

## THE ROLES OF JUVENILE HORMONE AND 20-HYDROXY- ECDYSONE DURING VITELLOGENESIS IN ISOLATED ABDOMENS OF *DROSOPHILA MELANOGASTER*

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**Abstract**—Both juvenile hormone and 20-hydroxy-ecdysone seem to be involved in the regulation of vitellogenesis in *Drosophila melanogaster*. It is the purpose of this paper to begin to define the functions of these two hormones. Although vitellogenin synthesis does not occur at a high rate in 1-day-old female abdomens isolated from the head and thorax before 0.75 hr after eclosion, both ZR515 (a juvenile hormone analogue) and 20-hydroxy-ecdysone can cause in these preparations vitellogenin synthesis and secretion into the haemolymph. The synthesis and secretion into the haemolymph of all three vitellogenins which are detectable by electrophoresis in sodium dodecyl sulphate-containing gels of polyacrylamide is promoted by both hormones. That result excludes the hypothesis that these two hormones regulate the synthesis of different vitellogenins. A dose-response curve showed that an injection of 0.2  $\mu$ l of a  $10^{-6}$  M 20-hydroxy-ecdysone solution was sufficient to promote vitellogenin synthesis and secretion in isolated abdomens. Ovaries from isolated female abdomens treated with juvenile hormone analogue showed nearly normal amounts of all three vitellogenins and morphologically normal advanced vitellogenic follicles, whereas ovaries from isolated abdomens treated with 20-hydroxy-ecdysone contained little vitellogenin and no vitellogenic follicles. We conclude that under the conditions used, juvenile hormone permits vitellogenin uptake into the oöcyte much more readily than does 20-hydroxy-ecdysone.

**Key word Index:** *Drosophila melanogaster*, vitellogenesis, 20-hydroxy-ecdysone, juvenile hormone, oögenesis, yolk protein synthesis.

### INTRODUCTION

TWO GENERAL processes are involved in vitellogenesis. The first is the synthesis of vitellogenin by the fat body and its secretion into the haemolymph, and the second is the sequestration of vitellogenin from the haemolymph and its deposition into yolk granules in the oöcytes (DOANE, 1973). Vitellogenin synthesis is regulated in different ways by various insect species. For example, in *Hyalophora cecropia*, vitellogenin synthesis is a programmed component of metamorphosis rather than an independently controlled process (PAN, 1977). In the two flies *Aedes aegypti* (HAGEDORN, 1974) and *Sarcophaga bullata* (HUYBRECHTS and DELOOF, 1977) either ecdysone or 20-hydroxy-ecdysone can induce vitellogenin synthesis, while in many other insects, juvenile hormone regulates vitellogenin synthesis (DOANE, 1973). In *Drosophila*, vitellogenin synthesis occurs in a mutant defective in juvenile hormone metabolism (GAVIN and WILLIAMSON, 1976) and there have been suggestions that ecdysteroids may be important in oögenesis (HODGETTS *et al.*, 1977; GAREN *et al.*, 1977). The present work is directed toward defining the roles of juvenile hormone and 20-hydroxy-ecdysone in *Drosophila* vitellogenesis.

We have recently shown that both 20-hydroxy-

ecdysone and juvenile hormone can cause vitellogenin synthesis in isolated female abdomens of *Drosophila melanogaster* (HANDLER and POSTLETHWAIT, 1978), although in those experiments, tube gels were used which did not resolve the vitellogenin region into the multiple bands detected in gradient slab gels (BOWNES and HAMES, 1977). There are several hypotheses that might account for the fact that two different hormones are each sufficient for the appearance of yolk protein in the haemolymph. The first possibility is that one hormone causes the other to appear, and it is the second hormone which actually stimulates vitellogenin synthesis. An alternative is that each hormone acts independently to induce a distinct protein species not resolved by the techniques previously used. The first purpose of this work is to test whether 20-hydroxy-ecdysone and juvenile hormone stimulate the synthesis of different vitellogenins in isolated female abdomens using high resolution slab gels to independently assay for each of the three vitellogenins.

It has been suggested that juvenile hormone is involved in the uptake of vitellogenin into the oöcyte on the basis of studies with female sterile mutants (POSTLETHWAIT and WEISER, 1973; GAVIN and WILLIAMSON, 1976; KAMBYSELLIS, 1977; POSTLETHWAIT and HANDLER, 1978) and surgically altered wild type flies (POSTLETHWAIT *et al.*, 1976; HANDLER and POSTLETHWAIT, 1977). The second purpose of this work was to test whether 20-hydroxy-ecdysone is also capable of causing vitellogenin to

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appear in oöcytes or whether the effects of 20-hydroxy-ecdysone and juvenile hormone analogue on isolated abdomens could be distinguished on this basis.

## MATERIALS AND METHODS

An Oregon R stock provided flies. Abdomens were isolated from the head and thorax of flies less than 0.75 hr old by ligation followed by removing the thorax with scissors. Age was determined from time of eclosion. The juvenile hormone analogue ZR515 (a gift from Zoecon Corporation) was dissolved in acetone to a concentration of 0.54 g/l and 0.3  $\mu$ l was topically applied to the tergites of experimental preparations. Controls received only acetone. 20-Hydroxy-ecdysone (Rohto Pharmaceutical) was dissolved at various concentrations in 10% ethanol in CHAN and GEHRING (1971) Ringer's solution and 0.2  $\mu$ l was injected into the ventral abdomen. Controls received only 10% ethanol in Ringers. Preparations were injected with 0.5 pCi of  $^3$ H-leucine (AMERSHAM) dissolved in 0.2  $\mu$ l Ringers for protein synthesis studies. Haemolymph was collected from the 6th tergite in a drawn out capillary tube. For SDS polyacrylamide gels, samples were dissolved in 1% SDS, 0.01%  $\beta$ -mercaptoethanol, 0.05 M Tris (pH 6.8) and boiled for 1 min, after which pyronin and glycerin were added at final concentrations of 0.2% and 6% respectively. A 5–20% gradient of polyacrylamide was used for the slab gels, employing the electrophoretic procedures described by O'FARRELL (1975). The autoradiographic procedures were those described by LASKEY and MILLS (1975). For sodium dodecyl sulphate (SDS) polyacrylamide tube gels, the procedure of SOBIESZEK and BREMEL (1975) was followed. Gels were sliced in 1 mm slices which were then incubated for 48 hr at 40 in toluene-based scintillation fluid with 2.5% Protosol, and counted in a Beckman scintillation counter. The haemolymph of ten preparations was pooled for each sample run on the gel. A total of 453 control and experimental isolated abdomens was used in the present study.

## RESULTS

### A. Hormonal control of vitellogenin synthesis

Our first objective was to find if 20-hydroxy-ecdysone and a juvenile hormone analogue cause different vitellogenin protein species to appear in the blood of isolated abdomens. To confirm the fact that none of the three vitellogenins is synthesized in abdomens isolated at the time of eclosion, two groups of isolated abdomens were prepared. The first group of abdomens was isolated at less than 0.75 hr after eclosion and allowed to age for 32 hr. The second group of abdomens was isolated from females 30 hr old to provide adult controls. When both sets of abdomens were 32 hr old, they were injected with  $^3$ H-leucine, and 2 hr later haemolymph was collected and proteins separated on denaturing polyacrylamide gels. Figure 1A and G show that abdomens isolated at 30 hr not only contained the three vitellogenin species but that they were also rapidly synthesizing those proteins.

In contrast, abdomens isolated at eclosion had at 32 hr reduced quantities of these vitellogenins (Fig. 1B), and had also an altered synthetic pattern (Fig. 1H). Only one slightly radioactive band was found in the yolk region. This band migrated slightly but consistently more rapidly than the larger molecular weight band. This experiment allows the conclusion that in abdomens isolated shortly after eclosion, the synthesis and secretion of vitellogenin is reduced or eliminated.

To find if a juvenile hormone analogue could cause the synthetic pattern to return to normal, we isolated female abdomens at less than 0.75 hr after eclosion and treated the abdomens with either juvenile hormone analogue or acetone at 24 hr followed by injection of  $^3$ H-leucine at 32 hr and collection of haemolymph at 34 hr. Figures 1C and I show that acetone treatment had no effect on the proteins present or the proteins synthesized by isolated abdomens (compare Fig. 1C and I and 1B and H). The juvenile hormone analogue on the other hand, caused the synthesis of all three vitellogenins (Fig. 1D and J). This experiment shows first, that the absence of vitellogenin synthesis in isolated abdomens is not due to an irreparable defect, but that these preparations are lacking a factor from the anterior that is required for vitellogenin synthesis. Second, this result indicates that a substance with juvenile hormone activity can replace the factor missing from isolated abdomens. These experimental results also rule out the hypothesis that juvenile hormone only controls the synthesis of one or two of the three vitellogenin proteins.

The effect of 20-hydroxy-ecdysone was tested by preparing isolated abdomens at less than 0.75 hr, injecting them with either  $10^{-4}$  M 20-hydroxy-ecdysone or control solution at 24 hr, injecting  $^3$ H-leucine at 32 hr, and finally, collecting haemolymph at 34 hr. Electrophoresis and fluorography (Fig. 1E and K) showed that ethanol-Ringers control solution did not stimulate vitellogenin synthesis, although the synthesis of some lower molecular weight species was enhanced. In contrast, 20-hydroxy-ecdysone stimulated rapid synthesis of all three vitellogenins (Fig. 1F and L). This result again rules out the hypothesis that ecdysteroids and juvenile hormone act by regulating the synthesis of separate vitellogenin proteins.

### B. Hormonal control of vitellogenin uptake

To test the effects of juvenile hormone analogue and 20-hydroxy-ecdysone on the uptake of vitellogenin into the oöcytes, we prepared isolated abdomens less than 0.75 hr after eclosion, treated them with either juvenile hormone analogue or  $10^{-4}$  M 20-hydroxy-ecdysone at 8 hr, and collected haemolymph and ovaries at 50 hr. In Fig. 2, 20 of these experimental ovaries were loaded to each of slots B, C, and D. The results show that juvenile hormone analogue caused all three vitellogenins to appear in the ovaries, although the faster migrating form was found in amounts somewhat less than normal. This extends our earlier work (HANDLER and POSTLETHWAIT, 1977) and shows that juvenile hormone analogue causes all three vitellogenins to appear in both haemolymph and ovaries. The ovaries of 20-hydroxy-ecdysone-treated isolated abdomens, on the other hand, contained much less vitellogenin (Fig. 2D). This result was

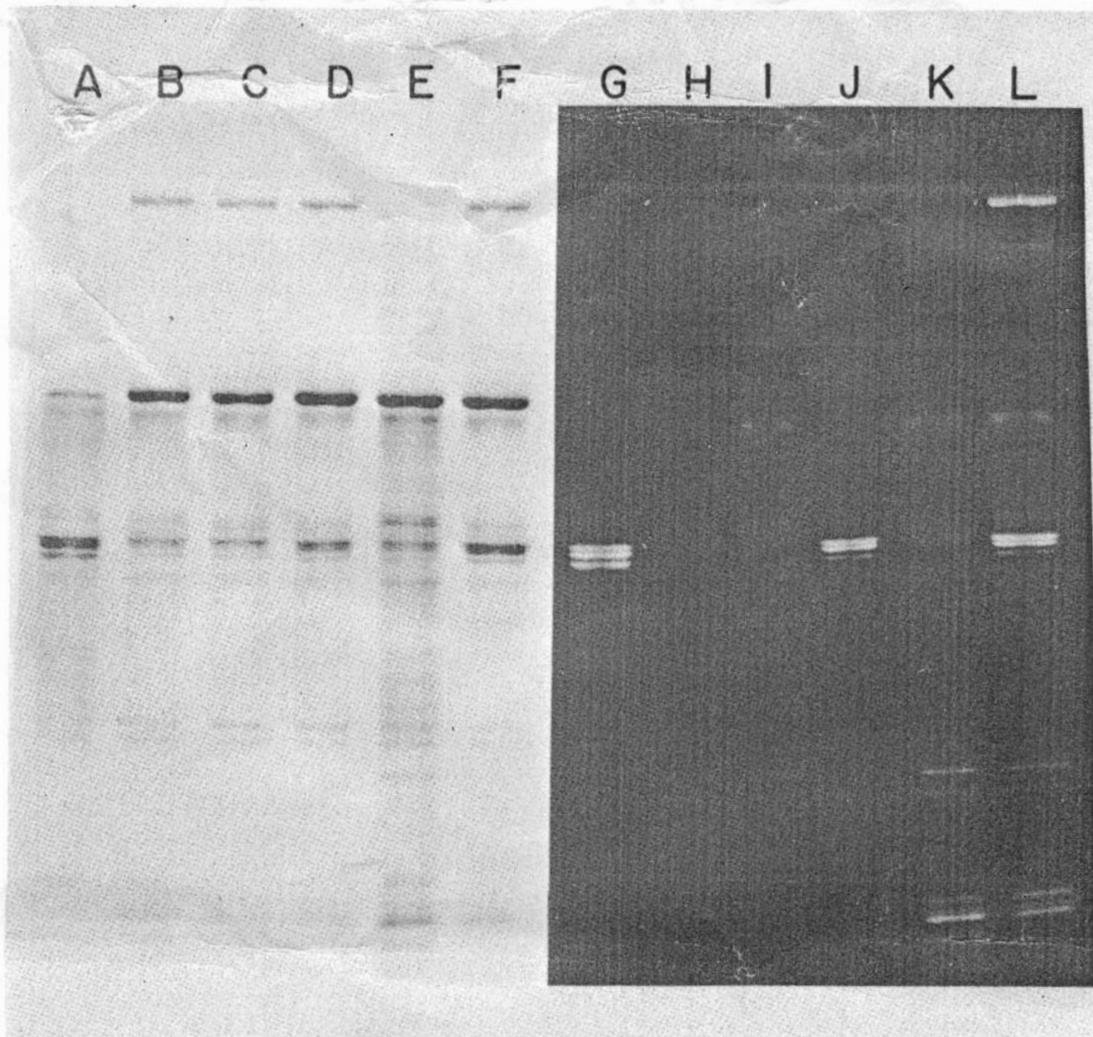


Fig. 1. Vitellogenin in haemolymph of isolated abdomens. A and G, abdomens isolated at 32 hr; B-F and H-L, abdomens isolated at less than 0.75 hr; B and H, no treatment; C and I, treatment with acetone; D and J, treatment with juvenile hormone analogue; E and K, injection with Ringers; F and L, injection with  $10^{-4}$  M 20-hydroxy-ecdysterone. For C to F and I to L, abdomens were treated with hormone or control solutions at 24 hr, injected with  $^3\text{H}$ -leucine at 32 hr, and haemolymph collected at 34 hr. A-F, gel stained for protein; G-L, autoradiograph. This experiment was conducted three times.

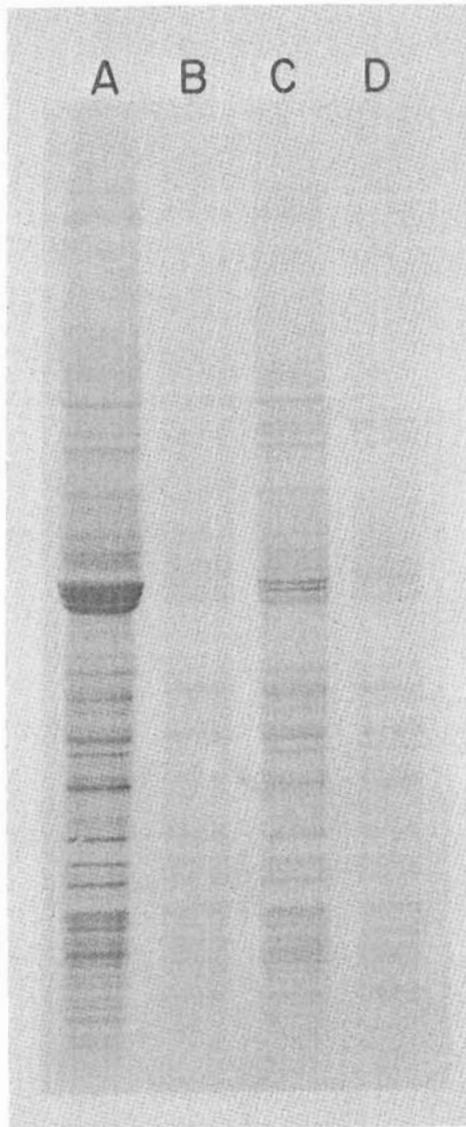


Fig. 2. Ovaries from control and hormone treated isolated abdomens. A. Four ovaries from 50 hr old untreated control females; B. abdomens isolated at less than 0.75 hr after eclosion and 20 ovaries dissected at 50 hr old; C. 20 ovaries from isolated abdomens treated with juvenile hormone analogue; D. 20 ovaries from isolated abdomens treated with  $10^{-4}$  M 20-hydroxy-ecdysone. The photograph is of the gel stained with Coomassie Blue. This experiment was conducted twice.

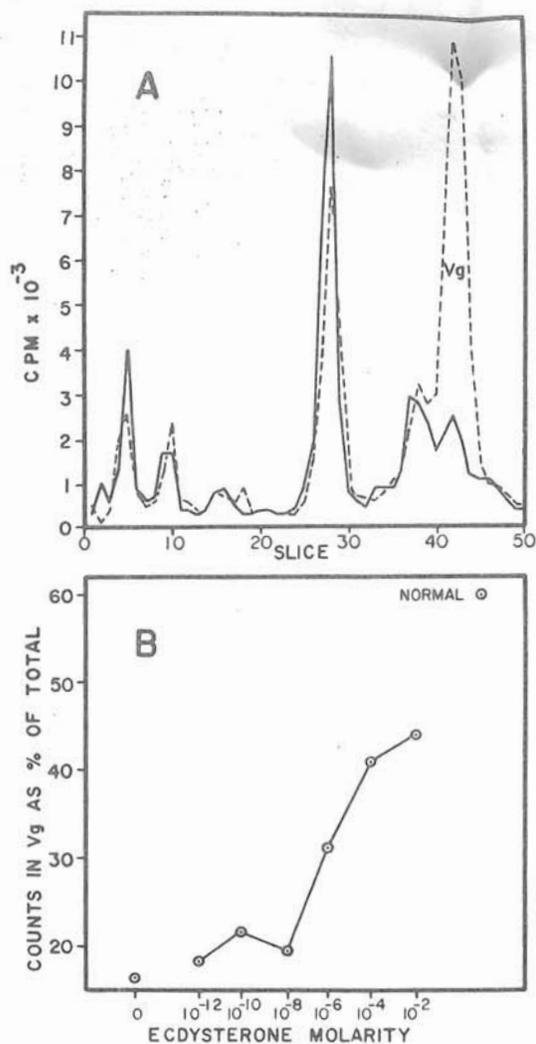


Fig. 3. Dose-response curve. A total counts incorporated into each 1 mm slice of the first half of gels of isolated abdomens injected with Ringers (solid line) or  $10^{-4}$  M ecdysterone (broken line), B per cent of counts in the vitellogenin peak for controls and various concentrations of ecdysterone (20-hydroxy-ecdysone). R Ringer injected controls; N, Normal females. This experiment was conducted three times.

confirmed by a direct visual observation of follicles in 20-hydroxy-ecdysone treated isolated abdomens. In 44 isolated abdomens treated with  $10^{-4}$ ,  $10^{-3}$ , or  $10^{-2}$  M concentrations of this hormone, no follicles were found over stage 7. This contrasts to the situation with juvenile hormone where 22 of 22 abdomens treated with juvenile hormone analogue contained vitellogenic stages including many morphologically mature stage 14 oöcytes (see also HANDLER and POSTLETHWAIT, 1977). Although 20-hydroxy-ecdysone can induce the appearance of vitellogenins in the blood, it does not cause these proteins to be sequestered in large amounts by the ovary.

#### C. Dose-response curve for 20-hydroxy-ecdysone

To find whether the moulting hormone is effective at physiological concentrations, we prepared isolated abdomens at less than 0.75 hr, and treated them at 24 hr with different concentrations of 20-hydroxy-

ecdysone. At 32 hr <sup>3</sup>H-leucine was injected into the preparations, and at 34 hr, haemolymph was collected and prepared for tube gel electrophoresis. Figure 3A shows the number of counts incorporated into different 1 mm slices after treatments with Ringers or high concentrations of 20-hydroxy-ecdysone. To obtain the data in the dose-response curve (Fig. 3B), we calculated the per cent of total counts incorporated into the vitellogenin peak. The data show that a response is first detectable at  $10^{-6}$  M.

## DISCUSSION

These experiments rule out the possibility that the synthesis of one or two of the three vitellogenins is regulated by juvenile hormone analogue while the complementary species is controlled by 20-hydroxy-ecdysone. Other hypotheses to account for this dual hormonal control of these three proteins have yet to be excluded. For example, juvenile hormone could cause an abdominal tissue to produce ecdysone or 20-hydroxy-ecdysone which then directly causes yolk protein synthesis (or *vice versa*). Alternatively, there might be two tissues that synthesize and secrete vitellogenin and each hormone might act on a different tissue. Or it may be that indeed one tissue can respond to either hormones by making all three proteins. These possibilities can be distinguished by *in vitro* culture experiments.

The concentration of 20-hydroxy-ecdysone required to cause an effect on vitellogenin synthesis can be compared to the amount required for *in vitro* effects. The minimal dose tested that gave an effect was  $0.2 \mu\text{l}$  of  $10^{-6}$  M hormone in isolated abdomens which represents about 60% of the weight of an intact fly. If the  $0.13 \mu\text{l}$  of haemolymph in a normal female (GAVIN and WILLIAMSON, 1976) is evenly distributed, then the final concentration of 20-hydroxy-ecdysone in the haemolymph would be about  $7 \times 10^{-7}$  M. Half maximal effect of 20-hydroxy-ecdysone on *Drosophila* tissues and cells in culture occur at  $10^{-8}$  to  $10^{-7}$  M (FRISTROM, 1972; ASHBURNER, 1973; CHERBAS *et al.*, 1977). Although not enough different concentrations were tested to accurately define the threshold and half maximal dose, it seems that this *in vivo* system requires only about one or two orders of magnitude greater concentration for an effect than the *in vitro* systems cited.

Although both 20-hydroxy-ecdysone and a juvenile hormone analogue can cause synthesis of the three vitellogenins, the functions of these hormones in the process of vitellogenesis are not totally overlapping. The present work shows that the juvenile hormone analogue causes both synthesis of vitellogenin and its sequestration into the oöcytes to form morphologically mature stage 14 oöcytes. The effects of 20-hydroxy-ecdysone are limited to promoting synthesis and secretion. No follicles in vitellogenic stages were seen, although a small amount of the three vitellogenins was detected in these ovaries by electrophoresis. We can therefore conclude that at hormone levels that cause equivalent amounts of vitellogenin synthesis, juvenile hormone analogue is much more effective at causing vitellogenin to appear in oöcytes, suggesting that juvenile hormone, but not

20-hydroxy-ecdysone, causes sequestration of vitellogenin.

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