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.

JERRY S. OLSON
HANS NIENSTAEDT*
Connecticut Agricultural Experiment Station, New Haven

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* Present address: Lake States Forest Experiment Station, U.S. Forest Service, St. Paul, Minn.

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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.

R. CREESE
D. B. TAYLOR
B. TILTON
Department of Pharmacology, University of California Medical Center, Los Angeles

References and Notes

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2. We are indebted to H. D. Baldridge, Naval Medical Research Institute, Bethesda, Md., for a specimen of the corresponding dichloro compound from which the labeled derivative was prepared by refluxing it with excess radioactive sodium iodide in acetone for 48 hours in accordance with a suggestion made by Seymour Freis, also of NMRI.
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3 December 1956

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.,

15 days after treatment. There had been light frosts with approximate low temperatures of −1, −2, and −4°C. Rainfall was adequate to keep the soil moist during the experiment.

Data on fresh and dry weights of clippings are given in Table 1. Determinations of dry weight were made after clippings had been air-dried for 10 days at 5 to 28°C in a loft and then further dried at 24°C in a vacuum.

It is apparent that, under the conditions of the test, gibberellic acid influenced significantly both the fresh and dry weights of the plants, especially when it was used in conjunction with fertilizer. Treated plants tended to contain more water, as evidenced by the comparison of fresh and dry weights. In this test, and as noted elsewhere (*1*), plants treated with gibberellic acid alone were yellowish-green. When fertilizer was used in conjunction with the gibberellic acid treatment, however, plants were bright green and appeared to offer a juvenile type of foliage that is common in this region in the spring and early summer.

Longer term effects of gibberellic acid, particularly with respect to winter injury, will be observed in the spring and during the subsequent seasons. Observations made since the bluegrass plots were harvested indicate that there may be some deleterious effects. Plants in plots treated with gibberellic acid continued to grow. Irrespective of the fertilization program, by 10 Dec. this new growth tended to be chlorotic and spindly. The ends of many of the leaves were dead, presumably because of the low temperatures that had prevailed. The crown region of the plants did not appear to have been injured, however, because new green tissue was produced on the advent of a short period of warm weather in late December.

These tests suggest that gibberellic acid may be useful for inducing the growth of grass in the fall and in the spring, and perhaps during the winter in warmer regions. When fertilizer is used in conjunction with gibberellic acid, the new growth appears to offer an excellent type of forage.

Table 1. Fresh and dry weights of clippings from field plots of bluegrass treated with gibberellic acid and fertilizer. Gibberellic acid was applied in water at a rate of about 100 gal/acre. Each entry for fresh and dry weight is based on 3 plots, each 96 ft².

Fertilizer (lb/acre)	Gibberellic acid (g/acre)	Av. fresh wt. (g)	Av. dry wt. (g)
0	0	481	231
0	28	854	345
0	56	740	294
0	112	808	321
215	0	595	246
215	28	999	357
215	56	1117	354
215	112	1208	414
645	0	754	300
645	28	1103	366
645	56	1276	431
645	112	1376	456
L.S.D., 0.01		281	171
L.S.D., 0.05		209	94

CURT LEBEN
Agricultural Research Center, Eli Lilly and Company, Greenfield, Indiana
LELA V. BARTON
Boyce Thompson Institute for Plant Research, Yonkers, New York

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2. We are grateful for the statistical assistance of E. V. King and for the aid given by the department of biochemical preparations of Eli Lilly and Company.

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Glucose Oxidase with Iodide-Iodate-Starch or o-Tolidine as a Specific Spray for Glucose

A specific spray test for chromatographically separated glucose has been lacking, because present tests depend on reactions of the carbonyl group, which is common to all reducing sugars, or depend on nonspecific reactions with strong oxidizing agents. We have found that glucose oxidase will catalyze the atmospheric oxidation of a glucose spot on a paper chromatogram to give gluconic acid and hydrogen peroxide (*1*). The formation of traces of gluconic acid on the chromatogram may be shown by a new application of the acid-iodide-iodate-starch reaction, and the peroxidase-catalyzed hydrogen peroxide-*o*-tolidine reaction may be used to demonstrate the formation of hydrogen peroxide.

Glucose oxidase has been used previously for the quantitative and qualitative determination of glucose in solutions (*2*), and its specificity for the oxidation of glucose and its mechanism of action have been established (*3*).

After irrigating a Whatman (*4*) No. 1 paper chromatogram with *n*-butanol, ethanol, and water (10/1/2) and air-drying, we sprayed the glucose area with a glucose oxidase solution prepared by dissolving 10 mg of Takamine Dee-0 in 10 ml of water. After the chromatogram had stood for 15 minutes at room temperature, it was sprayed with a freshly prepared reagent containing 1 percent soluble starch and 5 percent KI in aqueous solution and then with a 5-percent aqueous solution of KIO_3. A final spray, after the color was fully developed, with 5-percent aqueous $NaHCO_3$ delayed air oxidation of the background. With larger amounts of glucose, some of the hydrogen peroxide that is formed reacts with iodide to liberate iodine. However, the sensitivity of this test depends on the instantaneous reaction of gluconic acid at room temperature with the iodide-iodate system to liberate iodine, which in turn yields the familiar intensely blue color with starch (*5*).

As a confirmatory or alternative test for glucose, the hydrogen peroxide formed during the enzymatic oxidation was detected with *o*-tolidine. The air-dried chromatogram was sprayed with a glucose oxidase-peroxidase reagent (*6*) and then immediately with a 1-percent ethanolic solution of *o*-tolidine (*7*). The blue color resulting from the peroxidase-catalyzed reaction of *o*-tolidine with hydrogen peroxide reaches a maximum within a few minutes at room temperature and then gradually fades.

Ten micrograms of glucose that had moved 150 mm on a chromatogram gave a strong color with either of the aforementioned spray tests; 5μg gave a faint color. In order that either of the tests will be valid, it must be established that the color does not appear in the glucose area of the chromatogram in the absence of the glucose oxidase treatment.

The utility of glucose oxidase in detecting the presence of glucose on chromatograms suggests its use in combination with specific hydrolytic enzymes as a reagent for confirming the identity of glucose-containing polysaccharides on chromatograms. This enzyme is also useful as a reagent for removing glucose from solutions prior to chromatography.

LAWRENCE M. WHITE
GERALDINE E. SECOR
Western Utilization Research Branch, Agricultural Research Service, U.S. Department of Agriculture, Albany, California

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4. Mention of a manufacturer or a commercial product does not imply endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.
5. The iodide-iodate-starch system will detect as little as 0.05 μg of gluconic acid per square millimeter under the conditions employed. It is of general usefulness for detecting acid areas on chromatograms and neutral test papers, or