Various practical ways of protecting the soil surface against sudden drops in temperature in the late fall may be suggested. Threshing residues, chopped corn stalks, composts, or manure might be spread early in the fall after the row crop has been removed. Where these materials are not available, a fast-growing catch crop that forms a winter mulch might be interseeded at the last cultivation of the row crop.

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Distribution of C^{14} in Photosynthesizing Barley Seedlings[1]

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As part of a program for the biosynthesis of carbon-labeled biologically important compounds, especially sugars, the following exploratory work has been performed on the assimilation of radioactive carbon dioxide in the light by young barley plants.

Plants were grown in a greenhouse on Hoagland's nutrient solution until they averaged 6–7 inches in height. Upon removal to the laboratory, select plants were divided into two groups: (1) those from which primary and secondary roots and hypocotyl had been cut (hereafter referred to as "minus"); and (2) entire plants, except for secondary roots (hereafter referred to as "plus"). Fresh weights of each group were approximately equal. (The minus group therefore had considerably more leaf material, since the roots and hypocotyls of the plus plants constitute a large fraction of their weight.)

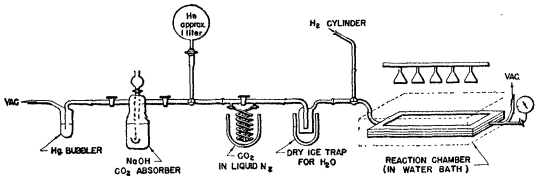

Fig. 1

The reaction chamber housing both groups of plants consisted of a rectangular brass frame sandwiched between two 1-inch glass panes; the free volume was approximately 1 l. The chamber was placed within a tank containing flowing water for cooling (ca. 25°) and illuminated from above, at a distance of 18 inches, with a bank of four GE Projector Spot, 250-watt lamps.

The setup in the experiment is depicted in Fig. 1. The CO_2 absorber, filled with NaOH, was present to catch any CO_2 escaping the spiral immersed in liquid nitrogen. The helium (or H_2) bulb, containing 2–5 p.s.i. (above atmospheric pressure) of the gas, flushed the spiral containing $C^{14}O_2$ into the chamber. At the beginning and end of the experiment, hydrogen flushed other gases out of the tissue. The dry ice trap was for water.

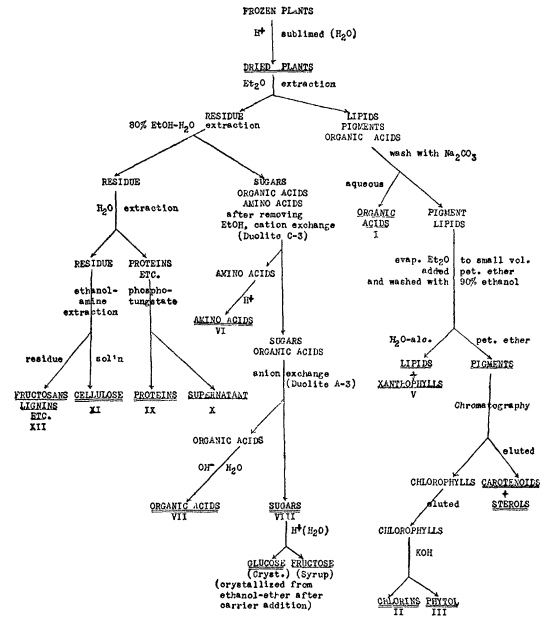

Fig. 2. The substances doubly underlined are materials upon which direct counts were obtained; those singly underlined, materials whose activity was obtained by difference.

TABLE 1

	With roots Total activity fixed (%)	Without roots Total activity fixed (%)
Dried whole plant	100*	100†
Ether extractable acids (I)	[12.5]‡	[7.9]‡
Chlorins (II)	6.1	0.8
Phytol (III)		0.9
Carotenoids (IV) (contaminated with sterols)	0.9	3.6
Other lipids (V)		
Amino acids (VI)	7.2	5.6
Acids (VII)	11.3	7.7
Sugars (VIII)	25.8	35.0
Soluble protein (IX)	0.7	6.9
Nonprotein (aqueous) (X)	[3.5]‡	
Cellulose (XI)	2.8	3.6
Lignins, etc. (XII)	[8.3]‡	[9.4]‡
	78.2§	79.7§

* The weight was 2.223 grams—containing 2.00×10^6 c/min.

† The weight was 1.871 grams—containing 0.58×10^6 c/min.

‡ Obtained by calculation rather than direct measurement.

§ Much of the loss is due to incomplete recovery of acids from regenerated adsorption columns.

The chamber was filled with hydrogen and evacuated 5 successive times to remove CO_2, after which the $C^{14}O_2$ (from approximately 45 mg. of $BaC^{14}O_3$) was swept in with the aid of

[1] This paper is based on work performed under Contract #W-7405-Eng-48 of the Atomic Energy Commission with the University of California, Berkeley.

[2] With the technical assistance of G. A. Hall.

helium to a vacuum of 23 inches, and finally, hydrogen was admitted to a vacuum of 5 inches. The light was then turned on, and the leaves permitted to photosynthesize for 60 minutes. At the end of the experiment the chamber was slowly evacuated, the remaining CO_2 being caught in the spiral and NaOH bubbler. Six flushings with hydrogen to remove as much CO_2 as possible, the final evacuation being to ca. 29 inches, consumed an additional hour. The respiration time was therefore two hours.

The plants taken from the chamber were placed immediately into liquid nitrogen and ground to a powder. The frozen powder was then treated as shown in Fig. 2.

The crystalline glucose was obtained by evaporating the hydrolyzed sucrose mixture to a syrup, dissolving this syrup together with several hundred milligrams of carrier glucose in the minimal amount of 95 per cent alcohol, and then adding ether to produce a slight cloudiness. Upon cooling, glucose crystallizes out.

It should be remembered, however, that the above distribution of activity is valid only for the specific set of conditions for photosynthesis given. It may be expected to be different for other conditions, *i.e.* time, O_2 pressure, CO_2 pressure, etc.

The activity measurements of the various fractions shown in the foregoing chart are given in Table 1.

IN THE LABORATORY

A Micromethod for the Determination of 1−(+) Lactic Acid[1]

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Lehmann (*2*) has presented a detailed description of a simple micromethod for determining 1−(+) lactic acid which appears to have been largely overlooked. We have found this basic method very useful and have devised certain modifications which eliminate interfering substances and permit final measurement to be made colorimetrically.

The method depends on the quantitative oxidation of lactic acid to pyruvic acid by means of the yeast enzyme, lactic dehydrogenase, in the presence of potassium ferricyanide, which acts as hydrogen acceptor. Lehmann titrated the resulting potassium ferrocyanide with ceric sulfate, using 1 per cent "setoglaucin-o" as the indicator. Diphenylamine sulfonic acid may likewise be used (*3*) as the indicator. According to Lehmann, the method is quite specific. Of a group of 52 substances investigated, which included carbohydrates, amino acids, α- and β-hydroxybutyric acids, intermediary metabolites, and substances related structurally to lactic acid, the only compounds which interfered with the determination were glycolic, α-hydroxybutyric, and glyceric acids, α-glycerophosphate, hexosediphosphate, and hexosemonophosphate. The first two compounds are not present in sufficient concentration to interfere in biological fluids. The last four can be removed by treatment with $CuSO_4$-$Ca(OH)_2$ according to the method of Friedemann and Kendall (*1*). Ascorbic acid which interferes by reacting directly with potassium ferricyanide can likewise be removed by this procedure. Preliminary treatment with $CuSO_4$-$Ca(OH)_2$ does not interfere with the enzymatic reaction providing the pH is properly adjusted before incubation with the enzyme.

[1] This work was supported by a grant from the Mallinckrodt Chemical Works.

Thus, the procedure used in this laboratory is similar to that described by Lehmann except for the use of $CuSO_4$-$Ca(OH)_2$ for the removal of interfering substances and the determination of potassium ferrocyanide by a colorimetric instead of a titrimetric method. A brief description follows.

Reagents necessary for the method are M/15 Na K phosphate buffer, pH 7.4; 5M/1,000 potassium ferricyanide in water; and ferric salt prepared as follows: Twenty grams of gum ghatti in a cheesecloth bag is soaked in 1 l. of water for 24 hours, and 5 grams of anhydrous $Fe_2(SO_4)_3$ and 75 cc. of 85 per cent H_3PO_4 + 100 cc. of water are added. After mixing, 15 cc. of 1 per cent $KMnO_4$ is added in order to destroy reducing materials in the gum ghatti. The solution is allowed to stand a few days before using.

Fleischmann's baker's yeast is powdered and dried at room temperature. It is then washed several times with distilled water (100 grams in 1 l. of water), following which it again is dried at room temperature and powdered. This dried yeast is the source of the enzyme, lactic dehydrogenase, and keeps indefinitely in the refrigerator. Before using, the yeast is washed once with phosphate buffer (50–100 mg. yeast, depending upon the activity, in 10 cc. of buffer), centrifuged, and resuspended in the same volume of buffer. The activity of the enzyme as well as the removal of intracellular substrate may be tested as suggested by Lehmann by incubating 1-cc. aliquots of the yeast suspension alone, and with 180 γ lactate, in the presence of 1 cc. of a solution of o-chlorophenol-indo-2:6-dichlorophenol (sodium 2,6-dichlorobenzenone-indo-3-chlorophenol (ortho), Eastman P 3467) (1:50,000) in an open test tube. In the presence of lactic acid the indicator should be decolorized in 3 minutes or less. The control should not be decolorized in 10 minutes and usually is not in an hour.

Procedure: The sample containing lactic acid is deproteinized with trichloracetic acid and centrifuged. The supernatant fluid is neutralized and diluted so that 1 cc. contains not more than 200 γ of 1-lactic acid. If ascorbic acid or phosphorylated hexoses be present, the sample is treated with $CuSO_4$-$Ca(OH)_2$ as described by Friedemann and Kendall. Treatment with trichloracetic acid is unnecessary when the $CuSO_4$-$Ca(OH)_2$ precipitation is used in unknowns with small amounts of