Effect of Ligation of Drosophila Larvae on Tumor Incidence

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Previous work (1) has shown that the introduction of the 1(3)gl gene, which causes the ring gland to be defective, into a tumor strain of Drosophila results in an increase in the number of tumors in comparison with siblings with normal ring glands. This suggests that the hormone responsible for metamorphosis may account for the usual regression of these tumors. Ligation also delays pupation and has been used as additional means for studying this inhibition of growth. If hormones in Drosophila are effective in influencing the growth of these tumors, then further studies on isolating them and testing their action on mammalian tumors would be justifiable.

METHODS

The tuwm strain was used in this investigation. Ten males and ten females were mated in each vial, containing stock medium with yeast growing on the surface, and were then removed after 48 hours. The larvae were observed carefully and in the late third instar, when they started to crawl up the sides of the vial, they were removed and ligated. A single knot of no. 00000 Deknatel, braided silk commonly used in vascular surgery, was tied about the larvae slightly posterior to the transverse tracheal trunks which serve as a guide for the location of the ring gland. Proper tension was obtained by suspending the larvae between the fingers and a weight of 25 gm. for 10 seconds. After cutting the ends of the silk near the knot, larvae were placed on moist filter paper in a Petri dish with streaks of live yeast on the surface of the paper (Fig. 1). The larvae tended to stay on the yeast and thus made scoring easier. At the end of 24 hours the number with tumors were counted and recorded. A control group was treated similarly, except that the larvae were not ligated. Cultures were maintained in an incubator at 25° C.

RESULTS

All larvae in these experiments (Table 1) were not sufficiently near the same age to behave similarly after ligation. The posterior end of each of the individuals classified as anterior pupae had the appearance of third instar larvae, whereas the anterior end was shortened, smooth, brown, and looked like the corresponding region of the control pupae (6). In those listed as pupae, the anterior part looked like the last group, but the posterior end was not shortened, the pupal segments remained, and a thin, light brown puparium was present. The posterior end thus seemed to be in a prepupal "instar."

Larvae in control experiments had pupated by the end of 18 hours (Table 1), and tumors appeared in 27 out of 181 of these larvae which were not ligated. In an unrelated experiment, approximately the same incidence of tumors, 66/401 or 16.4 per cent, was found. Of 352 larvae which were ligated, 334 survived, eighteen dying before 24 hours had elapsed. At the end of 24 hours 91 ligated larvae were still in the larval stage, and 39 of them had tumors. In 85 only the posterior end was in the larval stage. Forty-eight of these had tumors, an incidence of 56.4 per cent. In Figure 1 a number of these preparations may be seen, and a tumor is easily visible in one viewed under a higher power (Fig. 3). There were 55 with tumors among 135 individuals which had entered the puparium. In these three groups in which development of the posterior segment was delayed, the percentage of tumors was significantly higher (P < 0.01) than in the group without ligatures.

Only the anterior end was in the larval stage in 23 of those which had been ligated, and two of them had tumors. There was no difference in the incidence of tumors between this and the control group (P = 0.39). Larval movements sometimes

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made placement of ligatures less accurate than desired (the use of curare was abandoned as impractical), and almost invariably the ligature was very near the anterior end in those with anterior larval structures and the appearance of pupae posteriorly.

DISCUSSION

Tumors have been found in insects by at least two investigators as a result of endocrine studies. Following extirpation of corpora allata and cardiaca in Leucophaea maderae, Scharrer (7) noted neoplastic changes in the anterior portion of the alimentary canal, particularly in the midgut, in the salivary reservoir, and, in unusual instances, in the salivary glands. However, this was found to be due to severing the recurrent nerve rather than to ablation of the glands. Previously, Pflugfelder (5) found that growths with the appearance of tumors occurred in Dixippus nymphs after allatectomy. They also appeared in transplanted embryonic tissue in hosts which had corpora allata removed. These growths were prevented with implants of corpus allatum, but they also appeared when supernumerary corpora allata were implanted. Franks and co-workers (8) found that puation "hormone" isolated from Calliphora larvae by the method of Becker and Plagge inhibited the carcinogenic effect of methylcholanthrene on the skin of mice. In our hands preliminary work with a somewhat similar extract failed to inhibit the Walker tumor consistently. Recently, growth and differentiation hormone has been isolated for the first time in crystalline form from the prothoracic glands of silkworm pupae by Butenandt and Karlson (s). An account of hormonal control of metamorphosis in insects may be found in an excellent summary by Williams (11).

Since hormones which have been excluded in these experiments affect growth and differentiation and cause histolysis of larval cells, the effect of ligation on tumor incidence is of interest in the three classes of larvae in which metamorphosis has been delayed in the segment posterior to the ligature. The number of tumors appearing in the three classes with arrested differentiation of posterior end is significantly greater ($P < 0.01$) than in the control group or in those with only the anterior end in the larval stage. Previously (1), more tumors were found in the same strain if the ring gland was made defective by introducing the gene, $I(2)st$. Also, Oster$^1$ subsequently found independently that the gene, giant, on the first chromosome has a similar effect.

It is not possible to state whether hormones have a direct action on tumor cells, causing them to regress as the fat body and intestine of the larva do, or whether the increased incidence of tumors is a temporal, secondary effect of prolonging the larval stage and a favorable environment for development of more tumors. In either case, the humoral mechanism causing pupation is responsible for the behavior of the tumors, either acting primarily on the cell or through a secondary relationship. Perhaps this is not surprising, since the blood-forming organs, which have been suggested as the site of origin of tumors in Drosophila, normally disappear about the time the head is everted.

Pigmentation, which occurs frequently in the reaction to injury, occasionally may be confused with pigment in tumors. To minimize such an error, the period of observation of the larvae was shortened to 24 hours, during which time the two could be differentiated by location and appearance. Although it is recognized that the possibility of error cannot be entirely overcome in this type of experiment, the results are more significant when observations (1) on the same strain with defective ring glands (to which the same objection does not apply) are also taken into account.

Tumors in Drosophila are benign, cellular aggregates, probably originating from hemocytes, and the significance of their behavior in relation to mammalian tumors is not known. However, it is interesting to speculate whether the hormone causing insect metamorphosis may have some effect upon carcinogenesis or upon the growth of mammalian tumors, since insect hormones are not specific for the species in which they arise and certain invertebrate hormones act in vertebrates (4, 8, 10). Isolation of the puation hormone (9) makes this amenable to testing, and such studies are now in progress in our laboratory.

In any case, it seems clear that variation in the amount and distribution of metamorphosis hormone alters the incidence of tumors in Drosophila, and this is probably responsible either primarily or secondarily for the characteristic regression of these tumors.

SUMMARY

Larvae of the tu$^{+}$ strain which were ligated were found to have a higher incidence of tumors than individuals in which metamorphosis was not delayed by blocking the posterior passage of hormones in this manner.

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REFERENCES

Fig. 1.—Appearance of larvae immediately after ligation. Although still mobile they tend to stay on streaks of live yeast, facilitating subsequent scoring.
Fig. 2.—Appearance 24 hours after ligation, showing pupation of anterior end only

Fig. 3.—Differential pupation with visible tumor in posterior segment which is still in the larval stage
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