

Nonvitellogenic Female Sterile Mutants and the Regulation of Vitellogenesis in *Drosophila melanogaster*

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The genetic and endocrine regulation of vitellogenesis was investigated by studying 18 female sterile mutations that disrupt the development of normal vitellogenic follicles. Applications of exogenous juvenile hormone analog and reciprocal ovarian transplants between flies of different genotypes were employed to accomplish our first two objectives: to find (1) whether the mutation blocked development of the ovary directly, and (2) whether the mutation altered the hormonal milieu. In 15 of the mutants the developmental defect was localized to the ovary, but in the other 3 the ovary was competent to respond to a permissive environment. The internal milieu of these three mutants (*ap*⁴, *fs(3)A1*, *fs(2)A18*) was unable to provoke normal development in wild-type ovaries, suggesting that these mutations cause endocrine defects. Our third objective was to find whether an endocrine organ was itself defective in any of these mutants. The corpus allatum from two of the mutants was unable to provoke vitellogenesis in isolated wild-type abdomens, but corpora allata from wild-type females or from other mutants were able to promote maturation of ovarian follicles in isolated abdomens. Our fourth objective was to find whether any of the mutants were able to produce yolk proteins. Immunoelectrophoresis of fly hemolymph demonstrated that in all mutants tested vitellogenins were found in the blood. These experiments permit four main conclusions. First, they identify the first *Drosophila* mutants in which an endocrine gland is shown to be intrinsically defective during adulthood. Second, they show that the production of morphologically normal late previtellogenic follicles is not required for the induction of vitellogenin synthesis and secretion. Third, they show that juvenile hormone can cause ovarian follicles to sequester yolk in mutant flies. And finally, they show that mutants with defective corpora allata still synthesize and secrete vitellogenin. Taken together, these conclusions suggest that in *Drosophila melanogaster* the uptake of vitellogenin into follicles depends upon the availability of juvenile hormone, but that the synthesis and secretion of vitellogenin are independent of both normal ovaries and totally normal corpora allata.

INTRODUCTION

The development of a mature oocyte requires the combined efforts of a number of tissues besides the ovary. Ovarian maturation in insects involves a complex network of neural and neuroendocrine functions (for review, see Englemann, 1970; Doane, 1973; de Wilde and de Loof, 1973). Both the brain and the corpus allatum, a neurohormonal organ, play central roles during the maturation of ovaries in most insects, although in different insects these organs regulate ovarian development by different mechanisms. In order to understand the genetic

basis of this developmental process, we have begun to examine *Drosophila* mutants with defective oogenesis.

Recent experiments employing the classical tools of the endocrinologist have allowed a definition of the ovarian phenotype expressed by a *Drosophila melanogaster* female deprived of her anterior endocrine organs, including the brain, corpus allatum, and corpus cardiacum (Handler and Postlethwait, 1977). The follicles in such preparations develop normally up to stage 7, the last nonvitellogenic stage, and then cease developing. These experiments permit the designation of nonvitellogenic female sterile mutations as candidates for genes that

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affect endocrine function during oogenesis. A large number of such female sterile mutants have recently been produced (Kaplan *et al.*, 1970; Bakken, 1973; Gans *et al.*, 1975; Rice and Garen, 1975; Landers *et al.*, 1976; Mohler, 1977). We have used transplantation techniques to investigate some of these female sterile mutants that show the phenotype expected of altered endocrine function to determine (1) whether the mutant ovary is defective; (2) whether the mutant hormonal milieu is aberrant; and (3) whether the mutant corpus allatum is altered.

Developing oocytes of *Drosophila* accumulate specific yolk proteins (Gelti-Douka *et al.*, 1973; Gavin and Williamson, 1976a) and enzymes (Postlethwait and Gray, 1975; Sawicki and MacIntyre, 1975; Giorgi and Jacob, 1977). Since yolk proteins are present in the hemolymph and are sequestered by the developing oocyte, it is possible that some nonvitellogenic female sterile mutants may fail to undergo maturation because they lack yolk proteins in the blood. Thus, the fourth objective of this work was to find whether nonvitellogenic female sterile mutants can synthesize yolk proteins. Our preliminary results have been cited earlier (Postlethwait, J. H., cited in King and Mohler, 1975; Postlethwait *et al.*, 1976), as have those of Kambyzellis and Gelti-Douka (1974) and Kambyzellis and Craddock (1976).

MATERIALS AND METHODS

Animals. A collection of 18 autosomal nonvitellogenic female sterile mutants was obtained, 16 of which were produced by ethyl methane sulfonate mutagenesis. Three were discovered by Bakken (1973) (*fs(2)A18*, *fs(3)A1*, *fs(2)A17*), three by Landers *et al.* (1976) (*fs(3)L1-49*, *fs(3)L3*, *fs(3)L8^{sc}*), and two by Handler (1977) (*fs(3)H23*, *fs(3)H172*), and eight were obtained from flies mutagenized by R. Hardy and N. Orevi and kindly sent to us by Randy Smith (*fs(2)100A*, *fs(2)192A*, *fs(2)8B*, *fs(2)20B*, *fs(3)29A*, *fs(3)115A*, *fs(3)127A*, *fs(3)133A*).

In addition, we looked at *ap⁴* and *fs(2)B*, which are spontaneous mutations (see Butterworth and King, 1965; King *et al.*, 1961). All second-chromosome mutants were balanced over *SM5*, *al² Cy lt⁺ sp²*, and third-chromosome mutants were balanced over *TM3*, *Sb Ser*. An Oregon R stock maintained for many years at the University of Oregon provided wild-type animals. In some instances, heterozygous fertile siblings from the female sterile stocks were also used as controls. Since results from both kinds of controls were similar, they were combined.

Animals were cultured at 25°C throughout, except for the mutation *fs(3)L8^{sc}*, which is a temperature-sensitive female sterile. In experiments involving *fs(3)L8^{sc}*, all donors and hosts were cultured at the restrictive temperature (29°C) after eclosion.

Surgery. Ovarian transplants were performed as described by Ursprung (1967). Flies less than 2 hr old (8 hr before yolk is visible in follicles) (Handler and Postlethwait, 1977) served as both donors and recipients. Hosts were dissected at 72 hr and all vitellogenic follicles were counted in both host and donor ovaries. Donor ovaries are recognized since they fail to attach to the host's oviducts. The data in this paper come from over 500 transplanted ovaries.

The assay for corpus allatum function has been described earlier (Handler and Postlethwait, 1977). Oregon R females less than 15 min old were ligated around the first abdominal segment and the thorax was removed. These isolated abdomens were implanted with three corpus allatum-corpora cardiacum complexes obtained from 60- to 72-hr-old females of the genotype to be tested. Abdomens were opened 72 hr after eclosion and vitellogenic follicles were counted. Follicles were staged according to King (1970). Previtellogenic stages are stages 1-7; early vitellogenic stages are stages 8 and 9; intermediate, stages 10-12; and late, stages 13 and 14. Stage 14 is the mature oocyte.

Mutant animals were tested for their re-

sponse to ZR-515 (Zoecon Corp.), a synthetic juvenile hormone analog, by topical applications of 0.15 μg in 0.3 μg of acetone on the fly's abdomen. Some control animals received acetone alone, but since it was without effect, most controls were untreated.

Immunological techniques. Antiserum was prepared to soluble egg constituents after control serum was obtained from each rabbit. Fertilized eggs less than 3 hr old were homogenized in 0.85% NaCl and centrifuged to remove egg shells. This solution was mixed 1:1 with Freund's complete adjuvant and injected subcutaneously into 30 places along the backs of young female white rabbits. A boost was given 6 weeks later, and subsequently blood was obtained by heart puncture and serum was prepared by standard techniques.

Samples of ovaries were prepared for immunoelectrophoresis by the homogenization of 10 to 20 ovaries in 7 μl of 0.85% NaCl. After centrifugation at 27,000g in a refrigerated Sorvall centrifuge for 15 min, the supernatant was introduced into the well of an agar gel on a glass microscope slide, and immunoelectrophoresis was performed as described by Brewer *et al.* (1974). Hemolymph samples were prepared by puncturing a posterior tergite on the dorsal side with a finely drawn out 20- μl microcapillary and drawing up the hemolymph by capillarity. In some cases, capillaries were rinsed with phenylthiourea in acetone before use to inhibit the action of phenyloxidases.

RESULTS

The mutant phenotype. Since ovaries in wild-type flies lacking anterior endocrine centers fail to undergo vitellogenesis (Handler and Postlethwait, 1977), we have begun to look at mutant flies unable to develop mature ovaries in order to help elucidate the genetic control of this endocrine system. Our collection of mutants can be divided into three groups based upon ovarian morphology. The mutants of the

first type (designated Early Pre) contain ovaries in which follicles cease development at an early previtellogenic stage. When vitellogenic follicles do occur in Early Pre mutants, they are morphologically abnormal, of small size, and usually without respiratory horns. Mutants of the second type (designated Late Pre) develop normal follicles through stage 7, which then degenerate. Some Late Pre mutants accumulate many stage 7 follicles so that the ovary approaches normal size even though no vitellogenic follicles are usually present. Occasionally a stage 8, newly vitellogenic follicle will be seen, but no further development occurs. The third type of mutant (designated Poor Vit) begins vitellogenesis occasionally, but usually the process halts prematurely and only rarely do mature oocytes develop. When follicles do mature in Poor Vit mutants, they are morphologically normal. Table 1 (column 3) shows the average number of early, intermediate, and late vitellogenic follicles found in the female sterile mutants at 72 hr after eclosion. Except for *ap*⁴, which has no wings or halteres and is short-lived, and *fs(3)LS*^s, which is associated with a temperature-sensitive larval lethal affect and a non-conditional alteration in ommatidia organization, none of the mutants has a phenotypic effect other than female sterility.

The mutant ovary. Having categorized the ovarian phenotypes, we next proceeded with experiments designed to ask whether the genetic defect was inherent in the developing mutant ovary. To test the ability of the ovary to undergo vitellogenesis in a normal environment, we implanted ovaries from freshly eclosed mutant females into the abdomens of newly eclosed normal females. The results are shown in Table 1 (column 6), and they allow each mutant to be classified into one of two categories: (1) ovary autonomous or (2) ovary nonautonomous. Of the 18 mutants tested, only the ovaries of *ap*⁴ were able to develop substantially better in a normal host. The *ap*⁴ ovaries developed nearly as many mature fol-

TABLE 1
DEVELOPMENT OF MUTANT OVARIES EITHER *in Situ* OR AFTER TRANSPLANTATION INTO A WILD-TYPE HOST AT 72 HR AFTER ECLOSION (AVERAGE NUMBER OF FOLLICLES PER OVARY IN THE INDICATED STAGE)

(1) Genotype	(2) No. of ovaries	(3) Follicular stage <i>in situ</i>			(4) Morphological type ^c	(5) No. of ovaries	(6) Follicular stage after transplant.			(7) Ovary category ^b
		Early	Int.	Late			Early	Int.	Late	
<i>fs</i> (2)100A	12	0	0	0	EP	7	0	0	0.29 ^e	A
<i>fs</i> (2)192A	10	0	0	0	EP	3	0	0	0	A
<i>fs</i> (2)B	20	0	0	0	EP	—	—	—	—	A ^d
<i>fs</i> (2)8B	20	0	0	0	EP	3	0	0	0	A
<i>fs</i> (2)20B	24	0.2	0	0	EP	12	0	0	0	A
<i>fs</i> (3)H23	12	0	0	0	EP	9	0	0	0	A
<i>fs</i> (3)L3	20	0	0	0	EP	16	0	0	0	A
<i>fs</i> (2)A17	30	0	0	0	LP	17	0	0	0	A
<i>fs</i> (3)115A	22	0.1	0	0	LP	14	0	0	0	A
<i>fs</i> (3)127A	28	6.0	0	0	LP	8	0	0	0	A
<i>fs</i> (3)133A	20	0	0	0	LP	7	0	0	0	A
<i>fs</i> (3)L8 (29°C)	28	0.04	0	0	LP	8	0	0	0	A
<i>ap</i> ^a	34	0.06	0	0	PV	12	3.0	0.4	3.6	NA
<i>fs</i> (3)A1	28	0	0	0.07	PV	15	0.7	0	0	A
<i>fs</i> (2)A18	36	0.4	0	0	PV	22	0	0	0	A
<i>fs</i> (3)29A	20	0	0.1	0	PV	14	0	0	0	A
<i>fs</i> (3)H172	20	16.1	1.2	1.3	PV	10	0	0	0	A
<i>fs</i> (3)L1-49	16	1.4	0.9	0	PV	6	0.5	0	0	A
Oregon R	24	1.2	1.5	14.9	N	18	2.2	1.4	4.1	—

^a EP, oogenesis is aborted at early previtellogenic stages; LP, oogenesis is aborted at the latest previtellogenic stage, stage 7; PV, some vitellogenesis occurs, but it proceeds only poorly; N, normal vitellogenesis.

^b A, ovary autonomous in normal host; NA, ovary nonautonomous in normal host.

^c Morphologically abnormal.

^d Result according to King and Bodenstein (1965).

licles when implanted into normal flies as did the transplanted ovaries of Oregon R, thus confirming the earlier work of King and Bodenstein (1965). The other 17 mutants seem to be autonomous by this test, suggesting that the genetic lesion probably affects the ovary itself. In all cases, the ovaries of the normal host matured on schedule, indicating that none of the mutant ovaries adversely affects the host humoral system.

The mutant internal milieu. The previous experiments showed that most of the mutants caused defects in the ovary itself. That result still leaves open the question of whether the mutant internal environment is defective in addition, or if it can provide the proper humoral factors to support vitellogenesis. To test this possibility, we transplanted wild-type ovaries from newly eclosed females into young adult mutant

hosts. The mutants can be divided into three host categories on the basis of the data in Table 2 (column 2). The first category (Poor Host) is a group of three mutants that are unable to support completely normal development of wild-type ovaries. All of these mutants have ovaries of morphological type Poor Vit, which occasionally have an early or intermediate stage follicle. Apparently, these mutants do not provide proper humoral conditions for vitellogenesis.

The internal milieu of the second group of five mutants (Normal Host) permits wild-type ovaries to mature about as many late stage follicles as normal ovaries do when implanted into wild-type hosts. Mutants in the third category are Excellent Hosts, enabling wild-type ovaries to mature two to five times as many late stage oocytes as they do when implanted into normal

TABLE 2
DEVELOPMENT OF FOLLICLES IN NORMAL OVARIES TRANSPLANTED INTO MUTANT FEMALES (AVERAGE NUMBER OF FOLLICLES PER OVARY IN THE INDICATED STAGE)

(1) Genotype	(2) Normal donor ovary			(3) Mutant host ovary				(4) Host category ^a	
	No. of ovaries	Early	Int.	Late	No. of ovaries	Early	Int.		Late
<i>fs(2)100A</i>	4	4.3	0.7	5.7	8	0		0	N
<i>fs(2)192A</i>	2	3.5	1.5	26.0	4	0	0	0	E
<i>fs(2)B</i>	10	3.6	1.1	9.7	20	0	0	0	E
<i>fs(2)B8</i>	22	3.5	10.0	6	0	0	0	0	E
<i>fs(2)20B</i>	10	4.0	1.8	10.4	20	0	0	0	E
<i>fs(3)H23</i>	9	3.6	1.4	11.2	18	0	0	0.3 ^b	E
<i>fs(3)L3</i>	16	1.5	0.5	3.4	32	0	0	0	N
<i>fs(2)A17</i>	20	2.6	1.6	15.8	40	0	0	0	E
<i>fs(3)115A</i>	12	1.3	0.8	6.8	24	0.1	0	0	N
<i>fs(3)127A</i>	6	1.8	2.8	6.3	12	0.7	0	0	N
<i>fs(3)133A</i>	11	1.9	3.0	13.9	22	0	0	0	E
<i>fs(3)L8</i>	20	2.5	0.8	8.8	40	0.3	0	0.03	E
<i>ap⁴</i>	7	0.2	0	0	14	0	0	0	P
<i>fs(3)A1</i>	10	0.4	0	0.7	20	0	0	0	P
<i>fs(2)A18</i>	6	6.5	1.2	0.5	12	0.2	0	0	P
<i>fs(3)29A</i>	11	4.7	2.1	9.5	22	0	0	0	E
<i>fs(3)H172</i>	9	4.3	1.3	14.3	18	15.9	0.2	1.1	E
<i>fs(3)L1-49</i>	3	1.0	2.3	3.7	6	1.3	0	0	N
Oregon R	18	2.2	1.4	4.1	36	1.9	1.3	11.4	N

^a N, normal; P, poor; E, excellent.

^b Morphologically abnormal.

hosts. Five of the six mutants with ovaries of morphological type Early Pre, which seldom develop follicles even up to stage 7, are excellent hosts.

In some species, the ovary makes a hormone necessary for vitellogenesis. For example, the ovary of mosquitos produces ecdysone, which stimulates vitellogenin production (Hagedorn, 1974). To test whether any of the mutants we studied were defective in an ovarian hormone, we checked whether an implanted wild-type ovary could stimulate vitellogenesis in mutant hosts. The results (Table 2, column 3) showed that an implanted wild-type ovary did not cause substantial stimulation of vitellogenesis in any of the mutants tested. Apparently none of these mutants is defective in any humoral factor arising from the ovary.

A second way of examining the humoral conditions of the female sterile mutants is to find whether they can complement the

defect in an ovary nonautonomous mutant. To perform this test, we implanted *ap⁴* ovaries into the hemocoel of several of the other female sterile mutants. The data in Table 3 (column 2) show that the factor missing from *apterous* mutants can be provided by other female sterile mutants, particularly *fs(2)A17*. The mutants *fs(3)A1* and *fs(2)A18* are not very effective at curing the defect in *ap⁴* ovaries, and they also are not good hosts for wild-type ovaries (Table 2).

A more sensitive test for ovarian autonomy. The ovarian transplant experiments showed that *fs(2)A17* provides an especially favorable environment for vitellogenesis, allowing implanted normal ovaries to develop over three times as many mature follicles as they do in wild-type hosts. This can therefore provide the basis for a more sensitive test for ovarian autonomy in non-vitellogenic female sterile mutants. Therefore, ovaries from selected mutants of each morphological type were implanted into

TABLE 3

DEVELOPMENT OF FOLLICLES IN *ap*⁴ OVARIES TRANSPLANTED INTO VARIOUS MUTANT FEMALES (COLUMN 2) AND IN VARIOUS MUTANT OVARIES TRANSPLANTED INTO *fs(2)A17* FLIES (COLUMN 5) (AVERAGE NUMBER OF FOLLICLES PER OVARY IN THE INDICATED STAGE)

(1) Genotype	(2) <i>ap</i> ⁴ ovaries → indicated host				(3) Host category ^a	(4) Indicated ovaries → <i>fs(2)A17</i> hosts				(5) Ovary category ^b
	No. of ovaries	Early	Int.	Late		No. of ovaries	Early	Int.	Late	
<i>fs(3)L3</i>	7	2.1	0.9	1.7	N	8	0	0	0	AM
<i>fs(2)A17</i>	14	3.9	2.6	14.4	E	—	—	—	—	—
<i>fs(3)L8</i>	—	—	—	—	—	2	0	0	0	AM
<i>ap</i> ⁴	5	0	0	0	P	16	4.0	2.6	14.5	NAM
<i>fs(3)A1</i>	9	0.6	0.4	0.2	P	8	1.2	1.9	4.6	NAM
<i>fs(2)A18</i>	7	6.7	0.3	0.4	P	4	0.8	0.5	1.8	NAM
<i>fs(3)29A</i>	—	—	—	—	—	10	0	0	0	AM
<i>fs(3)L1-49</i>	2	2.0	1.0	4.0	N	6	1.0	0.8	0.2	AM
Oregon R	12	3.0	0.4	3.6	N	20	2.6	1.6	15.8	AM

^a N, normal; P, poor; E, excellent.

^b AM, ovary autonomous in a mutant host; NAM, ovary nonautonomous in a mutant host.

homozygous *fs(2)A17* hosts. The data in Table 3 (column 3) show that the ovaries of *fs(3)A1* and *fs(2)A18*, as well as *ap*⁴, behave nonautonomously when provided with the very favorable internal environment of *fs(2)A17*. Since all three of these mutants were poor hosts to wild-type and *ap*⁴ ovaries, it can be concluded that the major focus of their genetic defect does not reside in their ovaries, but rather in their internal milieu.

Juvenile hormone analog (JHA) treatments. The transplantation tests identified three mutants that failed to undergo vitellogenesis because of a nonpermissive internal environment. Since the juvenile hormone analog ZR-515 can reverse the lack of vitellogenesis in isolated wild-type abdomens (Handler and Postlethwait, 1977) and in *ap*⁴ (Postlethwait and Weiser, 1973; Gavin and Williamson, 1976b), we decided to test the effects of JHA on the nonvitellogenic female sterile mutants. The data in Table 4 show that the hormone analog had no significant effect on four of the mutants. In five of the ovary autonomous mutants tested, vitellogenesis was stimulated, but morphologically abnormal vitellogenic follicles were formed. These usually had no respiratory horns and were much shorter

TABLE 4

MATURATION OF OVARIAN FOLLICLES 3 DAYS AFTER TOPICALLY TREATING MUTANT FLIES WITH ZR-515, A JUVENILE HORMONE ANALOG

(1) Genotype	(2)				(3) Analog category ^a
	No. of ovaries	Early	Int.	Late	
<i>fs(3)H23</i>	24	0.1	0.1	0.5 ^b	VA
<i>fs(3)L3</i>	10	0	0	0	NE
<i>fs(2)A17</i>	12	0	0	0	NE
<i>fs(3)115A</i>	20	0.4	0	0	NE
<i>fs(3)127A</i>	18	5.8	0.2	0.2 ^c	VA
<i>fs(3)133A</i>	18	0.06	0	0.02 ^b	VA
<i>ap</i> ⁴	8	2.2	1.0	5.0	BV
<i>fs(3)A1</i>	26	0.1	0.04	1.5	BV
<i>fs(2)A18</i>	22	0.5	0.4	0.6	BV
<i>fs(3)29A</i>	18	0	0.1	0.2 ^b	VA
<i>fs(3)H172</i>	24	12.9	0.4	0.5	NE
<i>fs(3)L1-49</i>	12	6.6	0.2	0.5 ^b	VA

^a BV, better vitellogenesis; VA, vitellogenic but abnormal; NE, no effect.

^b Squat eggs without horns.

^c Vesiculated.

and more squat than normal stage 13 or 14 follicles. In contrast, the three nonautonomous mutants were able to form morphologically normal mature oocytes under the influence of JHA. This result suggests that the mutant phenotype may be due to decreased availability of juvenile hormone in the adult.

The mutant corpus allatum. A lower supply of juvenile hormone to the mutant ovaries could involve inadequate hormone transport, an increase in juvenile hormone degradation in the blood, or failure of the mutant corpus allatum to synthesize or secrete the hormone. To test the latter possibility, we employed the assay for corpus allatum function developed by Handler and Postlethwait (1977). This involves the transplantation of three mutant or wild-type corpus allatum-corpora cardiacum complexes from 3-day-old flies into an Oregon R wild-type abdomen isolated from the head and thorax less than 15 min after eclosion. The data in Table 5 show that the corpus allatum-corpora cardiacum complex functions nearly normally in four of the six mutants tested. Three of these mutants were shown by our previous experiments to possess an internal milieu capable of supporting normal vitellogenesis. Two of the mutants possessed corpus allatum-corpora cardiacum complexes that were defective in their ability to support ovarian maturation in isolated abdomens. In these mutants (*ap*⁴ and *fs(3)A1*) the corpora allata supported vitellogenesis in only 4% of the cases, compared to about 23% for the other mutants tested and 46% for wild-type corpora allata. Ovarian transplant experiments proved that these two mutants contained ovaries with a potential for normal vitellogenesis, but that their humoral conditions were un-

favorable for ovarian maturation. The present experiments suggest that *ap*⁴ and *fs(3)A1* are sterile due to defective corpora allata that either cannot synthesize or do not secrete juvenile hormone. The corpus allatum of the third nonautonomous mutant (*fs(3)A18*) is able to support normal vitellogenesis in this test system, and therefore the defect in this mutant, whether endocrine or nutritional, is not inherent to the corpus allatum.

The mutant hemolymph. The experiments reported thus far show that nearly normal vitellogenesis can be induced in the ovaries of three nonvitellogenic mutants by either transplantation to a permissive environment or treatment with a juvenile hormone analog. Yet the development of morphologically normal vitellogenic follicles does not ensure that normal proteins are sequestered from the hemolymph by the follicles. And likewise, the development of stage 14 oocytes in wild-type ovaries implanted into a female sterile host does not necessarily signify that the mutant hemolymph has provided the proper yolk proteins. Therefore, we decided to test whether the mutants do in fact contain bona fide yolk proteins in their hemolymph.

To do this, we prepared antiserum against the soluble proteins found in freshly oviposited eggs and tested experimental antigen preparations by immunoelectrophoresis. Figure 1b shows that three antigens

TABLE 5
VITELLOGENESIS IN ISOLATED WILD-TYPE ABDOMENS IMPLANTED WITH WILD-TYPE OR MUTANT CORPORA ALLATA

(1) Genotype of corpus allatum	(2) No. of surviving abdomens	(3) Vitellogenic preparations (%)	(4)			(5) Corpus allatum category ^a
			Early	Int.	Late	
<i>fs(3)L3</i>	25	20	0.10	0.06	0.06	N
<i>fs(2)A17</i>	29	21	0.22	0.10	0.03	N
<i>ap</i> ⁴	24	4	0	0	0.06	D
<i>fs(3)A1</i>	25	4	0	0	0.14	D
<i>fs(2)A18</i>	32	22	0.13	0.02	0.09	N
<i>fs(3)L1</i>	14	29	0.18	0	0.21	N
Oregon R	22	46	0.1	0.02	0.38	N
Ringer-injected control	12	0	0	0	0	--

^a N, normal; D, defective.

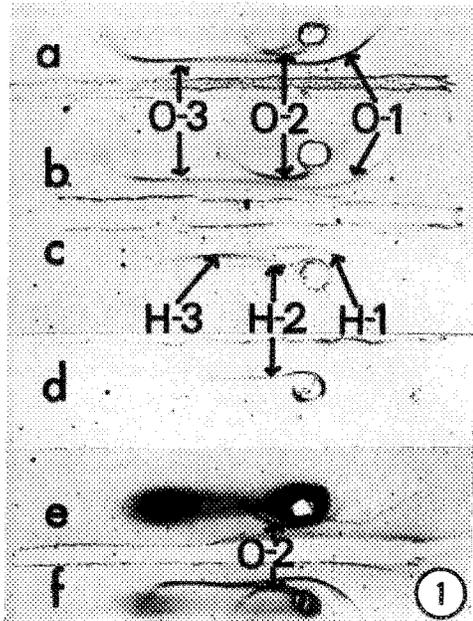


FIG. 1. Immunoelectrophoresis of (a) mature ovaries; (b) oviposited eggs; (c) hemolymph from mature females; (d) hemolymph from mature males; (e) immature ovaries; and (f) mature ovaries. O-1, O-2, and O-3—antigens from ovaries and oviposited eggs; H-1, H-2, and H-3—antigens from hemolymph.

present in oviposited eggs are detected by this procedure. These same three antigens are detected in mature ovaries (Fig. 1a). Arc O-2 is unrelated to arc O-3 since the two arcs cross each other. Arcs O-1 and O-3 have some antigenic sites in common since the two arcs fuse together. Arc O-3 contains antigenic sites that are missing from arc O-1 since arc O-3 extends beyond arc O-1 (Ouchterlony and Nilsson, 1973). Hemolymph contains two antigens (H-1 and H-3) that correspond in electrophoretic mobility to two of the antigens found in the ovaries (O-1 and O-3). Immunoabsorption experiments show that H-1 and H-3 correspond to O-1 and O-3 (Postlethwait and Handler, in preparation). In addition, female blood contains an antigen (H-2) that is not detected in these experiments in ovaries or eggs, but which is the only antigen present in male hemolymph (Fig. 1D) and is the only antigen detected by our procedure in the blood of freshly eclosed females

(Postlethwait and Handler, in preparation). Immature, nonvitellogenic wild-type ovaries contain only the O-2 antigen (Fig. 1e). These experiments permit the designation of antigens O-1 and O-3 as proteins that fulfill the classical definition of vitellogenins as outlined by Pan *et al.* (1969): Vitellogenins are female-specific proteins which are present in the blood and are ultimately taken up by maturing oocytes to form yolk.

Hemolymph from the female sterile mutants was tested by immunoelectrophoresis for the presence of the vitellogenin antigens. The results are shown in Fig. 2. All of the mutants showed the presence of arcs H-1, H-2, and H-3. Since H-1 and H-3 represent vitellogenins, this indicates that the lack of vitellogenesis in these mutants is not due to the inability of the mutants to produce vitellogenin. Since vitellogenin synthesis and secretion are not interrupted in any of these nonvitellogenic mutants, it seems that normal follicular development is not required for vitellogenin production.

In addition to the antigens found in wild-type fly hemolymph, 11 of the 16 female sterile mutants possessed a new arc (H4 in Fig. 2c) not found in the blood of normal

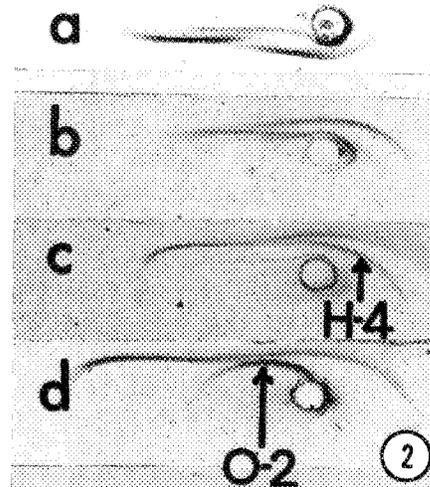


FIG. 2. Immunoelectrophoresis of normal and mutant tissues. (a) Wild-type hemolymph; (b) hemolymph of *fs(3)L3*; (c) hemolymph of *fs(2)A17*; (d) wild-type mature ovaries.

flies. Since arcs H-4 and H-3 merge, they apparently share antigenic determinants with each other. The significance of H-4 is unknown at this point, but it may represent a processed product of vitellogenins that appears in the blood when no ovaries capable of becoming vitellogenic are present to remove vitellogenins from the blood. This suggestion could be tested by two experiments. First, the H-4 band should disappear from the blood of female sterile flies into which wild-type ovaries are implanted; and second, ovariectomized wild-type flies should accumulate H-4.

Five of the female sterile mutants did not show antigen H-4 in their hemolymph. This group of genotypes (*fs(3)L3*, *fs(3)L8*, *ap⁴*, *fs(3)A1*, *fs(2)A18*, and Oregon R) includes the three nonautonomous mutants. Four of the five mutants lacking H-4 are leaky, in that they normally have an occasional vitellogenic follicle (Table 1, column 3). It thus appears that the ability to undergo even a small amount of vitellogenesis is in some way related to the lack of formation of antigen H-4.

DISCUSSION

The experiments reported here allow partial characterization of the stages at which vitellogenesis is blocked by the 18 nonvitellogenic mutations examined. Most of the mutants could not undergo vitellogenesis due to a genetic defect which directly affected the ovary. In seven of the mutants (morphological type Early Pre), the follicles became blocked prior to stage 7, the last nonvitellogenic stage. The result that follicular maturation was not substantially improved in these mutants by transplanting their ovaries into wild-type flies is consistent with the suggestion that no hormonal signal is required in the adult for maturation of ovaries up to stage 7 or, alternatively, that none of these mutants is defective in such a proposed hormone activity. Treating these mutants with a juvenile hormone analog did cause a few vitellogenic follicles to develop, but they were in each

case grossly abnormal in morphology. This suggests that Early Pre mutations affect a cellular process required for proper construction of the follicle. Ultrastructural examination of these mutants might reveal exactly which process is initially disrupted. Previous investigations of this sort have revealed extensive tumor cell formation and abnormal follicle cell function in the ovaries of *fs(2)B* (Koch and King, 1964).

Even though mutants of morphological type Early Pre do not make normal previtellogenic follicles, they do contain vitellogenins in their blood. This result shows that normal maturation of late previtellogenic follicles is unnecessary for the induction of yolk protein synthesis, secretion, and transport in the blood.

The finding that five of the six mutations causing Early Pre ovaries are excellent hosts corroborates Bodenstein's (1947) conclusion that there is competition among ovaries for vitellogenic factors. Bodenstein (1947) found that when one ovary was removed from a fly, the remaining ovary produced many more oocytes than a normal ovary, and that when flies were implanted with an extra ovary, each ovary produced fewer mature oocytes than unoperated controls. Since the ovaries of Early Pre mutants sequester no yolk, a single implanted normal ovary faces no competition for vitellogenic factors, and so can mature more eggs than it would when implanted into a normal female.

Autonomous Early Pre and Poor Vit mutants develop follicles up to stage 7, followed by either follicular degeneration or the maturation of a very few vitellogenic follicles. Since these mutants have a permissive internal environment, they must be defective in either juvenile hormone reception, interpretation, or execution of the developmental program leading to normal vitellogenesis in the ovary.

Three female sterile mutations were categorized as ovary nonautonomous from the transplantation experiments. These mutant ovaries are competent to respond

to humoral signals causing vitellogenesis in wild-type hosts or hosts of ovarian morphology type Early Pre, but the mutants possess internal environments that are not conducive to vitellogenesis in a wild-type ovary. Genetic alterations of the internal milieu might include aberrant juvenile hormone production, secretion, transport, or degradation, in addition to abnormal nutrition, protein synthesis, fat body function, or other defects.

There are precedents for female sterile mutants possessing aberrant corpora allata. The mutants *fs(2)adipose* and *fs(2)B* are ovary autonomous female sterile mutants that produce yolk-deficient eggs and follicular tumors, respectively (Doane, 1960; Koch and King, 1964). In these mutants, the corpus allatum hypertrophies abnormally in mated females, but this effect is not intrinsic to the corpus allatum since it is cured by implanting wild-type ovaries into the mutants (Doane, 1961; King, *et al.*, 1966). To test whether the corpus allatum was itself defective in our group of female sterile mutants, we transplanted mutant corpora allata into isolated wild-type abdomens. These tests for corpus allatum function revealed that two of the mutants possessed defective corpora allata. This might be due to a failure of the corpus allatum to be reactivated in the adult stage or to a defect in juvenile hormone synthesis or secretion. Since these two mutants metamorphose normally, we can conclude either that the adult and larval juvenile hormones are distinct qualitatively or quantitatively or that a genetically separate control mechanism activates the corpus allatum in the adult stage. This latter possibility is supported by results on a mutant of the tobacco hornworm, whose condition in some respects is the reciprocal of that of the *ap⁴* and *fs(2)A1* mutants in *Drosophila*. The black larval mutant of *Manduca* has a corpus allatum that is much less active than that of the wild type during the larval period (Safranek and Riddiford, 1975). Nevertheless, the mutant is just as fertile

on a per weight basis as the wild type, and so the adult must possess a corpus allatum that functions normally enough to allow for the critical step in oogenesis that juvenile hormone promotes in this moth (Nijhout and Riddiford, 1974). The study of conditional larval lethal mutants in *Drosophila* might disclose further mutants that have deficient larval corpora allata but normal adult allata or vice versa.

It is important to notice that the mutants with defective corpora allata nevertheless produce and secrete vitellogenin into the hemolymph. This result confirms and extends reports by Gavin and Williamson (1976b) and Kambysellis and Craddock (1976), and shows that high levels of juvenile hormone are not required for vitellogenin synthesis. Since juvenile hormone is required for vitellogenesis in wild-type (Handler and Postlethwait, 1977) and mutant (Postlethwait and Weiser, 1973; Gavin and Williamson, 1976b) females, it must act to stimulate yolk protein uptake. Whether the hormone acts directly on the ovary in this regard can be answered by *in vitro* organ culture experiments.

The third nonautonomous mutant possesses a corpus allatum that behaves normally in our test for gland function. This does not prove, however, that the gland functions normally *in situ*. A pair of nerves from the brain innervates the corpus allatum, and in other insects these nerves inhibit gland secretion (Sehnal and Granger, 1975). Transplanting mutant corpora allata removes them from this possible nervous inhibition, and thus may permit corpus allatum function. This suggestion could be tested by decapitating *fs(2)A18* females to sever nerve connections in order to test if gland function is restored and vitellogenesis begins. Alternatively, there may be humoral factors necessary for corpus allatum function that are missing in *fs(2)A18* but present in wild-type isolated abdomens. A final possibility exists that *fs(2)A18* is defective regarding some other as yet unknown humoral factor required for vitello-

genesis. Resolution of this question awaits further research.

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