PHYSIOLOGICAL GENETICS OF MELANOTIC TUMORS IN
DROSOPHILA MELANOGASTER. IV. GENE-ENVIRONMENT
INTERACTIONS OF *tu-bw* WITH DIFFERENT THIRD
CHROMOSOME BACKGROUNDS

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THE proportion of tumors found in an inbred population of the *tu-bw; st su-tu* strain of *Drosophila melanogaster* depends on the interactions of the second-chromosome tumor gene with its third-chromosome suppressor and with the environment, especially the nutritional conditions to which larvae are exposed immediately prior to the third instar (BURNET and SANG 1964b). Penetrance of the tumor character responds only to particular nutritional manipulations, which could operate by supplementing the action of the tumor gene itself, or by inhibiting the action of the suppressor, or both. Comparison of the reactions of two nonallelic tumor strains, one with a suppressor (*tu-bw; st su-tu*) and the other without (*tu-K*), led to the provisional conclusion that effective dietary treatments influenced the tumor system directly, and not the suppressor system. Data from crosses of these strains (BURNET and SANG 1964a) showed that the situation was not simple and that the remainder of the genotype also modified penetrance, as might be expected. The conclusion just stated could not therefore be taken as unequivocal. This paper is concerned with a direct comparison of the responses of the same *tu* gene (more exactly, the same second chromosome) in suppressed and unsuppressed strains. The question asked is: do both strains react similarly to the same nutritional manipulations? If they do, effective nutritional treatments influence the *tu* system and not the *su-tu* system, which, we may then conclude, is involved only in regulating the level of penetrance.

On the other hand, nutritional treatments may affect both the *tu* and the *su-tu* systems separately. It is then necessary to examine which treatments affect each system, and to determine whether the remainder of the genotype also plays a significant part in these reactions. Responses of the cross between the suppressed and unsuppressed strains, and of a similar cross carrying a balanced combination heterozygous for two different third chromosomes, one of which carried an allele of the *su-tu* gene, have therefore also been measured. Since the suppressor is recessive, the expectation is that both heterozygous strains will behave like the unsuppressed strain. Any differences among the three genotypes should then show how far penetrance may be altered by genes other than the suppressor.

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A comparison of the reactions of these strains to a comprehensive range of nutritional manipulations may be expected to give some indication of the metabolic systems most intimately involved in the action of the tumor gene with its suppressor. Although the complex interrelations of metabolic pathways make it unlikely that nutritional techniques alone can expose precisely which process, or processes, are defective in these mutants, this approach may indicate promising avenues for an attack on the problem at the biochemical level.

MATERIALS AND METHODS

The strains used are: (1) *tu-bw*; *st su-tu* and (2) *tu-bw*; $+^{su-tu}$, both kindly supplied by PROFESSOR BENTLEY GLASS (and which also carry the *er* and *Su-er* genes which are not considered here), (3) the F_1 between these two strains and (4) *tu bw*; *Sb* $+^{su-tu}/TM3$, *ri p^v bx^{34e} e^s Ser su-tu* which was derived from (1) for other purposes (BURNET and SANG 1964a). All these are isogenic for the same second chromosome carrying *tu* at locus 83.9 (GLASS 1954), and they have been maintained as brother-sister mated lines.

The technique of germ free culture has already been described, with details of the culture medium (SANG 1956; SANG and BURNET 1963a). Eight to ten replicate cultures, each inoculated with 40 larvae, were used for each treatment. Mixed cultures were sometimes employed when the responses of strains (1) and (2) were being compared since the genotypes could be distinguished by their eye colours. Fifty larvae in about equal numbers of the two genotypes were then seeded into the same culture vial. Tumor frequency was scored as the percentage of hatched adults and unhatched pupae carrying tumors in each culture. Differences in tumor penetrance with treatments was tested for significance by the usual 2×2 chi-square. All experiments included controls, and infected cultures were discarded.

RESULTS

Tumor frequency is different for each of the strains when reared on the germ-free, defined medium (Table 1), so that the extent of a treatment effect is most simply measured by the differences from these control values. In practice, there was some variability among controls in different tests (for reasons considered below) and each experiment has been assessed against its own control.

When compared with performance on live yeast, the germ-free, control medium decreases tumor penetrance significantly except for the suppressed strain (Table 1). This difference conveniently permits measurement of treatments which raise tumor frequency in the unsuppressed and heterozygous strains, which would be difficult using live yeast cultures. The *su-tu* genes do not completely suppress tumors even on live yeast. Table 1 also shows that the expectation that heterozygous strains would behave in the same way as the unsuppressed

TABLE 1
Percent tumor penetrance in flies reared on standard media

Medium	<i>tu-bw</i> ; <i>su-tu</i>	<i>tu-bw</i> ; $+^{su-tu}$	<i>tu-bw</i> ; $+^{su-tu}/su-tu$	<i>tu bw</i> ; <i>Sb</i> $+^{su-tu}/Ser su-tu$
Live yeast	7.5	95.0	81.0	99.5
Germfree defined	10.6	47.5**	75.5**	70.0**

** Significantly different from live yeast values at the 1% level.

strain is not fulfilled when the larvae are reared on defined media, whereas it is nearly true for live yeast cultures. Since there are more, not fewer, flies with tumors in the strains with one *su-tu* gene than in the unsuppressed strain, the strains must have different response thresholds to variations of nutrient balance, and the defined medium must be ill-balanced with respect to conditions for full tumor formation in all of the strains. Spot tests of the effects of dietary deficiencies may therefore be misleading, and apparently anomalous results have been confirmed by more extensive tests in the experiments which follow.

Major nutrients: Excess, as well as deficiency, of each of the major nutrients slows larval development (SANG 1956), so the effects of both levels were tested separately (Table 2).

Three of the four strains respond to a cholesterol deficiency (Table 2), with a significant increase of tumor frequency. Failure of the *tu-bw; Sb +^{su-tu}/Ser su-tu* hybrid to react corresponds to the situation found with the hybrid between *tu-K* and *tu-bw; su-tu* (BURNET and SANG 1964a). In the present instance it can be shown, however, that an even lower cholesterol provision (0.0008%) causes tumors to increase significantly. Only the unsuppressed strain is affected by excess cholesterol, and then by a reduction of tumor frequency, showing that the threshold of response of this strain is also different from the others. This very great difference in the extent of cholesterol reduction which the strains will tolerate before tumor frequency increases is of considerable interest, since cholesterol is an essential nutrient for *Drosophila*, apparently not synthesized by the growing larvae (VAN'T HOOG 1935). These, and previously published data (BURNET and SANG 1964a), suggest that the critical dietary levels for tumor formation are determined by genes other than *tu* and *su-tu*.

Lecithin deficiency also causes an increase of tumor frequency in the suppressed strain, but it has the converse effect on the unsuppressed strain. One

TABLE 2

Major nutrients: differences of percent tumor penetrance from control values

		<i>tu-bw; su-tu</i>	<i>tu-bw; +^{su-tu}</i>	<i>tu-bw; +^{su-tu}/su-tu</i>	<i>tu bw; Sb +^{su-tu}/Ser su-tu</i>
Deficient supply					
Cholesterol	0.0016%	+78.2**	+25.4**	+21.1**	— 8.8
Lecithin	0.0125	+17.8**	—19.8**	+18.0**	—25.2**
Sucrose	nil	— 5.6	—44.9**	—31.9**	—25.9**
Casein	2.5	+13.6**	+31.1**	— 0.8	+ 8.5
RNA	nil	— 3.3	—44.6**	—45.5**	—37.1**
Excess supply					
Cholesterol	0.128%	+ 0.4	—31.1**	+ 6.9	+ 7.6
Lecithin	0.80	+ 2.1	— 5.1	+ 7.7	—14.0*
Sucrose	4.0	— 8.0**	— 1.9	—16.6*	+ 8.8
Casein	10.0	— 6.3*	—43.8**	—24.2**	—42.3**
RNA	1.6	+12.5*	+41.5**	+15.0**	+13.0*

Significance of difference of tumor frequency from control levels tested by chi-square:

* Significant at the 5% level;

** Significant at the 1% level.

FIGURE 1.—Responses of unsuppressed (●) and suppressed (○) strains to choline added to the standard medium in place of lecithin. The broken line shows the difference between the two response curves. The normal lecithin supply provides more than 640 μ g choline.

FIGURE 2.—Responses of unsuppressed (●) and suppressed (○) strains to RNA. The standard medium contains 0.4% RNA.

heterozygous strain behaves like the unsuppressed strain but the other does not, even when lecithin is provided at only half the level shown in Table 2 (which results in a further 10% increase of tumors). This difference in the reactions of the two heterozygous strains again shows that genes other than *tu* and *su-tu* significantly affect the tumor response system, especially when account is also taken of the anomalous reaction of the *tu-bw*; *Sb* + *su-tu*/*Ser su-tu* strain to excess lecithin.

Lecithin is the source of choline in the diet, and it can be partly replaced by choline, which is the essential requirement (SANG 1956). The difference in responses of the suppressed and unsuppressed strains was therefore examined in greater detail by determining their dose responses to choline (Figure 1). When the supply of choline is less than the amount required for optimal growth (320 to 640 μ g per culture), there is a decrease in tumor frequency in the unsuppressed strain, whereas the suppressed strain shows increased penetrance over the same range. This relationship will be examined in detail in a subsequent paper, but it must be noted here that the suppressor containing chromosome acts as an "enhancer" of tumor penetrance at low choline levels.

Sugar-free diets reduce tumor penetrance for all genotypes, whilst excess sucrose tends to reduce penetrance significantly in the suppressed strain. Casein deficiency tends to raise penetrance, except in the heterozygotes, whilst excess reduces penetrance in all genotypes. That is to say, the amount of protein required for development without tumors is considerably more than the optimum growth rate requirement (ca. 5% casein) and this relationship involves the tumor gene rather than the suppressor. Variations in the supply of dietary ribose nucleic acid (RNA) affect all genotypes. High RNA raises tumor frequency, low RNA decreases it. The consequences of RNA deficiency are the opposite of those found for the *tu-K* strain (SANG and BURNET 1963a).

Previous work (BURNET and SANG 1964a) has shown that the suppressed strain was less responsive to dietary nucleotide imbalance than the *tu-K* strain, while the data of Table 2 show that the unsuppressed strain is not so insensitive to such treatments. This difference was examined further as an RNA dose-response for the two strains grown together (Figure 2). Both strains respond to increasing amounts of RNA with an increase of tumor frequency, but the presence of the suppressor gene reduces the dietary RNA sensitivity of the tumor system. The difference between the two response curves is such that the suppressor carrying chromosome acts as an enhancer of tumor frequency at the lowest RNA concentrations. The importance of genotype-environment interactions in determining the ranking of genotypes is well emphasized by this and the preceding figure, which could be compared with the similar effects of nutritional deficiencies upon growth relationships among inbred lines and crosses (SANG 1964).

Vitamin deficiencies: Provision of an excess of any of the vitamins required by *Drosophila* has no effect on growth or development (SANG 1956), nor does it alter tumor frequency (except in the situation noted below). On the other hand, deficiencies slow growth, and omission of individual vitamins leads to death at particular stages of development (see SANG 1954). Tests at vitamin levels which just permit survival show that some, but not all, such deficiencies also alter tumor frequency (Table 3), and these results are considered first in relation to the findings just described.

Since thiamine is involved primarily in sugar metabolism, low dietary thiamine might be expected to have the same effect as removal of sugar from the medium. This deficiency is found, on the contrary, to raise tumor frequency in the suppressed and unsuppressed strains and in the second heterozygote (Table 3). Low pyridoxine would be expected to restrict protein metabolism (see SANG 1962) and it does raise tumor frequency (Table 3), like a casein deficiency (Table 2). Its effect is the same on all genotypes, as are the consequences of alteration of the casein supply from excess to deficiency. Similarly, deficiencies of folic acid or biotin (which are essential requirements for synthesis of ribonucleotides *de novo*) would be expected to have similar effects to RNA deficiency, and where these have detectable consequences they do indeed reduce tumor penetrance (Table 3). The suppressed strain is not affected significantly in either case (cf. deficient RNA—Table 2). These results are particularly interesting since they are the opposite of those found with the *tu-K* strain (SANG and BURNET 1963a), and thus

TABLE 3

Vitamin deficiencies—differences of percent tumor penetrance from control values

Vitamin level in micrograms per culture		<i>tu bw</i> ; <i>su-tu</i>	<i>tu bw</i> ; + <i>su-tu</i>	<i>tu bw</i> ; + <i>su-tu</i> / <i>su-tu</i>	<i>tu bw</i> ; <i>Sb</i> + <i>su-tu</i> / <i>Ser su-tu</i>
Thiamine	0.8	+27.7**	+15.5**	+ 0.1	+12.4*
Riboflavin	1.5	— 3.1	—39.8**	—70.0**	—57.7**
Niacin	5.0	+14.4**	—18.3**	—62.5**	—62.1**
Pyridoxine	0.5	+30.5**	+48.8**	+23.2**	+17.5**
Ca Pantothenate	6.0	— 2.2	—30.7**	—54.5**	—44.7**
Biotin	0.005	+ 2.6	Lethal	—56.1**	—37.9**
Folic acid	nil	— 2.1	—12.7**	—17.3**	+ 4.1

* Significant at the 5% level;

** Significant at the 1% level.

agree with the responses of the strains to absence of dietary RNA. No significant effect of biotin deficiency on tumor penetrance in the unsuppressed strain could be detected over the range of sublethal deficiencies tested.

Reducing the dietary concentrations of the three remaining vitamins (riboflavin, niacin and pantothenic acid—Table 3) to fractions of the amounts necessary for normal growth causes a significant decrease of tumor frequency whatever the genotype, but the suppressed strain is again less sensitive to the treatments. An exception to this rule is the response of the suppressed strain to niacin: low niacin raises tumor frequency. That is, like choline, niacin deficiency has different effects on the suppressed and unsuppressed strains.

It is important to emphasize here that only two vitamin deficiencies, thiamine and pyridoxine, cause tumor penetrance to increase, and these deficiencies may be presumed to involve the *tu* system, either directly or indirectly.

Excess vitamins: In their studies on the *tu-e* strain of *Drosophila melanogaster*, FRIEDMAN, HARNLY and KOPAK (1955a) showed that addition of vitamins to a 2.5% killed yeast (i.e. nutritionally deficient) medium raised tumor penetrance. Thiamine, riboflavin, niacin, calcium pantothenate and biotin additions did so when provided singly or in various combinations, and analogues of thiamine, niacin and biotin countered the tumor promoting effects of their respective vitamins (FRIEDMAN, HARNLY and KOPAK 1955b). These findings have been checked to see if they also hold for the *tu-bw* strains.

In our experiments all vitamins are provided in excess (*ca.* tenfold) since these excesses have no detrimental effects on growth or development (SANG 1956). Ten times these standard amounts (i.e. about 100 times the growth requirements) had no effects on tumor frequency. Very high levels of pantothenate improved development rate and *lowered* tumor frequency in the suppressed strain, indicating that this strain has an atypically high requirement for the vitamin which is not shared by the unsuppressed strain.

The effects of antivitamins on the *tu-e* strain and of vitamin deficiencies on *tu-bw* also show one difference between them: thiamine deficiency raises tumor penetrance in *tu-bw*, pyriothiamine lowers penetrance in *tu-e*. However, this difference may reflect only the difference between the standard media employed

in the two experiments, since FRIEDMAN, HARNLY and KOPAK's (1955a) medium contained no sugars, other than those present in the killed yeast.

Magnesium: A new sample of agar had to be used for some of these experiments, and this raised tumor frequency in both suppressed and unsuppressed strains. Tests with other agars showed that "control" tumor levels varied with the agar employed. As reported elsewhere (SANG and BURNET 1963b), assessment of the ions present in these agars showed great variability among them, but suggested that tumor frequency was inversely proportional to their magnesium content, although not exactly so. Work was then restandardised on Oxoid agar No. 3, and Table 4 summarises the consequences of supplementing this medium with magnesium sulfate. Tumor frequency is high in both strains when no supplementary magnesium is provided. With increasing magnesium, penetrance falls to about the same extent in both the suppressed and unsuppressed strains. The magnesium sulfate could be replaced by magnesium chloride.

Table 4 also shows that development rate is improved by magnesium additions, but that maximum development rate is achieved at lower levels of magnesium than those which produce the minimum number of tumors. This partial correlation between development rate and tumor frequency is also found to hold within a culture, since the first flies to hatch tend to have fewer tumors than those emerging subsequently. It is not possible to decide from the evidence available at present whether the tumorigenic effect of magnesium deficiency is due to a shortage of magnesium ions as such, or an indirect effect due to general disturbance of ionic balance.

Interactions: All the strains homozygous for *tu-bw* respond in the same way to an excess or to a deficiency of a nutrient, except for lecithin (choline) and niacin which affect the suppressed and unsuppressed strains in opposite ways. With these exceptions, the data therefore imply that effective treatments operate by way of a *tu* gene controlled reaction. Not every genotype reacts significantly to each treatment, presumably owing to variation in response thresholds (as shown for cholesterol deficiency), and it is interesting that the heterozygotes are not the least sensitive genotypes (cf. SANG 1964). Nor is the extent of a response equivalent among reacting genotypes, emphasizing the important role of residual

TABLE 4
Responses to added Mg⁺, using Oxoid agar No. 3 in place of Kobe agar

Concentration MgSO ₄ · 7H ₂ O	<i>tu bw; su-tu</i>		<i>tu bw; +su-tu</i>	
	Percent tumors	Development time	Percent tumors	Development time
0	61.7	0.736	86.3	0.745
0.0075%	51.0	0.715	71.4	0.716
0.015%	47.9	0.706	59.5	0.740
0.030%	33.3	0.688	63.2	0.673
0.060%	20.5	0.697	54.5	0.707
0.120%	22.3	0.686	44.2	0.683

Development was measured over the larval period, in log days.

TABLE 5

Interactions of some effective nutrients—differences of percent tumor penetrance from deficient nutrient values (tu bw; +^{su}-tu)

Deficient nutrient	Excess nutrient			
	Thiamine—100 μ g	Pyridoxine—500 μ g	Cholesterol—0.12%	MgSO ₄ —0.124%
Thiamine 0.6 μ g	...	+ 4.9	+ 3.2	—15.7*
Pyridoxine 0.5 μ g	+24.6**	...	+ 8.0	+ 6.9
Cholesterol 0.002%	+ 3.6	— 3.9	...	+ 8.5
MgSO ₄ zero	+ 6.6	+ 2.2	+ 5.9	...

* Significant at the 5% level;

** Significant at the 1% level.

genetic variation, outside the second chromosome, in modulating the tumorigenic effects. This is not unexpected, but it implies that attention must be paid to this complicating circumstance in subsequent work.

Five deficiencies consistently raise tumor penetrance (Table 6) and it seems possible that two, casein and pyridoxine deficiencies, are closely connected. Casein is the only source of dietary amino acids in the synthetic medium, and pyridoxine is an important cofactor requirement in amino acid metabolism. In either case a dietary deficiency may be expected to have an effect on the balance and availability of amino acids for protein synthesis; a process also likely to depend on the availability of magnesium ions which are known to be essential for maintaining the integrity of functional ribosomal particles. The roles of the other effective treatments (thiamine and cholesterol deficiencies) are less obviously interrelated. Interactions between nutrients were therefore tested (Table 5) using the unsuppressed strain, which is the most responsive. Two interactions were found, both involving thiamine: excess thiamine raises tumor penetrance on low pyridoxine diets, but not, as already noted, on normal pyridoxine diets; extra Mg⁺⁺ lowers tumors on low thiamine diets. The first interaction is easily explained since the pyrimidine part of thiamine is known to act as an anti-vitamin with respect to pyridoxine (BRAUNSTEIN 1960). The interaction between thiamine and Mg⁺⁺, on the other hand, is open to a number of possible interpretations, some of which are currently being studied. The point of Table 5, however, is that, with this exception, effective deficiencies are found to operate independently of each other. For example, low thiamine does not raise tumor frequency by causing an effective lowering of available pyridoxine or of available cholesterol; and similarly for the other deficiencies. A number of nutritional states therefore affect tumor penetrance, and it is not surprising that different genetic backgrounds, which may affect these states in a variety of ways, modify responses to particular deficiencies.

DISCUSSION

The data presented here, together with those in preceding papers of this series, show that environmentally induced changes in the penetrance of *tu-bw* frequently involve a response threshold. Several of the nutritional treatments described in

Tables 2 and 3 are so close to lethal for some of the genotypes that more extreme deficiencies cannot be tested. Consequently, no significant response may be obtained if the threshold is not reached at a survival level permitting a reliable estimation of tumor penetrance to be made, and the data are incomplete on this account. Nonetheless, the results in Table 6 are adequate to support the general conclusion that, where there are significant changes in penetrance, the suppressed and unsuppressed strains respond to the same treatments in the same way. That is to say, that the treatments influence the metabolic reaction controlled by the tumor gene rather than by supplementing or inhibiting the action of its suppressor.

There are two exceptions to this generalization: lecithin (or choline, as Figure 1 shows), and niacin. In these cases the suppressed and unsuppressed strains respond in opposite directions when the diet is deficient in these nutrients. In the case of lecithin deficiency the direction of response is also strikingly inconsistent amongst the three phenotypically unsuppressed strains. These differences are almost certainly due to differences in the genetic contents of the third chromosomes employed in the various combinations. The *st su-tu* and *+su-tu* chromosomes differ in genetic background outside the suppressor locus. The suppressor allele in the *Ser su-tu* chromosome is of quite different origin from that in the *st su-tu* chromosome (BURNET and SANG 1964a); indeed these two chromosomes differ in the quality and arrangement of their gene contents. The three phenotypically unsuppressed strains also differ quantitatively in their response to identical conditions of dietary environmental stress, as is well illustrated in Tables 2 and 3, and these effects are also due primarily to differences in the genetic background provided by the various third-chromosome combinations. As we have already pointed out, the suppressed strain is usually least responsive to environmental variations; thus the suppressor gene has a general effect in raising the response

TABLE 6
Summary of nutritional deficiency effects

Deficient nutrient	<i>tu bw; su-tu</i>	<i>tu bw; +su-tu</i>	<i>tu bw; su-tu/+su-tu</i>	<i>tu bw; Sb +su-tu/Ser su-tu</i>	<i>tu-K</i>
Cholesterol	+	+	+	(+)	+
Lecithin	+	—	+	—	0
Sucrose	—	—	—	—	0
Casein	+	+	+	+	0
RNA	(—)	—	—	—	+
Thiamine	+	+	0	+	0
Riboflavin	0	—	—	—	0
Niacin	+	—	—	—	0
Pyridoxine	+	+	+	+	0
Pantothenic acid	(—)	—	—	—	0
Biotin	0	(0)	—	—	+
Folic acid	0	—	—	0	0
Mg ⁺⁺	+	+	not tested	not tested	+

The deficiency levels are those shown in Tables 2 and 3, except for the signs in brackets which are from the separate retests noted in the text. + indicates significant tumor increase, — a significant lowering of tumor frequency, and 0 is no significant response. Data for *tu-K* from SANG and BURNET (1963a and unpublished).

thresholds to a variety of conditions of dietary environmental stress, thereby increasing the stability of developmental canalisation.

BURNET and SANG (1964a) have suggested that the *tu* gene is responsible for a metabolic inadequacy based on a defective rather than a nonfunctional enzyme, so that those nutritional treatments which affect the penetrance of the gene may do so by altering metabolism in directions more or less favourable for the activity of the defective enzyme protein. What is surprising is the multiplicity of primary nutritional manipulations by which this can be achieved, and this fact seems to suggest that the suppressor locus itself is responsible for metabolic suppression of the effects of the tumor gene. An alternative possibility, namely that *tu-bw*; *su-tu* individuals may be capable of synthesising a truly wild-type enzyme would be excluded on the grounds that suppression can be reversed by dietary environmental manipulations, and even under normal environmental conditions suppression is incomplete. Indeed, several nutritional deficiencies reduce tumor penetrance in the unsuppressed strains; riboflavin and niacin deficiencies in particular are, quantitatively at least, equal in effect to homozygosity for the suppressor locus itself, and in so far as the metabolic conditions favourable for wild-type expression of the tumor gene can be achieved by a variety of means, it is likely that a number of independent mutant genes will prove to be capable of acting as suppressors of *tu-bw*.

The wild-type phenotype depends on a quantitative balance of essential nutritional requirements so that we might anticipate that those nutrients which have a profound effect on the level of penetrance might afford some inference, if only in very general terms, concerning the nature of the defective reaction responsible for the initiation of tumor formation. Five metabolites are relevant in this respect: cholesterol, casein, thiamine, pyridoxine and Mg^{++} (Table 5). Two of these, casein and pyridoxine, point to a disturbance of amino acid metabolism, and the observations of PLAINE and GLASS (1955) and BURNET and SANG (1964 a, b) on the effect of excess dietary tryptophan may be important here, a possibility which is now being examined in greater detail. However, thiamine, Mg^{++} and cholesterol deficiencies are not obviously connected with the others, and when we consider the dietary environmental responses of *tu-K* strain, cholesterol is found to be the sole deficiency so far known to raise tumor penetrance in all strains (Table 6). It is unfortunate that, although cholesterol is an essential nutrient for all insects (HOUSE 1962), little is known about its metabolism in *Drosophila*.

The fact that these two nonallelic tumor genes both give a similar response to at least one of the nutritional deficiencies tested in the present analysis calls for a certain amount of caution at this stage, when seeking for possible connections between the metabolic effects of the different treatments. All of the strains used in the present analysis are isogenic for the same second chromosome *tu-bw*. If this chromosome also carries an isoallelic mutant of the *tu-K* type which is penetrant only under suboptimal environmental conditions (SANG and BURNET 1963a), this would give rise to a mixed array of gene-environment interactions which would be difficult to disentangle. This possibility is by no means remote in the present instance, especially since certain nutritional treatments, including

cholesterol deficiency, induce tumors in the F_1 between the *tu-bw* and *tu-K* strains (BURNET and SANG 1964a). It is known that a number of loci in the second chromosome are capable of initiating tumor formation, and isoalleles of the *tu-K* type do appear to be present in certain other laboratory strains (unpublished data). These considerations point to the necessity for an analysis of possible interactions between *tu-bw* and the genetic background of the second chromosome.

Part of the purpose of this paper is to direct attention to the difference in the extent of the response of the various genotypes to nutritional imbalances of one sort or another. Previous work on inbred lines (SANG 1964) has suggested that strains differ in their growth response to dietary deficiencies or excesses. In this respect, subliminal variation within lines can be exposed by the treatments and followed through crosses. It is then found that different inbred lines have, indeed, different inherited reactions to malnutrition. If tumors are taken as an indicator character, the data presented here give additional support to this conclusion since the strains differ either in the threshold, or intensity, of their responses. Comparison of the *tu-bw* and *tu-K* strains also shows that the pattern of response of the latter is quite different from the former, and this would imply that the genetic lesions in the two involve different steps in the biochemical processes leading to tumor formation.

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SUMMARY

Only five dietary deficiencies (cholesterol, casein, thiamine, pyridoxine and Mg^{++}) increase the penetrance of *tu-bw*, and they are effective whether or not the third-chromosome *su-tu* gene is present. It is concluded that these treatments must modify the metabolic reactions involving the *tu* gene and that they do not act by influencing the suppressor system. Lecithin (or more exactly, choline) and niacin deficiencies affect the suppressed and unsuppressed strains in opposite ways, raising tumor penetrance in the suppressed and lowering it in the unsuppressed strain. However, the former deficiency has inconsistent effects on *tu* strains heterozygous for the *su-tu* gene due, it is assumed, to differences in their genetic backgrounds, and it cannot be concluded that these deficiencies operate by way of the suppressor system. Other deficiencies (sucrose, RNA, riboflavin, pantothenic acid, biotin and folic acid) which slow larval growth to extents similar to the effective treatments, tend to reduce tumor frequency, the extent of the effect depending on the remainder of the genotype. The data are therefore consistent with the conclusion that the *tu* gene causes a metabolic deficiency through forming a defective, rather than a nonfunctional, enzyme, and that the *su-tu* gene (like the nutritional manipulations) modifies the metabolic effects of the *tu* gene. There is no evidence that *su-tu* flies synthesise a wild-type enzyme.

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