TABLE 3

EFFECT OF 1-HR TREATMENT WITH β-PROPIOLACTONE ON *Neurospora* STRAIN 70,007–38,701 (adenineless)

| | 25° C | | | 35° C | | |
|---|---|---|---|---|---|---|
| | No. conidia tested | No. colonies formed after plating | No. mutations per $10^6$ conidia plated | No. conidia tested | No. colonies formed after plating | No. mutations per $10^6$ conidia plated |
| Control | $1.75 \times 10^6$ | 0 | 0 | $2.00 \times 10^6$ | 0 | 0 |
| 0.01% | $2.63 \times 10^6$ | 6 | 2.28 | $2.50 \times 10^6$ | 10 | 4.00 |
| 0.02% | $2.75 \times 10^6$ | 10 | 3.64 | $2.75 \times 10^6$ | 15 | 5.45 |
| 0.03% | $3.13 \times 10^6$ | 12 | 3.83 | $2.38 \times 10^6$ | 17 | 7.14 |

Mutagenicity of β-propiolactone against different loci in *Neurospora* seems now to be well established. Besides the evidence from reversions of the adenineless and inositolless strains, we find that treatment with the lactone substantially increases the number of canavanine-resistant colonies which appear when canavanine-sensitive spores are plated onto canavanine-containing medium. However, the spontaneous appearance of canavanine-resistant colonies is relatively high. And, in addition, some instances of appearance of growth spots on canavanine medium appear not to be due to gene mutation. We have, therefore, placed more reliance on tests with the adenineless and inositolless strains. It seems clear that mutation accounts for most of the reversions to growth-factor independence in these strains. Colonies that appear after plating have all been picked, established in separate culture, and then tested on minimal medium. All such cultures have shown the ability to grow on minimal medium. We have also made crosses of these cultures back to strains carrying the original mutant gene, but of opposite mating type. Ascospore isolates have been made from approximately one fifth of these crosses, and from all of the crosses wild-type progeny have been recovered.

Two phenomena of interest have been observed in these mutation experiments. One is the apparent effect of temperature on the mutagenic action of β-propiolactone, as indicated in Table 3. The other is the fact that in experiments so far, β-propiolactone has appeared to be considerably more effective in creating mutations in the adenineless strain than in the inositolless strain, although our ultraviolet treatments show the opposite effect. Both of these phenomena are under further investigation.

### References


1. OSTERGREN, G. *Botan. Notiser*, **4**, 376 (1948).
2. ERICKSON, R. O., and ROSEN, G. U. *Am. J. Botany*, **36**, 317 (1949).
3. KOLMARK, G., and WESTERGAARD, M. *Hereditas*, **35**, 490 (1949).
4. DICKEY, F. H., CLELAND, G. H., and LOTZ, C. *Proc. Natl. Acad. Sci. U. S.*, **35**, 581 (1949).
5. HOROWITZ, N. H., and SRB, A. M. *J. Biol. Chem.*, **174**, 371 (1948).


## Influence of Diethylstilbestrol on *Drosophila melanogaster* Tumors


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The capacity of estrogenic substances to induce, stimulate, or inhibit tumor development in several species of vertebrates suggested an investigation as to whether these substances exhibit similar properties in relation to invertebrate tumors.

Three different hereditary, benign-tumor-bearing *Drosophila melanogaster* stocks were raised in food cultures containing diethylstilbestrol. The stocks used were st sr; tu-36a, described and analyzed by Russell (*1*), which presents 4–5% tumors at laboratory temperature (20°–25° C); bw(tu), with a tumoral frequency which fluctuates around 50%, according to Hartung (*2*); se $e^{11}$; tu-49th, which was formed in our laboratory and which has a tumoral incidence of 40–50% (*3*).

Sterile eggs of the three stocks were cultivated in shell vials containing a sterile medium composed of water, agar, ammonium sulfate, magnesium sulfate, tartaric acid, monopotassic phosphate, and dead brewer's yeast, after the formula suggested by Birch (*4*); 1% diethylstilbestrol was suspended in the medium. Control flies were cultivated in an identical medium without the drug.

TABLE 1

EFFECT OF DIETHYLSTILBESTROL ON TUMOR INCIDENCE IN THREE STOCKS OF *Drosophila melanogaster*

| Stock | Treatment | No. individuals | No. tumors | % tumors |
|---|---|---|---|---|
| bw(tu) | DSB 1/100 | 255 | 73 | 28 |
| | Controls | 287 | 138 | 48 |
| se $e^{11}$; tu-49h | DSB 1/100 | 482 | 76 | 15.7 |
| | Controls | 1,058 | 489 | 46.2 |
| st sr; tu-36a | DSB 1/100 | 120 | 5 | 4.1 |
| | Controls | 223 | 14 | 6.2 |

The *D. melanogaster* eggs collected according to the method suggested by Schweitzer (*5*), were sterilized for 20 min in a 1/10,000 solution of sodium mercury thiosalicilate (Merthiolate "Lilly") in 70° alcohol. After being rinsed in 70° alcohol or saline solution, approximately 50 eggs were put into shell vials containing the medium. The treated and the control eggs were cultivated under the same environmental conditions.

As a result of the experiments (Table 1), it was observed that the flies raised in the medium containing diethylstilbestrol presented a lower percentage of tumors than those cultivated in the control vials. This lower number, nevertheless, was statistically significant only in the stocks of high tumoral incidence: bw (tu) and se $e^{11}$; tu-49h.

The experiments indicate that diethylstilbestrol,

under the conditions mentioned, interferes with the tumor development of *D. melanogaster* (at least in two of the three stocks), showing properties which could, up to a certain extent, be compared with some mammal tumors.

In the present stage of our investigations, it is impossible to give an explanation of the results. The action of the drug could be direct on the tumoral tissue; or the effect could be indirect, through some endocrine gland of the insect or through the modification of a chain of reactions controlled by these hormones; or, last, the action could be through a general toxic effect which influences the neoplasmic growth.

We are now working on experiments designed to answer some of these questions.


### References

1. RUSSELL, E. S. *J. Exptl. Zool.*, **84**, 363 (1940).
2. HARTUNG, E. W. *Science*, **107**, 296 (1948).
3. BRNCIC, D. *Biológica (Chile)*, **11**, 69 (1950).
4. BIRCH, M. M. Ph.D. Thesis, Wellesley College (1944).
5. SCHWEITZER, M. D. *Drosophila Inform. Service*, **4**, 65 (1935).


## Pteroylglutamic Acid Activity of Aminopterin in *Tetrahymena geleii*[1]


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During the course of some experiments on the interrelationships of steroids with pteroylglutamic acid (PGA) in the ciliated protozoan *Tetrahymena geleii,* an attempt was made to reverse the action of the PGA analog aminopterin (4-amino-PGA). Contrary to what was expected on the basis of observations in other organisms, it was found that aminopterin possesses high PGA activity for *Tetrahymena.*

Since aminopterin replacement of PGA has never, to the authors' knowledge, been previously reported,[2] it was felt that these data are significant, in that they may help to explain the structural conditions in the PGA molecule necessary for PGA action *in vivo.* This is the basis for this preliminary report.

TABLE 1

RESPONSE OF *T. geleii* TO AMINOPTERIN*

| Aminopterin added (mg%) | No PGA added | 6.0 μg% PGA added |
|---|---|---|
| 0 | .036 | .906 |
| 0.001 | .052 | .936 |
| 0.01 | .780 | .960 |
| 0.10 | .888 | .950 |
| 1.00 | .960 | .964 |
| 5.00 | .886 | .980 |

* Results shown represent optical densities of third serial transplant cultures.


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[2] G. W. Kidder, of Amherst College, has kindly informed us of similar studies made in his laboratory, the results of which are now in press.

TABLE 2

COMPOSITION OF BASAL MEDIUM*

| | | |
|---|---|---|
| Solution B (amino acids) | 14.0 | ml% |
| Solution C (vitamins)† | 6.0 | " |
| Solution D (salts) | 2.5 | " |
| Solution E (salts) | 0.6 | " |
| Solution F (phosphates) | 0.175 | " |
| Solution J (purines and pyrimidines) | 5.0 | " |
| Glucose‡ | 0.5% | |
| Sodium acetate | 0.2% | |
| Protogen§ | 2.0 | |
| Tween 80 | 2.4% | |
| pH 7.0 | | |

* The compositions of the solutions listed here are identical with those listed by Dewey *et al.* (*1*, 284).
† Solution does not contain PGA.
‡ Autoclaved separately and added aseptically.
§ Units/ml.

Results of a typical experiment are shown in Table 1. To obviate the possibility of PGA contamination of the aminopterin sample, the experiments were repeated with different aminopterin preparations. Results were essentially similar in all cases.

The ciliated protozoan *T. geleii* H was grown in pure culture. The base medium was essentially that of Dewey *et al.* (*1*), modified as indicated in Table 2. The organisms were grown in 50-ml Erlenmeyer flasks containing 5 ml of medium, according to the technique described by Hutner (*2*). All inoculations were made through a dilution flask containing only basal medium. Following inoculation, the flasks were kept at 25° C, and the cultures were harvested at the end of the logarithmic growth phase. Optical density of the harvested cultures was determined by the use of a Klett-Summerson photoelectric colorimeter with a green (#54) filter, following the procedure of Elliott (*3*).

From the data in Table 1, it is apparent that the addition of about 0.1 mg% of aminopterin equals the full effect upon growth of 6.0 μg% of PGA.


### References

1. DEWEY, V. C., PARKS, R. E., and KIDDER, G. W. *Arch. Biochem.*, **29**, 281 (1950).
2. HUTNER, S. H. *J. Gen. Microbiol.*, **4**, 286 (1950).
3. ELLIOTT, A. M. *Trans. Am. Microscop. Soc.*, **68**, 228 (1949).


## 3-(*p*-Chlorophenyl)-1,1-Dimethylurea—A New Herbicide


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In the course of a study dealing with materials having plant-regulating properties, 3-(*p*-chlorophenyl)-1,1-dimethylurea, a new chemical compound, has been synthesized and found to be very effective in killing many plant species. In greenhouse tests described in detail below, it has appeared particularly effective in killing both annual and perennial grasses.


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