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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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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trasting climates. Previous brief announcements of these conclusions (*1*) are supported by the following basic experiment and by later extensions that are summarized in subsequent paragraphs.

To study the breaking of bud dormancy, 2-year-old wild seedlings from near New Haven, Conn., were handled in four groups as follows: (i) one group was potted 1 Dec. 1952 after exposure to cool autumn weather and then kept in the greenhouse under natural short-day, long-night conditions; (ii) the second group was potted in early October 1952, before frost, but later was chilled indoors at 5° C for 5 to 15 weeks; (iii) the third group was potted in October as in group ii but was maintained in the greenhouse without chilling on a natural short-day, long-night cycle; (iv) the fourth group received treatment similar to that of group iii, except that supplementary incandescent light less than 200 lux reduced the dark period to 4 hours out of 24.

Buds on all chilled plants in groups i and ii promptly began growing when the seedlings were returned to favorable temperatures. Of the unchilled plants, none of those on long nights (group iii) opened their buds, while many on short nights (group iv) did so. This suggests the presence of bud dormancy, normally broken by brief chilling, and possible compensation for lack of chilling by short nights.

More detailed effects were shown by groups (ii) and (iii) after 9 Mar. 1953, when plants were moved to a 24°C (± 2°) growth room with different photoperiods. The plants treated with a short day and long night received 8 or 12 hours of continuous fluorescent light (4000 lux) followed by 16 or 12 hours of total darkness, respectively. Those treated with a long day and short night received 16 hours of light (3000 lux) followed by 8 hours of darkness or exposure to dim incandescent light (less than 200 lux) for 4 to 8 hours. The durations of total darkness were consequently 8, 4, or 0 hours, respectively.

Unchilled plants (group iii) on long nights still failed to break dormancy; those on short-night treatments (except for a few diseased plants) broke dormancy within 5 to 14 weeks (Fig. 1*A*). This all-or-none difference is a more drastic interaction of photoperiod and chilling in woody plants than most reports have previously indicated (*2*). Nevertheless, photoperiod only partially compensates for lack of chilling, as was shown by the number of weeks required for all buds to break dormancy (circled numerals in Fig. 1*A*). The period necessary was 2 to 3 times as great for unchilled plants on short nights as for chilled plants under the same conditions.

All the chilled plants (group ii) broke dormancy on all photoperiods within 3 to 5 weeks after removal from 5°C storage, regardless of the length of the chilling period (Fig. 1*A*).

Continued observation of chilled plants (group ii) showed that all those on long nights formed new buds within 10 weeks, whereas both chilled plants (group ii) and unchilled plants (group iii) which were under the short-night condition continued growth until at least the 15th week and then only gradually began forming buds (the boxed numerals in Fig. 1*B* indicate the number of weeks required for *all* plants to form buds). The 4-hour night produced noticeably greater elongation than either an 8-hour night or continuous light, as has been found also for elongation in Scotch pine (*3*). In hemlock, as in many other trees that do not have determinate growth, the amount of elongation is strongly conditioned by the length of the period of meristematic activity (*2–4*).

To show that duration of the light period is not the sole factor responsible for elongation, supplementary treatments were made by interrupting the dark periods of additional plants which had only short-day illumination at high intensity with dim incandescent light (less than 200 lux) as follows.

The 12-hour dark period was interrupted after 4 hours by subjecting the plants to 4 hours of incandescent light (4 + 4, Figs. 1*A* and 1*B*). The 16-hour dark period was interrupted after 4 hours by 2 hours of dim light followed by 4 hours of total darkness, then another 2 hours of dim light, and finally another 4 hours of total darkness (4 + 4 + 4, Figs. 1*A* and 1*B*). Short-day treatments with several short dark periods gave results comparable with those of the normal long-day, short-night treatments rather than with the combinations of short days with uninterrupted long nights. This was true both for the breaking of dormancy by the photoperiodic compensation for lack of chilling and for the delay in formation of terminal buds. The duration of the dark period controls the growth of other tree species (*3, 5*) and governs many other photoperiodic responses (*6*).

Other experiments (*6*) confirmed many of the present results and showed further the following. (i) The greatest difference in response came with about 8- and 9-hour nights, rather than with 10-, 11-, or 12-hour dark periods, for 30 seed sources from the area of Tennessee and North Carolina north to Nova Scotia and west to Minnesota. (ii) Seedlings with parents from areas with short growing seasons (about 100 days) went dormant progressively earlier than those from long-season regions (up to nearly 200 days), for each of 17 photoperiodic

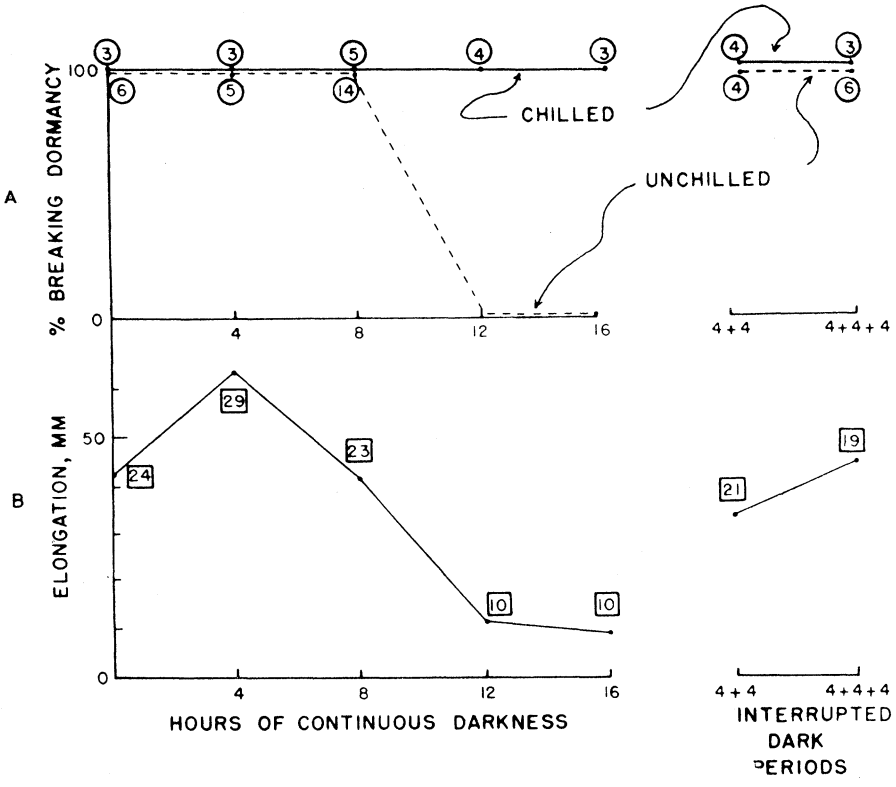

Fig. 1. Chilling and photoperiod as related to breaking of dormancy (*A*) and return to dormancy (*B*). The circled digits indicate the number of weeks required for all healthy plants to break dormancy. The boxed digits indicate the number of weeks required for all plants to return to dormancy.

and thermoperiodic treatments (compare Wareing, *4*). (iii) In forest and nursery plantings near New Haven, Conn., as indoors, long-season ecotypes grew faster than northern or mountain types, but this advantage was partly canceled by injury from early frost. (iv) Seed with 10 weeks or more of moist chilling germinated well at 12° and 17°C with either 0, 8, 12, or 16 hours of light, but, at higher temperatures or shorter periods of stratification, the photoperiod had a marked influence; optimal length of day for germination seemed to be longer at 27° than at 17°C (*7*).

The responses of seed and seedlings to light and temperature are pertinent to basic physiological problems of morphogenesis and to field ecology, forestry, and horticulture of *Tsuga* and other trees.

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Studies with Muscle Relaxant Labeled with Iodine-131

A search has been made for compounds that produce neuromuscular block and that can also be labeled with iodine-131 so that their movements in muscle may be studied in conjunction with their pharmacological effects (*1*). The compound, decamethylene 1,10-bis(2-iodoethyl dimethyl ammonium) dichloride has been prepared (*2*), and this may be regarded as a substitution product of decamethonium; it has been termed "iodocholinium." This compound resembles decamethonium in its action on the isolated guinea pig diaphragm. Decamethonium is known to produce an initial neuromuscular block which is followed by some recovery and the development of a slow secondary block that takes at least 3 hours for completion (*3*). Iodocholinium acts similarly but more slowly. In doses of 3 μg/ml, it gives a slow block which is still increasing even after 12 hours.

The uptake of the drug was studied by soaking diaphragm muscles from guinea pigs in saline containing subparalytic (3 μg/ml) doses of labeled compound, the muscles being removed at intervals for analysis. The temperature was 38°C, and the saline was renewed frequently. Radioactivity of the muscles expressed in counts per minute, per gram increased continuously for at least 12 hours, and this is consistent with the pharmacological findings. The uptake was surprisingly high, and it corresponds, after 1 hour, to 2.5 μg/g of muscle. After 12 hours it had reached 14 μg/g, and this indicates that each gram of tissue had concentrated an amount of drug contained in some 4.5 ml of external solution. The volume of extracellular space is less than 0.3 ml/g and cannot account for results of this magnitude.

The presence of *d*-tubocurarine in a paralytic dose of 5 μg/ml markedly altered the entry of labeled compound. Pairs of diaphragms were used to test this action. It was found that in every case (16 pairs) the uptake of labeled compound was markedly diminished by the presence of *d*-tubocurarine. This was apparent after 1 hour; after 12 hours, the uptake with curarine was less than half of that found in controls. Certain tissues on which the compound had no obvious pharmacological effect were also studied. In the case of rabbit bladder muscle and rabbit tendon, curarine had no significant effect on the uptake of the labeled compound.

The finding that curarine diminished the entry of labeled iodocholinium into guinea pig diaphragm may be of interest in discussions regarding the mechanism of action of curarine and the well-known antagonism between this drug and the depolarizing agents (*4*). The use of labeled compounds may also provide some direct information regarding the reaction between drugs and their receptor sites in muscle.

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References and Notes

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Effects of Gibberellic Acid on Growth of Kentucky Bluegrass

Crab grass [*Digitaria sanguinalis* (L.) Scop.] that was nearing the end of its seasonal development at Yonkers, N.Y., was stimulated to renewal of growth by a single application of gibberellic acid. Replicated plots of plants were sprayed until the foliage was moistened with aqueous solutions containing 10 and 100 μg of the acid per milliliter. The plants had been mowed and were about 8 cm tall when they were sprayed on 10 Sept. 1956. By 1 Oct., control plants that had been sprayed with water had assumed the usual autumnal red color and were about 13 cm in height. On the other hand, plants that had been sprayed with gibberellic acid remained green, and those that had been treated with 100 μg/ml had elongated to a height of 26 cm. No weight determinations were made on these plants.

After these observations had been made, an Australian patent application was received (*1*). It deals in part with the effects of gibberellic acid in several plant tests. Data are presented showing that gibberellic acid, applied in the spring as a spray to unspecified pasture plants in the field, produced an increase in dry weight, especially when it was used with fertilizer. Although details are not given, the application also states that gibberellic acid induced the growth of grass under conditions of low light intensity and low temperatures when growth was not expected.

These observations suggested that it would be of value to undertake additional tests with gibberellic acid for inducing the growth of grass, especially during an unfavorable time of the year (*2*). Plots of Kentucky bluegrass (*Poa pratensis* L.) at Greenfield, Ind., were fertilized with a granulated fertilizer (10-10-10) on 23 Oct. 1956, and sprayed once with water (control) or with freshly made solutions of gibberellic acid 3 days later. The plants were in the slow-growth stage common at this time of year.

Within 4 days, the grass that had been treated with gibberellic acid began to grow again as revealed by brightening of the green color and development of new shoots. Plants were harvested by clipping about 4 cm above the ground on 10 Nov.,