Reports

Growth-Inhibiting Effect of Irradiated Tryptophan

In an attempt to obtain competitive inhibitors, we have exposed crystalline L-tryptophan (*1*) to radiation (10^9 rep) produced by high-velocity electrons from a modified 1 Mev resonant-transformer-type x-ray unit previously described by Knowlton *et al.* (*2*). Care was taken to avoid increase in temperature above 65°C during the exposure. Chromatographic studies following exposure showed that a number of compounds were produced. None of these compounds have as yet been identified.

The irradiated tryptophan was placed in 100-percent methanol, and only the methanol-soluble fraction was used for microbiological studies. Varying amounts of this fraction were placed in test tubes, and the methanol was evaporated. To the residue was added 10 ml of tryptophan assay medium (*3*) containing 10 μg of untreated L-tryptophan.

Following sterilization for 15 minutes at 120°C, the test tubes were inoculated with (i) *Streptococcus mastitidis* V_9, (ii) *Streptococcus mastitidis* 68Cl, and (iii) *Lactobacillus arabinosis* 17-5. The bacterial cells had been washed twice with physiological saline and then diluted, so that the final suspension was only faintly cloudy. One drop of the bacterial suspension was added to each of the test tubes, which were then incubated for 18 hours at 37°C. Growth at the end of this period was recorded as 0 (no growth) to 4 (complete growth as in the control tubes).

We found that the irradiated tryptophan contained one or more compounds that were capable of inhibiting the growth of *Streptococcus mastitidis*. We also found that this inhibition could be overcome by addition of untreated L-tryptophan. Following these preliminary experiments, the methanol-soluble fraction was run through Al_2O_3 columns as described by Bumpus and Page (*4*). It was found that the first fractions passing through this column contained the inhibiting compounds.

To obtain more of the inhibitor and to study its adsorption properties, we extracted 20 g of irradiated L-tryptophan with methanol, concentrated the extracts to about 120 ml, filtered off some precipitated tryptophan, and poured the solution onto a column, 8 cm high by 9.5 cm in diameter, of Al_2O_3. The column was then washed with methanol. After the void volume of the column had been discarded, 22 fractions of filtrate (20 ml each) were collected. Tests of these fractions showed that all gave negative ninhydrin tests for α-amino acid. Fractions 6 to 14 showed positive tests for the α-unsubstituted indole nucleus with Ehrlich's reagent, and fractions 4, 5, and 6 contained an inhibitor for the growth of *Streptococcus mastitidis*. The dried solutes from fractions 1 to 22 varied considerably in odor, color, tarriness, and fluorescence. Previous chromatographic experiments had shown that no more inhibitor could be obtained by continuing the elution further with aqueous methanol.

The fractions containing the inhibitor (fractions 4, 5, and 6) were combined and extracted with acetone, and the extracts were poured onto a 35- by 180-mm column of silicic acid. The column was washed with 900 ml of acetone, followed by two 300-ml portions of 4/1 (by volume) acetone and methanol, followed by three 300-ml portions of 1/1 acetone and methanol. Only the first eluate fraction (4/1 acetone and methanol) contained the inhibitor, which showed a considerable effect upon growth of *Streptococcus mastiditis* V_9 and only a slight effect on *Lactobacillus arabinosis*. The inhibition could be overcome by addition of tryptophan to the cultures in amounts equal to, or in excess of, the weight of the inhibiting fraction.

The active fraction weighed 40 mg and was obtained in a very impure form amounting to less than 0.2 percent of the irradiated tryptophan. The fraction showed inhibiting effects on the following microorganisms: *Leuconostoc mesenteroides* 8042, *Escherichia coli* B/r, and *Staphylococcus aureus* P60; it showed no inhibiting effects upon *Streptococcus lactis* L21S, *Bacillus subtilis* S8, *Aerobacter aerogenes* 600, and *Sarcina*.

The following compounds were tested and showed no growth-inhibiting effects on *Streptococcus mastitidis*: indene, indolepropionic acid, phenol indophenol, *p*-aminoacetophenone, and indoleacetic acid. Experiments with other irradiated amino acids indicated the presence of growth-inhibiting compounds, especially in irradiated L-arginine, L-phenylalanine, and L-serine. We are now attempting to purify the compounds produced in these amino acids. We have been unable to produce growth-inhibiting fractions by prolonged exposure of L-tryptophan to ultraviolet radiation.

The active fraction obtained by passing irradiated tryptophan through Al_2O_3 and silicic acid columns is undoubtedly a mixture of compounds. We have not yet been able to isolate and identify the compound that inhibits the growth of *Streptococcus mastitidis*. Possibly there may be several compounds. It will be of interest to study the effects of the fractions obtained from irradiated amino acids on mammalian cells, both *in vivo* and in tissue cultures. Such studies are now under way (*5*).

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References and Notes

1. Nutritional Biochemicals Corp.
2. J. A. Knowlton, G. R. Mahn, J. W. Ranftl, *Nucleonics* 11, 64 (1953).
3. Difco Laboratories.
4. F. M. Bumpus and J. H. Page, *J. Biol. Chem.* **212**, 111 (1955).
5. We wish to thank J. S. Balwit for the radiation services required in this work and R. M. Mendenhall for chromatographic studies.

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Photoperiod and Chilling Control Growth of Hemlock

Eastern hemlock, *Tsuga canadensis* (L.) Carr., illustrates to an unusual degree the influence of photoperiod on the annual vegetative cycle of trees. Short nights not only prolong stem growth by delaying the formation of terminal buds but also result in a striking compensation for lack of chilling in the breaking of bud dormancy. These environmental effects apply throughout the range of this important forest tree, from the low southern Appalachian region to Canada, although they are modified by genetic differences that adapt ecotypes to con-

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