PHYSIOLOGICAL GENETICS OF MELANOTIC TUMORS IN *DROSOPHILA MELANOGASTER*. VI. THE TUMORIGENIC EFFECTS OF JUVENILE HORMONE-LIKE SUBSTANCES

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GENETICALLY determined melanotic tumors of *Drosophila* arise by the aggregation and subsequent melanisation of blood cells during larval life. They persist into the adult stage as apparently inert masses which either float freely in the haemolymph, or are associated with the fat body. The blood cells chiefly involved in tumorigenesis are the lamellocytes ("spindle cells"), which are derived by a transformation from the predominant blood cells, the plasmatocytes (ORTEDAL 1953; RIZKI 1960; SANG and BURNET 1963). RIZKI (1960) found that this transformation occurs in wild-type strains at about the time of metamorphosis, and he produced evidence to show that the transformation is under hormonal control. He showed that melanotic tumor strains are characterized by a premature blood cell transformation, and suggested that the metabolic defect underlying tumorigenesis may be in the hormonal system which controls metamorphosis. Support for this hypothesis comes from the finding that, in tumorous strains, tumor frequency can be altered by interfering with hormonal function by means of ligature (BURDETTE 1954; RIZKI 1960; see also SANG and BURNET 1963).

Any mutation involving the endocrine system of an insect would be extremely useful for the analysis of hormonal mechanisms, so it is important to explore the relationship between endocrine events and tumorigenesis in order to test RIZKI's hypothesis, and to delineate the type of defect, if one exists, in melanotic tumor strains. Several materials with hormonal activity for insects have recently become available, thus allowing a new approach to the problem, involving direct administration of these materials to the growing larvae as suggested by BURNET and SANG (1964b). The compounds we have examined are mimics of the juvenile hormone, and although there have been few reports of juvenile hormone effects on the higher Diptera (BRYANT and SANG 1968; SRIVASTAVA and GILBERT 1969), we have been able to show that many of these materials are very active in modifying the expression of melanotic tumor genes.

MATERIALS AND METHODS

The melanotic tumor gene with which we have been chiefly concerned is at locus 83.9 on the second chromosome (GLASS 1954) and is carried by the strain *tu bw; +^{su-tu}*. The tumor penetrance in this strain is approximately 90% when larvae are grown aseptically on defined medium

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under our conditions. The following tumor strains were also used in some experiments: *tu55g* (JACOBS, BOWMAN and WALLISER 1958); *tu^K* (SANG and BURNET 1963); and *tu^Wrc* (RIZKI 1960). Larvae were raised aseptically, following the procedure of SANG (1956). The constitution of the defined medium was based on medium C of this author, but differed from it in that (i) sucrose was used instead of fructose, to minimize the "browning" reaction with amino acids during autoclaving; (ii) the medium contained magnesium (as sulphate), a requirement demonstrated by SANG (1956) which is not satisfied by the magnesium content of Oxoid No. 3 agar (SANG and BURNET 1967); (iii) K_2HPO_4 replaced Na_2HPO_4 , to give a more optimal sodium/potassium ratio (BRYANT 1967). 5 ml or 2 ml of medium was dispensed into 15×2.5 cm test tubes, which were stoppered, autoclaved and cooled. Eggs were collected and sterilised following SANG's (1956) method. They were dechlorinated with saturated calcium hypochlorite solution, and sterilised by rinsing in 0.1% cetyl dimethyl benzyl ammonium chloride solution (a quaternary ammonium detergent), using for the latter purpose a reversing pump of the type described by SANG (1956). Sterile eggs were plated onto agar in Petri dishes and incubated at 25°C. At about 20 hrs after egg collection, the hatched larvae were transferred, using paper spoons under sterile conditions, into tubes containing defined medium. A standard number of 40 larvae was used for each culture, with five replicate cultures for each test. Cultures were raised at 25°C in a humid incubator and any which became infected were discarded. Dosages were measured in millimoles per litre (mM) and plotted on logarithmic scales; the maximum concentration tested was usually 20mM or less. Emerged adults were counted twice daily, and the average time of development to adult was computed for each test.

The manifestation of melanotic tumor genes has usually been quantified by measuring tumor penetrance; that is, the proportion of tumorous flies in the population (SANG and BURNET 1963; BURNET and SANG 1964a, 1964b; GLASS 1957). However, this method is useful only for populations in which the gene shows incomplete penetrance, and near the upper end of the penetrance scale, measurements become inaccurate unless very large populations are used. Many of the populations involved in the experiments to be described had very high tumor penetrance, and for this reason the number of tumors present in each adult fly was counted. SANG's (1966) fructose clearing method was used, since this renders tumors visible without dissection of the flies. The mean number of tumors per fly was used as the parameter of tumor gene manifestation, and this will be referred to as tumor expression.

RESULTS

Effects of hormonally active materials on tumor expression: Several juvenile hormone mimics, and some related compounds, were tested for their effects on tumor expression in the melanotic tumor strain, *tu bw*;+^{su-tu}, and the results of these experiments are presented in Figures 1-15. Developmental time to adult was also measured, as an indicator of non-specific effects on development, but these results will not be presented in detail. At concentrations which were sufficient to modify tumor expression, most of the materials tested caused some delay in development, but the extent of this delay was not related to the juvenile hormone activity of the material concerned. None of the materials was found to induce supernumerary larval moults, as some of them do in certain other insects (LEVINSON 1966; WIGGLESWORTH 1963).

It was found convenient to feed these compounds directly to the larvae, rather than to attempt to inject them, so they were mixed with the defined medium before autoclaving. Since most of the materials in question were not water-soluble, they probably existed in the medium as a solution in globules of lecithin, which is the dietary source of fat. Larvae were in continuous contact with the food; thus both epidermal and alimentary cells were directly exposed to the chemicals. That

this is an effective means of administration is shown by the fact that a farnesoate derivative, applied in this way, causes results reminiscent of true juvenile hormone effects in other insects (BRYANT and SANG 1968).

Farnesol, farnesal, farnesene, nerolidol, phytol, isophytol: Farnesol is a tri-terpene primary alcohol, while farnesal is the corresponding aldehyde, farnesene the corresponding alkene, and nerolidol the 3-hydroxy isomer. Phytol differs from farnesol in its larger size and greater degree of saturation, and isophytol is the 3-hydroxy isomer of phytol. The relative juvenile hormone activities of these compounds are not always consistent, as reported by different authors using various test species. However the activities of farnesol, farnesal, nerolidol and phytol are usually of the same order of magnitude, although farnesal is consistently more active than farnesol, which is usually more active than nerolidol (KARLSON 1963; SCHMIALEK 1963; SCHNEIDERMAN *et al.* 1965). Isophytol, apparently, has no juvenile hormone activity (SCHNEIDERMAN and GILBERT 1964), and that of farnesene is extremely low (SCHNEIDERMAN *et al.* 1965).

These materials were tested over extensive dose ranges, and the results are presented in Figures 1–5. They all lower tumor expression quite markedly when the concentration is 2.5 mM or above, with the exception of farnesene which has no effect on tumor expression at concentrations up to 12mM. Since the dose response curves for these effects are rarely straight lines, and since the shape of the curve varies between treatments, it is not possible to express relative activities of the compounds by simple numbers, as has been found possible with their juvenile hormone activities. However, the data show that the order of decreasing tumor-suppressing activity is farnesol>nerolidol>isophytol>phytol>farnesal>farnesene. This order of activities does not correspond with the order of juvenile hormone activities as reported by any of the authors mentioned above. For example, farnesal is much more active than nerolidol or phytol, when measured using *Tenebrio* (SCHMIALEK 1963) and is also more active than farnesol when measured using *Galleria* (SCHNEIDERMAN and GILBERT 1964). Hence a comparison of the relative activities of these materials indicates that their tumor-suppressing properties are not correlated with, and therefore are probably not a result of their juvenile hormone potencies.

Farnesyl ethers and farnesyl diethylamine: Farnesyl methyl ether and farnesyl diethylamine have repeatedly been found to show roughly equal juvenile hormone activity, at least ten times as great as that of farnesol (BOWERS and THOMPSON 1963; KARLSON 1963; ROMANUK, SLÁMA and ŠORM 1967; SCHMIALEK 1963; SCHNEIDERMAN *et al.* 1965; WIGGLESWORTH 1963). Their effects on tumor expression in *tu bw*;+*su-tu*, however, are completely different. Farnesyl methyl ether causes a significant elevation of tumor expression when the concentration is 0.5mM or above (Figure 6), and this compound can cause a much greater effect than is shown in the response curve when fed at a concentration of 4mM, especially in the *tu55g* strain, as will be shown later. Farnesyl diethylamine, on the other hand, causes a marked decrease in tumor expression when fed at 0.5mM (Figure 7), and probably would do so at a lower concentration. Thus it is more active in this respect than farnesol. This complete difference in activity between

FIGURES 1-15.—The relation between tumor expression and the concentration of various materials in the larval medium, for the *tu bw; +su-tu* strain. Tumor expression (T.E.) is the average number of tumors developed per fly, and the vertical bars represent $\pm$ the standard

error of this parameter. Dosages are millimolar concentrations except for Figure 9 where the dose is measured as % wt./vol.

the two compounds must indicate that their site of action for effects on tumors is remarkably specific, and that this specificity is different from that of their juvenile hormone effects in other insects. This conclusion is reinforced by the finding that farnesyl butyl ether has tumorigenic activity as great as that of the methyl ether (Figure 8) although its juvenile hormone effects are much weaker, at least in *Galleria* (SCHMIALEK 1963; SCHNEIDERMAN *et al.* 1965).

Farnesoate derivative: LAW, YUAN and WILLIAMS (1966) synthesized a material from farnesoic acid by treatment with ethanolic HCl, and this substance was found to have extremely high juvenile hormone activity, although it was not a pure compound. It is the only juvenile hormone mimic which has been reported as showing effects in Diptera. It causes arrest of metamorphosis at various times depending on the dose, in mosquitoes and in *Drosophila*, and it also prevents the ontogenetic rotation of the male genitalia in these organisms (BRYANT and SANG 1968; SPIELMAN and SKAFF 1967). A sample of this material was tested on the *tu bw*;+*su-tu* strain at three levels: 0.07%, 0.14% and 0.35% wt./vol. At these concentrations, this material causes arrest of metamorphosis, so that tumors were scored in pupae for this experiment. Figure 9 shows that the arrested pupae had an extremely high tumor expression, the value being 7.73 tumors per fly at the maximum concentration tested, which is the highest yet recorded for this strain. Hence the most potent juvenile hormone mimic available to us is the most potent agent we have found for increasing tumor expression in *tu bw*;+*su-tu*.

Straight-chain alcohols and ethers: SCHNEIDERMAN *et al.* (1965) showed that several long chain ethers had juvenile hormone activity in the *Galleria* wax test. The most active was dodecyl methyl ether, and decyl, undecyl, tridecyl and tetradecyl methyl ethers showed lower activity although the corresponding alcohols were completely inactive. Dodecyl ethyl ether had less activity than the methyl ether, and the butyl ether was almost inactive, indicating that the critical feature of the molecule was probably its size. Dodecyl methyl ether showed high activity in *Tenebrio* (BOWERS and THOMPSON, 1963), and dodecanol was also active in this species.

Most of these compounds have been tested for their effects on tumorigenesis in the *tu bw*;+*su-tu* strain, and the results are presented in Figures 10–13. Dodecyl

FIGURES 16–20.—Patterns of larval development. Solid lines represent the percentage of visible animals in the next instar at various times. Dotted lines represent the percentage of visible larvae with macroscopically visible melanotic tumors.

Figure 16. *tu bw*;+*su-tu* strain, grown on control medium.

Figure 17. *tu bw*;+*su-tu* strain, grown on medium containing 50mm L-tryptophan.

Figure 18. *tu bw*;+*su-tu* strain, grown on medium containing 0.001% cholesterol (control medium contains 0.03%).

Figure 19. *tu bw*;+*su-tu* strain, grown on medium containing 4mm farnesyl methyl ether.

Figure 20. *tu55g* strain, grown at 20°C on medium containing 6mm farnesyl methyl ether.

The results are based on sample sizes of 50–170 animals. The tumor expressions attained by the populations are indicated in the figures.

methyl ether and dodecanol affect the tumor system in opposite directions, just as do farnesyl methyl ether and farnesol. Dodecyl methyl ether is about as active as farnesyl methyl ether in increasing tumor expression, while dodecanol is even more active than farnesol in decreasing it. When the length of the longer chain of the ether is increased, there is a falling off of tumorigenic activity, just as there is a decrease of juvenile hormone activity; tridecyl and tetradecyl methyl ethers (up to 10mm) have no detectable effect on tumor expression, and pentadecyl methyl ether causes a lowering of tumor expression (Figure 12). However, an increase in the length of the short chain of the ether, although it results in loss of juvenile hormone activity (SCHNEIDERMAN *et al.* 1965), does not abolish tumorigenic activity, as is shown by the potent effect of dodecyl butyl ether (Figure 13). An exactly similar situation was noted above in the case of the farnesyl ethers.

Geraniol, linalool, squalene: Geraniol, farnesol, linalool and squalene are of interest in that they are related to intermediates in the biosynthesis of cholesterol in vertebrate tissues (BLOCH 1965). Insects do not synthesize cholesterol (CLAYTON 1964), but as juvenile hormone is structurally related to the terpenes, it may be synthesized via the cholesterol pathway. Geraniol and its 3-hydroxy isomer, linalool, have shown low juvenile hormone activity in some tests, and where both have been tested, linalool has shown greater activity than geraniol (GILBERT and SCHNEIDERMAN 1964; SCHMIALEK 1963). Geraniol (Figure 14) reduces tumor expression in the *tu bw*; +^{su-tu} strain, its effect being roughly comparable to that of phytol (Figure 4), but linalool has absolutely no effect on tumor expression, in spite of causing a retardation of development similar to that of geraniol. Hence the 1-3 isomerisation of geraniol results in loss of tumor-suppressing activity, but does not cause loss of juvenile hormone activity. It is surprising, therefore, that the same isomerisation of farnesol (to nerolidol) results in loss of neither activity (Figures 1 and 3), whereas a similar change in the phytol molecule (to isophytol) results in loss of juvenile hormone activity but has little effect on tumor-suppressing properties (Figures 4 and 5). Squalene also reduces tumor expression, its effectiveness being similar to that of phytol (Figure 15).

The time course of tumor formation: The above results add substantially to the already impressive array of environmental treatments which have been shown to be capable of influencing quantitatively the development of melanotic tumors (SANG and BURNET 1967). However, the fact that particular treatments can influence tumor penetrance or expression tells us little of the mechanism of these effects, and a search for qualitative differences in responses to various treatments seems to be required. We have begun such a study by examining the time of tumor formation under various environmental and genetic circumstances, and we have found that there are indeed marked variations in the time when tumors appear.

Environmental influences on the time of tumor formation: Figures 16-19 show the time of appearance of macroscopically visible melanotic tumors in the *tu bw*; +^{su-tu} strain grown under four different dietary conditions: control; supplementary tryptophan (50mm); cholesterol deficiency (0.001%); and farnesyl

methyl ether (FME), (4mm). The instar changes in these populations are plotted on the figures, and the tumor expression in the adults is also indicated.

In the control series (Figure 16), visible melanized tumors do not appear until late in the third larval instar, which is the usual time of tumor formation in strains carrying *tu bw* (BURNET and SANG 1964b) or other melanotic tumor genes (GUÉLÉLOVITCH 1958; JACOBS *et al.* 1958; OFTEDAL 1953; RIZKI 1960; SANG and BURNET 1963). However when tumor expression is increased by means of supplementary tryptophan or by cholesterol deficiency (Figures 17 and 18), tumors appear somewhat earlier during the third instar, and the more potent tumorigenic treatment (cholesterol deficiency) causes more precocious tumor formation. With the still more potent tumorigenic agent, FME, tumors appear even earlier than with cholesterol deficiency (Figure 19); in fact, out of the sample of 141 larvae, 4 showed typical melanotic tumors while still in the second larval instar. This finding is of particular interest in view of the suggestion (BURNET and SANG 1964b; RIZKI 1960) that the second ecdysis has some special importance in tumor development; this is evidently not the case with the second instar tumors induced by FME. Cholesterol deficiency or supplementary tryptophan did not induce second instar tumors in this experiment, but their tumorigenic effects are not as marked as that of FME. The only other treatment which has induced second instar tumors in *tu bw*; $+^{su-tu}$ is the farnesoate derivative (LAW *et al.* 1966) which has this effect at concentrations of 0.07% wt/vol. and above (see Figure 9).

Time of tumor formation as influenced by genotype: Table 1 compares two tumor strains and the hybrid between them with respect to tumor expression and time of tumor formation when grown on standard medium. In this table, T1 and T50 are the times when the first tumor appears, and when 50% of the larvae have tumors, respectively, both corrected to a standard larval development time of 96 hrs. Tumor expression in *tu55g* is higher than in *tu bw*; $+^{su-tu}$, and the hybrid is intermediate between the two homozygotes in this respect. The table shows that genetically determined differences in tumor expression are associated with differences in time of tumor development, in the same way as the environmental effects mentioned above; that is, high tumor expression is associated with early onset of tumor appearance. However, animals of these genotypes do not form melanotic tumors before the third instar under control conditions.

TABLE 1

Tumor expression and time of tumor formation under standard conditions for three genotypes

Genotype	T1	T50	Final tumor expression
<i>tu bw</i> ; $+^{su-tu}$	69	117	1.16
<i>tu bw</i> ; $+^{su-tu}$ $\times$ <i>tu 55g</i>	69	88	2.40
<i>tu55g</i>	65	75	4.26

T1 = time of appearance of first tumor

T50 = time when 50% of larvae have visible tumors
(Based on sample sizes of 50-170)

} all corrected to 96 hrs.

} median pupation time.

TABLE 2

Effect of 4mm FME on tumor expression in various tumor strains

Strain	Tumor expression	
	Control	FME, 4mm
<i>tu bw</i> ; + ^{su-tu}	1.16	3.41*
<i>tu 55g</i>	2.71	6.98*
<i>tu^K</i>	0.196	2.99*
<i>tu^w rc</i>	1.04	2.25*

* Indicates tumors appeared in 2nd instar.

We have also investigated the effects of FME on some tumor genes other than *tu bw*. As Table 2 shows, FME at a level of 4mm can increase tumor expression in the *tu bw*; +^{su-tu}, *tu^K*, and *tu^wrc* strains, all of which carry non-allelic tumor genes, as well as in the *tu55g* strain, which has a tumor gene allelic with *tu bw* (ERK and SANG 1966). It is significant that, in all of these strains, FME induces tumors to form during the second instar. That is, the effect of FME in increasing tumor expression and inducing second instar tumors is not restricted to *tu bw* but extends to other allelic and non-allelic tumor genes.

By using the *tu55g* strain, which has a high control level of tumor expression, and which is particularly sensitive to FME treatment (Table 3), tumors have been induced to form during the first larval instar, in a few instances. This was accomplished by growing the larvae at 20°C, which can be regarded as a tumorigenic treatment (BRYANT 1967) on medium containing 6mm FME. The time course of tumor appearance in this population is shown in Figure 20; the maximum number of first instar larvae recorded as showing tumors was nine in a sample of 146, the T1 and T50 for tumor formation were 22 and 27 hrs, respectively (on a 96 hr development time scale), and the final tumor expression was 7.24. It has not proved possible to induce first instar tumors in the *tu bw*; +^{su-tu} strain, even by using 0.35% wt./vol. of the farnesoate derivative, which gave a tumor expression of 7.73. It seems, therefore, that tumor expression and time of tumor formation are somewhat independent in their genetic control. It is not possible with the data available to decide if the differences between the two strains,

TABLE 3

Effect of FME on tumor expression, and on the time of tumor formation, in the tu 55g strain

Treatment	Tumor expression	Tumors visible in second instar
Control	4.56	0
FME 0.5mm	4.56	0
FME 1mm	5.17	+
FME 2mm	6.08	+

in tumor expression and in time of tumor appearance, are due to differences in genetic background, or in the tumor locus itself.

Specificity of treatments which induce second instar tumors: It has been shown above that, under standard conditions, tumors are formed earlier in *tu55g* than in any of the other strains tested. This strain has therefore been used in experiments to determine if the induction of tumors during the second instar is a specific effect of juvenile hormone mimics, or alternatively, if the effect is shown by other potent tumorigenic treatments. Table 3 shows that tumors can be induced to form in the second instar by treatment with FME at a concentration of 1mM, but not at 0.5 mM. The value of tumor expression, above which tumors become visible in second instar larvae, is therefore between 4.56 and 5.17 for the *tu55g* strain. Attempts to exceed this level of expression have been made using a variety of tumorigenic treatments, and it is indeed possible to exceed it by the use either of low temperature, or by replacing dietary cholesterol with dihydrocholesterol (shown to be tumorigenic by COOKE 1968). Low temperature gives a tumor expression of 5.87, and dihydrocholesterol gives 6.17, but in neither of these populations were tumors visible in second instar larvae. Therefore, second instar tumors are not merely a correlate of high tumor expression, but are a result of particular environmental treatments, namely potent juvenile hormone mimics such as FME and the farnesoate derivative.

Time of tumor formation in relation to developmental stage: All of the above observations of times of tumor formation were made on populations of larvae, and such experiments are limited in scope because the developmental stage of each larva is not known with precision. Hence these experiments cannot show if tumors appear at particular stages during each instar. This difficulty was overcome by growing larvae individually in separate tubes, and making observations at regular intervals of both instar changes and number of tumors present. The time of appearance of tumors was thus determined for each larva, in relation to the stage of development through each instar. Table 4 summarizes the results of such an experiment, using hybrid *tu bw*; +^{su-tu} × *tu55g* larvae grown on standard medium

TABLE 4

Time of tumor appearance in relation to developmental stage. Each larva grown individually on 0.1 ml defined medium. Strain tu bw; +^{su-tu} × tu55g

a) Average number of new tumors per individual which appear during developmental stages.										
Diet	Number of larvae observed	First instar	Second instar			Third instar			Total	Tumor expression in adults
			Early	Mid	Late	Early	Mid	Late		
Control	74	0	0	0	0	0.12	0.16	1.68	1.96	2.40
4mM FME	57	0	1.10	0.91	0.67	0.56	0.47	0.60	4.31	4.18
b) Percentage of larvae at different developmental stages which have visible tumors.										
Diet	Number of larvae observed	First instar	Second instar			Third instar			Penetrance in adults	
			Early	Mid	Late	Early	Mid	Late		
Control	74	0	0	0	0	4.1	10.9	77.1	89.0	
4mM FME	57	0	61.4	86.0	94.7	98.2	98.2	100.0	100.0	

and on medium containing 4mM FME; this genotype, as noted before, gives a tumor expression and time of tumor formation which is intermediate between the two homozygotes. The time spent in each instar is divided into three parts, and the data are presented in two ways: first, the percentage of larvae showing tumors at each stage of development, and second, the average number of new tumors per larva which appear during each stage. On the standard medium, the majority of tumors do not become visible until the late third instar, as noted before, although some appear during the early and middle stages of the third instar. On FME-supplemented medium however, 61.4% of the larvae develop tumors early in the second instar, and tumors continue to appear in the treated group during the rest of the second and third instars. After the sudden onset of tumor appearance following the first ecdysis (which may be related to the liberation of haemocytes at this time) the rate of tumor formation during subsequent stages is fairly uniform, and tumors appear with only slightly decreasing probability during the early, middle and late second instar, and in early, middle and late third instar. That is, under these conditions the time of appearance of tumors is not closely related to the larval molting cycle, in contrast to the effects of the non-hormonal treatments studied by BURNET and SANG (1964b).

Table 4 also shows that the number of tumors appearing during the late third instar is greater in the control group than in the treated group. The early appearance of tumors in the treated group, therefore, seems to decrease the probability of late tumor appearance; this may be due to a decrease in the number of blood cells available for tumor formation, although observation of blood samples from highly tumorous larvae revealed no obvious deficiency of blood cells. The average number of tumors in the adults from the treated series in this experiment was slightly lower than the number developed by the larvae; GUÉLÉLOVITCH (1958) has also reported that the tumor frequency in adults of the *tu vg bw* strain is lower than that of pupae. These results indicate a resorption or, more likely, a fusion of tumors during the pupal phase. However in the control series, the number of tumors in adults is greater than that in larvae. This increase probably occurred in very late third instars (after the last observation before pupation), although we cannot exclude the possibility of tumor formation during metamorphosis.

DISCUSSION

The main conclusion from this study is that some substances which have juvenile hormone activity when tested in insects other than Diptera, are remarkably tumorigenic when fed to some melanotic tumor strains of *Drosophila*. Until there is an unequivocal demonstration that juvenile hormone acts in Diptera we cannot conclude that tumorigenesis is a direct consequence of juvenile hormone action, but it seems possible that this will prove to be so since the effects of the farnesoic acid derivative (BRYANT and SANG 1968; SPIELMAN and SKAFF 1967) and of natural juvenile hormone (SRIVASTAVA and GILBERT 1969) support the assumption that the hormonal control of development in Diptera is similar to that of other insects. And it would be surprising if this were not so.

The correlation found here is that compounds with high juvenile hormone activity in non-Dipteran insects increase the expression of the melanotic tumor gene in the *tu bw*;+*su-tu* strain. Farnesyl methyl ether, dodecyl methyl ether and the derivative of farnesoic acid, all potent juvenile hormone mimics, are among the most active tumorigenic agents yet found. However, some related compounds which are less active, or inactive, as juvenile hormone mimics have no tumorigenic activity. For example, farnesene, tridecyl and tetradecyl methyl ether have no effect on tumorigenesis, while farnesol, nerolidol, farnesal, phytol, isophytol, pentadecyl methyl ether, geraniol and squalene actually reduce tumor frequency. There is one exception to the above generalisation; the butyl ethers of dodecanol and farnesol, which have negligible juvenile hormone activity (SCHNEIDERMAN *et al.* 1965), are as tumorigenic as the corresponding methyl ethers. This indicates that the specificity of the short chain of the molecule differs in its tumorigenic and its juvenile hormone functions, a conclusion which is reinforced by the finding that farnesyl diethylamine, whose juvenile hormone activity approaches that of farnesyl methyl ether, (SCHNEIDERMAN *et al.* 1965), actually reduces tumor expression. The specificity of the long chain of the molecule, on the other hand, is strikingly similar for the two activities, as is well demonstrated by the fact that dodecyl methyl ether has both tumorigenic and juvenile hormone activities, whereas tridecyl methyl ether has neither.

Although the correlation is not absolute, it seems reasonable to conclude that the tumorigenic effects reported here may be related to, or even due to, the high juvenile hormone activities of the materials concerned. The tumor-suppressing effects of the less active juvenile hormone mimics may be due to some other feature of the molecules, since some related materials with no juvenile hormone activity also show this effect.

These findings re-emphasize the conclusion, expressed by previous authors (BURDETTE 1954; BURNET and SANG 1964b; RIZKI 1960) that hormonal events are involved in melanotic tumor formation. But the nature of this involvement is far from clear. BURNET and SANG (1964b) discovered that the penetrance of the *tu bw* gene was modifiable by dietary treatments for only a short period which included the second larval molt, and furthermore that the haemocyte transformation leading to tumorigenesis was initiated in the time immediately following the dietary sensitive period. These authors, therefore, suggested that the tumor phenotype was due to an error in the reaction to hormonal changes at this time in development. The above evidence does not contradict this view, but it shows in addition that potent juvenile hormone mimics such as FME can cause tumor formation during earlier stages, which is unlikely to be related to humoral changes occurring at ecdyses. That is to say, tumorigenic treatments other than juvenile hormone mimics may indirectly affect the hormone balance at the second ecdysis, whereas FME for example may act directly on the haemocytes at any stage of development; larval molts would be of no special significance in the latter case. Unlike RIZKI (1960), we have been unable to find any abnormality of the ring gland of tumorous strains, which might explain why the second ecdysis is critical for the development of tumors in untreated larvae, or for the enhancement of

tumor frequency by dietary manipulations. The relative timing of larval and pupal molts is also normal in *tu bw* strains. Furthermore, we have been unable to induce melanotic tumor formation, using FME, in strains carrying no melanotic tumor genes. Therefore, it seems unlikely that melanotic tumors are merely the result of an endocrine abnormality which can be exaggerated or mimicked by feeding hormonally active materials. The genetic lesion in melanotic tumor strains must involve an abnormality of some system which is sensitive to the nutritional environment, and to the level of juvenile hormone. This implies, of course, that this system is normally regulated by the juvenile hormone, in which case it will be of obvious interest to examine the effects of the molting hormone, ecdysone, on melanotic tumor formation.

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SUMMARY

Several potent juvenile hormone mimics (farnesyl methyl ether-FME, dodecyl methyl ether, farnesoate derivative) are very effective in increasing tumor expression (the average number of tumors developed per fly) in the *tu bw*; +^{su-tu} strain, and several other tumor strains respond similarly to FME. Less active juvenile hormone mimics, however, and some related compounds, have no tumorigenic activity; in fact, many of these materials reduce tumor frequency. The specificity of the responses indicates that the tumorigenic effects may be related to juvenile hormone action, but that tumor-suppressing activity may be related to some other feature of the molecules. FME and farnesoate derivative produce one effect which is not seen with the other tumorigenic treatments described to date: that is, they cause tumors to form during the second or even the first larval instar, instead of during the third instar, which is usual. Hence, under these conditions, there is no phenocritical period at the time of the second larval molt, as there is with non-hormonal tumorigenic treatments. FME does not induce tumors in strains carrying no melanotic tumor genes, and we have been unable to find any abnormalities of the ring gland or of the timing of the molt process in tumor strains. The most obvious hypothesis, that FME operates by exaggerating a preexisting endocrine defect in melanotic tumor strains, is therefore not supported by our evidence. The melanotic tumor gene probably causes a defect in some system which is normally regulated by juvenile hormone.

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