene) and different surgical treatments on the follicle development and vitellogenin levels of hemolymph in both sexes of houseflies. Ecdysteroid levels in hemolymph and ovary were also studied with reference to the developmental stages of follicles and to the vitellogenin concentration in hemolymph.

MATERIALS AND METHODS

Animals. Two strains of the housefly, Musca domestica were used for experiments. The Lab em-7-em strain was established in the laboratory as a standard strain susceptible to insecticides (Hiroyoshi, 1964) and has an autogenous nutritional requirement (Agui et al., 1985). Misaki strain is resistant to organophosphorous insecticides (Hayashi et al., 1973) and also has an autogenous nutritional requirement (Agui et al., 1985). The larvae were reared in the same medium described in the previous paper (Agui et al., 1985) under 16L–8D at 25°C. Adult flies of Misaki strain were reared with a sugar cube and sugar solution (3%), whereas adults of the Lab em-7-em strain were reared with a baby milk powder and sugar solution. Ten to 20 flies treated with different surgery or hormone injection were kept an a petri dish (9 × 2 cm) containing a damp cotton ball to provide humidity.

Scoring of follicle development. Development of the ovaries was scored on the basis of the ten stages described by Adams (1974). In this method stage 1 is the germarium, stages 2–3 are the previtellogenic phase, and stages 4–8 and 9–10 are classified as vitellogenic and postvitellogenic phases, respectively. Yolk deposition in the follicles starts at stage 4 and eggs mature at stage 10.

Hormone treatment. Ecdysterone (purchased from Rhoto Pharmaceutical Co. Ltd., Japan) was dissolved in a saline (0.9% NaCl) solution and its concentration was adjusted to 0.25–5 μg/μl. One microliter of solution with or without hormone was injected into the lateral portion of the abdominal intersegmental membrane of flies anesthetized with ether. Juvenile hormone analog (methoprene, JHA, provided by Ohtsuka Pharmaceutical Co. Ltd., Japan) was dissolved in acetone and its concentration was adjusted to 0.5–1 μg/μl. JHA was applied topically to the ventral portion of the abdomen with a microsyringe. One microliter of acetone was applied in the same manner to controls.

Surgical methods. Decapitation was performed at the neck close to the thorax with fine scissors at different periods from day 0 to day 3 to investigate the effect of extirpation of the brain-ring gland complex on egg development at the first gonotropic cycle. Ovariectomy was performed on fresh female flies within 6 hr after eclosion: The ether-anesthetized fly was fixed dorsal side down with a stapler (X10-M, Max Co., Ltd., Japan) on a dissecting plate made of plasticine (Harbutt's Plasticine Ltd., England). The ovaries were then removed with fine forceps from both sides of the lateral portion of the intersegmental membrane between the 2nd and 3rd abdominal segments. Transplantation of the ovaries into male flies was done by making an opening at the abdominal intersegmental membrane between the 2nd and 3rd abdominal segment.

Quantitation of vitellogenin level. To quantify vitellogenin levels in the hemolymph, a single radial immunodiffusion test (SRID) was carried out by the method of Mancini et al. (1965) modified by Agui et al. (1985) using 0.15 M NaCl-0.01 M Na-phosphate buffer (pH 7.2), 0.1% NaN₃, 5% anti-vitelin IgG and 1 mm of gel thickness. Antivitelin IgG was separated from the serum of rabbit immunized with vitellin of mature
housefly egg protein. Extract from an ovary gravid with mature eggs was used as a standard antigen solution. This standard solution was estimated to be 7 mg protein/ml and 2.21 mg vitellin/ml (Agui et al., 1985). The original antigen solution was subjected to two-fold serial dilution and 3 μl of standard vitellogenin solution and either the same volume of standard antigen solution or hemolymph was placed in a 3 mm diameter hole. The plate was kept at 25°C and the diffusion ring was measured after 24 hr with a micrometer set in a binocular microscope (× 10) under reverse phase transparent illumination.

Withdrawal of hemolymph samples for SRID was carried out by the methods described in the previous report (Agui et al., 1985). An aliquot (3 μl) of each hemolymph sample was subjected to SRID.

Ecdysone measurement. To quantify ecdysteroids in the hemolymph and ovary, ecdysone-radiolmmunoassay (RIA) (Horn et al., 1976; Gilbert et al., 1977) was used. The antiserum used was prepared against a hemisuccinate derivative of ecdysone at the C-22 hydroxy group alone and then the labeled 3H-ecdysone (80 Ci/mmol, purchased from New England Nuclear) was used. The antibody had similar specificity for ecdysone and ecdysterone (Gilbert et al., 1977). Ecdysone was purchased from Eco-Chemical Intermediate (Division of Eco-Control, Inc.), USA. The hemolymph samples were mixed with a 30-fold volume of methyl alcohol and then volatized and centrifuged at 2,500 g for 20 min. The ovaries were homogenized with methyl alcohol:water (2:1) (1 mg/10 μl) using a microglass homogenizer and were centrifuged at 7,000 g for 5 min. An aliquot of each supernatant was subjected to ecdysone-RIA for assay of ecdysteroids.

The collection of hemolymph samples from adult flies for ecdysone-RIA was carried out by centrifugation: First, the alimentary organs such as crop and intestine were removed with fine forceps from the lateral part of the intersegmental membrane between the 2nd and 3rd abdominal segment. This operation assures that the collected hemolymph samples are free of severe contamination from the contents of the alimentary ducts. Second, a large wound was made in the prescutum with fine forceps, and the operated fly was then inserted into a disposable plastic pipette tip (5–200 μl) with its head at the top. The tip holding the fly was further inserted into a plastic microcentrifuge tube (0.4 ml) and centrifuged at 350 g for 2 min by a Microlabfluge (Sakuma, M-15) to collect the hemolymph. An aliquot (1 μl) of hemolymph was taken from the supernatant in each centrifuge tube, and three stocks of sample containing 10 μl of hemolymph were prepared from 30 flies for ecdysteroid extraction by methyl alcohol.

RESULTS

To investigate the effect of the head portion, in which the brain-ring gland complex is located, on follicle development, the decapitation of autogenous female flies was carried out on different days after eclosion. As shown in Table 1, the follicle of flies decapitated within 1 day after eclosion had not developed and the flies were still in the previtellogenic phase when inspected 5 days after eclosion. The follicle of flies decapitated 2 and 3 days after eclosion, on the contrary, had developed to stages 5 and 10 5 days after eclosion. However, a high mortality and follicular atresia were observed in females decapitated a longer time after eclosion. Thus, autogenous female flies decapitated within 6 hr after eclosion were used for the experiments to observe the effect of hormones on follicle development and vitellogenin levels.

Table 2 shows the effect of methoprene (JHA) on follicle development and vitello-
Table 1. Effect of decapitation on follicle development of autogenous adult houseflies

<table>
<thead>
<tr>
<th>Hours of decapitation after eclosion</th>
<th>No. of flies decapitated</th>
<th>No. of survivors</th>
<th>Follicle stage (x±SE)</th>
<th>Atria observed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>19</td>
<td>19</td>
<td>2.5±0.1</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>40</td>
<td>13</td>
<td>3.2±0.1</td>
<td>0</td>
</tr>
<tr>
<td>48</td>
<td>39</td>
<td>25</td>
<td>5.1±0.1</td>
<td>20</td>
</tr>
<tr>
<td>72</td>
<td>41</td>
<td>7</td>
<td>10.0±0.0</td>
<td>0</td>
</tr>
</tbody>
</table>

Autogenous flies of Misaki strain were decapitated at different times after adult eclosion and follicle stage was classified 5 days after eclosion by the scoring method of Adams (1974).

Table 2. Effect of juvenile hormone analog on vitellogenin levels of decapitated female houseflies

<table>
<thead>
<tr>
<th>JHA dose (µg)</th>
<th>No. of flies</th>
<th>Vitellogenic follicle (%)</th>
<th>Vitellogenin detected (%)</th>
<th>Hemolymph vitellogenin (x±SE, mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>26.7</td>
<td>100.0 (9)*</td>
<td>0.71±0.21</td>
</tr>
<tr>
<td>0.5</td>
<td>15</td>
<td>26.7</td>
<td>100.0 (7)</td>
<td>1.53±0.24</td>
</tr>
<tr>
<td>Acetone</td>
<td>10</td>
<td>0.0</td>
<td>28.6 (7)</td>
<td>0.07±0.04</td>
</tr>
<tr>
<td>0.0</td>
<td>15</td>
<td>0.0</td>
<td>73.3 (15)</td>
<td>0.35±0.10</td>
</tr>
</tbody>
</table>

Autogenous female flies of Misaki strain were decapitated at 6 hr of eclosion and topically applied with methoprene or acetone as control 48 hr after decapitation. Vitellogenin levels by SRID and follicle development were observed 24 hr after hormone treatment.

* No. of flies detecting vitellogenin by SRID.

Table 3. Effect of ecdysterone on vitellogenin levels of decapitated female houseflies

<table>
<thead>
<tr>
<th>Ecdysterone dose (µg)</th>
<th>No. of flies</th>
<th>Vitellogenic follicles (%)</th>
<th>Vitellogenin detected (%)</th>
<th>Hemolymph vitellogenin (x±SE, mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>16</td>
<td>50.0</td>
<td>100</td>
<td>1.99±0.21</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>40.0</td>
<td>100</td>
<td>1.35±0.54</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>13.3</td>
<td>100</td>
<td>0.95±0.19</td>
</tr>
<tr>
<td>0.5</td>
<td>14</td>
<td>14.3</td>
<td>100</td>
<td>0.82±0.17</td>
</tr>
<tr>
<td>0.25</td>
<td>14</td>
<td>7.1</td>
<td>100</td>
<td>0.84±0.26</td>
</tr>
<tr>
<td>0.0</td>
<td>15</td>
<td>0.0</td>
<td>73.3 (15)</td>
<td>0.35±0.10</td>
</tr>
</tbody>
</table>

Autogenous female flies of Misaki strain were decapitated at 6 hr of eclosion and injected with different doses of ecdysterone 48 hr thereafter. Vitellogenin levels by SRID and follicle development were observed 24 hr after hormone injection.

genein level in decapitated females. Vitellogenin in those treated with JHA increased 10–20-fold for acetone treatment and to 2–4-fold for blank as control. However, the follicles of approximately 30% of the flies developed from the previtellogenic to vitellogenic phase 24 hr after JHA application. In the hemolymph of a day 3 male, no vitellogenin was detectable 24 hr after JHA treatment.

Effects of different concentrations of ecdysterone on the follicle development and vitellogenin content in decapitated flies are shown in Table 3. The dose dependent
Effect of a hormone was observed in the vitellogenesis of the flies 24 hr after its application. Follicle development was observed maximally in 50% of females given the highest dose of ecdysterone. Vitellogenin content in the hemolymph of all females treated with ecdysterone increased drastically according to the dosage with amounts ranging from 5.7 to 2.4-fold that of control flies. Unexpectedly, a small quantity of vitellogenin was detected in over 70% of the day-3 decapitated females as control flies, although there were no flies with vitellogenic follicles. In 20–60% of the males a small amount of vitellogenin in the hemolymph was induced by the application of different concentrations of ecdysterone. Vitellogenin was never found in the hemolymph of intact males (Table 4). The amount of vitellogenin found in the treated male hemolymph was independent of the hormone dose and its level was less than that of the female treated hormone, except for male flies treated with 1 μg of ecdysterone.

To clarify the role of the ovary in the vitellogenin level of hemolymph, ovariectomy and ovary transplantation into male flies was performed and the vitellogenin level and follicle development of the ovary were then investigated. Table 5 shows that a large amount of vitellogenin was detectable in approximately 70% of the ovariectomized females when surgery was carried out within 7 hr after emergence and vitellogenin content was inspected 3 days thereafter. Male flies transplanted with a previtellogenic ovary of stage 2 had 93% of vitellogenic ovaries of stage 4–6. Interestingly, only a trace level

Table 4. Effect of ecdysterone on vitellogenin levels of male houseflies

<table>
<thead>
<tr>
<th>Ecdysterone dose (μg)</th>
<th>No. of flies</th>
<th>Vitellogenin detected (%)</th>
<th>Hemolymph vitellogenin (x±SE, mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>14</td>
<td>37.1</td>
<td>0.16±0.02</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>46.7</td>
<td>0.60±0.10</td>
</tr>
<tr>
<td>0.5</td>
<td>15</td>
<td>20.0</td>
<td>0.11±0.05</td>
</tr>
<tr>
<td>0.125</td>
<td>14</td>
<td>21.4</td>
<td>0.24±0.08</td>
</tr>
<tr>
<td>0.0</td>
<td>14</td>
<td>0.0</td>
<td>0.00±0.00</td>
</tr>
</tbody>
</table>

Sugar-fed male flies of Misaki strain were injected with different doses of ecdysterone 3 days after eclosion and hemolymph vitellogenin levels were determined by SRID 24 hr after injection.

Table 5. Effect of ovariectomy and ovary transplantation on the vitellogenin levels of female and male flies and on the follicle stage of ovary in males

<table>
<thead>
<tr>
<th>Treated</th>
<th>No. of flies</th>
<th>Vitellogenin detected (%)</th>
<th>Hemolymph vitellogenin (x±SE, mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovariectomy</td>
<td>14</td>
<td>71.4</td>
<td>1.03±0.27</td>
</tr>
<tr>
<td>Follicle stage</td>
<td>No.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previtellogenic</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Vitellogenic</td>
<td>4</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Ovary transplantation</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previtellogenic</td>
<td>5</td>
<td>3</td>
<td>Trace</td>
</tr>
<tr>
<td>Vitellogenic</td>
<td>6</td>
<td>2</td>
<td>0.04, 0.07</td>
</tr>
</tbody>
</table>

Ovariectomy of female flies and ovary transplantation into male flies were performed with Lab em-7-em strain with in 7 hr after eclosion. Follicle stage and hemolymph vitellogenin levels were assessed 3 days thereafter.
of vitellogenin was detectable in the hemolymph of males with vitellogenic follicles. Figure 1 shows the successive change of hemolymph ecdysteroid level during the first gonotrophic cycle. The successive change of vitellogenin level which was reported previously (Agui et al., 1985) and that of ecdysteroid levels were coincident making a similar sharp parabolic curve. Ecdysteroid was detectable at a very low level (~6 ng/ml) within 1 day after emergence in the previtellogenic phase and reached its maximum level (18 ng/ml) 3.5 days after eclosion in stage 7 of follicle development, then decreased drastically before the postvitellogenic phase. During the postvitellogenic phase (stage 9–10) ecdysteroid level was sustained at 1.6-fold that of the previtellogenic phase. No ecdysteroid surge appeared in the hemolymph of ovariectomized females (Fig. 2). Ecdysteroid level in the hemolymph of male flies was higher than that of females just after eclosion, but it decreased thereafter. Interestingly, the hemolymph taken from males which had ovaries transplanted at day 0 did not show any increase in ecdysteroid level. Such an increase in the intact ovary is reflected in the increase of ecdysteroid level of the hemolymph.

DISCUSSION

The use of an autogenous strain may offer a more tractable system to investigate the role of hormones in housefly vitellogenesis, since we can neglect any problem of nutritional deficiency caused by various surgical treatments such as decapitation, ligation or extirpation. Vitellogenesis of autogenous houseflies of the Misaki strain normally starts approximately 48 hr after eclosion and egg maturation is completed about 7 days thereafter at 25°C (Agui et al., 1985). However, the decapitation of adult female flies of this strain within 24 hr after eclosion completely prevented their follicle
development. This is the same result obtained in decapitation experiments of anautogenous houseflies just after eclosion (Sakurai, 1977). Based on our results, the critical period during which the egg development neurosecretory hormone (EDNH) is released from the brain-ring gland complex in Misaki strain houseflies seems to be within 24 hr of eclosion. In autogenous mosquitoes, *Aedes taeniorhynchus*, EDNH is released shortly after eclosion without any stimulus, but in anautogenous *A. aegypti*, it is released after blood feeding (Lea, 1970, 1972).

In the cyclorrhaphous flies, it has long been indicated that corpora allata is involved in the regulation of oogenesis (Thomsen, 1943; Possompès, 1955; Wilkens, 1968; Adams, 1970). Topical application of either JH or JHA analog to a female as a treatment for allactectomy was reported to induce ovarian maturation (Adams and HINTZ, 1969; Sakurai, 1977). JH analog application further progressed oogenesis in starved or decapitated houseflies as well as vitellogenesis in those sugar-fed (Sakurai, 1977). In the present experiments, the increase of vitellogenin in hemolymph in addition to the progress of oogenesis was observed immunologically in the decapitated autogenous female flies by the application of a 0.5–1.0 μg dose of methoprene, but vitellogenin was not induced in males by hormone treatment. Similar results demonstrated that vitellogenin was induced by the application of a comparable dose of JH or JHA analog in the female hemolymph of other flies, *Drosophila melanogaster* (Handler and Postlethwait, 1978; Postlethwait and Handler, 1979) and *Sarcophaga bullata* (Engelman et al., 1971), but not in the hemolymph of males (Postlethwait et al., 1980; Huybrechts and De Loof, 1982). Unlike the JH analog, methoprene at a dose of 2 μg has no stimulating effect on vitellogenin synthesis of the female *S. bullata* (Huybrechts and De Loof, 1982). In this species, however, more reliable data on the effect of JH may be obtained by in vitro experiments as described in *D. melanogaster* (Jowett and Postlethwait, 1980).

Dose-relation of ecdysterone and vitellogenin induction have been reported in *S. bullata* (Huybrechts and De Loof, 1981) and in *D. melanogaster* (Bownes, 1982). The clear dose-dependent effect of ecdysterone on follicle development, on the increase of vitellogenin content in hemolymph of female houseflies and on the induction of vitellogenin in male houseflies were also demonstrated in the present experiments. Furthermore, it was ascertained that the fat body of adult female of *D. melanogaster* responds to either ecdysterone or JHA by synthesizing and secreting vitellogenin into the culture medium, but the ovary can produce yolk proteins only by JHA application; this was determined when the isolated abdomens were treated with hormones and the stimulated organs then cultured in vitro with 35S-methionine (Jowett and Postlethwait, 1980). Similarly, the cultured adult fat body in female houseflies at the vitellogenic stage responds to ecdysterone and synthesizes and releases vitellogenin labeled with 35S-methionine into the culture medium. This was revealed by the fluorographic analysis of SDS-PAGE for the synthesized proteins in vitro (Agui and Izumi, in preparation). The induction of vitellogenin synthesis by ecdysterone is more directly proved through studies of molecular levels such as the cell-free translation system of yolk polypeptide m-RNA or the hybridization of cloned copies of yolk polypeptide genes to total RNA in male *D. melanogaster* (Shirk et al., 1983; Bownes et al., 1983).

Morphological observations showed that the follicle in the housefly ovary transplanted into an adult male host developed from previtellogenic to vitellogenic phase (Table 5), and further that a small number of eggs matured in the male host after several days.
of in vivo culture (Agui, unpublished data). Interestingly, only a trace level of vitellogenin was detected in the hemolymph of the male host holding a vitellogenic ovary, although one would expect the transplanted ovary to stimulate the vitellogenin synthesis in the male fat body by the secretion of an appropriate level of ecdysone; such a phenomenon was seen in A. aegypti (Hagedorn et al., 1979). Analogous observations reported that in D. melanogaster the transplanted ovary matured in the male host (Bodenstein, 1947; Kambsellis, 1977; Srdic and Jacobs-Lorena, 1978; Postlethwait et al., 1980), and that the transplanted ovary itself synthesized the yolk proteins in the male host for egg maturation (Srdic et al., 1979; Postlethwait et al., 1980).

The change of vitellogenin levels is reflected in the change of ecdysteroid levels in hemolymph of female houseflies during the first gonotrophic cycle: peaks of surge in both vitellogenin and in ecdysteroids appeared in the middle of the vitellogenic phase. The increase of ecdysteroid level in the hemolymph of female houseflies is also coincident with the increase of ecdysteroid level in the ovaries as described in S. bullata (Briers and De Loof, 1980). Meanwhile, although a considerable amount of vitellogenin can be detected, no conspicuous ecdysteroid surge appears in the hemolymph of the ovariectomized female houseflies. These results suggest that vitellogenin synthesis in the fat body of ovariectomized females is due to a very low level of hemolymph ecdysteroids (<4 ng/ml) which may originate other tissues than the ovary, and/or may depend on JH released into the hemolymph. The lack of large ecdysteroid surge in the hemolymph of intact male houseflies seems responsible for the absence of vitellogenin synthesis in the male fat body. However, the transplanted ovaries preceded egg maturation in the male host, although the ecdysteroid surge was not found in the hemolymph of male host houseflies with ovaries. The results strongly indicate that the ovary of the houseflies has no role in the ecdysteroid secretion into the hemolymph of a host male, and that the transplanted ovary itself synthesizes the yolk proteins for egg development. A possible yolk protein synthesis in the transplanted ovary in the male was further elucidated by the organ culture of vitellogenic ovary of housefly (Agui and Izumi, in preparation) as a similar result obtained by in vitro experiment in D. melanogaster (Postlethwait et al., 1980).

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REFERENCES


Hormonal Control of Housefly Vitellogenesis


