Dr. G. H. Bowen for construction of the phototube light-meter.

Literature Cited

1. Borthwick, H. A., Hendricks, S. B., and Parker, M. W., Action spectrum for inhibition of stem growth in dark-grown seedlings of albino and non-albino barley. Bot. Gaz. 113: 95–105. 1951.
2. Borthwick, H. A., Hendricks, S. B., and Parker, M. W. The reaction controlling floral initiation. Proc. Nat. Acad. Sci. Wash. 38: 929–934. 1952.
3. Borthwick, H. W., Hendricks, S. B., Parker, M. W., Toole, E. H., and Toole, V. K. A reversible photoreaction controlling seed germination. Proc. Nat. Acad. Sci. Wash. 38: 662–666. 1952.
4. Fischer, H., Riedmair, J., and Hasenkamp, J. Über Oxo-porphyrine: Ein Beitrag zur Kenntnis der Feinstruktur von Chlorophyll a. Ann. Chem. Justus Liebigs 508: 224–249. 1934.
5. Fischer, H. and Stern, A., in Fischer, H. and Orth, H. *Die Chemie des Pyrrols.* Leipzig, 1940. Vol. 2, part 2.
6. Flint, L. H. and McAlister, E. D. Wave lengths of radiation in the visible spectrum inhibiting the germination of light-sensitive lettuce seed. Smithsonian Inst. Publ. Misc. Coll. 94: 1–11. 1935; 96: 1–7. 1937.
7. French, C. S. and Young, V. K. The fluorescence spectra of red algae and the transfer of energy from phycoerythrin to phycocyanin and chlorophyll. Jour. Gen. Physiol. 35: 873–890. 1952.
8. Granick, S. Magnesium vinyl pheoporphyrin a_5, another intermediate in the biological synthesis of chlorophyll. Jour. Biol. Chem. 183: 713–730. 1950.
9. Hagenbach, A., Auerbacher, F., and Wiedemann, E. Zur Kenntnis der Lichtabsorption von Porphinfarbstoffen und über einige mögliche Beziehungen derselben zu ihrer Konstitution. Helv. Phys. Acta 9: 1–26. 1936.
10. Hendricks, S. B. Private communication.
11. Nicholas, R. E. H. and Rimington, C. Qualitative analysis of the porphyrins by partition chromatography. Scand. Jour. Clin. Lab. Invest. 1: 12–18. 1948.
12. Parker, M. W., Hendricks, S. B., and Borthwick, H. A. Spectral sensitivities for leaf and stem growth of etiolated pea seedlings and their similarity to action spectra for photoperiodism. Amer. Jour. Bot. 36: 194–204. 1949.
13. Parker, M. W., Hendricks, S. B., and Borthwick, H. A. Action spectrum for the photoperiodic control of floral initiation of the long-day plant *Hyoscyamus niger.* Bot. Gaz. 111: 242–252. 1950.
14. Parker, M. W., Hendricks, S. B., Borthwick, H. A., and Scully, N. J. Action spectrum for the photoperiodic control of floral initiation of short-day plants. Bot. Gaz. 108: 1–26. 1946.
15. Stern, A. and Molvig, H. Über die Lichtabsorption der Porphyrine X. Z. physik. Chem. A 178: 161–183. 1937.
16. Stern, A. and Wenderlein, H. Über die Lichtabsorption der Porphyrine II. Z. physik. Chem. A 174: 81–103. 1935.
17. Stern, A. and Wenderlein, H. Über die Lichtabsorption der Porphyrine IV. Z. physik. Chem. A 175: 405–437. 1936.
18. Stern, A. and Wenderlein, H. Über die Lichtabsorption der Porphyrine V. Z. physik. Chem. A 176: 81–124. 1936.
19. Stern, A. and Wenderlein, H. Über die Lichtabsorption der Porphyrine VII. Z. physik. Chem. A 177: 165–192. 1936.
20. U. S. Dept. Agr. Production and Marketing Admin. Rules and Regulations under the Federal Seed Act. Service and Regulatory Announcement No. 156. 1950.
21. Willstatter, R. and Stoll, A. Untersuchungen über Chlorophyll. Berlin. 1913.

POSITION OF C^{14} IN ALCOHOL AND CARBON DIOXIDE FORMED FROM LABELED GLUCOSE BY CORN ROOT TIPS[1, 2]

HARRY BEEVERS and MARTIN GIBBS

Department of Biological Sciences, Purdue University, Lafayette, Indiana, and Department of Biology, Brookhaven National Laboratory, Upton, New York

There are good reasons for supposing that the classical pathway of glycolysis (Embden-Meyerhof-Parnas, or E.M.P., sequence) plays an important role in the respiratory breakdown of glucose in higher plants. The evidence has come not only from experiments on cell-free systems capable of fermenting glucose to alcohol and CO_2 but also from work with intact tissues respiring in air and nitrogen (reviewed by Thomas, 17). The close analogy between fermentations induced by yeast and by higher plant tissues, and the recent isolation of some of the enzymes responsible for glycolysis from green plants (16) have justified the belief that the glycolytic mechanisms involved in the two cases are the same.

Recently, however, evidence has accumulated that alcohol and CO_2 may be formed during bacterial dissimilation of glucose by pathways (7, 10) other than the classical glycolysis, and, moreover, enzymes responsible for the reactions of one of these alternate pathways (the hexose monophosphate shunt or oxidative pathway) have been characterized in higher plant tissues (1, 2, 9). It was thus of interest to reexamine glucose breakdown in a plant tissue using methods which would reveal whether a mechanism other than the E.M.P. sequence was contributing to alcohol production and thus to alcohol precursors such as pyruvic acid.

The technique used was one which has been ap-

[1] Received January 22, 1954.

[2] Research carried out at the Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

plied successfully to determine the pathway of glucose dissimilation in yeast (12), fungi (8), and microorganisms (6, 7, 10). Glucose labeled with C^{14} in specific positions is metabolized by the organism, and the end products or intermediates are isolated and degraded in order to determine the location of the glucose carbon atoms. Thus if glucose breakdown was occurring by the E.M.P. sequence in the organisms being tested, the position of the tracer in the end product alcohol could be predicted, and if this sequence was operating to the exclusion of the direct oxidation pathway the predictions would be fulfilled. Clearly, if the locations of the glucose carbon atoms in the alcohol were different from those predicted, and in accordance with what is known about the reactions of the direct oxidation pathway (11), this would constitute evidence of its operation.

The possible occurrence of these pathways in young corn roots has now been studied in this way. The following substrates were used: a) glucose-1-C^{14} in a N_2 atmosphere and b) glucose-1-C^{14}, glucose-2-C^{14} and glucose-3,4-C^{14} in the presence of O_2 with sodium arsenite (which restricts the oxidation of pyruvate and diverts it to alcohol and CO_2). Following dissimilation of glucose, the two carbons of alcohol and that of the respired CO_2 were analyzed individually for radioactivity content to determine the distribution of isotopic carbon. The results are the subject of the present paper.

Materials and Methods

Plant Materials: Corn hybrid Wf 9×38-11 was soaked in water for 24 hours, drained and allowed to germinate at 25° C. When the primary roots had reached a length of 3 to 4 cm the tips (1 to 2 cm) were cut off, washed in distilled water and used without further treatment. Twenty tips per flask were usually used in the manometric experiments but in the gas stream experiments 2 to 4 gm of tissue was used as a sample.

Manometric Experiments: All experiments were carried out at 25° C using the conventional Warburg respirometer. The main compartment of the Warburg vessel contained the root tips, 1 ml of 0.03 M phosphate buffer at pH 5.0 and water to make a volume of 2.0 ml. The side arm carried the sugar sample (10 to 60 micromoles) while the center well contained 0.2 ml of 20 % KOH. In the anaerobic experiments the vessels were filled with prepurified N_2 and equilibrated before closing and adding the glucose from the side arms. At the end of the manometric experiments the KOH was removed from the center well and transferred to a flask filled with N_2. The center well was washed three times with CO_2-free water and the washings included with the KOH. The CO_2 was liberated by acid and passed into a known amount of 0.3 M $Ba(OH)_2$ and its amount determined by back titration with 0.1 N HCl. Usually the amount of CO_2 produced during the experiment was large enough to make the addition of carrier CO_2 unnecessary: i.e., sufficient $BaCO_3$ (about 20 mg) was produced to give a layer of near infinite thickness when it was transferred to a sintered glass disc for determination of radioactivity.

Gas Stream Experiments: The tissue was suspended in 8 ml of 0.03 M phosphate buffer, pH 5.0, in a 30 ml cylindrical, sintered glass funnel of medium porosity. 60 to 120 micromoles of glucose were added and a stream of CO_2-free air or prepurified N_2 was passed into the base of the funnel and through the solution bathing the roots. The gas stream was then passed through two absorption tubes in which the respired CO_2 was collected in 0.3 M $Ba(OH)_2$. In some of the experiments with arsenite the outgoing gas stream was first passed through 2 % sodium bisulfite to absorb acetaldehyde and then through half-saturated potassium permanganate before it entered the CO_2 absorption tubes. The amount of CO_2 produced was determined by titrating the excess $Ba(OH)_2$ with 0.1 N HCl and the $BaCO_3$ was transferred to sintered glass discs for determination of its radioactivity.

Alcohol Degradation: At the end of the experimental run an aliquot of the solution bathing the roots was removed and the amount of alcohol determined by means of yeast alcohol dehydrogenase (5). The rest of the solution was made alkaline to phenol red with 1 N KOH and the alcohol distilled with carrier alcohol. It was oxidized to acetic acid by heating at 95° C for 2 hours with 0.5 gm of $K_2Cr_2O_7$ in 4 N H_2SO_4. The acetic acid was distilled, neutralized with 0.1 N NaOH and evaporated to dryness. The sodium acetate was then degraded by the method of Phares (13).

Measurements of Radioactivity: All C^{14} samples were assayed as barium carbonate, using a methane-flow beta proportional counter (14).

Labeled Glucose: The glucose-1-C^{14} and glucose-2-C^{14} were kindly supplied by Dr. H. Isbell of the National Bureau of Standards. The glucose-3, 4-C^{14} was prepared from rat liver glycogen (18).

Results and Discussion

Some preliminary experiments were carried out in which 20 to 40 micromoles of uniformly labeled glucose (c. 20×10^{-9} curies) were applied to corn roots respiring in N_2 or in air. These established the important fact that, even though the glucose might not induce an increase in the high endogenous respiration rate, as judged from the measurements of gas exchange, nevertheless the exogenous glucose was utilized, as shown by the progressive release of $C^{14}O_2$. The aim was to supply to the respiring material a small amount of glucose (to limit dilution of radioactive material) and yet to provide an amount sufficient to give easily measurable amounts of radioactivity in the respiratory products.

Anaerobic Experiments with Corn Roots: Two types of experiments were carried out (table I): one of the gas stream type (experiment 1) and the other in Warburg vessels (experiment 2). In both experiments, it is clear that of the radioactivity recovered in the products, almost all of the aldehyde

TABLE I

THE LOCATION OF C^{14} IN THE CO_2 AND ETHANOL PRODUCED ANAEROBICALLY BY CORN ROOT TIPS * FROM GLUCOSE-1-C^{14}

EXPERIMENT	PRODUCT	SPECIFIC ACTIVITY **	TOTAL ACTIVITY	% OF TOTAL
		$m\mu$ c/mg C	$m\mu$ c	
I. Gas stream 4.5 hrs.	CO_2	0.10	0.34	4.6
	CH_2OH of alcohol	0.03	0.24	3.3
	CH_3 of alcohol	0.99	6.77	92.1
II. Manometric 4.25 hrs.	CO_2	0.16	0.21	7.6
	CH_2OH of alcohol	0	0	0
	CH_3 of alcohol	1.04	2.55	92.4

* In experiment I, 4-gm roots were suspended in 10 ml solution containing 120 micromoles glucose (specific activity 7.5 $m\mu$ c/mg C). 13.5 mg respired CO_2 was collected and no carrier was added. From the alcohol/CO_2 ratio of 0.92 observed previously with this tissue (4) it was calculated that 12.4 mg alcohol had been produced and 22.1 mg inactive alcohol was added as carrier before distillation.

In experiment II, a total of 160 root tips respired in 8 ml solution containing 60 micromoles glucose (specific activity 7.5 $m\mu$ c/mg C). 4.82 mg CO_2 were produced and Na_2CO_3 equivalent to 8.1 mg CO_2 was added as carrier. 22.8 mg inactive alcohol was added to the calculated 4.43 mg alcohol produced before distillation.

All figures are corrected for dilution by carrier.

** Specific activity expressed as millimicrocuries per milligram of carbon.

carbon of glucose was in the methyl group of alcohol; the carbinol group was essentially unlabeled and a trace of the isotope appeared in the CO_2. Since the location of the C^{14} was such as might have been expected from the E.M.P. scheme (12), it would appear that under anaerobic conditions, this was the only pathway of glucose breakdown being used by the corn roots.

AEROBIC EXPERIMENTS WITH CORN ROOTS AND ARSENITE: From Warburg experiments designed to find a concentration of arsenite which would prevent O_2 uptake and induce accumulation of pyruvate it soon became apparent that the corn tissues reacted differently from those of other species to which this method has been successfully applied in the past (6). The results are shown in figure 1. It will be seen that although O_2 uptake is strikingly reduced at concentrations as low as 10^{-3} M, the addition of more arsenite does not lead to a further steep decline in the curve, and at the highest concentrations used the residual O_2 uptake was still maintained at about 40 % of the control level. Even more significant, however, was the effect on R.Q. and CO_2 release. Whereas the control roots respired with an R.Q. of 1.08, those in the presence of increasing amounts of arsenite showed progressively higher R.Q.'s which reached a maximum of about 2.3. A calculation of the excess CO_2 over that which would have been expected from the O_2 uptake values if the R.Q. had remained at the control level yields curve "F". It is quite evident that excess CO_2 appears only when concentrations inhibitory to O_2 uptake are applied, and, as the O_2 uptake curve declines, so the "F" curve rises. Such effects of an inhibitor are reminiscent of others (3, 4) in which it has been shown that the excess CO_2 is due to induced aerobic fermentation. It has now been shown, in fact, that a similar explanation holds for the arsenite curves; alcohol and acetaldehyde accumulate as a result of arsenite poisoning. Aerobic fermentation is known to be induced by a variety of agents which for one reason or another might be expected to lead to an increased internal concentration of pyruvate, which does not accumulate in large amounts but is diverted towards alcohol through the action of carboxylase. Arsenite, it now appears, acts in a similar manner to induce aerobic fermentation; the accumulation of pyruvate, which results from the slowing down of its utilization by arsenite in other species, finds, as its counterpart in the corn root, the accumulation of alcohol and acetaldehyde.

Advantage was taken of this effect of arsenite in another group of experiments with differently labeled glucose samples (glucose-1-C^{14}, glucose-2-C^{14}, and glucose-3,4-C^{14}). The procedure was to collect the alcohol produced in air in the presence of M/200 arsenite and to determine the distribution of C^{14}. All of these experiments were of the gas stream type, and acetaldehyde was collected in some of these, only to confirm that it was produced (15); it was not degraded. In a 4 hour run with inactive glucose plus M/200 arsenite in which the three respiratory products were measured the following values were obtained: CO_2: 3.10 mg, acetaldehyde: 0.41 mg, alcohol: 1.88 mg. Since a considerable amount of aerobic respiration (as judged from O_2 uptake values in figure 1) persisted at this arsenite level, it was not surprising to find that activity appeared in the respired CO_2 from each of the labeled-glucose samples; as pointed out above, attention was focussed on the alcohol. Table II shows the results of the degrada-

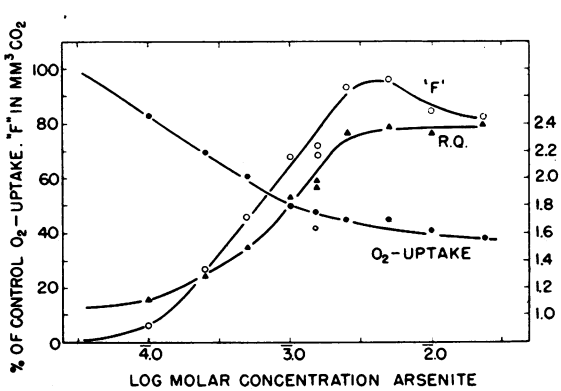

FIG. 1. The effects of graded concentrations of sodium arsenite on O_2-uptake and R.Q. of corn root tips. The "F" curve is calculated from the R.Q. and the O_2-uptake data by a method described in the text.

TABLE II

THE LOCATION OF C^{14} IN THE ALCOHOL PRODUCED AEROBICALLY BY CORN ROOT TIPS * RESPIRING LABELED GLUCOSE IN THE PRESENCE OF M/200 ARSENITE

	SUBSTRATE		
	GLUCOSE-1-C^{14}	GLUCOSE-2-C^{14}	GLUCOSE-3,4-C^{14}
Amount of glucose added (μM)	100	100	60
Amount of alcohol produced (mg)	2.9	2.4	3.4
Specific activity of glucose **	7.5	25.0	6.2
Specific activity of C in CH_3 of alcohol	1.08	0.15	0
Specific activity of C in CH_2OH of alcohol	0.04	5.03	0
Total radioactivity in CH_3 (mμ c)	1.61	0.19	0
Total radioactivity in CH_2OH (mμ c)	0.06	6.40	0
% of total in CH_3	97.5	2.8	0
% of total in CH_2OH	2.5	97.2	0

* In each case approximately 2-gm root tips were aerated with CO_2-free air in a solution containing the glucose sample and arsenite at pH 5.0. 22.1 mg carrier alcohol were added to each alcohol sample before distillation. The figures are corrected for carrier.

** Specific activity expressed as millimicrocuries per milligram of carbon.

tions. It will be seen that the fates of the constituent C atoms are very close to those which would have been predicted on the E.M.P. scheme. If triose which had arisen from the oxidative pathway had been diverted towards pyruvic acid and alcohol, a different pattern of labeling would have been expected (11); and the relative importance of such a pathway would presumably have determined the masking of the distribution expected on the E.M.P. scheme. The conclusion seems justified that in air, also, glucose dissimilation in corn roots is via the classical glycolytic sequence of reactions.

SUMMARY

From experiments on the fermentation of glucose-1-C^{14} by corn root tips, which produce alcohol and CO_2, the following has been found.

1. Carbon atom 1 (aldehyde carbon) of glucose gave rise to the methyl carbon of ethanol.

2. Both the carbinol carbon of alcohol and the CO_2 contained only traces of the isotope.

From experiments on the oxidation of glucose-1-C^{14}, glucose-2-C^{14} and glucose-3,4-C^{14} by corn root tips in which aerobic fermentation was induced by arsenite and evidenced by alcohol formation, the following has been found:

1. glucose-1-C^{14} yielded methyl labeled ethanol.
2. glucose-2-C^{14} gave rise to carbinol labeled ethanol.
3. glucose-3,4-C^{14} yielded unlabeled ethanol.

These results are compatible with the conclusion that glucose is dissimilated by corn root tips anaerobically as well as aerobically via the classical glycolytic sequence of reactions (Embden-Meyerhof-Parnas pathway).

LITERATURE CITED

1. AXELROD, B., BANDURSKI, R. S., GREINER, C. M., and JANG, R. The metabolism of hexose and pentose phosphates in higher plants. Jour. Biol. Chem. 202: 619–634. 1953.
2. BARNETT, R. C., STAFFORD, H. A., CONN, E. C., and VENNESLAND, B. Phosphogluconic acid dehydrogenase in higher plants. Plant Physiol. 28: 115–122. 1953.
3. BEEVERS, H. Malonic acid as an inhibitor of maize root respiration. Plant Physiol. 27: 725–735. 1952.
4. BEEVERS, H. 2,4-Dinitrophenol and plant respiration. Amer. Jour. Bot. 40: 91–96. 1953.
5. BONNICHSEN, R. K. and THEORELL, H. An enzymatic method for the micro-determination of ethanol. Scand. Jour. Clin. Lab. Invest. 3: 58–62. 1952.
6. ENTNER, N. and DUODOROFF, M. Glucose and gluconic acid oxidation of *Pseudomonas saccharophila*. Jour. Biol. Chem. 196: 853–862. 1952.
7. GIBBS, M. and DEMOSS, R. D. Ethanol formation in *Pseudomonas lindneri*. Arch. Biochem. Biophys. 34: 478–479. 1951.
8. GIBBS, M. and GASTEL, R. Glucose dissimilation by Rhizopus. Arch. Biochem. Biophys. 43: 33–38. 1953.
9. GIBBS, M. The respiration of the pea plant. Oxidation of hexose phosphate and pentose phosphate by cell free extracts of pea leaves. Plant Physiol. 29: 34–39. 1954.
10. GUNSALUS, I. C. and GIBBS, M. The heterolactic fermentation. II. Position of C^{14} in the products of glucose dissimilation by *Leuconostoc mesenteroides*. Jour. Biol. Chem. 194: 871–875. 1952.
11. HORECKER, B. L., GIBBS, M., KLENOW, H., and SMYRNIOTIS, P. Z. The mechanism of pentose phosphate conversion to hexose monophosphate. I. With a liver enzyme preparation. Jour. Biol. Chem. 207: 393–403. 1954.
12. KOSHLAND, D. E. and WESTHEIMER, F. H. Mechanism of alcoholic fermentation. The fermentation of glucose-1-C^{14}. Jour. Amer. Chem. Soc. 72: 3383–3388. 1950.
13. PHARES, E. F. Degradation of labeled propionic and acetic acids. Arch. Biochem. Biophys. 33: 173–178. 1951.
14. STEELE, R. and SFORTUNATO, T. Techniques in the use of C^{14}. Brookhaven Nat. Lab. Tech. Rept. T-6. 1949.
15. STOTZ, E. A colorimetric determination of acetaldehyde in blood. Jour. Biol. Chem. 148: 585–591. 1943.
16. STUMPF, P. K. Glycolytic enzymes in higher plants. Ann. Rev. Plant Physiol. 3: 17–34. 1953.
17. THOMAS, M. Plant Physiology, 3rd Ed. J. and A. Churchill, Ltd., London. 1947.
18. WOOD, H. A., LIFSON, N., and LORBER, V. The position of fixed carbon in glucose from rat liver glycogen. Jour. Biol. Chem. 159: 475–489. 1945.