PHYSIOLOGICAL GENETICS OF MELANOTIC TUMORS IN DROSOPHILA MELANOGASTER. II. THE GENETIC BASIS OF RESPONSE TO TUMORIGENIC TREATMENTS IN THE tu^K AND *tu bw*; *st su-tu* STRAINS

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THE first publication in this series (SANG and BURNET 1963) describes the effects of dietary-environmental treatments on the frequency of melanotic tumors in the tu^K strain of *Drosophila melanogaster*, when reared on chemically defined food media under germ-free conditions. Melanotic tumors occur infrequently on the complete medium, but the proportion of larvae with tumors (penetrance of the phenotype) is greatly increased when ribonucleic acid (RNA) is omitted from the diet, due to an absence of adenylic acid normally provided by RNA. A more detailed examination showed that the pattern of adenylic acid utilisation is influenced by the dietary level of cytidylic acid, the two pentose nucleotides acting antagonistically with respect to tumor formation. Furthermore, the amount of cholesterol and of biotin provided in the diet also affects tumor penetrance, and there is a regular quantitative dose-effect relationship for each treatment.

Using another strain of *D. melanogaster* carrying a *tu* gene on the second chromosome, PLAINE and GLASS (1955) showed that melanotic tumors can be produced by feeding an excess of L-tryptophan to larvae grown in nonsterile yeast cultures, and that the proportion of tumors induced is related to the dietary concentration of the amino acid. PLAINE and GLASS (1952) also induced tumors by X-irradiation of eggs, and GLASS (1957) has suggested that both tumorigenic treatments operate by blocking the action of a specific tumor suppressor located on the third chromosome of this stock. The following comparative study examines whether both tumor strains respond to the same environmental manipulations, or differ in ways which are related to genetic differences between them.

MATERIALS AND METHODS

A detailed description of the tu^K strain is given in the preceding paper of this series. The *tu bw*; *st su-tu* strain was kindly supplied by PROFESSOR BENTLEY GLASS and is kept as an inbred line in our laboratory. The strain carries two eye colour mutants, brown (*bw*, chromosome 2) and scarlet (*st*, chromosome 3), and a double mutant and suppressor system consisting of melanotic tumor (*tu*) and the suppressor of erupt on the second chromosome, and erupt (*er*) and suppressor of melanotic tumor on chromosome 3 (GLASS 1957). We shall be concerned here only with the tumor gene and its suppressor.

Two different second chromosomes carrying dominant marker genes were used for chromosome substitution analysis. These were *Cy* (2L) *dp*^{2} *b pr* and *SM5*, *al*^{2} *Cy lt*^{v} *sp*^{2} which both have Curly as the marker. *SM5* contains a complex inversion system which effectively suppresses crossing over in the second chromosome (MISLOVE and LEWIS 1955). Three third chromosomes containing dominant markers were used: *Mé Sb* (Moirée and Stubble), also a chromosome carrying *Sb* alone, and lastly *TM3*, *ri p*^{p} *bx*^{34e} *e*^{s} *Ser* with Serrate as marker. *TM3* contains a complex system of inversions which suppresses crossing over in the third chromosome (LEWIS 1960; TINDERHOLT 1960).

Experiments were carried out on germ free chemically defined media, of which full technical details are given by SANG (1956) and SANG and BURNET (1963). Embryos were irradiated on agar plates (kvp 140, 5 ma, distance 20 cm, 347 r/min). Tumor penetrance is the proportion of individuals with tumors expressed in percent, and for each treatment level is based on six to eight replicates, each containing 40 larvae, unless stated otherwise.

RESULTS

Comparison of the tu^{K} *and* tu bw; st su-tu *strains:* The effects of dietary-environmental treatments and of X-irradiation on the *tu*^{K} and *tu bw; st su-tu* strains are shown in Table 1. In addition, data for the F_1 generations of the reciprocal crosses between the two strains are shown; the results for reciprocals being combined since there was no difference between them. On the control medium, tumor penetrance is rather lower for the *tu*^{K} than for the *tu bw; st su-tu* strain and it is zero in the F_1, suggesting that genetic factors responsible for the spontaneous appearance of tumors are nonallelic in the two cases. When RNA is omitted from the medium, tumor penetrance rises to over 80 percent in the *tu*^{K} strain, but there is no significant rise in penetrance in either *tu bw; st su-tu* or in the F_1 over the control level. Low cholesterol medium causes a sharp increase in tumor penetrance in both *tu*^{K} and *tu bw; st su-tu*, but not in the F_1 ($\chi^2_1 = 3.68$, $P > 0.05$); addition of 0.9 percent L-tryptophan to the complete medium has a similar effect in all cases, resulting in the appearance of melanotic tumors in the majority of the treated larvae. The two strains differ with respect to the tumorigenic effect of X-irradiation. Twenty-two-hour old embryos were irradiated with 1000r in each case, and whereas this produced a rise in tumor penetrance in the *tu-bw; st su-tu* strain, neither *tu*^{K} nor the F_1 show a significant increase above the control level. A low frequency of morphoses involving the head, thorax or legs was found in each of the treated groups, and in addition to the increase in tumor penetrance the erupt phenotype also appeared among the irradiated *tu bw; st su-tu* group.

TABLE 1

Penetrance of melanotic tumors, expressed as percentage of individuals with tumors, in the tu^{K} *and* tu bw; st su-tu *strains and in their* F_1 *progeny*

Strain	Treatment				
	Control	RNA nil	Cholesterol 0.00156%	Tryptophan 0.9%	X ray 1000r
tu^{K}	1.3	81.4**	77.4**	83.0**	2.46
tu bw	12.2	12.6	84.5**	94.5**	87.4**
tu^{K} × *tu bw*	0	1.79	2.96	94.0**	1.81

** Significantly different from the control value at the 1 percent level of probability.

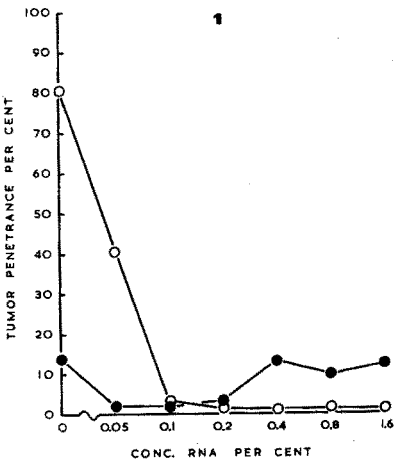
RNA and the pentose nucleotides: The effect of RNA deficiency was examined further to ascertain whether the difference in Table 1 is due to a greater ability of the *tu bw*; *st su-tu* strain to synthesise from simple precursors the pentose nucleotides normally available by hydrolysis of the whole RNA provided in the control medium. Figures 1 and 2 show that, in spite of the difference in tumor penetrance below 0.1 percent RNA, the effect of RNA deficiency on total larval development time does not differ markedly between the two strains, and in fact the *tu bw; st su-tu* strain has a slightly higher RNA requirement than the tu^K strain.

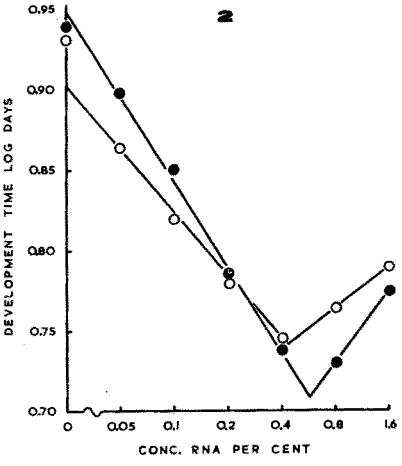
It must be borne in mind that the requirements of different systems need not be the same throughout development and that these two sets of response curves measure the effects of RNA concentration at different periods of larval development. In the tu^K strain the RNA requirement for tumor suppression must be satisfied by the end of the second larval stadium, which coincides with the time of action of the tumor gene (SANG and BURNET 1963), whereas larval growth rate is greatest during the third instar. Moreover, in this strain it is the relative molecular concentration of adenylic and cytidylic acids which determines the tumor response. Thus, if the rate of synthesis of these two nucleotides *de novo* differed between the two strains the rate of synthesis of adenylic acid might be great enough in the *tu bw*; *st su-tu* strain to achieve the necessary threshold concentration before the end of the critical period for tumor response, but insufficient to meet the demand for maximum larval growth during the third instar. Since dietary cytidylic acid increases the concentration of adenylic acid required to achieve tumor suppression, a reduced rate of cytidylic acid synthesis in the *tu-bw*; *st su-tu* strain could reduce the adenylic acid requirement for tumor suppression, allowing this to be met by the rate of *de novo* synthesis, and the deficiency of cytidylic acid could also prevent realisation of the maximum rate of growth during the third instar.

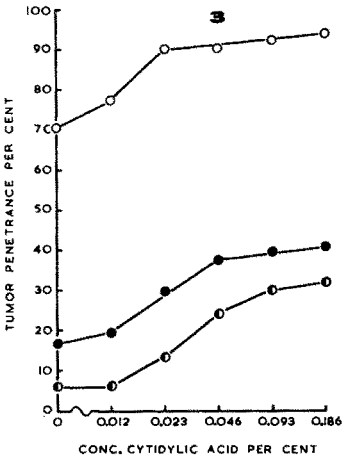
Figure 3 shows the effect on all three genotypes of supplementing a zero-RNA medium with cytidylic acid. Increasing the concentration of cytidylic acid causes a rise of tumor penetrance above the zero concentration, but the different genotypes maintain constant quantitative differences in their responses. Figure 4 shows dose-response curves for adenylic acid on media supplemented throughout with the optimal dietary supplement for larval growth (0.046 percent cytidylic acid). The points at the zero concentration of adenylic acid in Figure 4 corresponds, of course, to those at the 0.046 percent level on the cytidylic acid scale in Figure 3. Increasing the concentration of adenylic acid causes a reduction in tumor penetrance for each of the three genotypes, but the amount required by the F_1 is consistently lower than for either parental strain.

The differences in response between the tu^K and *tu bw*; *st su-tu* and their F_1 with respect to zero-RNA medium (Table 1 and Figure 1) is therefore not a qualitative, but a quantitative difference in sensitivity to ribonucleotide imbalance. All three genotypes react to this imbalance, but the tu^K strain is more sensitive than the *tu bw*; *st su-tu* strain and their F_1 is less sensitive than either parental strain.

Cholesterol: Compared with the parental strains the F_1 shows a marked differ-


1
TUMOR PENETRANCE PER CENT
100
90
80
70
60
50
40
30
20
10
0
0
0.05
0.1
0.2
0.4
0.8
1.6
CONC. RNA PER CENT


2
DEVELOPMENT TIME LOG DAYS
0.95
0.90
0.85
0.80
0.75
0.70
0
0.05
0.1
0.2
0.4
0.8
1.6
CONC. RNA PER CENT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TUMOR PENETRANCE PER CENT
100
90
80
70
60
50
40
30
20
10
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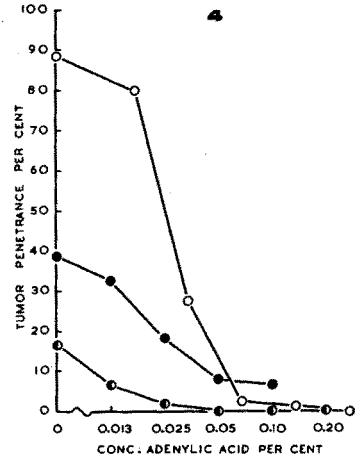
0
0.012
0.023
0.046
0.093
0.186
CONC. CYTIDYLIC ACID PER CENT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TUMOR PENETRANCE PER CENT
100
90
80
70
60
50
40
30
20
10
0
0
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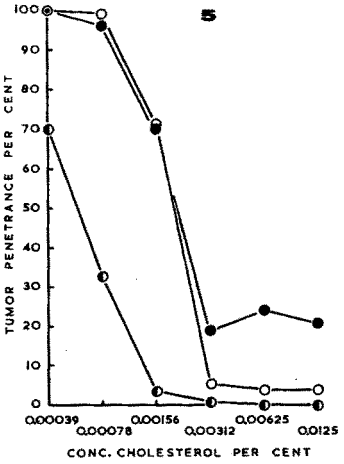
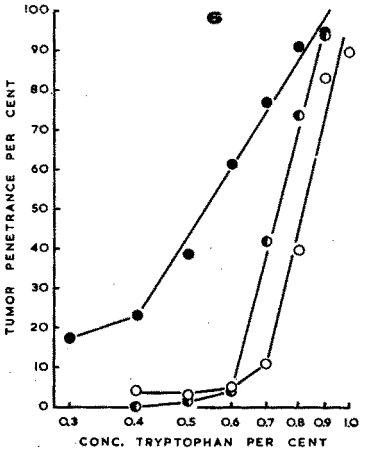
0.025
0.05
0.10
0.20
CONC. ADENYLIC ACID PER CENT


5
TUMOR PENETRANCE PER CENT
100
90
80
70
60
50
40
30
20
10
0
0.00039
0.00078
0.00156
0.00312
0.00625
0.0125
CONC. CHOLESTEROL PER CENT


6
TUMOR PENETRANCE PER CENT
100
90
80
70
60
50
40
30
20
10
0
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
CONC. TRYPTOPHAN PER CENT

ence in sensitivity to cholesterol deficiency at the level tested in Table 1. The three genotypes were reexamined in quantitative dose-responses in order to determine whether this difference was maintained at lower concentrations. The results in Figure 5 show that below 0.00156 percent cholesterol, tumor penetrance rose rapidly for the F_1, reaching a maximum at 0.00039 percent but falling short of 100 percent penetrance shown by both parental strains at this level. These extreme deficiencies adversely affected survival. Hardly any adult flies emerged successfully from the puparium below 0.00156 percent and no larvae survived on media containing less than 0.00039 percent cholesterol. All three genotypes respond to the treatment when it is made sufficiently severe. Both parent strains give a similar response, but the F_1 is more strongly buffered than either parental strain.

L-*Tryptophan:* The spot test shown in Table 1 conceals important quantitative differences in the response of the three genotypes to tryptophan, which become apparent only when a range of concentrations is considered, as in Figure 6. Effects on penetrance in the *tu bw*; *st su-tu* strain begin at concentrations exceeding 0.3 percent, whereas the tu^K strain does not show a response much below 0.7 percent. In contrast to the results obtained for the pentose nucleotides and cholesterol, the response of the F_1 falls between the two parental strains but closer to tu^K than to *tu bw*; *st su-tu.*

X-irradiation: Although X rays failed to produce a significant effect in the tu^K strain and the F_1 at the level tested in Table 1, the possibility of a higher threshold of response in these genotypes was examined with the extended dose range shown in Figure 7. Embryos were irradiated at 22 hours after oviposition coinciding with the mean time of eclosion from the egg. Penetrance rises rapidly in the *tu bw*; *st su-tu* strain and is close to 100 percent at a dose of 2000r. There is little evidence of a comparable response in the tu^K strain or the F_1 below 1000r, but at higher doses the response is rather greater for tu^K than for the F_1. Larval survival decreased over the range tested, and higher doses greatly reduced the proportion of viable pupae; no adult flies successfully eclosed from the puparium at doses exceeding 1000r. The majority of the inviable pupae showed developmental abnormalities, including melanised patches of epidermal tissue which made classification of the material rather unsatisfactory at the 4000r level. It seems that, at these

FIGURE 1.—The relation between tumor penetrance and the concentration of dietary RNA. tu^K strain hollow circles, *tu bw*; *st su-tu* strain solid circles. The control medium contains 0.4 percent RNA. FIGURE 2.—The relation between larval development time in log days and the concentration of dietary RNA. tu^K strain hollow circles, *tu bw*; *st su-tu* strain solid circles. FIGURE 3—.Relation between tumor penetrance and the concentration of cytidylic acid on a medium containing no RNA. tu^K strain hollow circles, *tu bw; st su-tu* strain solid circles, and F_1 half-solid circles. FIGURE 4.—Relation between tumor penetrance and concentration of dietary adenylic acid on a medium containing 0.0465 percent cytidylic acid. tu^K strain hollow circles, *tu bw*; *st su-tu* strain solid circles, and F_1 half-solid circles. FIGURE 5.—Relation between tumor penetrance and the concentration of dietary cholesterol. tu^K strain hollow circles, *tu bw*; *st su-tu* strain solid circles, and F_1 half-solid circles. The control medium contains 0.03 percent cholesterol. FIGURE 6.—Relation between tumor penetrance and the concentration of dietary L-tryptophan. tu^K strain hollow circles, *tu bw*; *st su-tu* strain solid circles, and F_1 half-solid circles.

extreme levels of treatment, melanotic tumors merge into a spectrum of generalised developmental disturbances where the distinction between teratogeny and specific response is difficult to make.

A comparison of the responses of the three genotypes to different tumorigenic treatments shows that both inbred strains and their F_1 react to the same environmental treatments, but that the quantitative pattern of response is not the same in each case. Such differences may be a consequence of nonallelic tumor genes in the *tu*^{K} and *tu bw; st su-tu* strains, which are responsible for different underlying metabolic defects, that nevertheless lead to identical changes in the phenotype. Alternatively they may derive from interactions of the same tumor gene with the residual genetic background of each of the separate lines, or from a combination of both possibilities. In addition the separate biochemical nature of the environmental treatments poses the question of whether the response elicited by different tumorigenic treatments has the same genetic basis in all cases. An approach to both of these questions can be made by chromosome substitution analysis under each of the conditions of treatment.

The chromosome location of tumor genes: Females from each inbred tumor strain were crossed to *Cy; Mé Sb* males of a dominant marker strain. Males from the F_1 progeny of this cross carrying all three dominant markers were backcrossed to virgin females from the inbred tumor strain, and the eggs from the backcross mating collected for culturing on different treatment media under germ free conditions. The adult progeny of the backcross form four phenotypic classes in which

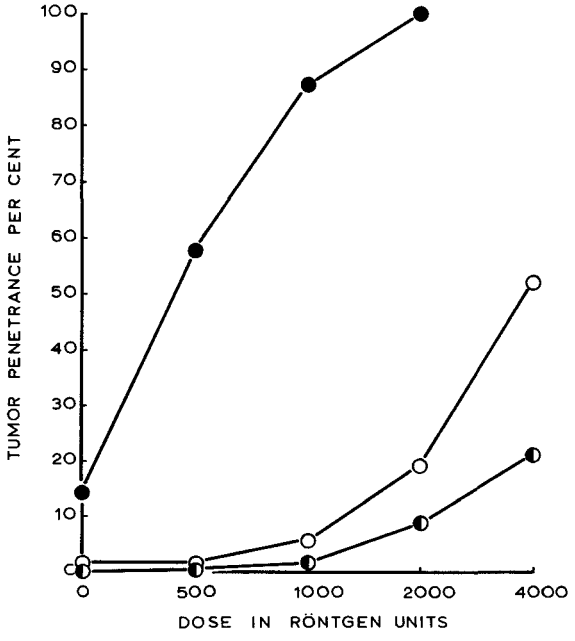


FIGURE 7.—Relation between tumor penetrance and X-ray dose to 22-hour old embryos. *tu*^{K} strain hollow circles, *tu bw*; *st su-tu* strain solid circles, and F_1 half-solid circles.

none, one second, one third, or one second plus one third chromosome of the original tumor strain is replaced by a chromosome containing a dominant marker gene. The tu^K strain carries the fourth-chromosome marker recessive eyeless (ey^K), but as the fourth chromosome was found to have no effect on the results, chromosome 4 classes are not shown separately in Table 2. Estimates of tumor penetrance are based on adult flies of both sexes only, and do not include unhatched pupae as in the data previously summarised in Table 1 and Figures 1 to 7. This should be borne in mind when assessing the results for the tu^K strain, in which tumor penetrance tends to be higher in pupae which fail to eclose than in adult flies for all tumorigenic treatments.

The results for the control medium (Table 2) show the presence of a melanotic tumor gene of low penetrance on the second chromosome of the tu^K strain. On the treatment media penetrance is low or zero when the second chromosome is made heterozygous. Substitution of the third chromosome also leads to a significant reduction of penetrance in each case, compared with the reconstituted tumor strain, revealing the presence of a recessive enhancer modifier ($e\text{-}tu^K$) on the third chromosome. The point of particular interest here is that this system is the same for each of the treatments tested. The control for the *tu bw; st su-tu* strain (Table 2) also shows the presence of a tumor gene of low penetrance on the second chromosome, and the results for the different treatments show that the tumor response is due to the second chromosome alone. Only in the case of cholesterol deficiency does the third chromosome substitution significantly reduce tumor penetrance ($P = 0.001$), disclosing the presence of a modifier specific to this treatment.

The occurrence of a small proportion of affected individuals in certain of the substituted second chromosome classes shows that neither the tu^K nor the *tu bw* gene is completely recessive in effect, but none of the treatments raises tumor penetrance to a level approaching that found in the F_1 between the two tumor

TABLE 2

Penetrance (percent) of melanotic tumors after backcross chromosome substitution in the tu^K *and* tu bw; st su-tu *strains. Each percentage is based on approximately 200 adult flies, in this and subsequent tables*

	Strain, and chromosome replaced							
	tu^K				tu bw; st su-tu			
Treatment	None	2	3	2 + 3	None	2	3	2 + 3
Control	1.48	0	0.26	0	5.06	0	7.9	0
RNA nil	51.1	0.81	31.7	0	...	...	...	...
Cytidylic acid 0.093 percent	81.6	9.2	61.0	1.84	49.0	1.65	51.2	6.95
Cholesterol 0.00156 percent	50.0	0	10.2	0	91.7	1.06	67.5	0
L-tryptophan 0.9 percent	69.1	9.2	13.1	0.54	62.0	4.2	68.0	8.6
X rays 1000r	...	...	...	...	89.5	0	91.5	0

strains (Table 1; Figures 3 to 6). Consequently, there is a special interaction between these two *tu* loci when both are present in single dose in the F_1. This could mean simply that the two *tu* genes are alleles of different potency at the same locus, but in that case we might expect the responses of the F_1 to be consistently intermediate between those of the two parental strains. The interpretation of chromosome substitution analyses of this type is complicated by the fact that the marker chromosome replacing an original chromosome of the inbred strain may itself introduce factors which influence those aspects of the phenotype which are being considered. One possibility is well illustrated by GLASS (1957), who showed that wild-type alleles of different potency at the erupt locus are present in strains of different geographical origin. In the present instance, wild-type alleles of low potency on the Curly second chromosome might explain the incomplete recessivity evident in Table 2, but the same result may equally be due to the contribution of this chromosome to the properties of the residual genotype. Nevertheless, the fact that tumors appear in heterozygotes only when environmental treatments are applied, indicates that the treatments themselves directly or indirectly influence the dominance relation of the mutant and wild-type loci.

The chromosome location of the suppressor: An important outcome of the results for the *tu bw; st su-tu* strain (Table 2) is that there is no indication of the effects of a third chromosome suppressor described by GLASS (1957). This would come about if the *st* chromosome of the tumor strain carries a dominant suppressor, or alternatively if both the *st* chromosome and the *Mé Sb* third chromosome used in the cross carry allelic recessive suppressors. The situation was examined further in order to distinguish between these possibilities.

Females of the *tu* strain were crossed to *SM5; TM3* males (MATERIALS AND METHODS) and to *Cy; Sb* males respectively, according to the following scheme, and reared on corn-meal-molasses-yeast medium.

$$P_1 \; ♂♂ \; SM5;TM3 \times ♀♀ \; tu \times Cy;Sb \; ♂♂$$
$$F_1 \qquad ♀♀ \; SM5;TM3 \times Cy;Sb \; ♂♂$$

The *SM5* second and *TM3* third chromosomes are marked with *Cy* and *Ser* respectively. The *Cy;Sb* males used in this cross derive from a different strain from the *Cy;Mé Sb* marker strain used for backcross chromosome substitution in Table 2. In the F_2, four recombination classes homozygous for the second chromosome of the *tu* strain are recovered, but with one or both third chromosomes from the dominant marker strain.

The results (Table 3) show that melanotic tumors appear spontaneously in nearly all larvae homozygous for the *tu bw* second chromosome in combination with *Ser / Sb* or *Sb / st*, whereas penetrance is no higher in *Ser / st* than in the reconstituted strain, *st / st*. In contrast, the results for the tu^K strain show no indication of an interaction of this kind, penetrance not being significantly greater in substituted third-chromosome classes than when both the original third chromosomes are present. This would not, of course, discount the suggestion that there is another recessive suppressor linked to tu^K on the second chromosome.

TABLE 3

Tumor penetrance (percent) after replacement of neither, one, or both third chromosomes of the tu^K *and* tu bw; st su-tu *strains*

Genotype:	$\frac{tu^K\ 3^K}{tu^K\ 3^K}$	$\frac{tu^K\ Ser}{tu^K\ 3^K}$	$\frac{tu^K\ Sb}{tu^K\ 3^K}$	$\frac{tu^K\ Ser}{tu^K\ Sb}$
Penetrance:	0.90%	1.76%	0.88%	2.32%
Genotype:	$\frac{tu\ bw\ st}{tu\ bw\ st}$	$\frac{tu\ bw\ Ser}{tu\ bw\ st}$	$\frac{tu\ bw\ Sb}{tu\ bw\ st}$	$\frac{tu\ bw\ Ser}{tu\ bw\ Sb}$
Penetrance:	7.5 %	8.15%	86.5 %	99.0 %

The results for the *tu bw*; *st su-tu* strain (Table 3) exclude the possibility of a dominant suppressor on the *st* third chromosome but could be explained by an allelic recessive suppressor (*su-tu*) on the *Ser* and *st* chromosomes, and a dominant inactive alternative linked with *Sb*. This point was further examined by testing third chromosomes from five different wild-type strains of separate geographical origin. Virgin females from each wild strain were crossed to *tu bw*; *Ser /Sb* males. F_1 males carrying *Sb* were then backcrossed to *tu bw; ser /Sb* females to give the F_2 combinations shown in Table 4. These results show that none of the strains tested carries a mutant allele of *tu bw*, and definitely establish the dominant inactive form of the suppressor ($+^{su-tu}$) as the wild-type allele at that locus. In addition, penetrance of *tu bw* is slightly lower in genotypes heterozygous for the suppressor (*Ser* /3 combinations), showing that dominance of the wild-type allele is not quite complete. Our findings, which have independent confirmation in the results of GLASS (1963 personal communication), contrast with the ubiquity of the erupt mutant, and the suppressor of erupt, in wild-type strains of diverse geographical origin (GLASS 1957).

We can reexamine the question of allelism between the two *tu* loci by making use of the fully penetrant *tu bw; Ser /Sb* strain and crossing to tu^K; *Ser /Sb*. The F_1 is heterozygous for both *tu* second chromosomes, whilst sharing the same bal-

TABLE 4

The effect of second and third chromosomes derived from inbred wild strains on the penetrance (percent) of tu bw

	Genotype			
	$\frac{tu\ bw\ Sb}{tu\ bw\ 3}$	$\frac{tu\ bw\ Ser}{tu\ bw\ 3}$	$\frac{tu\ bw\ Sb}{2\ \ 3}$	$\frac{tu\ bw\ Ser}{2\ \ 3}$
Strain				
Edinburgh	100	99.2	1.3	0
Pacific	100	94.6	0	0
Sao Paulo	100	85.0	0	0
Gabarros	99.1	95.2	0	0
Oregon-R	100	98.2	0	0

anced third chromosome combination as both parental strains. Of the 395 progeny of this cross none had any tumors, confirming that the two tumor genes are at different loci.

DISCUSSION

The nature of the treatments used in this analysis is such that they probably influence in a general way the pattern of nucleic acid, sterol and amino acid metabolism, rather than act selectively upon a particular reaction. Consequently, the delimited phenotypic response elicited by treatment must be due to differential sensitivity of a specific developmental pathway, relative to pathways leading to other phenotypic characters of the organism. In both of the strains examined, the effect has a definite genetic basis, depending on the influence of two nonallelic tumor genes *tu*^{K} and *tu bw*, the penetrance of which is conditional upon environmental conditions. The second chromosome location found in each case agrees with the results of other studies (BARIGOZZI and DI PASQUALE 1956; GHÉLÉOVITCH 1958) and adds to the already large number of loci in this chromosome known to be capable of initiating melanotic tumor formation.

Data recently published by BARIGOZZI, HALFER and SGORBATI (1962) add a new dimension to the complexity of the situation with respect to the genetic analysis of tumor strains. Their suggestion of an episomic controlling element introduces an unstable influence into an already variable system, and adds the possibility of maternal transmission through the egg. More definitive experiments are required to substantiate their analysis, since it is not possible to decide, without precise control of the environment, whether a strain carries a concealed or suppressed tumor gene, which is sometimes penetrant in yeasted cultures, or if there is additionally an episomic element also influenced in its effects by both genetic and environmental variables.

Although the phenotypic effect of the tumor genes appears to be indistinguishable, the underlying genetic mechanism controlling tumor penetrance in the two inbred strains is quite different. The *tu*^{K} gene is an isoallele in the sense that under optimal environmental conditions the phenotypic expression of this locus overlaps with the wild type. Indeed, the degree of developmental destabilisation which it produces is less than penetrance in the *tu*^{K} strain would indicate, because of the simultaneous presence of the unlinked enhancer modifier *e-tu*^{K}. It seems that the locus causes a subthreshold metabolic defect which only becomes manifest under particular conditions of environmental stress, in the manner already suggested by SANG and BURNET (1963). Contrasting with this situation, low penetrance of *tu bw* in the inbred strain is due to an interaction with the epistatic recessive suppressor *su-tu*. In the presence of the dominant wild-type allele + $^{su\text{-}tu}$, the phenotypic effect of the tumor locus is expressed even under optimal environmental conditions.

This situation has led PLAINE and GLASS (1955) and GLASS (1957) to suggest that tryptophan and X-ray treatments interfere directly with the action of the suppressor in the *tu bw*; *st su-tu* strain, thus allowing the phenotypic expression

of the tumor gene. But, with the exception of X-irradiation, treatments which increase tumor penetrance in the *tu bw; st su-tu* strain have the same effect in the tu^K strain in which no suppressor system has so far been detected. Thus, with the possible exception of X rays, tumorigenic treatments appear to influence the reaction controlled by the tumor gene, whereas X-irradiation might act through inhibition of the suppressor. But the hypothesis of a separate indirect role for the X-ray effect does not readily account for the results of studies which show that this treatment enhances the penetrance of other melanotic tumor genes, in genotypes in which no specific suppressors have been identified. In particular, HARTUNG (1942) observed a rise in tumor penetrance after X-ray treatment in the *l(1)7* strain (STARK 1918; RUSSELL 1940) and in two strains carrying the second chromosome tumor gene *tu 36a* (RUSSELL 1942), and more recently GHÉLÉLOVITCH (1961) obtained a similar result for the *tu 48a* (2-29.5) strain. Not every tumor gene responds readily to this treatment, as the results for the tu^K strain show. Thus the evidence tends to support the view that tumorigenic treatments have a more or less direct influence on the expression of the tumor gene in the *tu bw; st su-tu* strain, rather than on the operation of the suppressor.

The independent effect of tryptophan and X-ray treatments on the two *tu* strains is also relevant to the hypothesis (GLASS 1957) that both treatments influence the same biochemical reaction, namely the peroxidative conversion of L-tryptophan to formylkynurenine, catalysed by tryptophan pyrrolase. If this reaction is indeed the target of both treatments in the manner that GLASS suggests we should expect that both, or neither, would be effective in the tu^K strain. Moreover, this hypothesis does not in any obvious way account for the effects of cholesterol deficiency or suboptimal dietary ribonucleotide balances on either strain. It may be that each of the tumorigenic treatments reinforces an underlying metabolic inadequacy due to a defective enzyme, by altering the balance of different secondary reactions connected with it. This would be in line with SANG and BURNET's (1963) finding that the cholesterol and nucleotide balance effects are mutually independent, and the same reasoning would apply with respect to the tryptophan and X-ray effects. There is some indication that the action of X-irradiation is different from that of dietary treatments in that it is effective during the embryonic period, whereas the period sensitivity to dietary changes does not begin before the close of the second instar (BURNET and SANG, unpublished). A study of the phenocritical periods for each of the tumorigenic treatments, and of their effect on the unsuppressed *tu bw;* $+^{su-tu}$ strain, is now being undertaken to clarify some of these points.

The phenocopy effect, which consists of environmental induction of a phenotype characteristic of a known mutant gene, has been observed in a diversity of organisms (GOLDSCHMIDT 1938, 1955), but analysis of its genetic basis has rarely been attempted. The reason why this is so is to be sought partly in the assumption—for which the originator of the term was largely responsible—that treated individuals showing the phenocopy effect carried the wild-type allele of the mutant which was phenocopied. Following the work of SANG and MCDONALD (1954)

on the production of the eyeless phenocopy with boron salts, GOLDSCHMIDT and PITTERNICK (1957a, b) and GOLDSCHMIDT (1957) came to the conclusion that all phenocopies are due to the bringing to light of already present nonpenetrant, subthreshold or isoallelic mutants. That is to say that the genotype of individuals responding to the phenocopy treatment contains a subthreshold allele of the phenocopied mutant, and to that extent there is a latent tendency to develop in the direction of the mutant phenotype. Now developmental abnormalities of some sort can be induced in individuals irrespective of genotype, provided that the environmental stress is made sufficiently extreme. But the severity of the treatment has generally to be such as to be scarcely short of lethality, and the induced abnormalities tend to be of a rather generalised and variable nature, for which the term teratogeny seems most obviously appropriate. Certain genotypes give a characteristic phenocopy response, under conditions of environmental stress which permit survival of the majority of the treated individuals, and the question we are concerned with here, is whether differential sensitivity of the particular developmental pathway which is affected is due to the presence in these genotypes of a subthreshold allele, in the way that GOLDSCHMIDT suggests. The analysis which we have presented above is relevant to this question in that the tumorigenic effect of enviromental treatments is shown to be due to a tumor gene of low penetrance present in the genotype of each inbred strain, although the effects of the respective tumor genes are subthreshold for different reasons in the two cases. This gives us some insight into the mechanisms underlying the phenocopy effect, but it would be inadmissible to argue from the present evidence that all phenocopies necessarily depend on systems of the same kind in the way that GOLDSCHMIDT implies.

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SUMMARY

The inbred *tu*^{K} and *tu bw*; *st su-tu* strains of *Drosophila melanogaster* have a low incidence of melanotic tumors when cultured on a complete, defined medium under germ free conditions. Suboptimal balances of pentose nucleotides in the larval diet, cholesterol deficiency, an excess of L-tryptophan, and exposing embryos to X-irradiation, cause an increase in tumor penetrance in the two strains and their F_1, but there are quantitative differences between them.

The response of the *tu*^{K} strain to treatment is due to a gene of low penetrance (*tu*^{K}) on the second chromosome, and a recessive enhancer modifier (*e-tu*^{K}) on chromosome 3. The response of the *tu bw*; *st su-tu* strain is also due to a tumor gene (*tu bw*) on the second chromosome which in normal enviromental conditions is hypostatic to a recessive third chromosome suppressor (*su-tu*). It is fully penetrant in the absence of environmental treatments in genotypes containing the dominant allele (+ $^{su-tu}$) of the suppressor locus.

Dietary-environmental treatments which increase tumor penetrance are thought to influence the reaction controlled by the tumor gene, rather than to interfere with the action of a specific tumor suppressor.

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