Carbohydrate Metabolism of Sugar Beets I. Respiratory Catabolism of Mono and Disaccharides[1]

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Introduction

The respiratory activity of sugar beets is of interest to processors because of losses of sucrose from post-harvest beet roots during storage. Earlier, it has been reported (1)[3] that losses may be related directly to the respiratory activity of sugar beets. Stout and co-workers reported that the respiratory rate of sugar beets can be affected by temperature, bruising, CO_2 and oxygen contents of environmental atmosphere, surface area per unit weight, genetic varieties, and inhibitors (4, 5, 6). The nature of pathways of carbohydrate catabolism operative in beet roots is not yet elucidated.

In the present work the radiorespirometric method (2, 3) has been employed to examine the utilization of several C^{14} labeled monosaccharides and disaccharides by beet roots.

Materials and Methods

Sugar beets of the variety US 22/3 were shipped from Nampa, Idaho, to the laboratory by air immediately after harvest. Test samples, selected on the basis of uniformity in shape, weight and maturity, were cleaned thoroughly prior to experimentation. Each of the test samples weighed approximately 500 grams.

The C^{14} labeled compounds used in the present study were obtained from the National Bureau of Standards through the kind cooperation of Dr. H. S. Isbell and several other commercial sources. Purity of each of the labeled compounds was established by means of paper chromatography and radioautography.

The radioactive substrate in the form of an aqueous solution was administered to an intact root by means of the "well" method. According to this method, a cylindrical well 9.5 mm in diameter, was drilled into the crown of the root to a length of 1/3 of the beet by means of a sterilized glass tubing. Upon withdrawing the glass tubing from the root along with the tissue core inside the tubing, a well with a defined area was hence

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[2] A portion of this work is taken from the thesis presented by R. D. Barbour for the degree of Master of Science at Oregon State College, 1958.

[3] Numbers in parentheses refer to literature cited.

established. The radioactive substrate was then introduced into the well in the form of an aqueous solution having a given volume and prescribed concentration with respect to both amount and radioactivity. Approximately ⅔ of the previously removed tissue column was then inserted to cover the opening of the well and molten paraffin wax was applied to seal the entire opening.

Inasmuch as the size of the well is defined and the diffusion of the substrate solution into the root tissue is likely to follow a constant rate, one should, therefore, expect reasonably reproducible findings from replicate experiments. The nature of the substrate diffusion processes was examined by a radioautographic study of root sections following the administration of C^{14} labeled sucrose. It was observed that the diffusion of sucrose followed a defined and homogeneous pattern. In addition, satisfactory reproducibility of recovery data was realized with respect to respiratory $C^{14}O_2$ from a great number of beet roots administered with an equal amount of C^{14} labeled sucrose by the "well" method.

The utilization of monosaccharides and disaccharides by beet roots in respiration was studied by the radiorespirometric method reported earlier for similar studies with fruits (2, 3). The method relies on the examination of interval recoveries of respiratory $C^{14}O_2$ from a biological system utilizing C^{14} labeled substrate. The substrates employed in these experiments as well as their amounts and radioactivities are given in Table 1. In a typical experiment, labeled substrate was introduced into the beet root by the "well" method. The beet was then transferred into a respiration chamber, 8 liters in capacity and equipped with inlet and outlet tubes. CO_2-free air was introduced into

Table 1.—C^{14} Specifically labeled substrates used in radiorespirometric experiments.

Substrate	Weight, mg	Specific activity uc/mM	Total radioactivity uc
Sucrose-U-C^{14}	25	1.23	0.10
Maltose-1-C^{14}	20	18.00	1.00
Glucose-U-C^{14}	50	1.80	0.50
Fructose-U-C^{14}	10	18.00	1.00
Mannose-1-C^{14}	10	18.00	1.00
Galactose-1-C^{14}	10	18.00	1.00
Ribose-1-C^{14}	8	18.10	1.00
Glucuronate-6-C^{14}	20	6.77	0.70
Gluconate-U-C^{14}	28	2.31	0.28

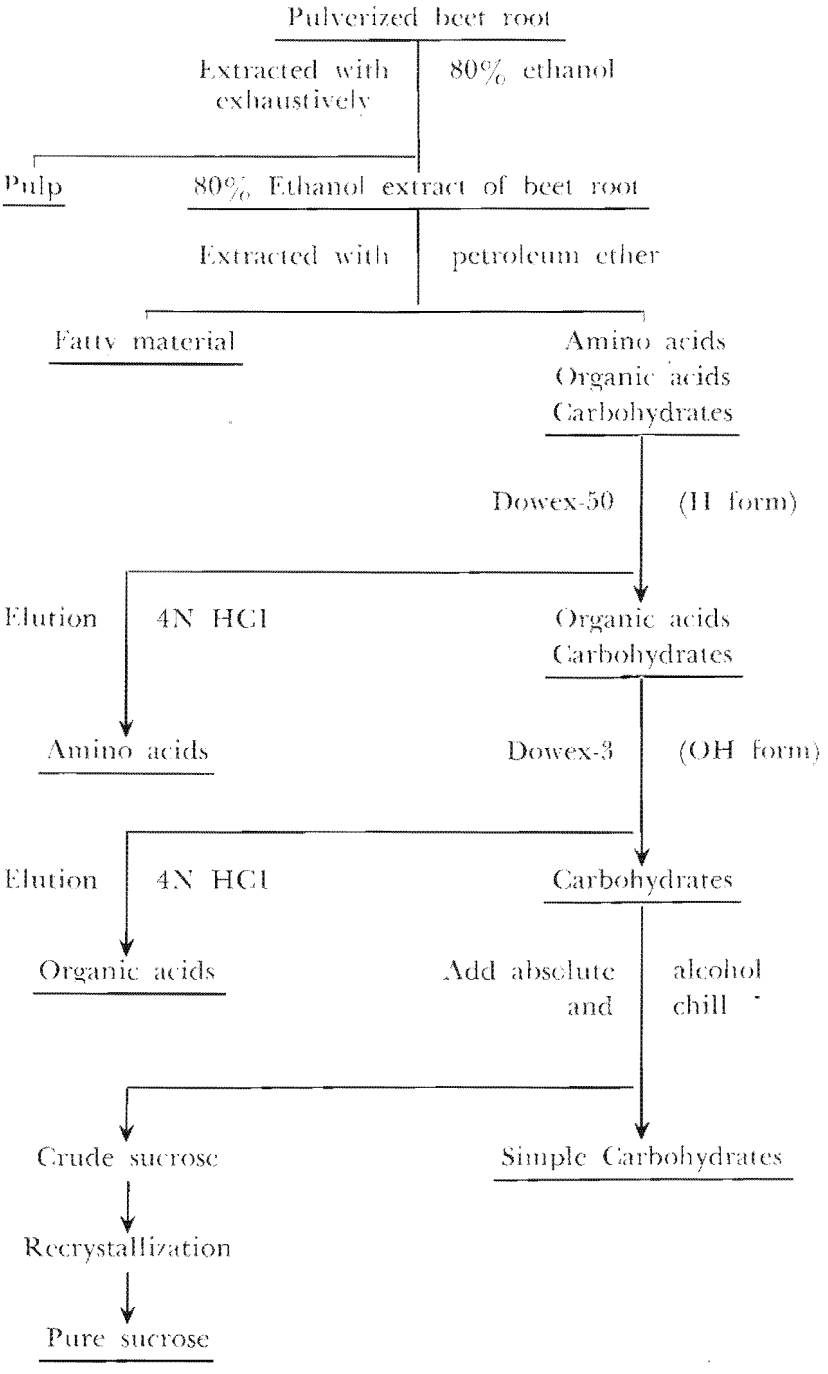

Figure 1.—Scheme for the isolation of beet constituents.

the chamber from a bypass manifold equipped with a flowmeter, thus permitting the control of air flow rate sweeping through the chamber. A flow rate of approximately 200 cc per minute was employed to insure a rapid turnover of atmosphere in the chamber. The respiratory CO_2 was swept into a CO_2 trap consisting of a sintered glass sparger immersed in 25 ml of 0.5 N CO_2-free sodium hydroxide solution. At prescribed time intervals, the solution in the trap was replaced and the CO_2 trapped was recovered as $BaCO_3$ by the addition of $BaCl_2$-NH_4Cl solution.

In a separate experiment, a relatively higher level of C^{14} labeled sucrose (9.1 μc in 100 mg) was administered to the beet weighing 831 grams, thus permitting the estimation of substrate incorporation into various constituents and metabolic products of beet root. Utilization of sucrose was followed radiorespirometrically for a period of 233 hours, at which time the specific activities of the respired CO_2 had declined and leveled to a steady value. The beet was then processed according to the scheme given in Figure 1 for the isolation of various groups of beet constituents for subsequent radioactive analysis.

The radioactivity of respiratory CO_2 and various beet constituents was determined by means of a Geiger-Muller gas flow counter. All counting samples were converted to $BaCO_3$, which in turn was mounted on aluminum planchets by the centrifugation technique. Countings were carried out to a standard deviation no greater than 1% and the counting data were corrected for background and self-absorption in the usual manner.

Results and Discussion

The radiorespirometric data on the utilization of C^{14} labeled monosaccharides and disaccharides given in Table 2 illustrates

Table 2.—Recoveries of $C^{14}O_2$ from intact beet root metabolizing C^{14} specifically labeled substrates.

Substrate	Cumulative percentage recovery of $C^{14}O_2$ at given hours				
	4 hr	8 hr	12 hr	16 hr	20 hr
Sucrose-U-C^{14}	0.3	0.4	8.6	11.5	13.6
Maltose-1-C^{14}	0.2	3.3	8.6	12.2	14.1
Glucose-U-C^{14}	1.5	5.8	9.2	12.4	14.7
Fructose-U-C^{14}	0.6	5.8	9.4	11.8	12.9
Mannose-1-C^{14}	0.1	0.6	1.5	3.0	4.2
Galactose-1-C^{14}	0.9	6.6	10.5	12.7	14.1
Ribose-1-C^{14}	0.5	4.6	11.4	18.5	21.8
Glucuronate-6-C^{14}	2.0	13.7	24.9	28.7	30.0
Gluconate-U-C^{14}	0.5	1.0	2.5	10.5	10.5

well the versatility of beet roots in metabolizing carbohydrate materials. At the end of 20 hours a considerable amount of substrate radioactivity was recovered in respiratory CO_2 from beet roots metabolizing C^{14} labeled sucrose, maltose, glucose, fructose, galactose, ribose, glucuronate and gluconate. Mannose was found to be metabolized only slightly indicating that mannose is not catabolized directly or converted rapidly to other readily catabolized hexoses such as glucose and fructose.

It is also noteworthy that 25% of the C^{14} activity in the administered glucuronate-6-C^{14} was converted to CO_2 in a matter of 12 hours. This fact may indicate the active operation of a direct decarboxylation process of glucuronate leading to the formation of xylose. Since a significant amount of $C^{14}O_2$ was produced in the gluconate-U-C^{14} experiment, it is possible that the phosphogluconate decarboxylation process may be operative in beet roots possibly as another mechanism for pentose production. It should be emphasized that the data presented here served only as evidence for the presence of enzyme systems responsible for the metabolism of the respective carbohydrates. Variation with respect to the rates of substrate diffusion in beet root does not permit one to elucidate the exact nature of the pathway nor to compare the findings with different labeled substrates.

The fate of sucrose in beet metabolism was traced by a radiorespirometric study of the utilization of sucrose-U-C^{14} with higher specific activity. The radiorespirometric data collected in this experiment are given in Figure 2. It is noted that the specific activity of $C^{14}O_2$ rises sharply in the earlier phase evidently reflecting the active metabolism of the substrate prior to its diffusion away from the site of administration. The progress of diffusion is indicated by the decline of the specific activity

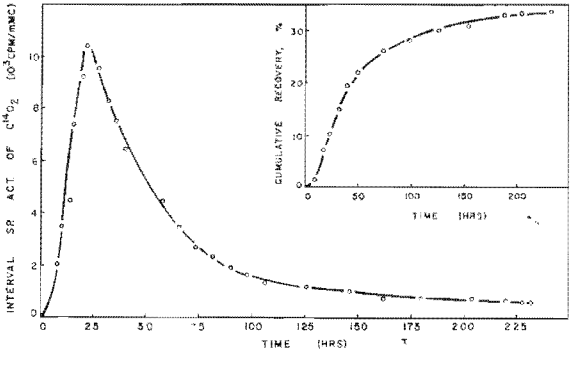

Figure 2.—Radiorespirometric pattern of sucrose-U-C^{14} catabolism in beet roots.

of $C^{14}O_2$ beginning at 25 hours after substrate administration and continuing until approximately 220 hours. The beet root was found to be still in normal physiological state and at the end of the experiment the final specific activity of the respiratory $C^{14}O_2$ was 619 cpm/mM of carbon. The beet root was then processed to isolate various fractions for radioactive assays. The distribution of substrate activity in these fractions are given in Table 3.

Table 3.—Distribution of activity of sucrose-U-C^{14} in beet root fractions.

Carbon dioxide	34.0%
Pulp	23.5%
Amino acids	5.6%
Organic acids	2.2%
Sucrose	11.2%
Neutral fraction (Simple carbohydrates, etc.)	13.2%

It is interesting to note that as much as 24% of the administered sucrose was found to be incorporated into the insoluble pulp fraction. The significance of this observation is yet to be elucidated. Examinatoin of the specific activity of beet sucrose in this experiment also permits one to correlate the origin of respiratory CO_2 to sucrose catabolism. The activity of sucrose was found to be 528 cpm per mM of carbon, a value that approaches the observed specific activity for the respiratory CO_2 (619 cpm/mM of carbon) at the end of the experiment. This fact led us to believe that sucrose is the principal, if not the exclusive, source of carbon for the production of respiratory CO_2 in beet roots.

The conclusion is drawn from the following considerations: (a) toward the end of the sucrose-U-C^{14} experiment, the specific activity of $C^{14}O_2$ has reached a steady level (Figure 2) and hence represents the specific activity of the carbon atoms of the carbonaceous compounds directly involved in the respiratory mechanism; (b) despite the fact that it is unlikely that the administered labeled sucrose had mixed homogeneously with beet sucrose, the specific activity of $C^{14}O_2$ reflected directly the $C^{14}O_2/C^{12}O_2$ ratio of the entire beet root under study; (c) the specific activity of sucrose isolated in the end of the experiment represented the sucrose-U-C^{14}/sucrose-C^{12} ratio of the entire beet root under study; (d) if the respiratory CO_2 is derived from carbon sources other than sucrose one would expect a noticeable dilution of $C^{14}O_2$ from $C^{12}O_2$ resulting in a significant re-

duction in the specific activity of $C^{14}O_2$ below that of the beet sucrose. The finding is in agreement with that reported earlier by Barr et al. (1) using a different method of estimation.

The nature of the pathways involved in the catabolism of beet sucrose has been studied and the findings are reported elsewhere (7).

Summary

In beet roots, sucrose is the most important carbon source for the production of respiratory CO_2. Simple carbohydrates and their derivatives such as maltose, glucose, fructose, mannose, galactose, ribose, glucuronate and gluconate can also be catabolized by sugar beet root.

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