THE EFFECT OF TRYPTOPHAN ON THE PENETRANCE OF TUMOROUS HEAD IN CROSSES AMONG SELECTED STOCKS OF *DROSOPHILA MELANOGASTER**

JOHN R. SIMMONS¹ AND ELDON J. GARDNER

Utah State University, Logan, Utah

Received June 10, 1957

THE tumorous head phenotype and genetic mechanism have been described by several investigators (GARDNER 1948; GARDNER and WOOLF 1949; NEWBY 1949; GARDNER and RATTY 1952). Two major genes associated with the trait were located from linkage data in the first and third chromosomes (STOTT and GARDNER 1952). A maternal effect (GARDNER and GARDNER 1953) was detected from reciprocal outcrosses. Modifiers (GARDNER and STOTT 1951; GARDNER, STOTT and DEARDEN 1952) were found to be widespread in laboratory stocks and wild populations. Alleles of the sex-linked gene were identified in three laboratory stocks. Increased temperature (GARDNER and WOOLF 1950) from 18° C to 30° C was associated with a progressive increase in penetrance. A temperature effective period was identified during the first 24 hours of development.

PLAINE and GLASS (1955) showed that L-tryptophan added to a complete food medium increased the expression of larval melanotic tumors and also the erupt eye phenotype which is similar to one expression of tumorous head. Other investigators (HINTON *et al.* 1951; MITTLER 1952) have also reported that tryptophan in high concentrations produces phenotypic abnormalities including multiple melanotic tumors and eye malformations. Hydrogen peroxide (PLAINE 1955a) increased oxygen tension and X-rays (PLAINE 1955b; GLASS and PLAINE 1952; PLAINE and GLASS 1952) also increased tumor incidence in the suppressor-erupt stock. The action of X-rays on tryptophan was more pronounced when combined with increased oxygen tension. A considerable amount of information on the metabolism of tryptophan has been accumulated (KIKKAWA 1950, 1953; MEHLER 1955; PLAINE 1955; WHITE *et al.* 1954). The present study was undertaken to determine the effect of L-tryptophan and related compounds on the tumorous head phenotype and the maternal effect.

MATERIALS AND METHODS

The inbred tumorous head (*tu-h*) stock maintained in this laboratory for several years was used in the experiments. Other laboratory stocks involved in the crosses were Canton and Stephenville. Canton had been shown to behave like most other stocks when crossed with *tu-h*. Stephenville had been shown to carry an allele (*tu-1*^s) of one of the *tu-h* genes (*tu-1*). A derived Stephenville stock

* Aided by grants from the American Cancer Society, Damon Runyon Memorial Fund and Utah State University Research Funds.

¹ Present address: California Institute of Technology, Pasadena, California.

which did not carry *tu-1^s* was also used in the experiments. The Stephenville stock carrying *tu-1^s* was identified as Stephenville¹ and the stock without *tu-1^s* was designated Stephenville².

Before testing the effect of L-tryptophan and other chemicals it was necessary to determine the present penetrance level of tumorous head under normal laboratory conditions. It was also necessary to explore the relation between the age of the culture and the penetrance level. First a series of six cultures was prepared from the inbred *tu-h* stock and maintained in half pint bottles at room temperature (23°–28° C) on the standard corn meal-molasses-agar medium. The progeny were removed at 24-hour intervals and examined under a magnification of 45×. Flies showing any irregularity of the eyes or other parts of the head were classed as abnormal. Some bottles were held from 27 to 43 days to observe the effect of the age of the culture on penetrance.

For the second series, Petri dishes were prepared with the standard laboratory medium. Large numbers of males and females expressing the tumorous head trait were selected from the inbred stock bottles and placed in ring laying cages 7.5 cm in diameter and 1 cm high, similar to those used by KING (1955). The ring was covered with dacron netting which was fine enough to retain the flies but allowed access to the food for oviposition. Cultures were kept moist in a constant temperature incubator at $25 \pm 1^\circ$ C. At 24-hour intervals the parent flies were transferred to fresh dishes. The adult flies which emerged from the cultures were etherized and examined in the usual way. By use of the laying cages it was possible to maintain the same group of parents for the 41-day period of the experiment and thus to insure a uniform genetic background for all experimental cultures. Environmental conditions were controlled as well as possible to maintain uniform conditions and thus eliminate many environmental variables.

For the treatment with tryptophan and other chemicals, brewer's yeast was used in the medium and live yeast was not added. Brewer's yeast was shown by PLAINE (1952) to supply a complete medium for *Drosophila*. The control medium was prepared with 5 percent brewer's yeast and 1.5 percent agar. Compounds to be tested were added to this medium. A 0.5 percent concentration of tryptophan was used, higher concentrations, in solution, having been shown in preliminary experiments and by PLAINE and GLASS (1955) to be toxic. Indole was added at 0.01 percent, DL-serine at 0.5 percent and L-cysteine at the 0.1 percent level.

Crosses employed to test the effects of the chemicals on the penetrance of tumorous head and the maternal effect were as follows (female listed first): Canton $\times$ Canton, Canton $\times$ *tu-h*, *tu-h* $\times$ Canton, Stephenville¹ $\times$ Stephenville¹, Stephenville¹ $\times$ *tu-h*, *tu-h* $\times$ Stephenville¹, Stephenville² $\times$ Stephenville², Stephenville² $\times$ *tu-h*, *tu-h* $\times$ Stephenville², and *tu-h* $\times$ *tu-h*. Four to six replications were included in each experiment.

EXPERIMENTAL RESULTS

The results of experiments designed to determine the present penetrance level of the tumorous head stock are summarized in Table 1. From the total of 2382

flies classified from the same six cultures at daily intervals for 13 days an average of 93.4 percent showed the tumorous head phenotype. Among the 1097 females 93.5 percent were abnormal compared with 93.3 percent for the 1285 males. Considerable variation was observed in the penetrance represented by the daily counts of individual cultures. Penetrance values above the mean and those considerably below were usually associated with small numbers of flies. When averages were considered over the 13-day period, the penetrance levels were consistent.

TABLE 1

Penetrance in inbred tu-h cultures at room temperature. Averages of six separate cultures from which all flies were removed daily

24-hour interval	Total flies classified	Avg. % abn. (6 cultures)
1.....	42	90
2.....	271	96
3.....	308	94
4.....	373	96
5.....	333	93
6.....	237	93
7.....	146	94
8.....	168	95
9.....	143	83
10.....	126	92
11.....	95	93
12.....	71	93
13.....	69	93
	2382	93.4

When the flies were not removed from the cultures each day but were allowed to remain in the bottles for long periods of time, the penetrance decreased. Bottles 27 days old had an average of 74 percent abnormal flies. Those 38 days old showed an average of 67 percent and those 43 days old 61 percent. None of the individual cultures 27 days old or older had a penetrance exceeding 76 percent.

The next experiment, designed to investigate the cause of the decrease in penetrance associated with culture age, was carried out with laying cages. Parent flies from the inbred *tu-h* stock were transferred daily for 41 days to new food and allowed to deposit eggs. The number of eggs laid in the different 24-hour intervals varied considerably, but sufficient numbers were obtained during each period to provide meaningful penetrance values. From the total of 2664 adult flies classified from the 41 cultures, an average of 96.1 percent was abnormal. Among the 1278 females 96.6 percent and among the 1386 males 95.7 percent was abnormal. The cultures in the lower range were distributed through the 41-day period. Average penetrance levels were as high at the end of the experiment as at the beginning, indicating that the age of the parents had no effect on the penetrance level in the progeny.

For testing the effects of L-tryptophan, indole, DL-serine, and L-cysteine, reciprocal crosses involving *tu-h* and wild stocks, Canton and Stephenville, were carried along with the four inbred line crosses; i.e., Canton, Stephenville¹, Stephenville², and *tu-h*. Reciprocal crosses were used to detect any influence which the chemicals might have on the maternal effect which would be observable in the F_1 . The results of the control crosses maintained on the brewer's yeast-agar medium are summarized in Table 2. The maternal effect was indicated in all crosses in which *tu-h* or Stephenville¹ females were involved. From the cross between *tu-h* females and Canton males for example, 39 percent of the progeny was abnormal. No abnormal flies were observed in the results of the reciprocal cross between Canton females and *tu-h* males.

TABLE 2

Penetrance of tumorous head among F_1 progeny from different crosses maintained on control medium

Cross†	Females		Males		Total	
	n*	%‡	n	%	n	%
Can × Can	311	0	278	0	589	0
Can × <i>tu-h</i>	453	0	507	0	960	0
<i>tu-h</i> × Can	487	42	417	35	904	39
Steph ¹ × Steph ¹	442	0	490	0	932	0
Steph ¹ × <i>tu-h</i>	395	46	452	47	847	47
<i>tu-h</i> × Steph ¹	206	62	156	41	362	53
Steph ² × Steph ²	477	0	435	0	912	0
Steph ² × <i>tu-h</i>	417	0	391	0	808	0
<i>tu-h</i> × Steph ²	257	51	210	33	467	43
<i>tu-h</i> × <i>tu-h</i>	216	96	320	94	536	95

† The female parent is listed first in each cross.

*n = total number of flies.

‡% = percentage of abnormal flies to the nearest whole number.

When Stephenville¹ females were mated with the *tu-h* males 47 percent of the progeny was abnormal. From the reciprocal cross between the *tu-h* females and the Stephenville¹ males 53 percent was abnormal. When *tu-h* females were crossed with Stephenville² males 43 percent of the progeny was abnormal. No abnormal flies resulted from the reciprocal cross between Stephenville² females and *tu-h* males. The inbred *tu-h* females crossed with males from the same stock produced 95 percent abnormal progeny. The development of the flies on the brewer's yeast-agar medium was normal, with eclosion occurring at the usual time. Ample food was available in each culture throughout the developmental period. Penetrance levels in the *tu-h* × *tu-h* control were comparable with those of the preliminary experiments cited above.

When the same crosses were made and the cultures were maintained under the same environmental conditions except that 0.5 percent L-tryptophan was added to the medium, the penetrance was significantly decreased. The results are summarized in Table 3. Averages of 22, 21, 28, 30, and 71 percent penetrance were

obtained from the crosses: *tu-h* × Canton, Stephenville¹ × *tu-h*, *tu-h* × Stephenville¹, *tu-h* × Stephenville², and *tu-h* × *tu-h*, respectively. From the crosses involving *tu-h* or Stephenville¹ females the penetrance levels were about 20 percent below those of the controls with no additive in the medium. By using 2 × 2 contingency tables, the results were tested with the chi-square method for goodness of fit. The differences were significant at the one percent level. It is, therefore, improbable that the differences between the control and treatment percentages were due to chance alone. The addition of 0.5 percent L-tryptophan to a complete medium decreased the penetrance of the tumorous head phenotype.

About half of the tryptophan treated progeny from the Stephenville × Stephenville crosses had melanotic bodies in their abdomens. These dark bodies were also observed in the abdomens of about ten percent of the tryptophan treated progeny of outcrosses involving Stephenville. The bodies were subcutaneous and scattered throughout the abdominal region. The size and number varied considerably. The

TABLE 3

Penetrance of tumorous head among F₁ progeny from different crosses maintained on control medium with 0.5 percent L-tryptophan added

Cross†	Females		Males		Total	
	n*	%‡	n	%	n	%
Can × Can	135	0	168	0	303	0
Can × <i>tu-h</i>	274	0	245	0	519	0
<i>tu-h</i> × Can	279	29	240	13	519	22
Steph ¹ × Steph ¹	39	0	43	0	82	0
Steph ¹ × <i>tu-h</i>	220	19	157	24	377	21
<i>tu-h</i> × Steph ¹	79	33	66	23	145	28
Steph ² × Steph ²	233	0	214	0	447	0
Steph ² × <i>tu-h</i>	376	0	333	0	709	0
<i>tu-h</i> × Steph ²	169	37	181	24	350	30
<i>tu-h</i> × <i>tu-h</i>	250	73	346	70	596	71

† The female parent is listed first in each cross.

*n = total number of flies.

‡% = percentage of abnormal flies to the nearest whole number.

dark bodies could be identified in late third instar larvae and early pupal stages and also in the adult flies. Some mature flies with dark bodies were transferred to the usual laboratory corn meal-molasses-agar medium. The melanotic bodies were maintained in these flies but did not appear in any of their progeny. As long as the Stephenville flies were kept on the medium with the tryptophan additive, the dark bodies persisted generation after generation. They were not observed in cultures other than Stephenville which were treated with tryptophan. The melanotic bodies were thus dependent on something in the Stephenville strain and were expressed only in the presence of excess tryptophan.

When indole was added to the complete medium in a concentration of 0.01 percent the penetrance level was not altered. The statistical analysis based on the chi-square method indicated that the deviations from the controls were not signifi-

cant. Likewise, the addition of 0.5 percent DL-serine to the medium produced no significant effect on the penetrance of tumorous head. Treatment with L-cysteine at 0.1 percent concentration gave a varying effect. The crosses *tu-h* × Canton and *tu-h* × Stephenville showed no statistically significant differences as compared with the controls. The effect of L-cysteine on the *tu-h* × *tu-h* crosses was more pronounced. The average percentage of abnormal flies was 79 percent as compared with 95 percent for the controls. The chi-square test indicated that the difference was significant at the one percent level.

DISCUSSION

When the tumorous head stock was first investigated, a penetrance of 64 percent was reported. Following close inbreeding and selection over a two-year period, the penetrance was increased to 79 percent. The present experiments place the penetrance level at 93 percent. The best explanation for the increase following inbreeding and rigid selection is based on the accumulation of modifiers favorable to the expression. Modifiers of tumorous head have been shown to be widespread in wild populations and laboratory stocks. Continued selection, required to maintain this stock, would tend to concentrate the modifiers favorable to the expression. Close inbreeding would tend to raise the frequency of favorable modifiers and they would become homozygous. Segregation of heterozygous modifiers could in part, explain the variability which has been observed in the expression of the tumorous head phenotype in stock cultures and outcrosses.

The results obtained from the experiments on penetrance in the inbred stock emphasize the importance of the environment in determining the penetrance level. Given abundant food, uncrowded cultures, favorable temperature and humidity the penetrance level is high and there is a relatively small variation from culture to culture. In older cultures where there is crowding and competition for the available food, the penetrance level is lower and highly variable. In view of the low viability of *tu-h* as compared with wild flies, (GARDNER and RATTY 1952) it is probable that the decrease in the percentage of abnormal flies found in older cultures is due primarily to the failure of the tumorous head flies to survive until the time of classification rather than an increase in the number of normal flies hatched.

The appearance of melanotic growths in the Stephenville flies is interesting in view of the observations of HINTON *et al.* (1951), MITTLER (1952) and PLAINE and GLASS (1955) that tryptophan in excess increases the incidence of certain types of melanotic growths in particular stocks studied by these investigators.

In the *tu-h* inbred stock and in the results and outcrosses involving *tu-h* and Stephenville¹ females the incidence of the tumorous head abnormality was significantly lowered when tryptophan was added to the medium. It would be presumptuous, without further investigation, to attribute this lowered penetrance to a direct effect of tryptophan on the genetic-physiological mechanism of the *tu-h* phenotype. In view of the lower viability of the *tu-h* strain as compared with wild stocks, it is quite possible that the decreased penetrance could result from a dif-

ferential toxic effect of tryptophan on the flies expressing the tumorous head phenotype. This possibility is being investigated.

While tryptophan was not found to increase the penetrance of the tumorous head phenotype, there is a possibility that other substances might. Compounds of the relatively simple nature of L-tryptophan could hardly be expected to influence all tumor-like growths in *Drosophila melanogaster*. The recent report of BURTON, HARNLY and KOPAC (1956) of an extract from a melanotic tumor-bearing stock which is effective in inducing growths in non-tumorous strains indicates that substances of a more complex nature may be involved in the production of some types of abnormal growths.

SUMMARY

1. The present penetrance level of the inbred tumorous head stock was found to average 93 percent when the cultures were maintained on the standard medium at room temperature.

2. The age of the parental flies did not affect the penetrance level, but in cultures allowed to develop for an extended period without removal of flies the penetrance level decreased. The lowered penetrance in old cultures probably resulted from the inability of the tumorous head flies to compete favorably against those of normal phenotype in the same cultures.

3. When flies were reared on a brewer's yeast-agar medium to which 0.5 percent tryptophan was added, the penetrance of tumorous head was significantly reduced.

4. When 0.5 percent tryptophan was added to the medium of flies from the Stephenville stock, about half of the progeny had melanotic bodies in their abdomens. In the same medium about 10 percent of the progeny from outcrosses involving Stephenville had similar melanotic bodies.

5. Indole at 0.01 percent concentration and DL-serine at 0.5 percent did not significantly affect the penetrance of the tumorous head phenotype.

6. L-cysteine at a concentration of 0.1 percent did not significantly affect the results of the outcrosses tested but the penetrance of the tumorous head phenotype was significantly reduced in the inbred *tu-h* flies when 0.1 percent L-cysteine was added to the medium.

LITERATURE CITED

- BURTON, L., M. H. HARNLY, and M. J. KOPAC, 1956 The activity of a tumor factor in *Drosophila* development. *Cancer Research* **16**: 402-407.
- GARDNER, E. J., 1948 Head tumors in *Drosophila melanogaster*. (abstr.) *Utah Acad. Sci. Proc.* **25**: 164.
- GARDNER, E. J., and M. D. GARDNER, 1953 Further evidence for maternal effect and modifiers of tumorous head genes in natural populations of *Drosophila melanogaster*. *Cancer Research* **13**: 689-693.
- GARDNER, E. J., and F. J. RATTY, 1952 Penetrance and expressivity of tumorous head in *Drosophila melanogaster* and relative viability of flies carrying tumorous head genes. *Genetics* **37**: 49-61.

- GARDNER, E. J., and G. H. STOTT, 1951 Genes producing a maternal effect and modifiers of tumorous head in "wild" and tumor bearing stocks of *Drosophila melanogaster*. *Genetics* **36**: 72-83.
- GARDNER, E. J., G. H. STOTT, and D. M. DEARDEN, 1952 Modifiers of tumorous head genes in natural populations and laboratory stocks of *Drosophila melanogaster*. *Genetics* **37**: 451-456.
- GARDNER, E. J., and C. M. WOOLF, 1949 Maternal effect involved in the inheritance of abnormal growths in the head region of *Drosophila melanogaster*. *Genetics* **34**: 573-585.
- 1950 The influence of high and low temperature on the expression of tumorous head in *Drosophila melanogaster*. *Genetics* **35**: 44-55.
- GLASS, B., and H. L. PLAINE, 1952 The role of oxygen concentration in determining the effectiveness of X-rays on the action of a specific gene in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.* **38**: 697-705.
- HINTON, T., D. T. NOYES, and J. ELLIS, 1951 Amino acids and growth factors in a chemically defined medium for *Drosophila*. *Physiol. Zool.* **24**: 335-353.
- KIKKAWA, H., 1950 Tryptophan synthesis in insects. *Science* **111**: 495-496.
- 1953 Biochemical genetics of *Bombyx mori* (silkworm). *Advances in Genet.* **5**: 107-140.
- KING, R. C., 1955 A method for studying successive batches of eggs laid by treated or control *Drosophila*. *Am. Naturalist* **89**: 369-370.
- MEHLER, A. H., 1955 Metabolism of tryptophan. pp 882-908. *Symposium on Amino Acid Metabolism*. Edited by W. D. McELROY and B. GLASS. Johns Hopkins Press, Baltimore.
- MITTLER, S., 1952 Influence of amino acids upon incidence of tumors in *tu*^{50j} stock of *Drosophila melanogaster*. *Science* **116**: 657-659.
- NEWBY, W. W., 1949 Abnormal growths on the head of *Drosophila melanogaster*. *J. Morphol.* **85**: 177-196.
- PLAINE, H. L., 1952 Non-growing brewer's yeast as the sole medium for rearing *Drosophila* larvae. *Drosophila Inf. Ser.* **26**: 133.
- 1955a The effect of oxygen and of hydrogen peroxide on the action of a specific gene and on tumor induction in *Drosophila melanogaster*. *Genetics* **40**: 268-280.
- 1955b The counteraction by cysteine of the effects of X-rays and of tryptophan on the action of specific suppressor systems in *Drosophila melanogaster*. *Cancer Research* **15**: 151-158.
- PLAINE, H. L., and B. GLASS, 1952 The effect of oxygen concentration upon the induction by X-rays of melanotic tumors in *Drosophila melanogaster*. *Cancer Research* **12**: 829-833.
- 1955 Influence of tryptophan and related compounds upon the action of a specific gene and the induction of melanotic tumors in *Drosophila melanogaster*. *J. Genet.* **53**: 244-261.
- STOTT, G. H., and E. J. GARDNER, 1952 The location of the two major genes responsible for the inheritance of tumorous head in *Drosophila melanogaster* (abstr.) *Utah Acad. Sci. Proc.* **29**: 50.
- WHITE, A., P. HANDLER, E. L. SMITH, and D. STETTEN, 1954 *Principles of Biochemistry*. McGraw-Hill Book Co., Inc., New York.