wedge of the tubing was cut out so that it could be clipped onto the pipette and used as a movable scale to measure the volume of the injection. Injections were made in the right side of the ventral abdomen, medial to the 5th or 6th sternites. No leakage of fluid was observed after injection. No toxic effects were seen, and fertility was not obviously affected.

To ensure that all stages of meiosis were assessed, the treated males were placed with fresh +/I .(FM6) virgins (3 to 6 days old) on 3 successive occasions lasting 3 days each, and on a fourth occasion lasting 5 days. The females that had been mated were subsequently transferred to new bottles and allowed to lay additional eggs for a time equal to the times that they had been with the males. An exception was the second 5-AzaCdR-treated brood whose females were inadvertently discarded prior to the second egg lay.

Lethal mutations, detected by the absence of adult males, were subsequently made heterozygous with FM6 balancers and confirmed in the fourth generation.

Results: Lethal mutations/chromosomes tested

Broods	1	2	3	4	Total	%
Treatments:						
Control	0/311	0/371	1/233	0/227	1/1142	0.09
5-AzaCdR	0/431	0/260	0/229	1/233	1/1153	0.09

Our results indicate that injections that should lead to an initial concentration greater than 2 mM 5-AzaCdR in the haemolymph are relatively nontoxic and do not cause mutations in the germ line. This apparent lack of mutagenicity agrees with the studies on mammalian cell culture. Since our results are negative, they are not unambiguously interpretable. For example, the metabolism of 5-AzaCdR may be different in Drosophila than it is in mammalian cells.

We do not plan to pursue this work further at this time.

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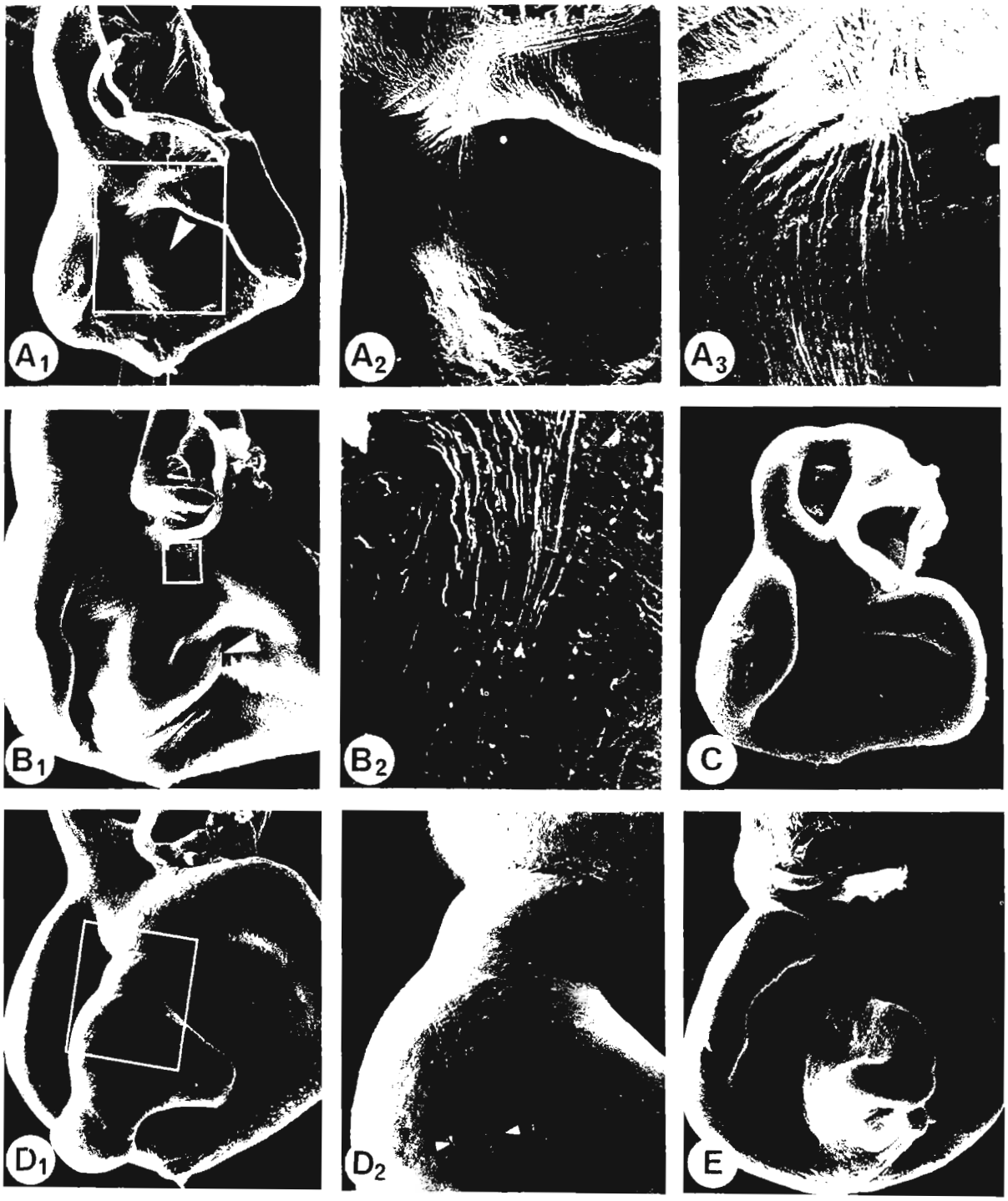
Zůst, B. and J. Wůest. Université de Geneve, Switzerland. The tumorous-head mutant of **Drosophila melanogaster:** examination by scanning electron microscopy of eye imaginal discs.

In flies of the homoeotic mutant tumorous-head (tuh) of **Drosophila melanogaster** head structures are partially replaced by abdominal, genital or undefined "amorphous" outgrowths (Newby 1949; Postlethwait et al. 1972; Kuhn et al. 1981). Kuhn and collaborators described the aldehyde oxidase (aldox)-staining patterns in the eye imaginal discs of tuh larvae (Kuhn & Cunningham 1976; Kuhn & Walker 1978) and found it likely that "the changes in aldox distribution correlate with the transformed portion of the disc affected by the homoeotic mutation". These transformed portions of tuh discs, isolated and cultured in vivo, differentiated autonomously (Kuhn et al. 1979). In this study tuh eye discs with visible abnormal regions were analysed by scanning electron microscopy (SEM).

Imaginal eye discs of third instar larvae of a tuh strain (Arizona State University strain; kindly provided by D.T. Kuhn) were dissected in Ringer's solution to which 2% glutaraldehyde in 0.1M cacodylate buffer (pH 7.3) was slowly added. They were fixed in pure 2% glutaraldehyde for 2h at room temperature, then washed, and in some cases stored up to 5 days in 0.1M cacodylate buffer (pH 7.3) at 4°C. Postfixation was done in 2% aqueous OsO_4 for 1h. The osmium-fixed discs were stored in 70% ethanol and later prepared for SEM. Eye discs of **D.melanogaster** Colmar strain served as controls.

Results and Discussion. 52 eye discs carrying transformed areas have been grouped according to size of their outgrowths: 14 small (27%; Fig. A: arrow), 14 medium (27%; Fig B1: arrow) and 24 large ones (46%; Fig. D1. Fig. E). Some of these areas had uniform shape (20/52 = 38%; Fig. A1), others appeared lobed (26/52 = 50%; Fig. D1), or twisted (6/52 = 12%; Fig. E). No correlation between size and shape has been found. Certain of these abnormal growths appeared clearly delimited from their unaltered supporting tissue (Fig. D1), while in others no clear limit could be detected (Fig. A1, B1). In the 52 discs analysed, 43 growths were located in the central region of the disc (region 2: giving normally rise to many of the eye facets), one in each lateral part of the disc (region 1 = ventral and 3 = dorsal) and in 7 cases the region could not be determined (for more details on the eye disc regions (see Kuhn & Walker 1978).



Figures: Imaginal eye discs of 3rd instar **D.melanogaster** larvae. C from Colmar control strain, A, B, D, E, from tuh mutant larvae with outgrowth in region 2. A1 disc with small outgrowth (arrow) of uniform shape, not clearly delimited from unaltered disc tissue, A2, A3 details with folds. B1 disc with medium sized outgrowth (arrow), slightly lobed, only in part delimited from unaltered disc tissue, B2 detail with folds. C control disc. D1 disc with large outgrowth, lobed, clearly delimited from unaltered tissue, D2 detail with folds (arrows). E disc with large outgrowth, twisted, only in part clearly delimited from unaltered disc tissue. Position of enlarged regions represented in Fig. A2, B2, D2 are indicated in Fig. A1, B1, D1. A1, B1, C, D1, E = 280x. A2, D2 = 700x. A3, B2 = 2100x.

A striking feature of these discs are folds at their surface which occur in the region of contact between the outgrowth and the underlying disc. Of the 52 discs studied, 39 (75%) showed such folds (Fig. A, B, D, E). In 31 discs these folds are oriented parallel to the anterior-posterior axis of the disc and they connect disc and transformed area (Fig. B2, D2); in 5 discs the folds ran parallel to the boundary between the disc and transformed area and in 3 cases they ran at an angle (Fig. A2). These folds are found in transformed regions of all sizes and shapes. Since the transformed areas of discs without visible connecting folds do not appear to be different from transformed areas having such folds, the role of these folds is not clear. Folds may be caused in part by mechnical stress, but not exclusively since in the disc represented in Fig. D2, for example, they run along a convex region of the abnormal outgrowth. The folds seem to comprise only the peripodial membrane. Occasionally such folds occur in areas other than the border region between disc and outgrowth.

The present study shows different connections between the eye disc and its transformed area in homoeotically transformed tuh mutant larvae. From these it appears likely that Kuhn et al. (1979) isolated and transplanted mainly those outgrowths (small, medium or large) which appear clearly delimited. Whether such a selection might influence the frequencies with which the various homoeotic differentiations occurred in these experiments is not known.

Kuhn & Walker (1978) also state that after dissection "aldox positive areas revealed, in each case, that a smooth boundary uniformly surrounds the entire area". That the transformed areas are functionally only little integrated into the normal part of the eye disc is also suggested by an observation by Stocker who could not show any nerves projecting into the transformed regions of the flies (pers. comm.).

It is interesting to compare the frequencies of abnormal outgrowths in eye discs of tuh larvae with the frequencies of abnormalities in the head of the tuh imago. Kuhn & Cunningham (1976) report that 80-90% of adult flies show head abnormalities (derived from eye and antennal discs). In 3rd instar larvae aldox staining revealed abnormalities in one or two eye discs in 63% of all animals. Since antennal discs stain positively in mutated and non-mutated larvae, their transformed areas cannot be recognized, which accounts for some of the differences between the frequency of the abnormalities in adults and larvae. In 84 3rd instar larvae analysed for visible abnormalities in their eye discs, 100% had them in one or two discs. Of 303 of their metamorphosed siblings, however, 11% did not show any visible eye abnormalities. These figures suggest on the one hand that aldox staining does not allow identification of all the transformed areas before their differentiation during metamorphosis, and on the other hand that either not all the abnormalities in tuh discs are caused by the genes responsible for the tuh trait or that a few transformations are not recognizable on unsectioned metamorphosed eyes.

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Zullo, S. Southern Illinois University, Carbondale, Illinois USNA. The influence of singed (sn^3) in **Drosophila melanogaster - D.simulans** hybrids.

The outcome of interspecific crosses between **Drosophila melanogaster** females and **D.simulans** males is well-known, producing sterile hybrid females (Sturtevant 1920). During investigations conducted with a field-collected strain of **D.simulans** (from Niles, Michigan USNA) (Figure 1), bristle abnormalities were noted in hybrids from matings with fertile, singed (sn^3) **D.melanogaster** females (Figure 2).

Six virgin y, sn^3, lz^{50e30}, v females were mated with 6 **D.simulans** males in each of 7 cultures (standard agar, yeast, molasses medium). Only 4 cultures produced progeny, 284 sterile hybrid females. One or more bristles of all the hybrids except one were misshapen (Figure 3). The posterior scutellars were most often affected. Occasionally the dorsocentrals, supra-alars, post-alars, and verticals also demonstrated a singed influence. The microchaetae showed a slight singed influence. The bristles appeared slightly thicker than in the hybrids with wild-type parents. The lone fly without a misshapen bristle did have thick bristles with a slight waviness. I noted no definite singed bristles in the hybrids.

While missing bristles were noted in hybrids from interspecific crosses between wild-type **D.melanogaster** females and these **D.simulans** males, as reported by others (Sturtevant 1920; Biddle 1932), no abnormal bristles were found on over 500 sterile hybrid females.