INTERACTIONS BETWEEN SEX-TRANSFORMATION MUTANTS OF *DROSOPHILA MELANOGASTER*. I. HEMOLYMPH VITELLOGENINS AND GONAD MORPHOLOGY.

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ABSTRACT

In Drosophila, vitellogenins (yolk protein precursors) are synthesized by the female fat body, secreted into the hemolymph and subsequently taken up by the developing oocytes. The male fat body, on the other hand, does not do this even when immature ovaries are transplanted into the body cavity and grow. Thus, the hemolymph vitellogenins serve as an easily detectable sexually dimorphic biochemical marker.——We have examined hemolymph vitellogenins by SDS polyacrylamide gel electrophoresis in flies carrying various sex-transformation mutants (*dsx*, *tra*, *tra-2* and *tra-2^{OTF}*) singly and in all possible combinations. Chromosomal females homozygous for *tra* or *tra-2* have no detectable hemolymph vitellogenins, while those homozygous for *tra-2^{OTF}* exhibit appreciable levels of these proteins. Flies homozygous for *dsx*, both *X/X* and *X/Y*, have hemolymph vitellogenins, although the amount is consistently smaller in the latter. Indeed, *X/Y*; *dsx/dsx* is the only genotype in which hemolymph vitellogenins are detected in the *X/Y* flies. A clear hierarchy of epistasis exists among these sex-transformation mutants when they are examined in various combinations: *dsx* > *tra*, *tra-2* > *tra-2^{OTF}*. Moreover, an interaction between *tra-2^{OTF}* and *tra* was seen in these experiments: *X/X*; *tra-2^{OTF}/tra-2^{OTF}* flies show the presence of only a trace of hemolymph vitellogenins when they are made heterozygous for *tra*. These results, combined with observations on gonad morphology, are discussed with respect to the BAKER and RIDGE (1980) hypothesis of sex determination.

MECHANISMS of sex determination and differentiation in Drosophila have received renewed interest in recent years (NÖTHIGER, DÜBENDORFER and EPPER 1977; MARSH and WIESCHAUS 1978; SCHÜPBACH, WIESCHAUS and NÖTHIGER 1978; FUJIHARA, KAWABE and OISHI 1978; CLINE 1979), and a proposal has been submitted providing explanations for the actions of various sex-transformation genes in a single scheme (BAKER and RIDGE 1980). Examinations of these genes so far have relied heavily on the morphological characteristics and centered about the actions of null mutants. In an attempt to contribute to the elucidation of these problems, we have studied the interactions between various sex-transformation mutants, paying special attention to a leaky allele, *tra-2^{OTF}* (FUJIHARA, KAWABE and OISHI 1978), in terms of both morphological charac-

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teristics and a sexually dimorphic biochemical characteristic, vitellogenin synthesis by the fat body. The idea behind this is that, since the leaky allele produces an incomplete sex transformation, the interactions with other sex-transformation mutants may result in modification of the expression that should be easy to detect.

In the present communication, we present results on the levels of hemolymph vitellogenin and the gonad morphology of sexually transformed flies. While our work was in progress, a paper appeared describing the effect of some sex-transformation mutants on vitellogenin synthesis (POSTLETHWAIT, BOWNES and JOWETT 1980). Our results confirm their observations and extend them farther to show the interactions between various sex-transformation mutants.

In Drosophila, vitellogenins (yolk protein precursors) are produced by two organs: the fat body and the ovary. The adult female fat body synthesizes vitellogenins (3 protein species), which have been extensively characterized (BOWNES and HAMES 1977; POSTLETHWAIT and KASCHNITZ 1978; WARREN and MAHOWALD 1979; WARREN, BRENNAN and MAHOWALD 1979; POSTLETHWAIT, BOWNES and JOWETT 1980). These proteins are secreted into the hemolymph and are then taken up by the developing ovaries. Immature ovaries transplanted into the body cavity of young adult males can grow and accumulate yolk proteins. This is due to the production of yolk proteins by the ovary itself, not to the induction in the male fat body of vitellogenin synthesis. Implanted and mature ovaries secrete vitellogenins into the culture medium *in vitro*, but, if it also happens *in vivo*, sequestration back into the ovaries is apparently so rapid that the vitellogenins are not detectable in the host male hemolymph (BOWNES and HAMES 1978; POSTLETHWAIT, BOWNES and JOWETT 1980). These three vitellogenin polypeptides are coded for by genes on the *X* chromosome (POSTLETHWAIT and JOWETT 1980; BARNETT *et al.* 1980). Our interest is in the regulation of these genes: *X* chromosomal genes being expressed in the female fat body but not in the male, and the expression being regulated by various autosomal sex-transformation genes.

MATERIALS AND METHODS

Sex-transformation mutants: Four sex-transformation mutants representing three loci were examined: *tra-2* (*2–70*, WATANABE 1975), its allele *tra-2^{OTF}* (FUJIHARA, KAWABE and OISHI 1978), *tra* (*3–45*, STURTEVANT 1945), and *dsx* (*3–48.1*, HILDRETH 1965). *tra-2* and *tra* are similar in their actions: both transform *X/X* individuals into morphologically nearly normal males. These were shown to be null mutants by BAKER and RIDGE (1980). *tra-2^{OTF}* is leaky in its action since various structures are female-like in transformed *X/X* individuals (FUJIHARA, KAWABE and OISHI 1978) and heterozygotes with null alleles or deficiencies exhibit more complete transformation (BAKER and RIDGE 1980). *dsx* is unique in its action: both *X/X* and *X/Y* flies are transformed into intersexes. This was also shown to be a null mutant (BAKER and RIDGE 1980). These mutants were examined separately and in all possible combinations.

The following chromosomes were used to construct and/or to maintain the stocks: *SM1*, a balanced lethal chromosome *2* marked with the dominant gene *Cy*; *TM6*, a balanced lethal chromosome *3* marked with the dominant gene *Ubx*; *Pm*, a chromosome *2* marked with the dominant gene *Pm*; *Sb*, a chromosome *3* marked with the dominant gene *Sb*; *B^{S}Y*, a *Y* chromosome marked with the dominant gene *B^{S}*. This last chromosome was used to distinguish *X/Y* flies

from X/X transformed flies. For the chromosomes and mutants without direct citations, see LINDSLEY and GRELL (1968).

Flies were reared at 24 ± 1° on a standard medium (per liter of water: sugar 55 g, dried yeast 35 g, agar 20 g, with propionic acid, 3–4 ml, added as a mold inhibitor).

Detection of vitellogenins: In the present study, we determined the presence or absence of vitellogenins in the hemolymph. Fresh hemolymph samples (total 0.2 μl each) were collected from several flies, using glass microinjection pipettes through the ventral side of the thorax. Each sample was then added with 15 μl of an SDS-sample buffer (10% w/v glycerol, 5% v/v 2-mercaptoethanol, 2.3% w/v SDS, 0.0625 M Tris-HCl, pH 6.8), and heated for 15 min at 90°. Samples were subjected to SDS-polyacrylamide slab-gel electrophoresis in 10% running gels with 3% stacking gels, using the method of LAEMMLI (1970), either immediately or within 2 weeks following storage at 0°. Gels were stained with 0.1% Coomassie Brilliant Blue in a solution containing 50% methanol and 10% acetic acid and destained in 10% acetic acid.

Gonad morphology: Dissection, observation and photographic preparations were made as previously described (FUJIHARA, KAWABE and OISHI 1978).

RESULTS AND DISCUSSION

Figure 1 shows the SDS-polyacrylamide gel patterns of hemolymph proteins from various genotypes with respect to *tra* and *dsx*. Three bands marked YP1–3 are hemolymph vitellogenins. Although we did not make any further attempts to identify these bands positively as vitellogenins, the methods employed are those that have been used extensively to resolve vitellogenins (WARREN and MAHOWALD 1979; WARREN, BRENNAN and MAHOWALD 1979; BARNETT *et al.* 1980) and serve as a reliable assay. Two bands marked H1–2 are of unknown character. It was noted, however, that both bands were prominent in the hemolymph from flies up to 4–5 days after eclosion and that H1 decreased greatly in amount in flies 6 days old or older. We attempted to use the same volume of hemolymph for each sample insofar as possible, using calibrated micropipettes. Certain variation in the density of stained bands probably represents the technical limits. Preliminary observations indicate that, if samples were taken from flies of the same age and run on the same gel, the relative densitometric readings of bands YP1–3/H2 were always quite similar.

Experiments were done with various genotypes, including wild-type females and males (Oregon-R), but we present here only the pertinent results. Results in Figure 1 are in good agreement with those of POSTLETHWAIT, BOWNES and JOWETT (1980): *tra*-transformed (X/X; *tra/tra*) flies showed no detectable vitellogenins in their hemolymph, and *dsx*-transformed (X/X; *dsx/dsx*) flies had significant amounts of hemolymph vitellogenins. Our results extend the observations of POSTLETHWAIT, BOWNES and JOWETT (1980) by demonstrating the presence of hemolymph vitellogenins, though in a reduced amount, in X/Y; *dsx/dsx* flies. Since it has been shown that the amount of hemolymph vitellogenins in females is dosage dependent (POSTLETHWAIT and JOWETT 1980), it is possible that this observed difference reflects the dosage of the X-linked vitellogenin genes, *i.e.* nondosage compensation of these genes. If dosage compensation for the X-linked genes in *Drosophila* came about by a "piecemeal evolution of a compensatory mechanism," as suggested by LUCCHESI (1978), this explanation may not be unreasonable. Under normal conditions, vitellogenin genes are not expressed in

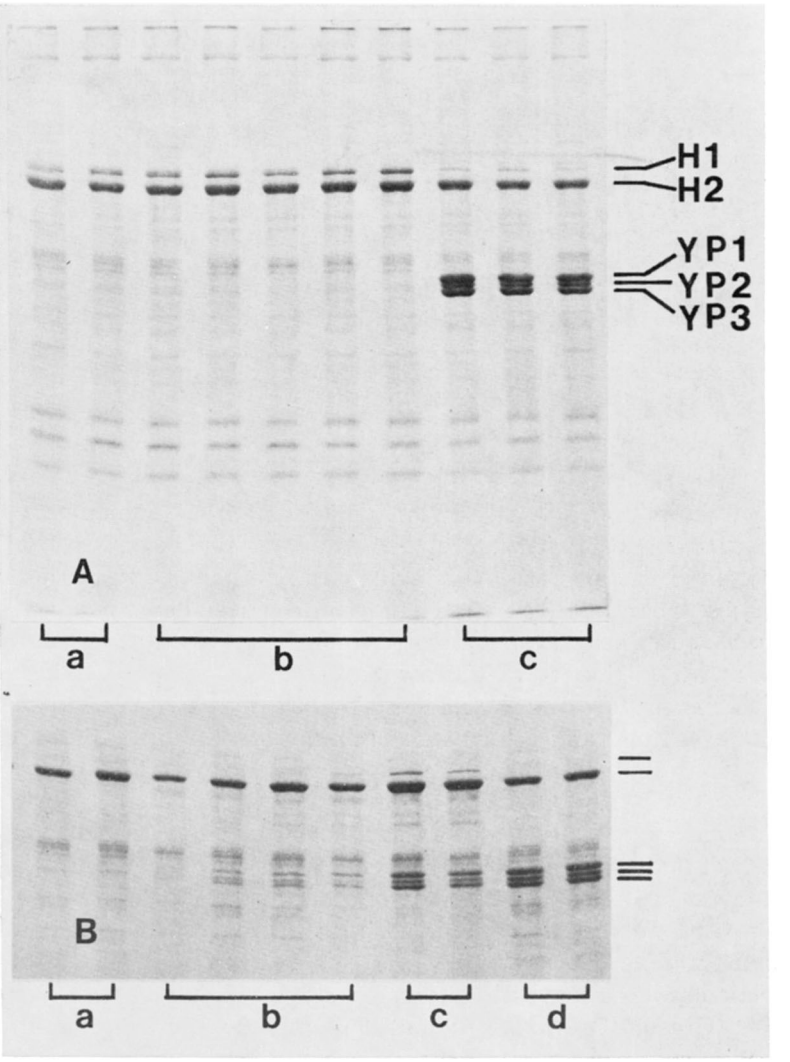

FIGURE 1.—SDS-polyacrylamide gel patterns of hemolymph proteins from flies carrying *tra* or *dsx* mutations (Coomassie blue stain).
(A) a: *X/Y; tra/tra*, b: *X/X; tra/tra*, c: *X/X; tra/+*. (B) a: *X/Y; dsx/+*, b: *X/Y; dsx/dsx*, c:*X/X; dsx/dsx*, d: *X/X; dsx/+*. All samples are from 12–14 day-old flies. Bands YP1–3 are hemolymph vitellogenins and H1–2 are hemolymph proteins of unknown character (see text).

1*X*2*A* flies and, thus, may not have evolved the dosage compensation mechanism needed by most other *X*-linked genes, which are expressed in both sexes. It should be pointed out that homozygosity for the *dsx* mutant does not affect the dosage compensation of other *X*-linked genes that have been measured (SMITH and LUCCHESI 1969). Our preliminary observations show that relative densitometric readings of YP1–3 *versus* H2 from *X/X; dsx/dsx* and *X/Y; dsx/dsx* flies come close to 2 : 1, thus giving support to the possibility of nondosage compensation.

Figure 2 shows the gel patterns of *tra-2-* and *tra-2^{0TF}*-transformed flies. *tra-2* was quite similar to *tra* and produced no vitellogenins in the hemolymph of *X/X*; *tra-2/tra-2* flies. On the other hand, *tra-2^{0TF}* was leaky, and *X/X*; *tra-2^{0TF}/tra-2^{0TF}* flies had hemolymph vitellogenin levels that were similar to those of normal females (*X/X*; *tra-2^{0TF}/+*). The heteroallelic genotype, *X/X*; *tra-2/tra-2^{0TF}*, showed trace amounts of hemolymph vitellogenins if samples were taken from 2–4 day-old flies, but showed distinct bands, although still small in amount, if flies were 6–7 days post-eclosion or older. Clearly, the leakiness of *tra-2^{0TF}*, as previously shown in morphological characteristics (FUJIHARA, KAWABE and OISHI 1978), is also reflected in this sexually dimorphic biochemical character. Table 1 summarizes the observations on hemolymph vitellogenins in flies homozygous or heterozygous for single sex-transformation mutants.

tra-2^{0TF}-transformed flies have rudimentary ovaries in contrast to *tra-2*-transformed and *tra*-transformed flies, both of which have rudimentary testis-like structures. *tra-2/tra-2^{0TF}*-transformed flies have rudimentary ovotestis-like structures (FUJIHARA, KAWABE and OISHI 1978). The gross morphological appearance of rudimentary gonads, however, is not completely correlated with the presence or absence of hemolymph vitellogenins. For example, *tra-2^{0TF}*-transformed flies (which at a low frequency carry nearly mature-sized ovaries) were separated into those with rudimentary ovaries and those with large ovaries and the hemolymph samples were examined separately. No difference in the amount of hemolymph vitellogenins was detected (data not shown).

Results summarized in Table 2 demonstrate various aspects of the actions of combinations of sex-transformation mutants with respect to the production of vitellogenins and gonad morphology. The action of *dsx* is epistatic to that of *tra-2* and *tra*. Both *X/X*; *tra-2/tra-2* and *X/X*; *tra/tra*, which produce no vitellogenins in the hemolymph, had hemolymph vitellogenins when homozygous for *dsx*. This hierarchy of epistasis is the same as that determined by BAKER and RIDGE (1980),

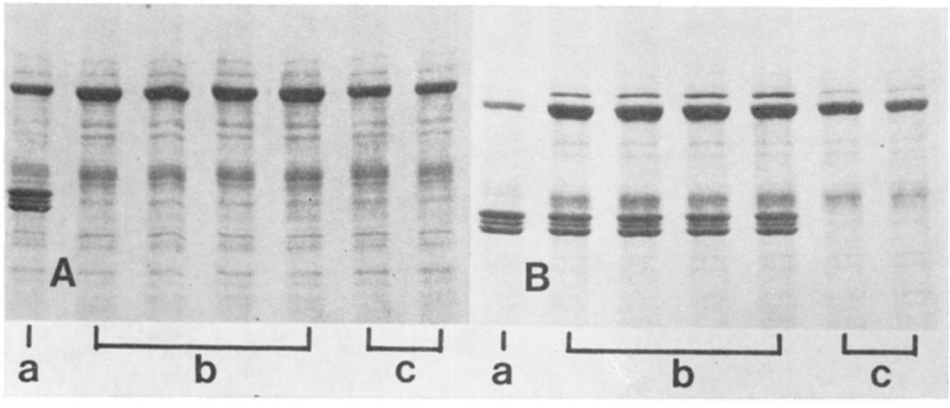

FIGURE 2.—Stained gel patterns of hemolymph proteins from *tra-2- or tra-2^{0TF}*-transformed flies.
(A) a: *X/X*; *tra-2/+*, b: *X/X*; *tra-2/tra-2*, c: *X/Y*; *tra-2/tra-2*. (B) a: *X/X*; *tra-2^{0TF}/+*, b: *X/X*; *tra-2^{0TF}/tra-2^{0TF}*, c: *X/Y*; *tra-2^{0TF}/tra-2^{0TF}*. All samples are from 12–14 day-old flies.

TABLE 1

Hemolymph vitellogenins in flies with various sex-transformation mutants

Genotype	Phenotypic sex*	Gonad†	Hemolymph vitellogenins‡ Days after eclosion (2–4)	(6–8)	(12–14)
X/X ; +/+§ ; *tra*/+‖	♀	ovary		+++	+++
X/X ; +/+ ; *tra/tra*	♂	testis-like		—	—
X/B^{S}*Y*; +/+ ; *tra/tra*	♂	testis		—	—
X/X ; +/+ ; *dsx*/+	♀	ovary		+++	
X/X ; +/+ ; *dsx/dsx*	♂	ne¶		+++	
X/B^{S}*Y*; +/+ ; *dsx/dsx*	♂	ne		++	
X/X ; *tra-2*/+ ; +/+	♀	ovary	+++	+++	+++
X/X ; *tra-2/tra-2* ; +/+	♂	testis-like		—	—
X/B^{S}*Y*; *tra-2/tra-2* ; +/+	♂	testis		—	—
X/X ; *tra-2*^{OTF}/+ ; +/+	♀	ovary	+++	+++	+++
X/X ; *tra-2*^{OTF}/*tra-2*^{OTF}; +/+	♂	ovary-like**	++	+++	+++
X/B^{S}*Y*; *tra-2*^{OTF}/*tra-2*^{OTF}; +/+	♂	testis		—	—
X/X ; *tra-2/tra-2*^{OTF}; +/+	♂	ovotestis-like**	±	++	++
X/B^{S}*Y*; *tra-2/tra-2*^{OTF}; +/+	♂	testis	—	—	—

* Details of external morphology have been published (BAKER and RIDGE 1980 and references therein. Certain external characteristics that were not dealt with by BAKER and RIDGE (1980) will be presented elsewhere (OTA *et al.*, in preparation).

† Morphological details have been published, see BROWN and KING (1961) for *tra*, HILDRETH (1965) for *dsx*, and FUJIHARA, KAWABE and OISHI (1978) for *tra-2* and *tra-2*^{OTF}.

‡ Arbitrary units, see Figures 1–3. § *SM1* or *Pm*. ‖ *TM6* or *Sb*. ¶ Not examined. ** See Figure 4, text and figure legends for details. Brackets indicate that the various genotypes came from the same cross.

TABLE 2

Hemolymph vitellogenins and gonad morphology in flies that carry two or more sex-transformation mutants

Genotype	Phenotypic sex*	Gonad†	Hemolymph vitellogenins‡ Days after eclosion (2–4)	(6–8)	(12–14)
X/X ; tra-2/+§ ; *tra/+*‖	♀	ovary		+++	
X/X ; tra-2/+ ; tra/tra	♂	testis-like		—	
X/X ; tra-2/tra-2 ; tra/+	♂	testis-like		—	
X/X ; tra-2/tra-2 ; tra/tra	♂	testis-like		—	
X/X ; tra-2/+ ; dsx/+	♀	ovary		+++	+++
X/X ; tra-2/+ ; dsx/dsx	♀'	ne¶		+++	
X/X ; tra-2/tra-2 ; dsx/+	♂	testis-like		—	—
X/X ; tra-2/tra-2 ; dsx/dsx	♀'	ne		+++	
X/B^{S}Y; tra-2/+ ; dsx/dsx	♀'	ne		++	
X/B^{S}Y; tra-2/tra-2 ; dsx/dsx	♀'	ne		++	
X/X ; tra-2^{OTF}/+ ; tra/+	♀	ovary	+++	+++	+++
X/X ; tra-2^{OTF}/+ ; tra/tra	♂	testis-like	—	—	—
X/X ; tra-2^{OTF}/tra-2^{OTF}; tra/+	♂	testis-like** ‡‡	±	±	+
X/X ; tra-2^{OTF}/tra-2^{OTF}; tra/tra	♂	testis-like	—	—	—
X/X ; tra-2^{OTF}/tra-2^{OTF}; dsx/+	♂	ovary-like**		+++	
X/X ; tra-2^{OTF}/tra-2^{OTF}; dsx/dsx	♀'	ne		+++	
X/B^{S}Y; tra-2^{OTF}/tra-2^{OTF}; dsx/dsx	♀'	ne		++	
X/X ; tra-2/tra-2^{OTF}; tra/+	♂	testis-like**		—	±
X/X ; tra-2/tra-2^{OTF}; tra/tra	♂	testis-like		—	

TABLE 2—Continued

Genotype	Phenotypic sex*	Gonad†	Hemolymph vitellogenins‡ Days after eclosion (2–4)	(6–8)	(12–14)
X/X ; tra-2/tra-2^{OTF}; dsx/+	♂	ovotestis-like*•		++	++
X/X ; tra-2/tra-2^{OTF}; dsx/dsx	♀	ne		+++	
X/B^{S}Y; tra-2/tra-2^{OTF}; dsx/+	♂	testis		—	
X/B^{S}Y; tra-2/tra-2^{OTF}; dsx/dsx	♀	ne		++	
X/X ; +/+ ; tra dsx/+ +	♀	ovary		+++	
X/X ; +/+ ; tra dsx/tra dsx	♀	ne		+++	
X/B^{S}Y; +/+ ; tra dsx/+ +	♂	testis		—	
X/B^{S}Y; +/+ ; tra dsx/tra dsx	♀	ne		++	
X/X ; tra-2/+ ; tra dsx/+ +	♀	ovary		+++	+++
X/X ; tra-2/+ ; tra dsx/tra dsx	♀	ne		+++	
X/X ; tra-2/tra-2 ; tra dsx/+ +	♂	ne		—	—
X/X ; tra-2/tra-2 ; tra dsx/tra dsx	♀	ne		++	
X/B^{S}Y; tra-2/+ ; tra dsx/tra dsx	♀	ne		+	
X/B^{S}Y; tra-2/tra-2 ; tra dsx/tra dsx	♀	ne		++	
X/X ; tra-2^{OTF}/+ ; tra +/+ dsx	♀	ovary	+++	+++	+++
X/X ; tra-2^{OTF}/tra-2^{OTF}; tra +/+ dsx	♂	testis-like** ††	—	+	+
X/B^{S}Y; tra-2^{OTF}/tra-2^{OTF}; tra +/+ dsx	♂	testis	—	—	
X/X ; tra-2/tra-2^{OTF}; tra +/+ dsx	♂	testis-like**		±	+

Explanations as in Table 1 except for the following: † Testis-like structures in *tra-2*- and *tra*-transformed flies are very similar. The testis-like structures here indicate the similar structures. †† Variations in morphology are somewhat extensive. See text and figure legends for further details.

who used the external morphology as their criterion of sexual phenotype. In the present study, we did not attempt to examine the morphology of gonads in any combinations in which *dsx* was homozygous since the internal reproductive organs in *dsx*-transformed flies are quite variable (HILDRETH 1965). A second observation in this experiment is the finding that *tra-2^{OTF}* interacts in some way with *tra*: *X/X*; *tra-2^{OTF}/tra-2^{OTF}* flies, which had hemolymph vitellogenins in an amount comparable to that of normal females, exhibited a smaller amount of hemolymph vitellogenins when they were made heterozygous for *tra* (Figure 3). Similar reductions in the amount of hemolymph vitellogenins were also observed in *X/X*; *tra-2/tra-2^{OTF}*; *tra/+* flies. These results were always accompanied by changes in gonad morphology (Figure 4). *X/X*; *tra-2^{OTF}/tra-2^{OTF}*; *+/+* flies had rudimentary ovary-like colorless structures (Figure 4b). When they were made heterozygous for *tra*, they had typically testis-like (yellow colored) structures (Figure 4c), but some had rudimentary gonads, one of which was testis-like and the other ovotestis-like (yellow colored bleb with a colorless structure attached anteriorly). (Double homozygotes had no hemolymph vitellogenins, and they had rudimentary gonads that resembled those of *X/X*; *tra/tra*.) The gonads of *X/X*; *tra-2/tra-2^{OTF}*; *+/+* flies were ovotestis-like (Figure 4f), but, when flies were made heterozygous for *tra*, the gonads became testis-like (Figure 4g). (*tra*-type rudimentary testis-like structures were observed in the *X/X*; *tra-2/tra-2^{OTF}*; *tra/tra* flies.) The effect of heterozygous *tra* on *tra-2^{OTF}/tra-2^{OTF}* and *tra-2/tra-2^{OTF}* flies was also clearly detectable in some external morphological characteristics (OTA *et al.*, in preparation).

Although the regulation of vitellogenin synthesis by the fat body through hormones has been well established (JOWETT and POSTLETHWAIT 1980; POSTLETHWAIT, BOWNES and JOWETT 1980 and references therein), it is clear that the system is somehow under the control of sex-determination mechanisms. That it

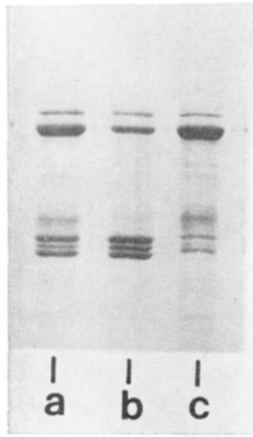

FIGURE 3.—Stained gel patterns of hemolymph proteins showing the effect of a single dose of *tra* on *tra-2^{OTF}/tra-2^{OTF}*.
a: *X/X*; *tra-2^{OTF}/tra-2^{OTF}*, b: *X/X*; *tra-2^{OTF}/+*; *tra/+*, c: *X/X*; *tra-2^{OTF}/tra-2^{OTF}*; *tra/+*. All samples are from 7–8 day-old flies.

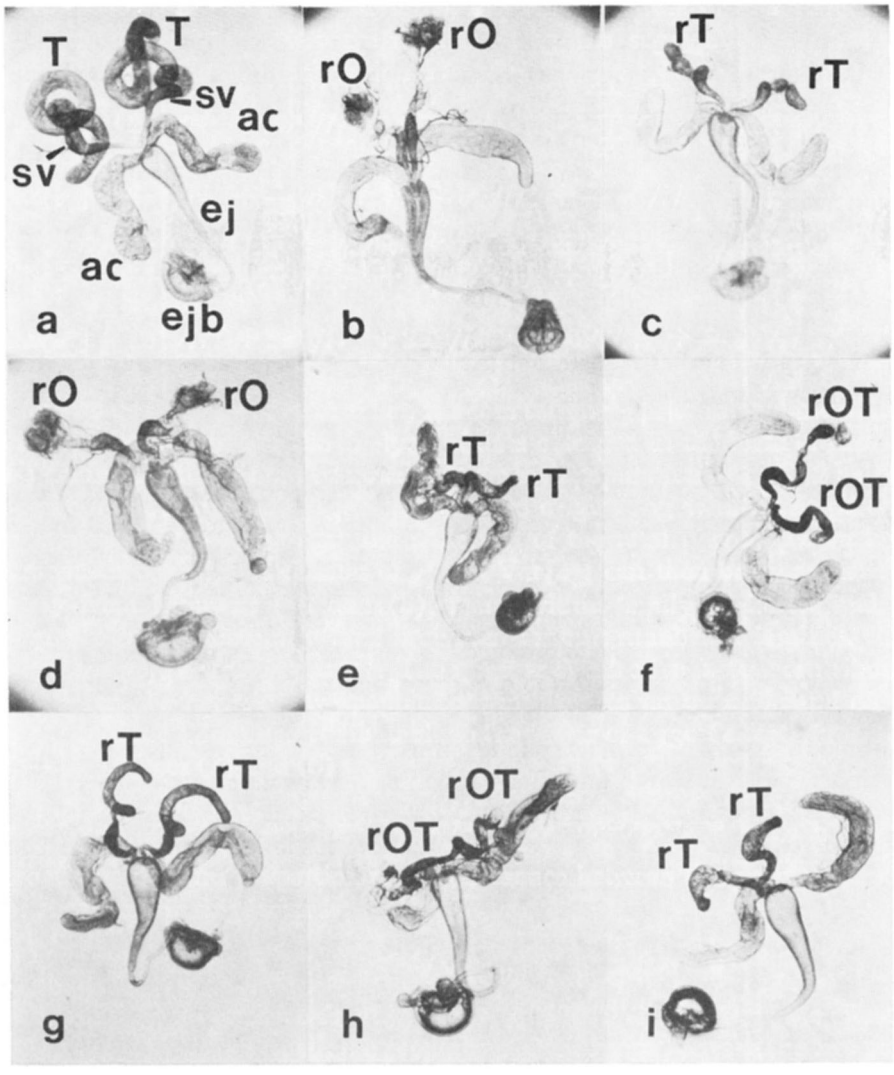

FIGURE 4.—Internal reproductive organs of various genotypes. Abbreviations: T, testis; rT, rudimentary testis-like structure; rO, rudimentary ovary-like structure; rOT, rudimentary ovotestis-like structure; ac, accessary gland (paragonium); sv, seminal vesicle; ej, ejaculatory duct; ejb, ejaculatory bulb.

(a) Control fertile male. (b) *X/X*; *tra-2*^{0TF}*/tra-2*^{0TF}. Note rudimentary gonads are colorless amorphous structures (rudimentary "ovary-like" structures). (c) *X/X*; *tra-2*^{0TF}*/tra-2*^{0TF}*; tra/+*. Rudimentary gonads are generally "testis-like" (yellow colored), but some show gonads, one of which is "testis-like" and the other "ovotestis-like" (yellow colored bleb with colorless amorphous structure attached anteriorly). (d) *X/X*; *tra-2*^{0TF}*/tra-2*^{0TF}; *dsx/+*. (e) *X/X*; *tra-2*^{0TF}*/tra-2*^{0TF}; *tra +/+ dsx*. Note that the effect of a single dose of *tra* can be seen. (f) *X/X*; *tra-2/tra-2*^{0TF}. Rudimentary gonads are ovotestis-like. (g) *X/X*; *tra-2/tra-2*^{0TF}; *tra/+*. (h) *X/X*; *tra-2/tra-2*^{0TF}; *dsx/+*. (i) *X/X*; *tra-2/tra-2*^{0TF}; *tra +/+ dsx*. Note again that the effect of a single dose of *tra* is seen.

is not the *X:A* balance *per se* that is responsible for the production of vitellogenins, but rather the action of sex-transformation loci such as *tra*, *tra-2* and *dsx*, is suggested by our demonstration that *X/Y*; *dsx/dsx* flies synthesize vitellogenins. Whether it is a primary effect on some tissue (complete or incomplete transformation in the fat body) or a secondary effect through the formation or transformation of certain (hormone secreting ?) tissues (*e.g.*, gonads, ring glands, etc.) by the sex-transformation mutants remains to be seen.

In vitro experiments have shown that the male fat body can be induced to synthesize vitellogenins only by 20-hydroxyecdysone, while the female fat body synthesized vitellogenins upon stimulation by either 20-hydroxyecdysone or a juvenile hormone analog (JOWETT and POSTLETHWAIT 1980; POSTLETHWAIT, BOWNES and JOWETT 1980). Complete transformation of the fat body of *X/X*; *tra/tra*, for example, into maleness and presumptive unresponsiveness to juvenile hormone analogs may not be sufficient for the absence of hemolymph vitellogenins unless the hormonal milieu also changes. The presented here are in overall agreement with the conclusion of POSTLETHWAIT, BOWNES and JOWETT (1980) that "the production of yolk polypeptides is inversely related to the degree of transformation to the male phenotype shown in the sex mutants", but more specifically we note a correlation between the levels of hemolymph vitellogenins and sexual differentiation of the gonads. *X/X*; *tra-2^{OTF}/tra-2^{OTF}* flies have a complete set of well-developed male genitalia, but rudimentary ovary-like gonads, and they have abundant hemolymph vitellogenins. Both *tra-2*- and *tra*-transformed flies have similarly well-developed male genital structures, but rudimentary testis-like gonads; these flies do not have vitellogenins in their hemolymph. The *dsx*-transformed flies (both *X/X* and *X/Y*) have underdeveloped female and male gonads, as well as genital structures (HILDRETH 1965), but they have hemolymph vitellogenins. Furthermore, heterozygosity for *tra* in *tra-2/tra-2^{OTF}* and *tra-2^{OTF}/tra-2^{OTF}* has no further effect on the already completely male external genitalia, but both gonad morphology and hemolymph vitellogenin amount become more male-like.

BAKER and RIDGE (1980) proposed a mechanism of sex determination in Drosophila: (1) The *dsx*$^+$ locus can be expressed in either of two modes depending on the *X:A* balance. (2) In *1X2A*, *dsx*$^+$ takes the basal mode of expression (female differentiation repressed) in which the *dsx*$^+$ product acts to repress a battery of genes for female differentiation functions. (3) In *2X2A*, *tra-2*$^+$ and *tra*$^+$ are activated and their products change the mode of *dsx*$^+$ expression from the basal (male) state to the other (female) state. In this case, the *dsx*$^+$ product suppresses a battery of genes for male differentiation functions. According to this hypothesis, we can explain the present results as follows. The *dsx* mutant (null mutant, BAKER and RIDGE 1980) cannot receive the signal from the *X:A* balance, and cells become "neutral". The expression of vitellogenin genes, which normally occurs only in *X/X* individuals, then escapes repression. The smaller amount of vitellogenins in *X/Y*; *dsx/dsx* flies might simply be a reflection of gene dosage. In the absence of *tra-2*+ or *tra*$^+$ function in *2X2A*, *dsx*$^+$ remains in the basal male mode: thus vitellogenin genes are regulated as though they were in *1X2A* and are not

expressed. Our results with X/X; *tra-2*^{OTF}/*tra-2*^{OTF}; *tra*/+ suggest that the products of *tra-2*$^+$ and *tra*$^+$ do not act independently, but act together, to regulate the mode of *dsx*$^+$ expression, thus adding one small detail to the hypothesis.

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