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REGULATION OF VITELLOGENIN SYNTHESIS IN DROSOPHILA BY ECDYSTERONE AND JUVENILE HORMONE

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ABSTRACT The hormonal regulation of vitellogenin (Vg) synthesis in Drosophila was investigated by studying the incorporation of ^{3}H-leucine into hemolymph proteins of normal mature females and in isolated female abdomens either untreated or treated with solutions of a juvenile hormone analog (JHA), ecdysterone, or control solutions. It was found that the synthesis of Vg was reduced in untreated isolated abdomens and in isolated abdomens treated with control solutions, but that in isolated abdomens treated with either JHA or ecdysterone, Vg synthesis was specifically stimulated.

A Drosophila female can lay eggs amounting up to 60% of her weight each day (David and Merle, '68). A large part of this prodigious biosynthetic activity is directed toward producing vitellogenins (Vg), female-specific proteins that are made by the fat body, secreted into the blood, and sequestered by the developing oocyte to form yolk (Kambysellis, '77). It has been known for some time that the process of yolk deposition is under hormonal control in Drosophila (Vogt, '40) as well as in other insects (Engelmann, '70). In Drosophila, it has recently been shown that juvenile hormone is required for uptake of Vg into oocytes (Postlethwait and Weiser, '73; Gavin and Williamson, '76b; Kambysellis and Craddock, '76; and Postlethwait and Handler, in press). But the factors that induce the synthesis of Vg in Drosophila are still unknown. Vg synthesis is induced in cockroaches by juvenile hormone (Engelmann, '70) and in mosquitos by ecdysone (Hagedorn et al., '75). There are recent indications that ecdysones may also be important

in the ovarian maturation of Drosophila (Hodgetts et al., '77; Garen et al., '77). In the experiments reported here, we tested whether the synthesis of vitellogenin could be induced in isolated abdomens of Drosophila melanogaster by ecdysterone (β-ecdysone) or by a juvenile hormone analogue (JHA).

MATERIALS AND METHODS All animals came from an Oregon-R stock, maintained on standard cornmeal-molasses-agar media. Abdomens were isolated at times indicated by tying off the abdomen from the thorax and head using a nylon ligature. Abdomens were topically applied with either 0.2 μg of ZR-515 (a gift from Zoecon Corp.) dissolved in acetone, or acetone by itself. ZR-515 is a potent juvenile hormone analog for Drosophila (Postlethwait, '74). Approximately 0.3 μl of 10^{-12} M or 10^{-4} M ecdysterone (Rohto Pharm.) dissolved in 10% ethanol in Ringers was injected into isolated abdomens. Animals and isolated abdomens were each injected with 0.45 μC of ^{3}H-leucine (Amersham) dissolved in 0.3 μl Drosophila Ringers in groups of 10 to 15 individuals. Hemolymph was collected by puncturing the dorsal sixth tergite with a finely drawn out glass capillary. SDS-polyacrylamide gels were run according to Sobieszek and Bremel ('75), sliced in 1 mm slices, and counted in a toluene based scintillation fluid in 2.5% protosol.

RESULTS Vg has been identified by electrophoresis on sodium dodecyl sulfate (SDS) containing gels of polyacrylamide in tubes as a band of approximately 48,000 d (Kambysellis, '77; Gavin and Williamson, '76a; Handler and Postlethwait, '77) (fig. 1A). In order to study the synthesis of this Vg band independent from its uptake into the oocyte, we have concentrated on the appearance of Vg in the hemolymph. The blood of female flies taken 24 hours after eclosion contains four major electrophoretic bands. These have apparent molecular weights of 48,000, 55,000, 76,000, and 160,000 d as determined by mobility in 7.5% polyacrylamide gels in SDS, and they will be

here designated Vg (=48K), 55K, 76K, and 160K (fig. 1). In order to study the relative rates of synthesis of these proteins in normal females, we allowed freshly eclosed flies to age for 24 hours on standard Drosophila medium, and then injected them with ^{3}H-leucine. Hemolymph was collected four hours later. Figure 1A shows that more label was incorporated into Vg (39% of the total counts on the gel) than into any other blood protein over this time interval, although there was often more Coomassie Blue staining material in 76K than in Vg (gel scans not shown).

In order to test hormones for their ability to stimulate Vg synthesis, we first needed a condition in which Vg is synthesized only at a low rate. The anterior endocrine organs, which we suspected of providing hormones necessary for Vg synthesis, were separated from the fat body and ovaries by tying a ligature between the thorax and abdomen of flies less than one hour old, followed by removing the head and thorax with scissors. At 24 hours after eclosion, we injected the isolated abdomens with ^{3}H-leucine and four hours later, hemolymph was collected and prepared for electrophoresis. The results (fig. 1A) showed that the amount of label incorporated into Vg was substantially decreased in isolated abdomens. Of the counts in the first half of the gel, 41% were incorporated into Vg in control flies, while only 13% were at the Vg position in isolated abdomens. These experiments show that some factor from the anterior is necessary for Vg synthesis in isolated abdomens.

We next considered whether a juvenile hormone analogue (JHA) could repair the defect in isolated abdomens. Our earlier experiments had shown that isolated abdomens treated with JHA contained, by three days, mature stage 14 oocytes possessing Vg (Handler and Postlethwait, '77). It remained to be shown, however, that vitellogenesis in these preparations was due to

de novo synthesis of Vg. Therefore, in the present work we have isolated abdomens from freshly eclosed flies and at 24 hours after eclosion treated them topically with JHA dissolved in acetone or with acetone alone. At 30 hours we injected them with ^{3}H-leucine and finally collected blood at 34 hours after eclosion. The results (fig. 1B) showed that the JHA stimulated Vg synthesis since 36% of the counts in the first half of the gel were incorporated into Vg in isolated abdomens treated with JHA, compared to only 18% in controls. This result shows that JHA can induce an increased rate of Vg synthesis in isolated Drosophila abdomens. Since our earlier results (Handler and Postlethwait, '77) showed that JHA can cause the ovaries in isolated abdomens to sequester Vg from the blood, we can conclude that juvenile hormone is all that is necessary for isolated abdomens to synthesize Vg, secrete it into the blood, and sequester it into oocytes.

Since ecdysterone induces Vg in a mosquito, it seemed prudent to test whether it was also effective in causing the synthesis of Drosophila Vg. To find whether ecdysterone can stimulate Vg synthesis, we injected isolated abdomens with either solvent or various concentrations (10^{-12} to 10^{-2} M) of

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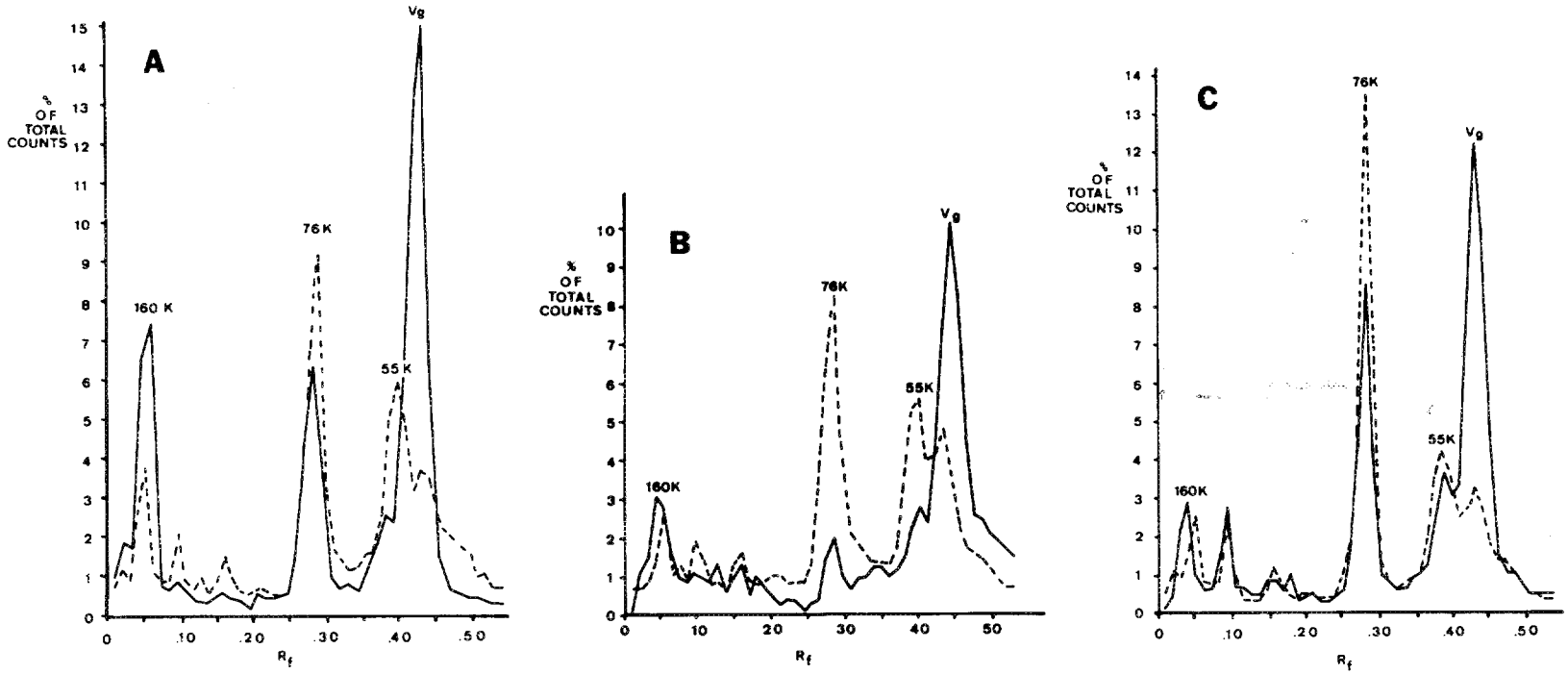
FIGURE LEGENDS

1 Synthesis of vitellogenin. A. Hemolymph from mature 24-hour-old females (solid line) and from isolated abdomens (dashed line). B. Hemolymph from acetone-treated control isolated abdomens (dashed line) and from isolated abdomens treated topically with 0.2 µg of ZR-515, a juvenile hormone analogue (solid line). C. Hemolymph from isolated abdomens injected with solutions of 10^{-12} M (dashed line), or 10^{-4} M ecdysterone (solid line) in 10% ethanol in Ringers. Since 80-90% of counts on the gels appear in the first half, only data for the first half of each gel are shown. The first half of each gel presented had from 34,000 to 91,000 total counts (average 57,000). Each of the experiments represented by the six characteristic gels in figure 1 was conducted between three and six times. Rf is the mobility relative to the tracking dye.


A
% OF TOTAL COUNTS
160 K
76K
55K
Vg
Rf
B
% OF TOTAL COUNTS
160K
76K
55K
Vg
Rf
C
% OF TOTAL COUNTS
160K
76K
55K
Vg
Rf

ecdysterone at 24 hours after eclosion. At 30 hours we injected the preparations with ^{3}H-leucine, and at 34 hours, collected hemolymph. The results (fig. 1C) showed that ecdysterone caused stimulation of Vg synthesis in isolated abdomens, since 38% of the counts in the first half of the gel were incorporated into Vg in isolated abdomens treated with 10^{-4} M ecdysterone, compared to only 12% with 10^{-12} M ecdysterone. Concentrations of 10^{-6} M or higher gave stimulation of Vg synthesis. When the blood of ecdysterone-treated isolated abdomens was subjected to immunoelectrophoresis, Vg was also detected, although it was absent from Ringer-injected controls (Handler, '77). Although isolated abdomens treated with ecdysterone rapidly synthesized Vg, their ovaries remained without visible yolk even after two days following ecdysterone injection. Apparently the presence of Vg in the blood is not a sufficient condition for Vg sequestration into oocytes.

DISCUSSION The present experiments show that both ecdysterone and JHA can cause an increase in the rate of Vg synthesis and secretion in preparations surgically deprived of anterior endocrine organs. JHA, but not ecdysterone, can cause in addition, Vg to be sequestered from the blood into the oocyte (Postlethwait and Weiser, '73; Gavin and Williamson, '76b; Kambysellis and Craddock, '76; Handler and Postlethwait, '77; Postlethwait and Handler, in press). There are at least three hypotheses which might account for these observations. First, it is possible that each hormone acts independently and directly on the fat body, the source of Vg, to modulate Vg synthesis. Alternatively, one hormone may act indirectly by causing the production of the other hormone which then directly effects Vg synthesis. Since we have shown that either the corpus allatum (Handler and Postlethwait, '77) (a gland that synthesizes juvenile hormone and is located in the thorax) or a JHA are sufficient to induce normal Vg synthesis, secretion, and sequestration, it

may be that juvenile hormone acts prior to an ecdysome. The reverse, however, is not ruled out. We would like to propose here that JH could act on a tissue in the abdomen to cause it to synthesize an ecdysone. The ecdysone could then affect the fat body to stimulate Vg synthesis and secretion while JH could directly influence the ovary to promote Vg uptake into oocytes. The third possibility is raised by the recent findings that the single Vg band detected in SDS tube gels can be resolved by immunoelectrophoresis (Handler, '77) and by gradient slab gels (Bownes and Hames, '77) into 2 or 3 components, respectively. It could be that one hormone stimulates the synthesis of one of these components and the other hormone regulates synthesis of the remaining proteins. These three hypotheses are currently being tested.

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