

Physiological genetics of melanotic tumours in *Drosophila melanogaster*

VII. The relationship of dietary sterols to tumour penetrance

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SUMMARY

A strain of *Drosophila*, homozygous for the variably penetrant gene *tu bw*, which causes the formation of abnormal masses of melanizing haemocytes (melanotic tumours), has been reared on defined axenic diets containing various sterols, both singly and in pairs. Both optimal and deficient nutritional levels of sterol have been employed, as well as certain sterols inadequate by themselves to support development. The effect of these diets upon probability of tumour formation has been studied in relation to their nutritional adequacy, as defined by the growth-rate, survival, and in one case, adult body-weight.

The results demonstrate a rather complex pattern of interaction between dietary sterols in determining the variables of the phenotype produced, under circumstances suggesting that all the sterols investigated have entered the developing larvae. There is only a partial overall correlation, and occasionally an inverse relationship, between tumour suppressant and growth-promoting properties of particular sterols. Within single molecules, structural sterol features tend to exert their characteristic effects additively upon the phenotype, except for an inadequacy in utilization of molecules containing the Δ_7 double bond, which dominates at low concentrations. With pairs of dietary sterols, however, non-additive or 'saving' effects are sometimes seen.

It is suggested that the *tu bw* allele allows the resolution of several discrete developmental functions for sterols and/or their immediate metabolic products in *Drosophila*, which cannot synthesize its own sterol. However, the molecular nature of these functions is little understood.

1. INTRODUCTION

Melanotic tumours in *Drosophila* are aggregates of haemocytes which form about the time of the second larval ecdysis due to the precocious transformation of these cells from the rounded plasmatocyte form into flattened lamellocytes (Rizki, 1960). Melanization of the mass occurs later, just prior to pupation, and the discrete 'tumours' can readily be scored in pupae or adults. Whether or not these melanomas form depends on the presence of one or other of a number of non-allelic tumour (*tu*) genes (Lindsley & Grell (1968)) and on the larval environment prior to the end of second instar (Burnet & Sang, 1963). The system is thus a complex one, with

responding cells (haemocytes) undergoing a morphogenetic change at a specific time in an environmentally and genetically modifiable metabolic milieu. It is a typical developmental system, but one which is amenable to experimental manipulation, and to the quantitative measurement of response. One purpose of this paper has been to explore how such a system reacts to specific treatments, since we have few models on which to base our ideas about the regulation of morphogenetic changes.

Penetrance of melanotic tumour genes is readily modified by alterations in the amounts of some, but not all, constituents of the larval diet (summary in Sang, 1969). Cholesterol is one such essential nutrient affecting the *tu bw* strain: a deficient supply raises tumour penetrance. However, as van't Hoog (1935) first reported, developing larvae can use other sterols than cholesterol, and Cooke & Sang (1970) have recently shown that they grow best when fed phytosterols. As shown below, they then have fewer tumours. So tumorigenesis depends on the quality as well as the quantity of dietary sterol, and this relationship has been examined for a number of sterols. The initial assumption was that a knowledge of the interrelation between sterol structure and tumour penetrance might indicate the role sterols play in tumour formation. For instance, it seemed likely that certain sterols would be more readily converted than others to the hormone ecdysone (Robbins, Kaplanis, Svoboda & Thompson, 1971), and that the levels of this hormone might determine tumourigenesis, as Burdette (1954), Rizki (1960) and others have suggested. Unfortunately, too little is yet known about the significance of sterols in insect metabolism for this expectation to be fulfilled. Our results suggest that sterols have more, and probably more complex, functions than have yet been elucidated by conventional studies, and in this respect, examination of the sensitive parameter of mutant gene penetrance has been more revealing than measures of growth and survival.

2. MATERIALS AND METHODS

The *tumour brown* strain (*tu bw*) was used. Larvae were reared on axenic diets as described by Cooke & Sang (1970), except that dietary lecithin was replaced by 0.004 % choline. When Δ , sterols were tested, 0.1 % ascorbic acid was included in the diet; it had no effect on larval growth. Each treatment involved setting up 50 germ-free larvae in each of five replicated cultures. The adults which hatched from the cultures were cleared in fructose solution (Sang, 1966) and a record made of those with tumours. The tumour penetrance was scored as the percentage of individuals in the total population carrying tumours, and the significance of differences was assessed by the usual χ^2 test. Development was measured for the larval period in log-days, for reasons already given (Sang, 1956), and differences between populations gauged by *t*-test.

Table 1. *Effects of feeding different sterols on tumour penetrance, and on development rate*

| Sterol | Tumour penetrance (%) | Development rate | Structural departure from cholesterol |
|-------------------------|-----------------------|------------------|--|
| 1. Cholesterol | 58 | +++ | — |
| 2. Cholestanol | 92 | ++ | Δ_5 saturated |
| 3. 7-Dehydrocholesterol | 12 | +++ | Δ_7 added |
| 4. Lathosterol | 37 | ++ | Δ_7 added and Δ_5 saturated |
| 5. Ergosterol | 1 | ++++ | Δ_7 , Δ_{22} , and C_{24} methyl added |
| 6. β -Sitosterol | 3 | ++++ | C_{24} ethyl added |
| 7. Stigmasterol | 17 | ++++ | C_{24} ethyl added and Δ_{22} added |
| 8. Ostreasterol | 20 | ++ | C_{24} methylene added |
| 9. Desmosterol | 26 | + | Δ_{24} added |
| 10. 25-Norcholesterol | 26 | ++ | C_{25} methyl replaced by keto group |

All sterol samples were fed at 0.03% of the diet. All tumour percentages are significantly different from the cholesterol control level. Development rates are taken from Cooke & Sang (1970), where +++ is the rate with cholesterol and improvement indicated by an additional +, and slower growth by fewer +'s.

3. RESULTS

The ten sterols listed in Table 1 all support larval growth, but when fed in the same amounts they do so with different efficiencies; the three phytosterols being superior, and the remainder inferior, to cholesterol and 7-dehydrocholesterol, which are equivalent. The individual sterols induce more or less characteristic tumour levels when fed as sole dietary sterol, and there is a rough relationship between the larval development rate which they support and tumour frequency. Prolonged development is associated with high tumour percentages, and vice versa.

The consequences of modifications of the cholesterol molecule can be assessed more exactly by comparing pairs of molecules having only single differences between them (Table 2). Saturation of the C_5 double bond slows development and raises tumour penetrance by about 30% average. A double bond at C_{22} also raises tumour frequency, but without causing a significant growth rate alteration. All the other listed modifications lower tumour penetrance: the C_7 double bond addition reduces tumours by 50% (but has no effect on development rate), addition of substituents, or of a double bond, at C_{24} also lowers tumour penetrance, but may either slow, or improve, growth rate. The keto group substitution at C_{25} slows growth and lowers tumour incidence. Thus there is no direct connexion between growth rate and tumour penetrance, but an array of characteristic consequences of particular molecular structures. The data (Table 2) also suggest that these structural alterations act additively when combined in one molecule (see also ergosterol in Table 1), but the range tested is insufficient to prove this.

Since we are concerned essentially with alterations of the cholesterol molecule at C_5 , C_7 and C_{24} , subsequent experiments have paid particular attention to

Table 2. *The influence of individual structural changes of the cholesterol molecule on tumour penetrance and on development*

| Structural change | Comparison | Percentage effects on tumours | Development rate |
|---|------------|-------------------------------|------------------|
| C ₅ double bond saturated | 2-1 | + 34 | — |
| | 4-3 | + 25 | — |
| C ₇ double bond added | 3-1 | - 46 | 0 |
| | 4-2 | - 55 | 0 |
| C ₂₂ double bond added | 7-6 | + 14 | 0 |
| C ₂₄ double bond added | 9-1 | - 32 | - - |
| C ₂₄ ethyl added | 6-1 | - 55 | + |
| C ₂₄ methylene added | 8-1 | - 38 | — |
| C ₂₅ methyl replaced by keto | 10-1 | - 32 | — |

Comparisons are of the sterols as numbered in Table 1. Development is improved (+), little affected (0), slowed (-) or greatly slowed (- -) by the structural change. The pair of C₅ double bond saturation values are not significantly different from one another, nor is the pair of values for C₇ double bond additions. All the pair comparisons are statistically significant at the 1% level.

cholestanol (dihydrocholesterol), 7-dehydrocholesterol and β -sitosterol, each of which has a simple, single modification at the indicated positions (Table 1). There is evidence that part of the intake of the two latter sterols is converted to cholesterol by *Drosophila* larvae, but there is no evidence for the converse, or that cholestanol is changed (Cooke & Sang, 1970). Dose responses to these sterols are given in Fig. 1.

Reduction of the amount of each sterol fed to larvae increases tumour frequency, and the relationship is curvilinear with dose. Three of the sterols behave similarly, each having its own characteristic response level: the response to 7-dehydrocholesterol is different in character (Fig. 1A). The same pattern is followed with respect to larval development (Fig. 1B), and it is worth noting that about 0.004% β -sitosterol is almost as effective for growth as 0.064% cholesterol. Likewise, about 0.004% cholesterol is about as good as 0.064% cholestanol. And approximately the same relationships hold for tumour penetrance. Consequently there is linear relationship between development rate and tumour penetrance (Fig. 1C), up to levels where most flies are tumourous. This result implies that the three sterols are required for some common function, and that they have different capabilities for meeting it, β -sitosterol being superior to cholesterol which is better than cholestanol. However, a deficiency of one sterol is equivalent to some deficiency of another, both for development rate and for tumours. This common function cannot be just 'a cholesterol rôle' since it seems satisfied by cholestanol which is not converted to cholesterol; that is, it is more likely to be a general sterol function (bulk role) which can be met by all three sterols. They would then also have a particular function dependent on their structural characteristics.

The dose response to 7-dehydrocholesterol follows a different pattern. Low dietary supplies, up to about 0.008%, give mostly tumourous flies and a slow

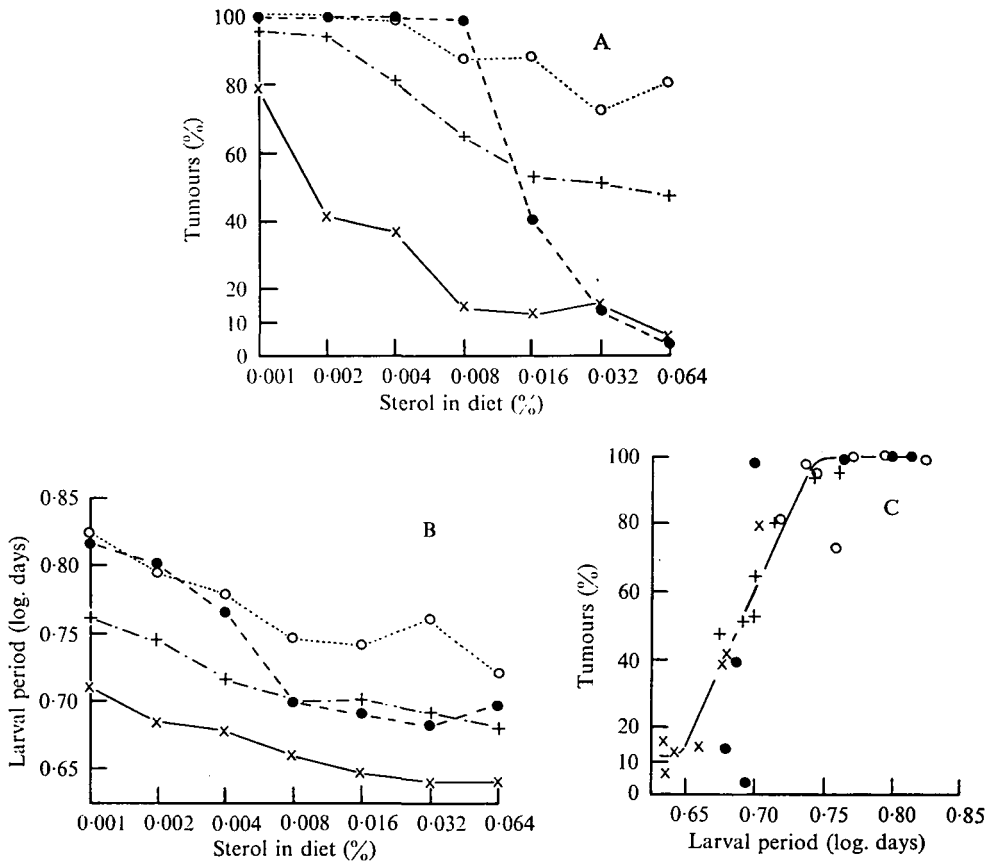


Fig. 1. The effects of sterol provision on growth rate and on tumour penetrance. The sterols were provided as sole sterol in a lecithin-free medium. + = cholesterol, ● = 7-dehydrocholesterol, ○ = cholestanol and × = β -sitosterol. (A) Tumour penetrance responses, (B) larval development rate responses and (C) the relationship between larval development rate and tumour penetrance.

larval development, but amounts greater than this dramatically lower tumour frequency and improve growth rate. At the highest level tested tumours are as infrequent as when β -sitosterol is fed, and development is as rapid as with cholesterol (but not as with β -sitosterol). This again implies that the system we are concerned with has at least two functional components. The dose-response to ergosterol has the same form as that for 7-dehydrocholesterol, confirming that the C₇ double bond is the cause of the difference from the other sterols (Fig. 2). However, ergosterol gives faster growth than cholesterol, and is equivalent to β -sitosterol in this respect. So the methyl (or ethyl) group at C₂₄ is important for growth, which it improves (see also Table 1).

These results suggested that mixtures of two sterols might not have average properties, as assayed by growth rate and by tumour frequency. Table 3 summarizes the outcome of tests using combinations of the four typical sterols each

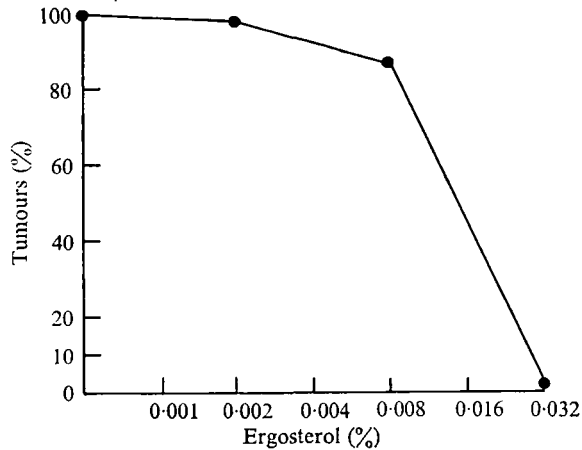


Fig. 2. Tumour response to various levels of dietary ergosterol.

Table 3. *The effects of feeding mixtures of sterols*

| Sterol and level fed | Tumour frequency (%) | | Development time (log days) | |
|-------------------------|----------------------|------------|-----------------------------|------------|
| | 0.008 % | 0.004 % | 0.008 % | 0.004 % |
| 1. Cholesterol | 55.2 | 72.5 | 0.768 | 0.785 |
| 2. 7-Dehydrocholesterol | 81.2 | 94.4 | 0.786 | 0.825 |
| 3. Cholestanol | 92.4 | 97.6 | 0.890 | 0.813 |
| 4. β -Sitosterol | 12.9 | 26.8 | 0.738 | 0.757 |
| Sterol mixture | Found | Calculated | Found | Calculated |
| 1 and 2 | 61.3 | 68.2 | 0.764 | 0.777 |
| 1 and 3 | 82.8 | 73.8 | 0.805 | 0.829 |
| 1 and 4 | 32.6 | 34.1 | 0.735 | 0.753 |
| 2 and 3 | 65.2** | 87.2 | 0.768* | 0.838 |
| 2 and 4 | 13.1** | 47.5 | 0.719* | 0.762 |
| 3 and 4 | 33.8** | 52.6 | 0.744* | 0.814 |

The sterol mixtures contained 0.004 % of each sterol, and are therefore compared with the calculated average for the values of single sterols fed at 0.008 %.

* = significant at the 5 % and ** at the 1 % level in this, and subsequent, tables.

provided at the sensitive level of 0.004 %. Combinations which included cholesterol were indistinguishable from the calculated average, either for development rate or for tumours. That is, these combinations apparently behave as simple mixtures (suggesting no significant differences in the uptake of the sterols) giving additive effects. The three remaining mixtures give faster growth and fewer tumours than the calculated average, and thus are not additive in action. The most interesting of the three is the mixture of 0.004 % 7-dehydrocholesterol and 0.004 % β -sitosterol, which gives a growth rate and tumour frequency indistinguishable from 0.008 % β -sitosterol. That is, 7-dehydrocholesterol and β -sitosterol are interchangeable with respect to some sterol function(s) in this combination. This interchangeability is not found for either sterol in combinations with cholesterol or with cholestanol.

Table 4. *Cholestanol-sterol interactions*

| Sterol | Tumours (%) | Development time (log days) |
|---|-------------|-----------------------------|
| 0.008 % Cholestanol | 82.3 | 0.721 |
| 0.008 % Cholestanol + 0.02 % cholestanol | 90.2 | 0.729 |
| 0.008 % Cholestanol + 0.02 % cholesterol | 75.2 | 0.691* |
| 0.008 % Cholestanol + 0.02 % 7-dehydrocholesterol | 57.6** | 0.684* |
| 0.008 % Cholestanol + 0.02 % β -sitosterol | 51.2** | 0.711 |
| 0.002 % Cholestanol | 100.0 | 0.761 |
| 0.002 % Cholesterol | 81.3 | 0.738 |
| 0.002 % 7-Dehydrocholesterol | 75.0 | 0.723 |
| 0.002 % β -Sitosterol | 88.9 | 0.755 |
| 0.006 % Cholesterol - control | 54.9 | 0.682 |

Only significant differences from 0.008% cholestanol indicated, as before.

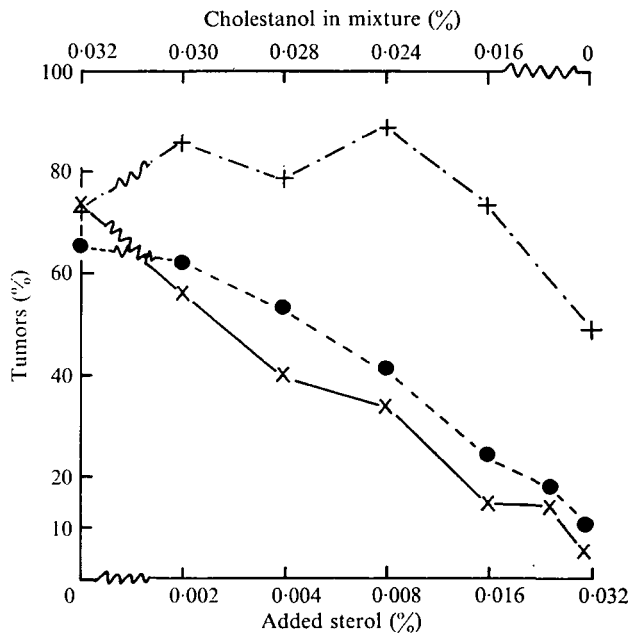


Fig. 3. Responses to 7-dehydrocholesterol and β -sitosterol in the presence of cholestanol, the total medium and sterol content being kept at 0.032%. ● = 7-dehydrocholesterol, x = β -sitosterol and + = cholesterol.

However, it is interesting that combinations of 7-dehydrocholesterol with cholesterol or cholestanol (1 and 2, 2 and 3) give identical results, as do combinations of β -sitosterol with these two sterols (1 and 4, 3 and 4). Both results imply that cholesterol and cholestanol can perform some function equally, and that 7-dehydrocholesterol and β -sitosterol are then used for another function intimately related to improved growth and few tumours. As before, Table 3 indicates a correlation between tumour development and growth rate.

Both β -sitosterol and 7-dehydrocholesterol are converted to cholesterol by

Table 5. *Effects of addition of some non-nutrient sterols to a low-cholesterol medium*

| | Tumours (%) | Survival (%) |
|---|-------------|--------------|
| 1. Control | 79.8 | 57.6 |
| 2. + Cholesterol | 66.9 | 74.0 |
| 3. + Cholesta-5en-3one | 81.7 | 61.2 |
| 4. + Pregnenalone | 70.8 | 61.3 |
| 5. + Cholesteryl chloride | 89.5** | 52.8 |
| 6. + Coprostanol | 98.8** | 49.8 |
| 7. + Δ_7 -coprostanol | 96.8** | 61.6 |
| 8. + Cholesta-3 β -5 α -6 β -triol | 100.0** | 55.2 |

The control contained 0.004% cholesterol and all additions were of the same amount. 250 larvae were set up for each test and survival was measured as the proportions of adults emerging. Tumours in pupae and adults were scored.

Drosophila larvae (Cooke & Sang, 1970). Consequently, we cannot be certain how far the interactions detailed in Table 3 depend on this conversion, and to what extent on the properties of the sterols themselves. Certainly the data strongly suggest that second possibility rather than the first. Since cholestanol is not converted to cholesterol in any quantity, it is possible to examine this problem further, and more sensitively, by adding small amounts of each sterol to a fixed amount of cholestanol. If the previous deductions are correct, we should expect little effect from adding cholesterol, and considerable tumour reduction from 7-dehydrocholesterol and β -sitosterol supplements. Table 4 shows that this is, indeed, the case. The addition of cholesterol at 20% of the mixture does not lower tumours significantly, although it does improve development rate somewhat. Both 7-dehydrocholesterol and β -sitosterol, at the same level, have marked effects on tumour penetrance, but only 7-dehydrocholesterol improves development. The action of each sterol must therefore depend on its molecular structure and be independent of any conversion to cholesterol. Conversely, Table 3 also shows that an equal provision of cholestanol permits 7-dehydrocholesterol and β -sitosterol (but not cholesterol) to function as tumours suppressing sterols.

These data imply that cholestanol can meet one sterol function but not another, and that this second function may be the one relevant to tumourigenesis. They also suggest that only relatively small amounts of the anti-tumourigenic sterols may be needed to 'cure' tumours when the first sterol function is being satisfied with cholestanol. This second possibility was further examined by feeding a range of mixtures, the total sterol provision being kept at 0.032% (Fig. 3). Responses to both 7-dehydrocholesterol and β -sitosterol are then proportional to the log amount of the dietary supply of each; that is, it is not the case that only a limited provision of either is needed to lower tumours. However, in neither case does the sterol behave as if it were a mixture with cholestanol giving an average tumour level; in both cases tumours are always lower than this prediction (e.g. at 0.016%). When compared with Fig. 1A, the response to β -sitosterol is less in the mixture than with β -sitosterol alone, showing that cholestanol competes with the latter to

Table 6. Interactions between Δ_7 -coprostanol and the four test sterols

| | Nil addition | | With Δ_7 -coprostanol | |
|----------------------|----------------|-----------------|------------------------------|-----------------|
| | Tumours (%) | Survival (%) | Tumours (%) | Survival (%) |
| Cholesterol | 39.7 | 76.8 | 48.2* | 64.2* |
| 7-Dehydrocholesterol | 11.3 | 81.3 | 7.3 | 80.0 |
| Cholestanol | 75.3 | 60.8 | 93.6* | 58.6 |
| β -Sitosterol | 18.4 | 69.7 | 18.1 | 76.6 |

The major sterol was provided in optimal amount (0.06%) and the Δ_7 -coprostanol at 0.008%. Significant differences at the 5% level are starred.

Table 7. Responses to desmosterol

| Sterol (%) | Tumour penetrance (%) | Development time (log days) | Male body weight (mg) |
|--------------------------------------|-----------------------------|-----------------------------------|--------------------------------|
| Cholesterol control 0.06 | 61.0 | 0.847 | 0.48 \pm 0.01 |
| Desmosterol 0.06 | 22.0 | 0.921 | 0.51 \pm 0.01 |
| Cholesterol 0.002 | 95.0 | 0.911 | 0.39 \pm 0.02 |
| Desmosterol 0.06 + cholesterol 0.002 | 41.5** | 0.859* | 0.50 \pm 0.01 |

The significance of the cholesterol addition to desmosterol only is indicated. All tumour penetrances are different. There is no growth rate difference between desmosterol 0.06% and cholesterol 0.002%, or between the cholesterol control and desmosterol + cholesterol.

an extent, but perhaps more importantly, that (as judged by tumour penetrance) small amounts of β -sitosterol alone (0.002–0.008%) can meet all needs as effectively as when supplemented with cholestanol. This is not the case with 7-dehydrocholesterol, which is more effective in the presence of cholestanol. Thus, β -sitosterol and 7-dehydrocholesterol, although they behave similarly, can be shown to act differently (see also Sang, 1973). Mixtures with cholesterol are even more strikingly different (Fig. 3); cholesterol as half the mixture has no tumour lowering effect.

Some sterols are not used by *Drosophila* when provided as sole sterol source (Cooke & Sang, 1970). A number of these were fed along with cholesterol, which was provided at a level giving a sensitive tumour frequency (Table 5). Pregnenalone and cholesta-5en-3one were without action, and all other additions raised tumour frequency, but without influencing survival (except for coprostanol). That is, these sterols are utilized in the presence of cholesterol and are tumourigenic. The coprostanols are the most interesting of this group since they have a different steric configuration from the others. For this reason it seemed possible that they might be competing with cholesterol and functioning by blocking its utilization. So a further test was run using optimal amounts of the major sterols (Table 6). As before the Δ_7 -coprostanol raised tumours and had an effect on survival when fed along with cholesterol. More interestingly, however, it had a greater effect when provided with cholestanol, and no effect with 7-dehydrocholesterol or with β -sitosterol. So Δ_7 -

coprostanol, itself tumorigenic, can compete with major sterols: the effective order being the inverse of that previously listed.

We have ignored sterols which slow growth but lower tumour penetrance. Desmosterol is particularly interesting in this context since it appears to be an intermediate in the conversion of β -sitosterol to cholesterol in some insects (Thompson, Svoboda, Kaplanis & Robbins, 1972). Unlike β -sitosterol, desmosterol prolongs larval growth compared with cholesterol (Table 7). Thus it seems unlikely that the β -sitosterol \rightarrow desmosterol \rightarrow cholesterol pathway is found in *Drosophila melanogaster*. However, desmosterol is used, and the flies formed are normal in size and have relatively few tumours. Generally, slow development (as with 0.002% cholesterol in Table 6) is associated with small size and high tumour penetrance. Desmosterol has thus two of the properties of β -sitosterol, but not the third. A small addition of cholesterol (one thirtieth) corrects this, to give more or less normal development and size, but at the expense of a significant increase of tumour penetrance. Unfortunately, the sample of desmosterol used in these tests was insufficient to allow the problem to be pursued further.

4. DISCUSSION

The starting point of this study was the observed dependence of tumour penetrance upon the amount of cholesterol fed to the *tu bw* strains of *Drosophila*. This observation has been confirmed and extended to three related sterols, when lowering the provision always raises tumour penetrance (Fig. 1). Consequently, we may conclude that insufficient utilizable dietary sterol raises the probability of tumour formation. Since there is then also a correlation between tumour penetrance and larval growth rate, both characteristics might be explained by the formation of inadequate sterol-dependent structures, or an insufficient sterol supply for particular processes. However, the kind of sterol fed also affects both parameters, and we are therefore concerned with both the quantity and the quality of sterol fed, which is also likely to imply defective structures or processes. Apparently these are of no importance for tumourigenesis in the absence of the *tu bw* gene, since even low doses of the most tumourigenic sterol (cholestanol) have no effect on wild type larvae (Ore R), although such larvae grow very slowly. Hence, we are dealing with an event determined by the *tu bw* gene and modulated by the sterol provision, and which is growth independent, *sensu stricto*.

The importance of sterol structure is well emphasized by Table 2 which shows, for instance, that addition of a C₇ double bond to the cholesterol molecule lowers tumours without affecting growth rate, that a C₂₄ ethyl group similarly lowers tumour penetrance but improves growth, that a C₂₄ double bond lowers tumours but slows growth, and so on. Non-nutrient sterols fed along with a minimal amount of cholesterol (Table 5) make the same point, namely, that sterol functions with respect to growth and tumourigenesis can be separated. Finally, Table 7 (desmosterol) suggests that larval size, growth rate and tumour penetrance may all be divorced from one another. Different sterols have different capabilities as judged by the phenotypic character studied (i.e. by function).

When sterol molecules differ from one another at more than one structural site, our limited evidence (Tables 1 and 2) suggests that the structures behave additively with respect to tumour penetrance. This is not the case for mixtures of sterols. Mixtures show that if one function is met by one sterol, a second function (as measured by tumour penetrance) may be disproportionately modified by a small supplement of another sterol (Tables 5 and 6). This implies that sterols satisfy macro- and micro-rôles, as has been suggested from purely nutritional studies with insects (Clark & Bloch, 1959; Clayton, 1964); but it also suggests some active partitioning of sterols in metabolism, about which we know nothing (Sang, 1973). Multiply modified sterols are not handled in this way, apparently, so they must function largely as intact molecules. Taken together, these results indicate that sterols modulate tumour penetrance by way of their micro-rôle, and that they may then function as intact molecules.

There are two main possible micro-rôles, one structural (and hypothetical) and the other as a precursor of some active product required in small amount. The only likely candidate for the second class is the hormone ecdysone (Sang, 1970) which would be expected to act antagonistically to juvenile hormone, which is already known to be tumourigenic (Bryant & Sang, 1969). Tests with ecdysone and ecdysterone fed to larvae, either *ab initio* or just prior to the time when tumours are formed (48 h of larval life according to Burnet & Sang, 1963), have given ambiguous results, implying either that the hormone is broken down if fed, or that it is not involved in tumour formation. This latter conclusion is not in agreement with Burdette's (1954) report that head ligation of larvae (i.e. reduction of ecdysone to the body) raises tumour penetrance. So the former conclusion is more probable, and the significance of ecdysone for tumour formation is still uncertain.

Ecdysone is synthesized *in vivo* from cholesterol or 7-dehydrocholesterol (Galbraith, Horn, Middleton & Thomson, 1970), and Thompson *et al.* (1972) suggest that dietary cholesterol is first converted to 7-dehydrocholesterol, which is the direct precursor of the ecdysones. This might imply that feeding 7-dehydrocholesterol would give fewer tumours than cholesterol, as is found. But it is not compatible with the high tumour penetrances formed at low dietary levels of 7-dehydrocholesterol, compared with cholesterol (Fig. 1), or with the anti-tumour effect of β -sitosterol.

Thompson *et al.* (1972) have reviewed sterol metabolism in insects, particularly of species like *Drosophila* which can use phytosterols. Their elegant studies with *Manduca sexta* show that β -sitosterol, campesterol and stigmasterol may all be converted to cholesterol, via desmosterol, and thence to 7-dehydrocholesterol: and they view this as the general case. The phytosterols, on the previous argument, might thus be expected to be more tumourigenic than cholesterol and 7-dehydrocholesterol, which they are not. Although β -sitosterol can be converted to cholesterol (Cooke & Sang, 1970), our incomplete data suggest that this complex pathway is not found in *Drosophila* (Table 7), since desmosterol is not equivalent to any of the sterols listed. Further, the improved growth rate of larvae fed phytosterols (Table 1) and the low tumour levels then found, can be accounted for only

if we assume that they are directly used. The interactions with other sterols (and of desmosterol with cholesterol) suggest that this involves a sterol structural rôle. As we have already noted, this would probably be a micro-rôle and not a bulk function. The various rôles of sterols in insects await study (see also Clayton, 1964), but the data presented here suggest that they are important with respect to size, development rate, and tumour penetrance.

Our results with cholestanol support these conclusions. When it is provided as sole sterol, larval development is slow, tumour penetrance high, and the emerging adults lay inviable eggs in small numbers: cholestanol is an unsatisfactory nutritional sterol, which is apparently not converted in any amount to other more useful sterols. A small addition of 7-dehydrocholesterol, or of β -sitosterol to cholestanol lowers tumour penetrance, whereas cholesterol does not, but only 7-dehydrocholesterol improves growth. Thus, micro-amounts of particular sterols are again effective, but variously for different functions. The inadequacy of cholestanol is increased when the supply is reduced, and the same is true of the other sterols. Inadequate sterol structures may be formed either from poor sterols or through an insufficient sterol provision; both will lead to slow growth and to a proneness to tumour formation.

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