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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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

for detecting microgram quantities of acid on the spot plate.

6. One hundred milligrams of Dee-O was dissolved in 10 ml of acetate buffer of *p*H 5.3 to 5.6, and about 1 g of scraped raw potato pulp was added. After it had stood 15 to 20 minutes, the reagent was filtered through glass wool. The raw potato is a convenient, essentially glucose-free source of peroxidase.
7. When *o*-tolidine dihydrochloride was used, 1 g was stirred with 0.45 g of KOH (85 percent pure) in 100 ml of 95-percent ethanol until it was dissolved, and the precipitated KCl was removed by filtration.

12 December 1956

Infrared Spectra of Mixtures of α- and β-D-Glucose Pentaacetate

Infrared spectra have been extensively used to identify sugars and their derivatives (*1*). The α- and β-anomers can also be differentiated through the presence or absence of certain characteristic peaks in their infrared spectra (*2*). In connection with some studies undertaken in this laboratory, it was proposed to use the intensity of an absorption peak characteristic of a particular anomer to determine the concentration of that anomer in solution.

In order to see whether or not this was feasible, we first investigated the infrared spectra of mixtures of the two anomers, α-D-glucose pentaacetate—mp. 112°C; $[\alpha]^{21}D$, +101° (EtOH, *c.*, 0.5) (*3*)—and β-D-glucose pentaacetate—mp, 133–134°C; $[\alpha]^{21}D$, +2° (EtOH, *c.*, 0.5) (*3, 4*)—in chloroform and acetone solutions. The α- and β-D-glucose pentaacetates were mixed in the proportions 1 α/4 β, 1 α/1 β, and 4 α/1 β at a total concentration of 1.00 percent (wt./vol.), and the infrared spectra were recorded with a Beckman IR3 recording spectrophotometer. This instrument permits one to obtain infrared spectra of compounds in solution without the spectrum including any contributions from the solvent.

Table 1 shows a series of absorption peaks found in the 8- to 15-μ region. The majority of those shown are apparently diagnostic of either the α- or β-anomer. The intensity of absorption is indicated by the figures in parentheses, which represent the percentage absorption, taking the absorption at 5.68 μ as 100 percent. The absorption at 5.68 μ was the same for both the anomers and their mixtures and was one of the most intense bands. There are also shown, for comparison, two peaks that are found in both α- and β-D-glucose pentaacetate. The α-anomer shows a slightly more intense absorption at 8.7 μ than the β-anomer. Both the anomers show essentially the same absorption at 11.15 μ.

Certain absorption peaks, such as that at 8.95 μ which is apparently characteristic of the β-anomer or that at 9.86 μ which is apparently characteristic of the α-anomer, decrease in intensity as the concentration of the anomer is decreased. On the other hand, the absorption peak at 9.65 μ which is characteristic of the β-anomer or the peak at 11.00 μ which is characteristic of the α-anomer rapidly disappears in the presence of the opposite anomer. It should be noted that the intensity of absorption is apparently not a factor. The absorption at 9.65 μ is very intense, while that at 11.00 μ is rather weak. Other examples will be apparent from Table 1. Essentially the same spectra were obtained when the solvent was allowed to evaporate and the solids run as films.

Since certain of the absorption peaks characteristic of an anomer behave as might be expected, and since the optical rotation of the mixtures was as calculated, we do not believe that there has been any interconversion between the α- and β-anomers. A possible explanation is that an association takes place in solution between the α- and β-anomers that suppresses the vibrations responsible for the infrared absorption characteristic of the anomer.

It would seem that considerable care should be taken when infrared analysis is used either for the identification or quantitative determination of sugars, particularly if there is a possibility that anomers are present (*5*).

F. A. H. Rice
L. C. Browning
Research and Development Department, U.S. Naval Powder Factory, Indian Head, Maryland

Table 1. Infrared absorption at several wavelengths of chloroform or acetone solutions of α- and β- and mixtures of α- and β-D-glucose pentaacetate. Total concentration, 1.00 percent (wt./vol.); wavelength in microns. The figures in parentheses represent the amount of absorption as compared with the absorption at 5.68 μ (percentage). The adsorption at 5.68 μ was the same for each anomer and for the mixtures.

β	4β/1α	1β/1α	1β/4α	α
8.70 (43)	8.65 (43)	8.65 (60)	8.65 (60)	8.65 (65)
8.95 (55)	8.95 (53)	8.95 (46)	8.90 (42)	
9.65 (92)	9.62 (94)			
	9.85 (50)	9.87 (73)	9.87 (73)	9.86 (78)
10.15 (26)	10.15 (26)	10.18 (25)	10.15 (25)	10.15 (24)
			10.35 (16)	10.35 (18)
10.43 (21)	10.45 (18)	10.45 (19)		
10.65 (62)	10.65 (49)	10.65 (53)	10.65 (23)	
10.75 (52)	10.75 (41)	10.75 (44)	10.80 (24)	
			11.00 (35)	11.00 (37)
13.45 (9)	13.45 (7)	13.45 (8)		
	14.05 (6)	14.05 (5)	14.15 (10)	14.17 (13)
14.45 (11)	14.45 (10)	14.42 (9)		

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Glycolysis by Tumor Mitochondria and the Action of Insulin

The cardinal role of glycolysis (formation of lactic acid from glucose) in the metabolism of living cancer cells has been recognized for well over 30 years (*1*). A high rate of glycolysis may also be readily demonstrated in cell-free homogenates prepared from tumors. However, the view is still widely held, despite evidence to the contrary (*2*), that the glycolytic enzymes of tumor cells (and of normal cells) are localized primarily in the nonparticulate fluid fraction of the cell cytoplasm. Mitochondria are regarded, at best, as potential stimulators of the glycolysis of the fluid fraction or else as playing some obscure part in the integrated functioning of the combined cell fractions. Mitochondria are generally considered not to possess the full complement of enzymes required to convert glucose into lactic acid.

During the course of investigations on hormonal regulation of the subcellular glycolysis of tumors, we have obtained new evidence of high intrinsic rates of anaerobic glycolysis by tumor mitochondria, provided that the mitochondria have been supplemented with supernatant fraction that has been enzymatically inactivated. Thus, when the supernatant fraction of a tumor homogenate