



# Vitellogenesis in diapausing and mutant *Drosophila melanogaster*: further evidence for the relative roles of ecdysteroids and juvenile hormones

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## Abstract

The juvenile hormones (JHs) have long been believed to be key elements of the regulation of vitellogenesis in *Drosophila melanogaster*. This essential role for JH was challenged in Richard et al. (Journal of Insect Physiology 44 (1998) 637) in a novel model of the endocrine control of vitellogenesis. Further evidence supporting this proposed model and for understanding yolk protein (YP) production and uptake in JH-deficient conditions is presented here. Pre-vitellogenic diapause in the Canton-S strain was terminated within 4 days by the injection of 0.1 ng 20-hydroxyecdysone; the application of 1 µg JH III failed to elicit a response suggesting once more that ecdysteroids may be the more important agent. Nevertheless, this dose of JH III did reverse the delay associated with the onset of reproductive development of the JH-deficient mutant *ap<sup>56f</sup>* in a manner consistent with the proposed role for JH of stimulating early YP synthesis by ovarian follicle cells. Similarly, JH III application to *ap<sup>d</sup>* females also stimulated a degree of ovarian development. A high affinity JH III binding factor ( $K_D=1.5$  nM) in whole body extracts was quantified by equilibrium dialysis. Binding levels were greater in Canton-S females than in *ap<sup>56f</sup>* females though in *ap<sup>56f</sup>* binding could be stimulated within 18 h of eclosion by the application of 1 µg JH III. Ovaries from *ap<sup>56f</sup>* and Canton-S failed to produce any JH-like compounds. These data are discussed in the context of our model for the endocrine control of vitellogenesis in *Drosophila*. © 2001 Elsevier Science Ltd. All rights reserved.

**Keywords:** *Drosophila melanogaster*; 20-Hydroxyecdysone; Juvenile hormone; Vitellogenesis; Yolk protein

## 1. Introduction

Juvenile hormone (JH) and ecdysteroids have long been believed to play positive gonadotropic roles in reproduction in adult insects. Juvenile hormone synthesized by the corpus allatum (CA) of *Drosophila melanogaster* stimulates yolk protein (YP) synthesis and uptake by the developing oocytes; ecdysteroids produced by follicle cells in the ovary, and by other tissues, stimulate fat body YP synthesis (Koeppel et al., 1985; Bownes et al., 1993; Kelly, 1994). This model is supported by observations of the JH-deficient mutant, *apterous<sup>d</sup>*, in which little vitellogenesis occurs; a condition partially

rescued by the application of the JH analog methoprene (Postlethwait and Weiser, 1973). Indeed, CA from these females fail to produce normal levels of JH in vitro (Altaratz et al., 1991). Furthermore, when methoprene was applied to starved wild-type adults or isolated abdomens, under these conditions YP synthesis would be low, YP transcript became elevated (Jowett and Postlethwait, 1980). Methoprene also stimulated fat body YP transcript levels in fed flies (Bownes et al., 1987) further supporting the postulate that JH is required for vitellogenesis. In Richard et al. (1998), we presented evidence that this may not be due to the direct effect of the JH-analog upon YP synthesis by fat body, but rather due to JH-stimulation of ovarian ecdysteroid synthesis, the latter in part responsible for YP production and uptake. Bownes et al. (1996) presented evidence that 20-hydroxyecdysone not JH regulated YP gene expression via *cis*-acting sequences, further suggesting that JH does not directly affect YP transcription in the fat body.

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This widely accepted role for juvenile hormone was challenged by observations of another JH deficient mutant (*apterous<sup>56f</sup>*) in which near normal vitellogenesis occurs in the absence of normal levels of JH production by isolated CA (Altartatz et al., 1991; Richard et al., 1998). However, ovaries from these flies produce above normal levels of ecdysteroids (Richard et al., 1998). The only apparent changes in reproduction as compared to wild-type females are a delay in YP uptake (Altartatz et al., 1991) and altered sexual activity (Ringo et al., 1991). We believe that the delay is the result of absent early YP synthesis due to sub-threshold levels of JH production and supporting data will be presented in this paper.

Many insects enter an over-wintering diapause condition in response to changes in photoperiod and temperature, apparently under the control of the endocrine system which can disrupt the synthesis of JH and/or ecdysone (see Denlinger, 1985). This exhibits itself in *D. melanogaster* as a pre-vitellogenic arrest in ovarian development (Saunders et al., 1989). No YPs are deposited in the developing oocytes even though they are present in the hemolymph (Saunders et al., 1990) and both JH and ecdysteroid synthesis are depressed throughout (Richard et al., 1998). In some species, female reproductive diapause is thought to result from a block in JH production by the CA (De Wilde and De Boer, 1961; Hodkova, 1977). Indeed, the application of JH III or JHB<sub>3</sub> (Richard et al., 1989a) to abdomens of diapausing females did restore vitellogenesis (Saunders et al., 1990). However, diapause termination by warming the flies from 11 to 25°C involves an increase in ecdysteroid, but not JH synthesis, within 8 h of transfer (Richard et al., 1998). The injection of 1 µg 20-hydroxyecdysone into diapausing females can also initiate vitellogenesis. In other words, ecdysteroids, but not JHs are apparently active in diapause termination.

In this paper, we will present further evidence that a role for JH is to regulate early YP synthesis and uptake from follicle cells, and that in the absence of JHs, late YP synthesis by the fat body is possibly under the control of ecdysteroids. This naturally presupposes that the CA is the sole source of JH within the adult female fly. We shall present data supporting this supposition. The presence and concentration of JH binding factors in developing wild-type and mutant females will also be discussed in the context of a limited role for JH in the regulation of vitellogenesis.

## 2. Materials and methods

Wild-type *D. melanogaster* (Canton-S strain) were maintained under a L12:D12 photoperiod at 25°C unless otherwise indicated. Diapausing animals were obtained by allowing adults to eclose under an L12:D12 photop-

eriod at 12°C (Saunders et al., 1989). Stocks of *ap<sup>4</sup>/GlaBcElp* and *ap<sup>56f</sup>* mutant animals were raised under L12:D12 at 25°C. All animals were reared on artificial *Drosophila* medium (Carolina Biological) supplemented with dried baker's yeast. Flies were anaesthetized on ice prior to dissection or injection/application of hormones.

20-Hydroxyecdysone (Sigma) solutions were injected in 23 nl of Eagles minimal essential medium MEM (Sigma) into the abdomens of flies using an oil-displacement nanoliter injector (World Precision Instruments). Methylene blue was included in the MEM (1 mg/ml) as a visual indicator of successful injection in both experimental and control injections (no 20-hydroxyecdysone). One microgram of JH III (Sigma) in 1 µl acetone, or 1 µl acetone controls was applied to the abdomens of diapausing and *ap<sup>56f</sup>* females using a 10 µl Hamilton syringe.

Equilibrium dialysis binding assays (Klotz, 1989; modified by Park et al., 1993) involved dialyzing tissue extract against buffer containing radiolabeled JH III (17.4 Ci/mM, 54,000 dpm per tube, New England Nuclear). Two hundred milligrams of staged females were homogenized on ice in 2 ml PBS+I (comprising PBS pH 7.4 containing the protease inhibitors: EDTA, 1 mM; Leupeptin, 0.5 µg/ml; PMSF, 0.2 mM; Pepstatin, 0.7 µg/ml; Aprotinin, 1 µg/ml; Antipain, 5 µg/ml; Trypsin Inhibitor, 50 µg/ml). One milliliter of a 100,000 g cytosolic extract, diluted to 5 mg equivalents/ml in PBS+I, was enclosed in a length of PEG-treated 8000 Da MW cutoff dialysis tubing (Spectrapour Inc.), and the tube incubated overnight at 4°C in a 5 ml PEG-treated shell vial containing 1 ml of PBS+I supplemented with the radiolabeled JH III and unlabelled competitor JH III (Sigma) as appropriate. Levels of radioactivity in 0.5 ml aliquots of the extract and the dialysis buffer were determined using a Beckman LSC 6500.

The radiochemical assay for JH production in vitro, based on that developed by Tobe and Pratt (1974) was carried out with isolated third instar larval brain-ventral ganglion-ring gland complexes, whole abdomens and isolated ovaries in MEM (minus methionine) supplemented with L-[<sup>3</sup>H-methyl]-methionine (Amersham, specific activity 81 Ci/mmol) as the methyl-group donor, and with the JH-esterase inhibitor octyl-1,1,1-trifluoropropanone (OTFP) to a concentration of 0.5 µM (Hammock et al., 1984) as described previously (Richard et al., 1989a; Altartatz et al., 1991). Immediately prior to use, the tritiated methionine was washed 10 times with 50 volumes of hexane to remove lipid soluble contaminants that would otherwise interfere with the assay. Whole disrupted abdomens, single pairs of ovaries and third larval instar brain/ring gland complexes were incubated for 2 h at 25°C in the presence or absence of 20 µM JH-precursor farnesoic acid (FA) (kindly supplied by Dr Stephen S. Tobe, University of Toronto). Pooled

hexane fractions were subjected to silica gel thin-layer chromatography with a JH III standard visualized under UV light as described previously (Richard et al., 1989a).

### 3. Results

Ovarian volume was calculated using the equation  $\pi 0.5LW$  where  $L$  is the ovary length and  $W$  is the ovary width (Richard et al., 1998). The ovarian maturity index (OMI) was calculated by multiplying ovarian volume by the stage of the most mature vitellogenic oocyte (determined according to King, 1970). If no yolk was observed, the vitellogenic state was designated as stage 7. The use of OMI as a measure of development allowed the rapid quantitative screening of large numbers of ovaries during the course of these experiments.

In Richard et al. (1998) we reported that the injection of 1  $\mu\text{g}$  20-hydroxyecdysone into diapausing females resulted in the termination of diapause as determined by the appearance of yolk in the developing oocytes. Fig. 1 demonstrates that dosages of 20-hydroxyecdysone as low as 0.1 ng are able to terminate diapause, resulting in an increase in OMI from the diapausing level of between 200 and 300 to approximately 1000 within 7 days. The time course of diapause termination is shown in Fig. 2, where the injection of 11 ng 20-hydroxyecdysone resulted in a significant increase in OMI within 4 days ( $P < 0.001$ ,  $t = -4.55$  with 27 df). MEM-injected and non-injected females did not show the same increase. Stage 13/14 oocytes were present in the injected, but not in the untreated or MEM-injected, females after 4 days. Females to which 1  $\mu\text{g}$  JH III was applied on day 9 did not show an increase in OMI.

The ability of JH III to stimulate reproductive pro-

cesses is demonstrated by the rescue of the 6 h delay associated with  $ap^{56f}$  vitellogenic development by the application of 1  $\mu\text{g}$  JH III in 1  $\mu\text{l}$  acetone at 6 h post eclosion. The flies were dissected at 2 h intervals thereafter. Stage 14 oocytes were found 6 h earlier in the JH III-treated than in either the acetone-treated or untreated females (Fig. 3). The application of a similar dose of JH III to  $ap^4$  homozygous females 1 h after eclosion also has a significant stimulatory effect on OMI ( $P = 0.008$ ;  $t = 2.71$  with 13 df) as measured at 18 h post eclosion at 25°C (Fig. 4). However, this OMI increase was due primarily to changes in ovary volume as little visible accumulation of YPs was noted. The nature of this volume increase is uncertain.

Scatchard analysis (Fig. 5A) of tritiated JH III binding to 1 ml of a 5 mg/ml 24 h female Canton-S *D. melanogaster* 100,000 g cytosolic extract was performed. JH III was added to the assays as a competitor over a 0.14–140 pmol concentration range. This demonstrated that a JH III binding factor was present with a saturable affinity of binding ( $K_D$ ) of 1.55 nM and a binding site concentration ( $R_T$ ) of 0.0162 nmol/mg extract.

Homogenized extracts of newly eclosed Canton-S wild-type females (as described above) bound three times as much JH III as equivalent aged  $ap^{56f}$  females, whereas by 4 h post eclosion and thereafter, they bound only twice as much (Fig. 5B).

The ability of topically applied JH III to induce increases in JH III binding capacity in  $ap^{56f}$  females was investigated by equilibrium dialysis of extracts of 18 h post-eclosion females to which 1  $\mu\text{g}$  JH III had been applied 6 h post eclosion (Fig. 6). Significant increases in JH III binding were observed as compared to acetone-applied controls ( $P = 0.013$ ;  $t = 2.299$  with 34 df). Equivalent increases were not noted upon the application of JH III to wild-type females.

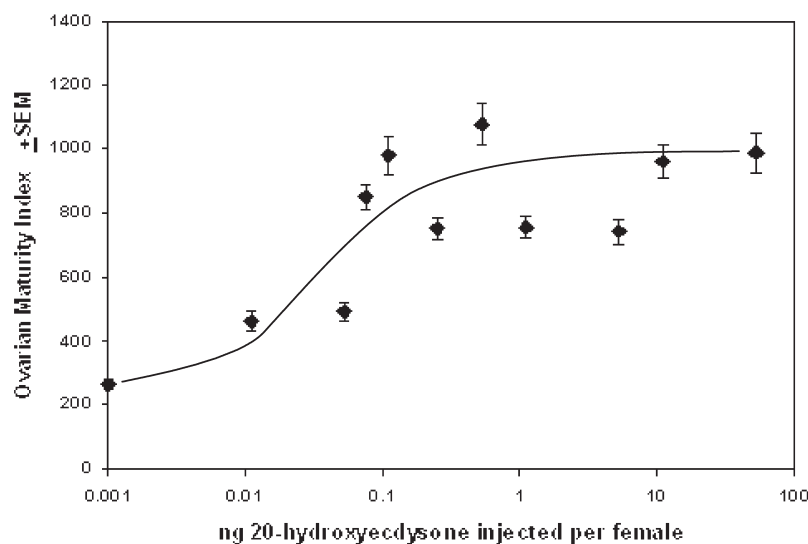


Fig. 1. The dose-dependent effect of 20-hydroxyecdysone injection (in 23 nl MEM supplemented with 1 mg/ml methylene blue) on the ovarian development (OMI) of diapausing Canton-S *D. melanogaster* females 14 days after eclosion in L12:D12 at 12°C. Flies were dissected and evaluated 7 days after injection. OMI of MEM injected females and uninjected females 21 days post eclosion were  $370 \pm 57$  and  $354 \pm 22$ , respectively;  $n = 7-14$ .

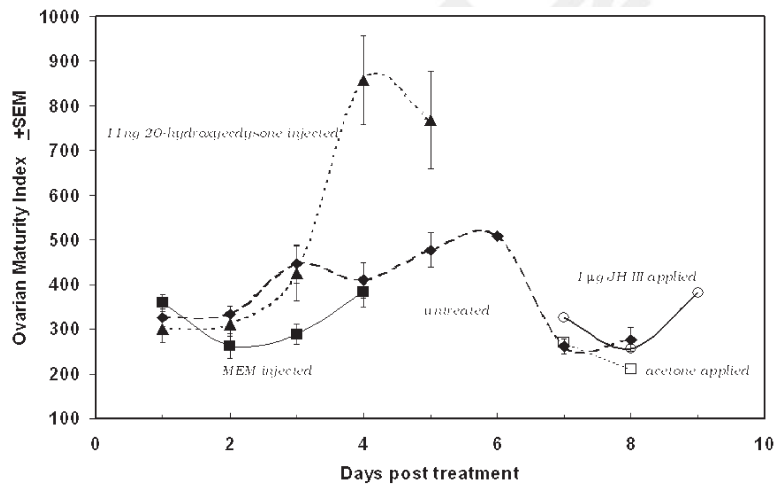


Fig. 2. The time course of diapause termination in day-14 Canton-S female *D. melanogaster* following injection of 20-hydroxyecdysone (11 ng in 23 nl MEM supplemented with 1 mg/ml methylene blue — filled triangles); MEM (23 nl supplemented with 1 mg/ml methylene blue — filled squares); or application of JH III (1 µg in 1 ml acetone — open circles) or acetone (open squares). Untreated females shown by open diamonds. Females were treated and then dissected for the evaluation of ovarian development (OMI) at the time points shown;  $n=16-30$ .

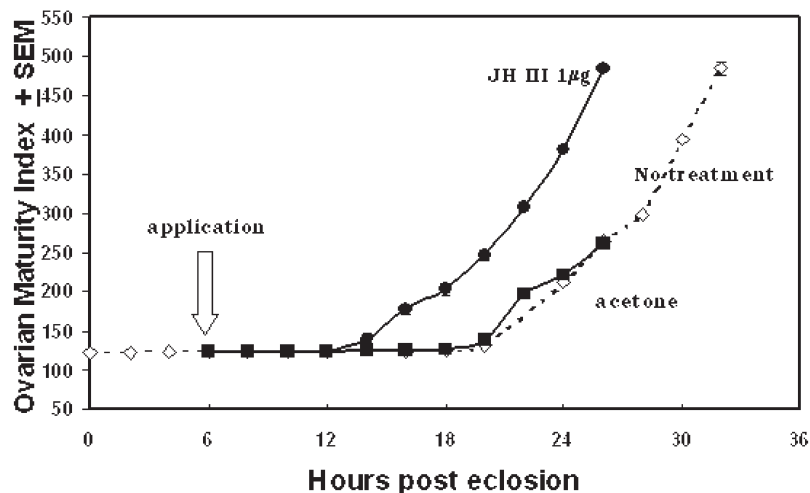


Fig. 3. The effect of application of 1 µg JH III in 1 µl acetone (filled circles) or 1 µl acetone (filled squares) 6 h after eclosion on the ovarian development (OMI) of *ap<sup>56f</sup>* female flies. Females were dissected at 2 h intervals thereafter. Untreated females are shown by open diamonds. The numbers in parentheses denote the mean number of stage-14 oocytes (King, 1970) per female;  $n=12-14$ .

The apparent level of JH production (Fig. 7) by both Canton-S and *ap<sup>56f</sup>* 24 h post-eclosion ovaries in vitro as measured by radiochemical assay in the absence of the JH precursor FA was comparable to published levels of JH production by isolated ring glands and adult CA (Richard et al., 1989b; Altaratz et al., 1991). However, these levels did not increase in the presence of FA, and even decreased in the case of *ap<sup>56f</sup>* ovaries. Brain-ventral ganglion-ring gland complexes produced expected levels of radiolabel incorporation in both the absence and presence of FA. When the pooled hexane fractions from the farnesic acid-treated incubations were analyzed by thin layer chromatography (Fig. 8), a factor consistent with JHB<sub>3</sub> was noted only with the brain-ventral ganglion-ring gland complexes (JHB<sub>3</sub>, Rf=0.68, JH III

Rf=0.81). No JH-like factor was noted in either the FA treated or untreated ovaries (data not shown). Likewise, when whole, disrupted abdomens were incubated in the presence or absence of FA, no JH-like compounds were produced (data not shown). The identity(ies) of the hexane soluble radiolabel from the ovarian incubations shown in Fig. 7 remains unknown.

#### 4. Discussion

The data presented here support a model (Richard et al., 1998) in which ecdysteroids play the more important role in the regulation of vitellogenesis in *D. melanogaster*. JHs may play a less essential role as YP synthesis

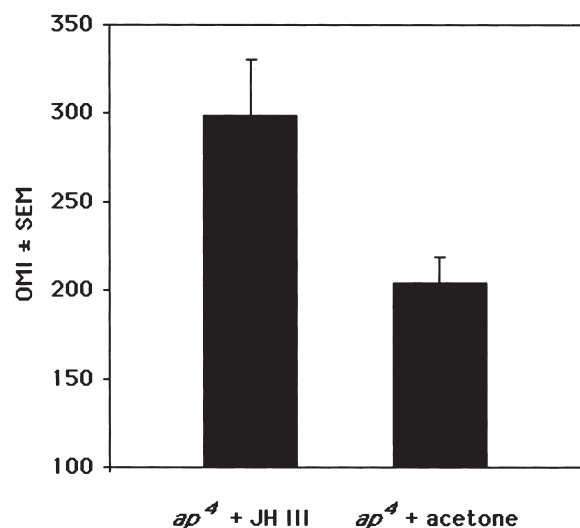


Fig. 4. The effect of JH III application (1  $\mu$ g in 1  $\mu$ l acetone) on the ovarian development (OMI) of *ap<sup>4</sup>* females at 25°C. JH III was applied 1 h post eclosion, dissection and evaluation at 18 h post eclosion;  $n=10-12$ .

and uptake can occur in their absence. This model was based upon observations of the JH-deficient *ap<sup>56f</sup>* mutation in which vitellogenesis occurred with only a delay in its onset, and observations of diapausing Canton-S females. Our interest in diapause is two-fold: (1) the processes by which this overwintering condition is induced and terminated, and (2) as a tool by which normal endocrine function can be interrupted conditionally in a wild-type background. Since some endocrine-associated genes have proven to be pleiotropic in nature, for example *apterous* (Cohen et al., 1992) and the temperature-sensitive allele *ecdysoneless<sup>1</sup>* (Redfern and Bownes, 1983), it is useful to disrupt hormonal production in a wild-type fly without worrying that other genetic influences or non-specific effects of temperature elevation may be having an effect.

Diapause in many insects is characterized by stage specific arrested development resulting from pre-programmed endocrine lesions (Denlinger, 1985). In *D. melanogaster*, diapause is exhibited as a pre-vitellogenic arrest under short-day photoperiods at low temperatures (Saunders et al., 1989). JH synthesis by isolated corpora allata (Saunders et al., 1990) and ovarian ecdysteroid synthesis (Richard et al., 1998) are low during diapause. However, during diapause termination, only ecdysteroid synthesis is elevated, JH production remains below the limits of detection (Richard et al., 1998). Nevertheless, the application of JH III or JHB<sub>3</sub> to day-14 diapausing females can result in the termination of diapause in some circumstances (Saunders et al., 1990). Furthermore, Richard et al. (1998) reported that the injection of 1  $\mu$ g of 20-hydroxyecdysone into similar diapausing females resulted in the termination of diapause as noted by an increase in ovarian volume and in mean oocyte stage

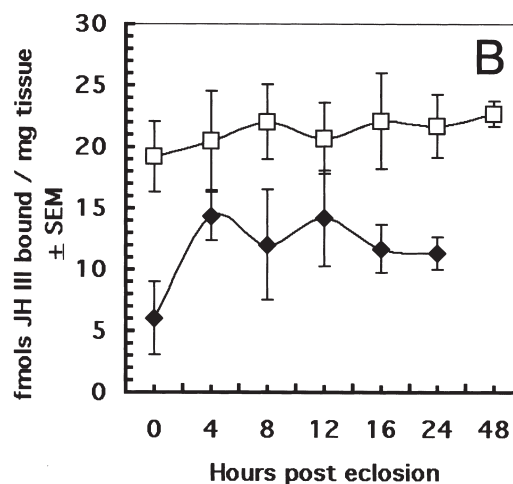
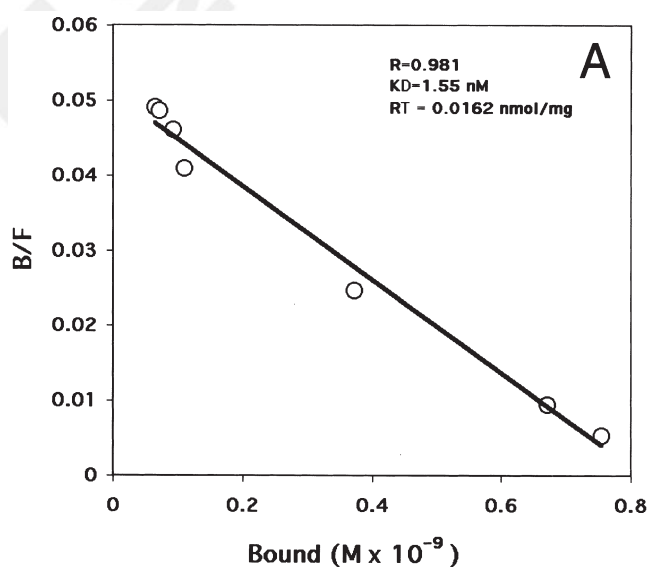


Fig. 5. (A) Scatchard plot of JH III binding to 1 ml of a 5 mg/ml 24 h female Canton-S 100,000 g cytosolic extract (tritiated JH III, New England Nuclear, 0.14 pmol of 17.4 Ci/mM JH III, 54,000 dpm per assay). Unlabeled JH III (Sigma) was added to the assays as a competitor over a 0.14–141 pmol concentration range. The dissociation constant ( $K_D$ ) and total binding capacity ( $R_T$ ) were calculated from the regression line ( $r$ =correlation coefficient). (B) JH III binding in Canton-S (squares) and *ap<sup>56f</sup>* (diamonds) females during the 48 h following eclosion at 25°C. Binding measured by equilibrium binding of 1.4 pmol of 10 Ci/mM tritiated JH III (46,000 dpm) with 1 ml of 5 mg/ml 100,000 g cytosolic extract (in PBS plus protease inhibitors) of 24 h females;  $n=3-6$ .

(King, 1970). Here we report that diapausing females are even more sensitive to ecdysteroids than previously demonstrated. The injection of only 0.1 ng 20-hydroxyecdysone is sufficient to elicit major responses by the ovaries; ovarian volume and developmental stage both increase significantly. This level of injected hormone is close to the physiological range (2–10 pg/ovary/5 h; Richard et al., 1998) of ecdysteroid production by isolated wild-type ovaries in vitro and to the published concentration of immunoreactive ecdysteroids in adult hemolymph at eclosion of 10 pg/ $\mu$ l (Handler, 1982).

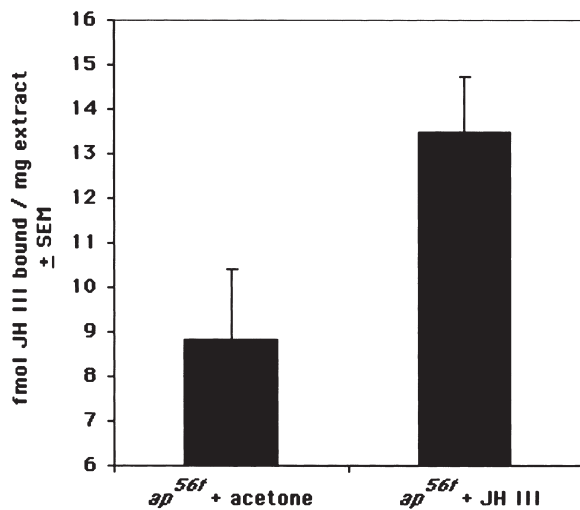


Fig. 6. The inducibility of JH binding in 18 h post-eclosion *ap<sup>56f</sup>* females by 1  $\mu$ g JH III in 1  $\mu$ l acetone applied at 6 h post eclosion to 500 flies ( $n=35$ ). Acetone was applied as a control to 100 flies ( $n=16$ ). Binding assayed by equilibrium dialysis as described in Fig. 5A.

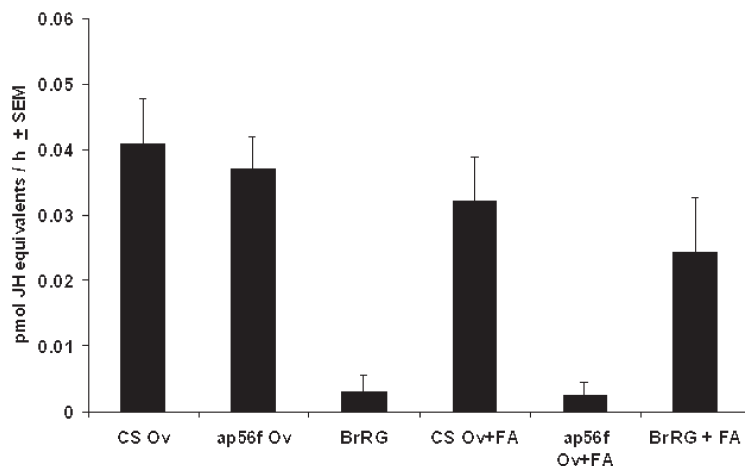


Fig. 7. The in vitro production of radiolabeled hexane soluble material (in pmol JH equivalents/h) by isolated ovaries from 24 h post-eclosion Canton-S and *ap<sup>56f</sup>* females, and by isolated brain-ventral ganglion-ring gland complexes from third instar Canton-S larvae, in the presence and absence of 20  $\mu$ M FA. Incubation was for 2 h at 25°C;  $n=6-12$ .

We also report the extensive use of a novel method of characterizing ovarian development, the OMI. This takes into consideration both changes in ovarian volume (Richard et al., 1998) and the maximum developmental stage and as such provides a useful, rapid and quantitative means of comparing separate samples.

The time course of diapause termination following injection of 20-hydroxyecdysone and application of JH III suggests a greater temporal sensitivity to the steroid. The OMI increased to vitellogenic levels by 4 days post-injection, thereafter many stage 14 oocytes were present. No significant increase in OMI following JH application was noted even by 9 days post application. This surprising result failed to reproduce the data reported in Saunders et al. (1990) in which a similar dose of either JH III or JHB<sub>3</sub> stimulated diapause termination as measured

after 7 days. The possibility that the amount of JH III being applied to the diapausing females was insufficient to elicit a response, perhaps due to degradation, was discounted by the use of the same JH III in *ap<sup>56f</sup>* experiments discussed below in which the hormone was effective at the same concentration. Another possibility that remains to be addressed experimentally is the method of anaesthetizing the females prior to the application of the JH or the injection of the 20-hydroxyecdysone. In Saunders et al. (1990) we etherized flies prior to treatment whereas in the present study, and in Richard et al. (1998), we cooled the adults on ice. Organic solvents (notably hexane) have pupal-diapause terminating effects in both flies (*Sarcophaga crassipalpis*) and moths (*Manduca sexta*) (Denlinger et al., 1980). It is possible therefore that the ether sensitized the diapausing *Drosophila* females to JH application in a way not achieved by the less physiologically invasive method of chilling on ice. Diapause termination by JH application as we reported in Saunders et al. (1990) may therefore not accurately reflect the physiological condition but rather

be a pharmacological effect. This view is strengthened when the processes of receptor mediated endocytosis are examined during diapause termination. In normal vitellogenesis, the endocytosis proteins clathrin,  $\alpha$ -adaptin and the putative YP receptor are seen by immunofluorescent localization both lining the boundary between follicle cells and the oocyte during early YP uptake, and in nurse cells during late YP uptake (Richard, unpublished observations). This follicle cell based endocytosis is not seen during diapause termination, consistent with the observation that JH production does not rise during this process (Richard et al., 1998), and therefore with the model in which JHs only regulate early YP synthesis and uptake in non-diapausing females. Since JHB<sub>3</sub> has been demonstrated to stimulate ovarian ecdysteroid production in newly eclosed females (Richard et al., 1998),

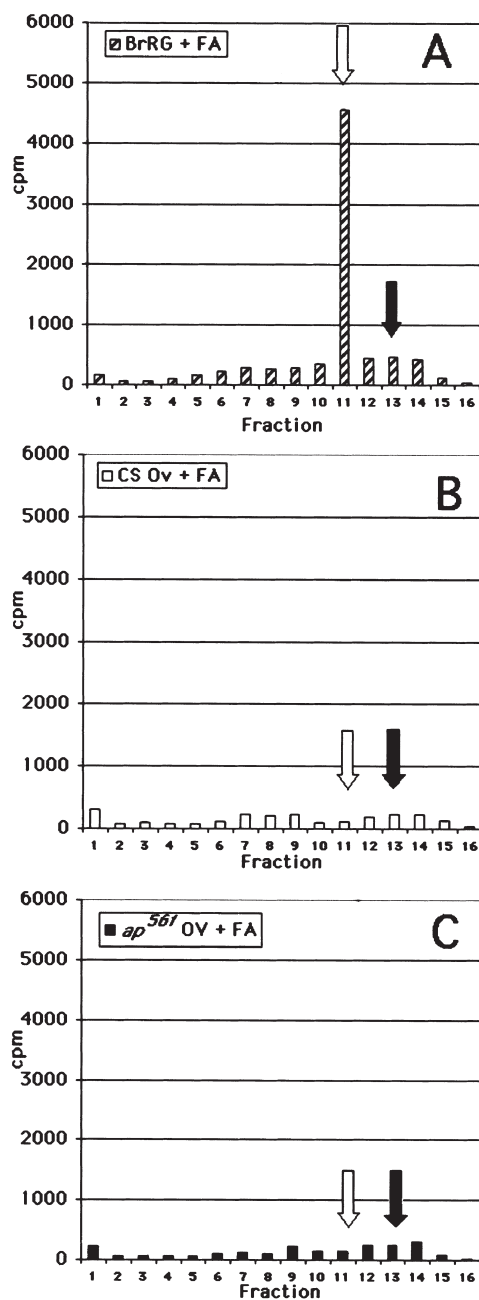


Fig. 8. Thin-layer chromatographic analysis (Kodak silica gel F plates developed with 3:2 hexane:ethyl acetate) of selected radiolabeled products from Fig. 7. (A) Brain–ventral ganglion–ring gland complex plus 20  $\mu$ M FA; (B) 24-h post-eclosion Canton-S ovaries plus 20  $\mu$ M FA; (C) 24-h post-eclosion *ap<sup>56f</sup>* ovaries plus 20  $\mu$ M FA. The filled arrow denotes the position of a JH III standard ( $R_f=0.81$ ), the open arrow denotes the position of putative JHB<sub>3</sub> ( $R_f=0.68$ ).

it remains an additional possibility that the potentially ether-sensitized females (Saunders et al., 1990) responded to JH application by increasing ovarian ecdysteroid production, and that it was the ecdysteroids that subsequently terminated diapause.

We proposed (Richard et al., 1998) that the JH deficiency in *ap<sup>56f</sup>* females resulted in the absence of

early YP synthesis by ovarian follicle cells but that late fat body YP synthesis and trafficking still occurred under the control of ecdysteroids produced by the ovaries themselves (Garen et al., 1977; Rubenstein et al., 1982), and possibly by other tissues as well (Bownes et al., 1984). The lack of follicle cell YPs, therefore, may have resulted in the delay associated with this mutation reported in Altaratz et al. (1991). In the present paper, the application of 1  $\mu$ g of JH III in 1  $\mu$ l acetone to the abdomens of cold-anaesthetized *ap<sup>56f</sup>* females rescued this delay as determined by both an OMI increase and an increase in the number of stage 14 oocytes. These observations were based upon the application of the same dose of JH III to chilled *ap<sup>56f</sup>* females that failed to elicit a response when applied to day-14 diapausing Canton-S females. Interestingly, when 1  $\mu$ g JH III was applied to 1 h post-eclosion *ap<sup>4</sup>* homozygotes, these too showed an increase in reproductive condition as determined by OMI, suggesting that JH may restore partial function to even these severe mutants before premature death ensues. Both alleles therefore show a delay of ovarian development, possibly associated with lower early YP synthesis by follicle cells caused by an absence of JH. In both cases, this can be rescued by JH III application, though to varying degrees.

As shown, *ap<sup>56f</sup>* females have low levels of JH III production yet they remain responsive to stimulation by JH application. Presumably, therefore, there must be binding protein/receptor systems in place, or at least inducible to working concentrations in order for such response to occur. Juvenile hormones are believed to act via nuclear-binding protein receptors (Riddiford, 1994), much like the ecdysteroid receptor (Koelle et al., 1992). When JH III binding was examined by equilibrium dialysis of whole body extracts of 24 h Canton-S females, a binding factor with a saturable affinity of binding ( $K_D$ ) of 1.55 nM was noted. Such high affinity binding is characteristic of receptor proteins and is identical to a 400 kDa JH binding protein ( $K_D=1.5$  nM) reported by Shemshedini and Wilson (1988). JH III binding in staged Canton-S females was steady over the 48 h following adult eclosion (this paper). However, *ap<sup>56f</sup>* female JH III binding was only one-third of the wild-type level at eclosion and approximately one-half thereafter. Interestingly, when JH III was applied to *ap<sup>56f</sup>* females 6 h after eclosion, and then JH III binding levels examined 12 h later, a significant increase in binding was noted as compared to the presumed developmental increase seen with acetone-only applications. These profiles are consistent with receptor levels that may be both developmentally regulated and be induced by the presence of the ligand such that in the absence of circulating JH III binding, binding may be lower. This supports our proposal that low circulating levels of JH in *ap<sup>56f</sup>* result in lower than normal levels of binding protein/receptor. Nevertheless, the presence of low levels of this putative

receptor would be essential to account for the response of *ap<sup>56f</sup>* to JH III in terms of the induction of early follicle cell YP synthesis, as noted above. The downstream effects of JH action would therefore appear to include the stimulation of YP synthesis, possibly by follicle cells, and the accelerated deposition of these YPs into the developing oocytes. It should, however, be acknowledged that these binding studies were performed on whole body extracts rather than extracted ovaries so such proposals remain tentative at present.

These conclusions are largely based upon the observation that the apparently JH-deficient *ap<sup>56f</sup>* females are not completely deficient in vitellogenic development. This presupposes that there is no source of JH in the insect other than the corpus allatum. The possibility that the ovaries themselves, or other abdominal tissues, are a source of JH was tested by the incubation of isolated 24 h post-eclosion Canton-S and *ap<sup>56f</sup>* ovaries or disrupted abdomens, in radioactive methionine in the JH radiochemical assay developed by Tobe and Pratt (1974) and modified for *Drosophila* by Richard et al. (1989a). The absence of JH production by ovaries or abdomens from either Canton-S or *ap<sup>56f</sup>* is consistent with the model proposed for the endocrine regulation of vitellogenesis (Richard et al., 1998). It remains to be seen whether other adult female tissues might be synthesizing JHs, though there seems little evidence to suggest that this might be the case. It is interesting to note that the ovaries do incorporate the radiolabel from methionine into hexane soluble material. However, TLC demonstrated conclusively that this material is not an identifiable JH. Brain–ventral ganglion–ring gland complexes from third instar larvae produced expected amounts of JHB<sub>3</sub>.

Whole body JH measurements (conducted by gas chromatography/mass spectroscopy according to the method of Bownes and Rembold, 1987), showed no correlation between the level of JH and overall fertility (M. Bownes, personal communication of unpublished information). Infertile *ap<sup>4</sup>* homozygous females contained as much JH (1.8 pmol/g) as Oregon-R females, and considerably more than the fertile *ap<sup>56f</sup>* females (1.2 pmol/g). The source and significance of this JH remains unknown as the CA of both *ap<sup>4</sup>* and *ap<sup>56f</sup>* fail to produce normal levels following eclosion (Altaratz et al., 1991). Nevertheless, these data support our proposal that vitellogenesis can occur in the absence of normal levels of JH production, and that JH levels themselves are not key controllers of vitellogenic development.

The mechanism by which YPs are subsequently translocated to the developing oocyte appears to be via receptor mediated endocytosis involving clathrin,  $\alpha$ -adaptin and a putative YP receptor (Richard, unpublished data). Early YPs are apparently trafficked across the follicle cell/oocyte boundary and late YPs from the fat body appear to be sequestered from the hemolymph by those

nurse cells immediately adjacent to the oocyte and then translocated via ring canals to the oocyte. These mechanisms remain a focus of our investigations in both diapausing and mutant females.

This paper provides supporting evidence for our previously published model for vitellogenesis in *D. melanogaster* in which JHs stimulate early YP synthesis by follicle cells, and ecdysteroids stimulate late YP synthesis by the fat body (Richard et al., 1998). While these observations advance our understanding of female reproductive processes, they are as yet incomplete. The involvement of other factors such as the 36 amino acid sex peptide or mating (Soller et al., 1997; Moshitzky et al., 1996) in these processes remains to be assessed experimentally in the context of our proposed model.

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