

Yolk Protein Synthesis in Ovariectomized and Genetically Agametic [X^{87}] *Drosophila melanogaster*

J. H. POSTLETHWAIT,* G. LAUGÉ,† AND A. M. HANDLER¹

*Department of Biology, University of Oregon, Eugene, Oregon 97403 and †Laboratoire d'Entomologie, Université Paris-Sud, 91405 Orsay Cedex, France

Accepted October 1, 1979

The steroid 20-hydroxyecdysone can stimulate yolk protein synthesis in *Drosophila*, and an ecdysteroid is produced by the ovaries in several insect species. In this work we test the hypothesis that normal ovaries are necessary for yolk polypeptide synthesis by studying the incorporation of [35 S]methionine into hemolymph yolk protein precursors in (1) flies surgically ovariectomized at eclosion; (2) isolated female abdomens experimentally deprived of anterior endocrine organs at eclosion; and (3) females genetically agametic (X^{87}). The results show that, while isolated abdomens have low rates of yolk polypeptide synthesis a day after eclosion, ovariectomized flies continue to synthesize rapidly yolk polypeptides at least a week after the operation. Likewise, agametic X^{87} flies also synthesize yolk polypeptides for many days. These experiments show that, although an anterior factor is required for high levels of yolk polypeptide synthesis, no ovarian factor is necessary after 30 min past eclosion, and neither the oocyte nor the nurse cells are needed for yolk polypeptide synthesis at any stage in the life cycle.

A remarkable parallel exists in the hormonal regulation of yolk production between vertebrates and insects (Hagedorn, 1974; Tata, 1976). In some species of both groups a steroid hormone produced by the ovary stimulates the synthesis and secretion of yolk protein precursors (vitellogenins) by the liver or its analog, the fat body. Vitellogenin is then sequestered from the circulation by the developing oocyte to form mature yolk protein (vitellin). It is the purpose of these experiments to test whether the ovary and/or the germ cells are necessary for vitellogenin synthesis in *Drosophila*.

It was shown many years ago that the process of yolk deposition (vitellogenesis) is also under hormonal control in the fruitfly *Drosophila* (Vogt, 1940; Bodenstein, 1947), but the precise endocrine interactions remain obscure. Although recent work has indicated that the juvenile hormone (JH) is necessary for sequestration of yolk protein

precursors, into the developing oocyte (Postlethwait and Weiser, 1973; Kambysellis and Heed, 1974; Gavin and Williamson, 1976) the synthesis of yolk protein (YP) precursors (Bownes and Hames, 1977; Warren and Mahowald, 1979) appears to be regulated by two hormones: Both JH and 20-hydroxyecdysone (20-HE) are able to stimulate YP synthesis in isolated *Drosophila* abdomens (Handler and Postlethwait, 1978; Postlethwait and Handler, 1979). At least two hypotheses might account for this result. First, each hormone might act independently to stimulate vitellogenin synthesis and secretion. Alternatively, one of the hormones might cause the synthesis and/or secretion of the second hormone which in turn actually induces YP synthesis.

The corpus allatum, an endocrine organ which in *Drosophila* resides in the thorax, secretes JH in adult insects (Wigglesworth, 1965), and the ovary contains or produces ecdysteroids in the adults of a number of insects (Hagedorn *et al.*, 1975; Legay *et al.*, 1976; Laverdure *et al.*, 1977; Delbecque *et al.*, 1978; Bollenbacher *et al.*, 1978). Ec-

¹ Current address: Developmental Biology Center, University of California, Irvine, Calif. 92664.

dysone, secreted from the ovaries, stimulates the synthesis of vitellogenin in mosquitoes (Hagedorn, 1974). It was determined by radioimmunoassay that adult female *Drosophila* contain more ecdysteroids than males (Hodgetts *et al.*, 1977), and that most of it is in the ovaries (Garen *et al.*, 1977).

Finally, the transplantation of *Drosophila* ovaries into hosts lacking YPs, such as pupae or mature males (Bodenstein, 1947; Kambysellis, 1977), results in an accumulation of yolk in the implanted ovary. Since ovaries apparently can make an ecdysteroid, since an ecdysteroid stimulates YP synthesis in isolated abdomens, and since it has been suggested by Kambysellis (1977) that implanted ovaries induce YP synthesis in males, the hypothesis suggests itself that in isolated *Drosophila* abdomens JH may act on the ovary to effect the secretion of ecdysone, which in turn induces vitellogenin synthesis in the fat body. It is the purpose of this paper to test the hypothesis that ovaries are required for yolk protein synthesis in *Drosophila*. The production of yolk proteins was monitored in female flies which were surgically ovariectomized and in mutant flies which possess no germ cells.

MATERIALS AND METHODS

Oregon R flies 15 to 30 min after eclosion were ovariectomized by removing the ovaries with forceps through a slit torn in the lateral abdominal wall between the fourth and fifth tergites. Four or seven days later the flies were injected with 0.9 μCi [^{35}S]methionine in 0.3 μl *Drosophila* Ringer's (Chan and Gehring, 1971). Hemolymph was collected into a drawn out capillary 3 hr later. Isolated abdomens were prepared from Oregon R flies less than 30 min after eclosion by tying a ligature between the thorax and abdomen and removing the head and thorax with scissors. Thirty hours later preparations were labeled as described above. Abdomens isolated at less than 30 min after eclosion were treated topically at 24 hr with 0.2 μg ZR-515, a JH analog for *Drosophila* (Postlethwait, 1974). At 30 hr hormone-treated abdomens were radioactively labeled as described above. Agametic female flies were obtained from the offspring of homozygous X^{87} females (Thierry-Meig, 1976; Laugé *et al.*, 1977). Agametic animals were aged for 14 days and then radioactively

labeled as described above. Hemolymph proteins were separated by electrophoresis in polyacrylamide gels in the presence of sodium dodecyl sulfate (SDS-PAGE) after the method of O'Farrell (1975) and then autoradiographed (Lasky and Mills, 1975). The behavior of the three *Drosophila* YPs on SDS-PAGE has been extensively documented and it serves as a positive identification for these polypeptides in the hemolymph (Bownes and Hames, 1977; Postlethwait and Kaschnitz, 1978; Warren and Mahowald, 1979).

RESULTS AND CONCLUSIONS

To test whether the ovary is continuously required after eclosion for YP synthesis, we removed either one or both ovaries from freshly eclosed Oregon R flies and 4 or 7 days later examined the incorporation of radioactive amino acids into circulating YP precursors. Removal of one (Fig. 1c) or even both (Fig. 1b) ovaries only slightly diminished the ability of a fly to synthesize vitellogenins. All three YPs were found to be labeled in the hemolymph of these flies and in nearly normal quantities (Fig. 1a). The synthesis of a number of polypeptides, other than YPs, seems to be stimulated by the surgery when uni- and bilaterally ovariectomized flies are compared to operated controls (Figs. 1a-c). Since the same amount of label is distributed among more bands in the operated animals, the relative incorporation into YPs is somewhat higher in the control. The conclusion however remains clear that ovariectomy at eclosion does not prevent the normal increase in YP synthesis that occurs after emergence. This result is in concordance with that obtained after ovariectomy in several other insect species (Telfer, 1954; Bell, 1969; Wilkens, 1969; De Loof and De Wild, 1970; Engelmann, 1978), but is different for the result obtained after ovariectomy in mosquitoes (Hagedorn and Fallon, 1973).

To show that hormonal signals are still required for YP synthesis at the time of ovariectomy, we removed by ligation the anterior of the fly, which contains the corpus allatum and other endocrine organs. This treatment blocks most, but not all, of the increase in vitellogenin synthesis seen

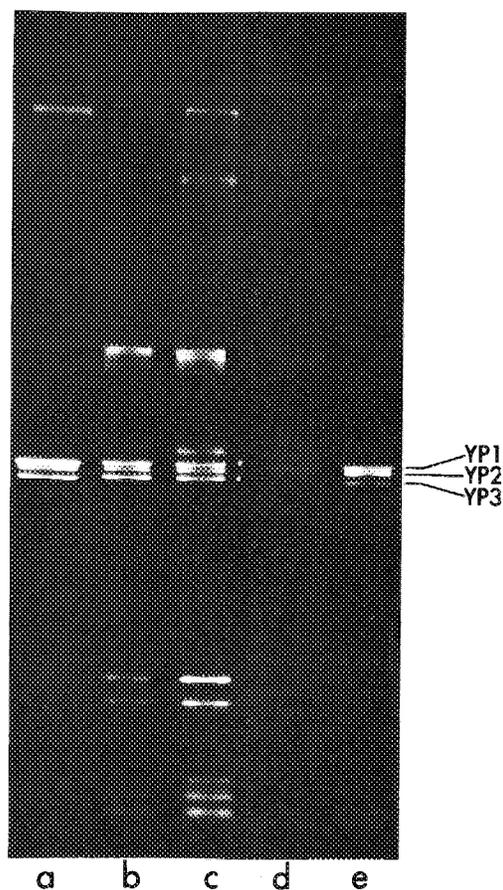


FIG. 1. Synthesis of yolk protein precursors in ovarietomized females and in isolated female abdomens. Hemolymph from (a) 10 control females; (b) 10 bilaterally ovarietomized females; (c) 10 unilaterally ovarietomized controls; (d) 10 isolated abdomens; (e) 10 isolated abdomens treated with ZR-515. The figure is an autoradiograph of the gel.

after eclosion (compare Figs. 1a and d), demonstrating that vitellogenin synthesis is not yet fully autonomous by 30 min after eclosion, but requires input from the fly's anterior. Vitellogenin synthesis can be regained by treating isolated abdomens with a JH analog (Fig. 1e). Although the levels of JH analog used were likely greater than the natural JH titer, implantation of corpora allata into isolated abdomens can cause complete vitellogenesis, including not only YP synthesis, but uptake as well (Handler

and Postlethwait, 1977), thus showing that the naturally occurring hormones can act in the same way. The experiments reported here show that removal of the anterior endocrine organs, but not extirpation of the ovaries, results in significant diminution of the ability to synthesize yolk protein precursors. Apparently continued presence of the ovaries is unnecessary for the increase in YP synthesis that occurs after eclosion.

These experiments thus rule out the hypothesis that JH triggers the ovary to produce or release ecdysone, which then acts on the fat body as the sole stimulator of YP synthesis after eclosion. It is still conceivable that an ovarian factor plays a role in vitellogenin production prior to 30 min after eclosion. This is an especially relevant question since it has been shown that ovaries in culture secrete YPs (Postlethwait and Bownes, in preparation) and that ovaries contain YP mRNA (Bownes and Hames, 1978). Although we have not been able to successfully bilaterally ovarietomize flies prior to eclosion, we have made use of the X^{87} mutant. Females homozygous for X^{87} produce eggs that frequently have defects in pole cell development so that no pole cells populate the gonads. These eggs develop into daughters whose ovaries contain no germ cells, and only a rudimentary mesodermal component (Laugé *et al.*, 1977). Hence the original mutant has no grandchildren. Penetrance of X^{87} is incomplete, so hemolymph from each fly was collected separately and then her ovaries were dissected and examined for the presence of germ cells. Homozygous mutant animals in which one or both ovaries were populated with germ cells served as controls. The results showed that X^{87} ovaries with no germ cells contained no YPs (Fig. 2a), but mutant ovaries containing germ cells possessed the normal complement of YPs (Fig. 2d). When the hemolymph samples were examined, a remarkable concentration of vitellogenins and elevated levels of a few other hemolymph

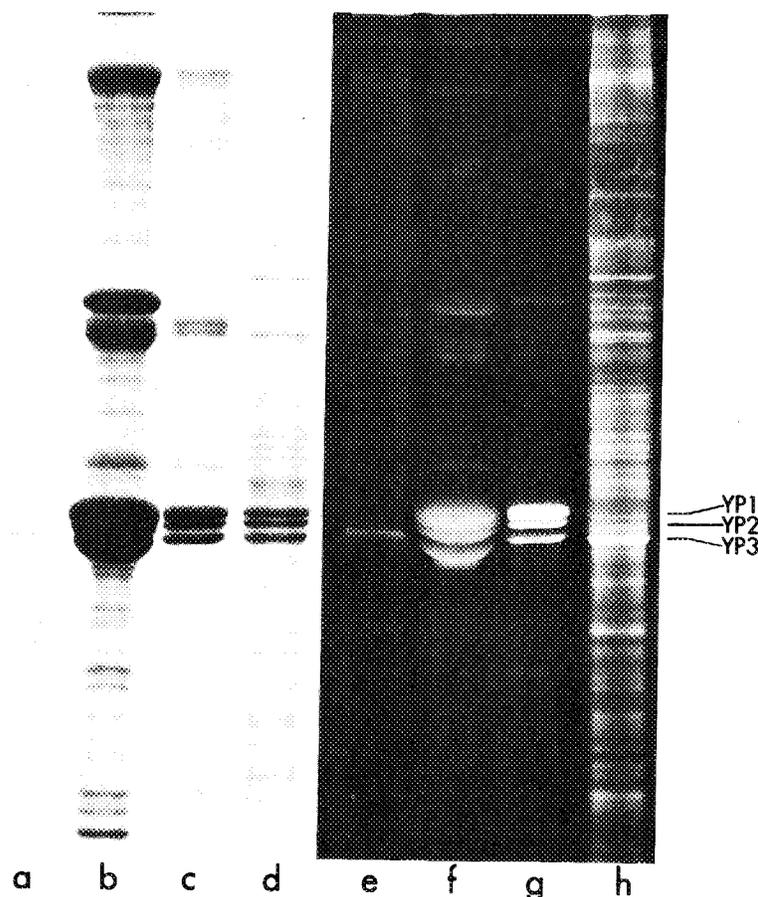


FIG. 2. Synthesis of yolk protein precursors in offspring of homozygous X^{N7} females. (a and e) Ovaries from six agametic mutant flies; (b and f) hemolymph from six agametic mutant flies; (c and g) hemolymph from six vitellogenic mutant flies; (d and h) ovaries from one vitellogenic mutant fly. Tracks a-d are the gel stained for protein and tracks e-h are the autoradiographs of the gel.

proteins were found in the agametic flies (Fig. 2b), but normal amounts of vitellogenins appeared in the hemolymph of siblings that contained developing oocytes (Fig. 2c). The autoradiographs confirmed that agametic flies continued to synthesize and secrete vitellogenins even 14 days after eclosion (Figs. 2e-h).

Our results prove that there is no dependence in any way on the germ cells for the induction or continuation of YP synthesis. In addition, the prodigious quantity of YPs in the hemolymph of agametic flies indicates that either there is no feedback mechanism regulating vitellogenin synthesis

when vitellogenin levels in the hemolymph exceed usual concentrations, or if such a mechanism exists, it is mediated through the germ cells. Kambyzellis (1977) has also reported high titers of YP in the hemolymph of some genetically nonvitellogenic flies with defective germ cells. It may prove to be that deposition of yolk in the oocytes is the only means of decreasing the hemolymph concentrations of vitellogenins.

At this point it is unknown whether the agametic daughters of X^{N7} contain ecdysone. It has recently been shown that in locusts ecdysone reaches remarkable con-

centrations in the follicle cells (Glass *et al.*, 1978) and that it may be synthesized in the follicle cells (Lagueux *et al.*, 1977). Since the follicle cells are present, albeit not completely normal, in the ovaries of agametic offspring of X^{87} females, it is possible that these cells could provide a source of ecdysone in agametic females even though our ovariectomy experiments indicate that postulation of such ovarian ecdysone is unnecessary for the stimulation of YP synthesis seen after eclosion. The oenocytes have also been suggested as a site of ecdysone synthesis (Locke, 1969; Romer *et al.*, 1974; Huybrechts and De Loof, 1977).

While these experiments show that no ovarian factor is necessary for YP synthesis after 30 min past eclosion, and no germ cell-derived factor is required at any time in development, the precise role of an ecdysteroid in *Drosophila* vitellogenesis and its source is still in question. Experiments involving radioimmunoassay of various surgically altered flies for ecdysone are underway.

ACKNOWLEDGMENTS

We wish to thank D. Sears and J. A. Postlethwait for technical assistance and the U.S. National Institutes of Health for support.

REFERENCES

- Bell, W. J. (1969). Dual role of juvenile hormone in the control of yolk formation in *Periplaneta americana*. *J. Insect Physiol.* **15**, 1279–1290.
- Bodenstein, D. (1947). Investigations on the reproductive system of *Drosophila*. *J. Exp. Zool.* **104**, 101–152.
- Bollenbacher, W., Zvenko, H., Kumaran, A., and Gilbert, L. (1978). Changes in ecdysone content during post-embryonic development of the wax moth *Galleria mellonella*: The role of the ovary. *Gen. Comp. Endocrinol.* **3A**, 169–179.
- Bownes, M., and Hames, B. D. (1978). Analysis of the yolk proteins in *Drosophila melanogaster*: Translation in a cell free system and peptide analysis. *FEBS Lett.* **96**, 327–330.
- Bownes, M., and Hames, B. (1977). Accumulation and degradation of three major yolk proteins in *Drosophila melanogaster*. *J. Exp. Zool.* **200**, 149–156.
- Chan, L.-N., and Gehring, W. (1971). Determination of blastoderm cells in *Drosophila melanogaster*. *Proc. Nat. Acad. Sci. USA* **68**, 2217–2221.
- Delbecque, J., Lanzrein, B., Bordereau, C., Imboden, H., Hirn, M., O'Connor, J., Noirot, C., and Luscher, M. (1978). Ecdysone and ecdysterone in physogastric termite queens and eggs of *Macrotermes bellicosus* and *Macrotermes subhyalinus*. *Gen. Comp. Endocrinol.* **36**, 40–47.
- De Loof A., and De Wilde J. (1970). The relationship between haemolymph proteins and vitellogenesis in the Colorado beetle, *Leptinotarsa decemlineata*. *J. Insect Physiol.* **16**, 157–169.
- Engelmann, F. (1978). Synthesis of vitellogenin after long-term ovariectomy in a cockroach. *Insect Biochem.* **8**, 149–154.
- Garen, A., Kuvar, L., and Lepesant, J. (1977). Roles of ecdysone in *Drosophila* development. *Proc. Nat. Acad. Sci. USA* **74**, 5099–5103.
- Gavin, J. A., and Williamson, J. H. (1976). Juvenile hormone-induced vitellogenesis in apterous¹, a nonvitellogenic mutant in *Drosophila melanogaster*. *J. Insect Physiol.* **22**, 1737–1742.
- Glass, H., Emmerich, H., and Spindler, K.-D. (1978). Immunohistochemical localization of ecdysteroids in the follicular epithelium of locust oocytes. *Cell Tissue Res.* **194**, 237–244.
- Hagedorn, H. (1974). The control of vitellogenesis in the mosquito, *Aedes aegypti*. *Amer. Zool.* **14**, 1207–1217.
- Hagedorn, H. H., and Fallon, A. M. (1973). Ovarian control of vitellogenin synthesis by the fat body in *Aedes aegypti*. *Nature (London)* **244**, 103–105.
- Hagedorn, H., O'Connor, J. D., Fuchs, M. S., Sage, B., Schlaeger, D. A., and Bohme, M. K. (1975). The ovary as a source of α -ecdysone in an adult mosquito. *Proc. Nat. Acad. Sci. USA* **72**, 3255–3259.
- Handler, A., and Postlethwait, J. H. (1978). Regulation of vitellogenin synthesis in *Drosophila* by ecdysterone and juvenile hormone. *J. Exp. Zool.* **206**, 247–254.
- Handler, A. M., and Postlethwait, J. H. (1977). Endocrine control of vitellogenesis in *Drosophila melanogaster*: Effects of the brain and corpus allatum. *J. Exp. Zool.* **202**, 389–401.
- Hodgetts, R. B., Sage, B., and O'Connor, J. D. (1977). Ecdysone titers during post embryonic development of *Drosophila melanogaster*. *Develop. Biol.* **60**, 310–317.
- Huybrechts, R., and De Loof, A. (1977). Induction of vitellogenin synthesis in male *Sarcophaga bullata* by ecysterone. *J. Insect Physiol.* **23**, 1359–1362.
- Kambysellis, M. (1977). Genetic and hormonal regulation of vitellogenesis in *Drosophila*. *Amer. Zool.* **17**, 535–549.
- Kambysellis, R., and Heed, W. (1974). Juvenile hormone induces ovarian development in diapausing cave-dwelling *Drosophila* species. *J. Insect Physiol.* **20**, 1779–1786.

- Lagueux, M., Hirn, M., and Hoffman, J. A. (1977). Ecdysone during ovarian development in *Locusta migratoria*. *J. Insect Physiol.* **23**, 109–119.
- Laugé, G., Sauphanor, B., and Randrianandrianina, L. (1977). Étude histologique des gonades d'un mutant de stérilité à effet retardé de *Drosophila melanogaster* Meig. *C. R. Acad. Sci. Paris* **284**, 1187–1189.
- Laverdure, A., Lagueux, M., and Hoffman, J. A. (1977). Ecdysone et développement ovarien chez la femelle adulte de *Tenebrio molitor*. *Bull. Soc. Zool. Fr.* **102**, 311–312.
- Legay, J. M., Calvez, B., Hirn, M., and DeReggi, M. L. (1976). Ecdysone and oocyte morphogenesis in *Bobyx mori*. *Nature (London)* **262**, 489–490.
- Locke, M. (1969). The ultrastructure of the oenocytes in the moult/intermoult cycle of an insect *Calpodes ethlius* Stall. *Tissue Cell* **1**, 103–154.
- O'Farrell, P. (1975). High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **250**, 4007–4021.
- Postlethwait, J. H. (1974). Juvenile hormone and the adult development of *Drosophila*. *Biol. Bull.* **147**, 119–135.
- Postlethwait, J. H., and Handler, A. (1979). The roles of juvenile hormone and 20-hydroxyecdysone during vitellogenesis in isolated abdomens of *Drosophila melanogaster*. *J. Insect Physiol.* **25**, 455–460.
- Postlethwait, J. H., and Kaschnitz, R. (1978). The synthesis of *Drosophila melanogaster* vitellogenins *in vivo*, in culture, and in a cell-free translation system. *FEBS Lett.* **95**, 247–251.
- Postlethwait, J. H., and Weiser, K. (1973). Vitellogenesis induced by juvenile hormone in the female sterile mutant apterous-four in *Drosophila melanogaster*. *Nature New Biol.* **244**, 284–285.
- Romer, F., Emmerich, H., and Nawock, J. (1974). Biosynthesis of ecdysones in isolated prothoracic glands and oenocytes of *Tenebrio molitor* *in vitro*. *J. Insect Physiol.* **20**, 1975–1987.
- Tata, J. R. (1976). The expression of the vitellogenin gene. *Cell* **9**, 1–14.
- Telfer, W. H. (1954). Immunological studies of insect metamorphosis II. The role of a sex-limited blood protein in egg formation by the *Cecropia* silkworm. *J. Gen. Physiol.* **37**, 539–558.
- Thierry-Mieg, D. (1976). Study of a temperature-sensitive mutant grandchildless-like in *Drosophila melanogaster*. *J. Microsc. Biol. Cell* **25**, 1–6.
- Vogt, M. E. (1940). Zur Ursache der unterschied zwischen gonadotropen wirkung der Ringdrüsen von *Drosophila funebris* and *Drosophila melanogaster*. *W. Roux Arch. Entwicklungsmech. Organ.* **140**, 525–546.
- Warren, T., and Mahowald, A. P. (1979). Isolation and partial chemical characterization of the three major yolk polypeptides from *Drosophila melanogaster*. *Develop. Biol.* **68**, 130–139.
- Wigglesworth, V. (1965). The juvenile hormone. *Nature (London)* **208**, 522–524.
- Wilkins, J. L. (1969). The endocrine control of protein metabolism as related to reproduction in the fleshfly *Sarcophaga bullata*. *J. Insect Physiol.* **15**, 1015–1024.