Carbohydrate Metabolism of Sugar Beets
II. Catabolic Pathways for Acetate, Glyoxylate, Pyruvate, Glucose and Gluconate[1]

C. H. Wang and Royal D. Barbour[2]

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Introduction

In a previous paper (3), the utilization of sucrose, fructose, mannose, galactose, ribose, glucuronate and gluconate in the respiratory functions of sugar beets was demonstrated by a series of radiorespirometric experiments. However, the nature of the pathways for the catabolism of carbohydrates is yet to be elucidated. In the present work, C^{14} specifically labeled acetate, glyoxylate, pyruvate, glucose and gluconate were used as substrates to identify and estimate major catabolic pathways operative in sugar beets.

Materials and Methods

Sugar beet roots of the US 22/3 variety were used exclusively as test samples, and they were selected on the basis of uniformity in shape, weight, and maturity. Average weight of cleaned beet roots was approximately 500 grams.

The C^{14} specifically labeled acetate, pyruvate and glucose were obtained from the National Bureau of Standards through the kind cooperation of Dr. H. S. Isbell and from commercial sources except in the case of glucose-3 (4) -C^{14} which was prepared in this laboratory by the method of Wood et al. (16). Glyoxylate-1 and -2-C^{14} was prepared by the OsO_4 oxidation method of Weissbach and Sprinson (14) from fumarate-1,4-C^{14} and fumarate-2-3-C^{14} respectively. Potassium gluconate-1, -2, -3 (4), -6 and -U-C^{14} were prepared according to the method of Moore and Link (11).

The radiorespirometric experiments on the utilization of C^{14} specifically labeled substrates were carried out in a manner similar to that described previously (3). The "well" method (3) was employed exclusively as the means for the administration of labeled substrates.

Radioactivity of respiratory CO_2 samples were determined by means of a mica end-window Geiger-Muller counter using $BaCO_3$ exclusively as the counting form. Countings were carried out

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to a standard deviation no greater than 1% and counting data were corrected for background and self-absorption in the conventional manner.

Results and Discussion

The application of the radiorespirometric method for the identification and estimation of catabolic pathways operative in a given biological system relies heavily on the reproducibility of $C^{14}O_2$ recovery data from C^{14} specifically labeled substrates within a given series of experiments. It follows that the method employed for the administration of labeled substrates constitutes an important factor in this regard. The degree of reproducibility of $C^{14}O_2$ recoveries from the beet roots administered with specifically labeled substrates such as glucose is indicated by the data presented in Table 1. It can be seen that the average deviation among replicate experiments is reasonably small and the data are, therefore, valid for pathway participation studies on either the qualitative or the quantitative basis.

Table 1—Reproducibility of $C^{14}O_2$ yields from beet roots metabolizing specifically labeled glucose samples.

Substrate	Substrate weight, mg	Radioactivity, μc	Percent recovery at 20 hours
Glucose-1-C^{14}	50	0.5	15.8
Glucose-1-C^{14}	50	0.5	17.5
Glucose-1-C^{14}	50	0.5	14.6
Glucose-2-C^{14}	50	0.5	13.0
Glucose-2-C^{14}	50	0.5	12.6
Glucose-2-C^{14}	50	0.5	13.0
Glucose-3,4-C^{14}	50	0.5	21.5
Glucose-3,4-C^{14}	50	0.5	21.0
Glucose-6-C^{14}	50	0.5	10.5
Glucose-6-C^{14}	50	0.5	12.2
Glucose-6-C^{14}	50	0.5	10.1

Identification of Catabolic Pathways.

In view of the extraordinary abundance of sucrose in beet root, and the role played by beet sucrose and in beet respiration (3), it is reasonable to believe that an active mechanism must be operative for the degradation of sucrose to CO_2 with glucose and fructose serving as the obvious intermediates. Efforts were, therefore, made to elucidate the catabolic pathways responsible for the conversion of glucose to smaller fragments, for biosynthetic functions, and to the respiratory CO_2 for energy production.

In studies of this type it is often advantageous to first understand the mechanism involved in the catabolism of degradation products derived from glucose such as pyruvate, acetate, and glyoxylate. The radiorespirometric pattern, i.e., time course plots of interval $C^{14}O_2$ recoveries or cumulative $C^{14}O_2$ recoveries for beets metabolizing C^{14} specifically labeled pyruvate are given in Figure 1. The extensive conversion of C-1 of pyruvate to CO_2 undoubtedly reflects the oxidative decarboxylation of pyruvate giving rise to the formation of a C_2 compound. That the C_2 compound is in the nature of acetate can be concluded from a comparison of the radiorespirometric patterns for C-2 and C-3 of pyruvate with that for C-1 and C-2 of acetate given in Figure 2. Not only do the time courses of the respective plots bear a close resemblance to each other, the ratio of recoveries for C-2/C-3 of pyruvate at the end of experiment was calculated as 3.0, a value approaching closely the analogous ratio of C-1/C-2 for acetate, 3.5. The exact nature of the mechanism for acetate catabolism cannot be defined by the present work, however, the preferential conversion of C-1 of acetate to CO_2 as compared to that of C-2

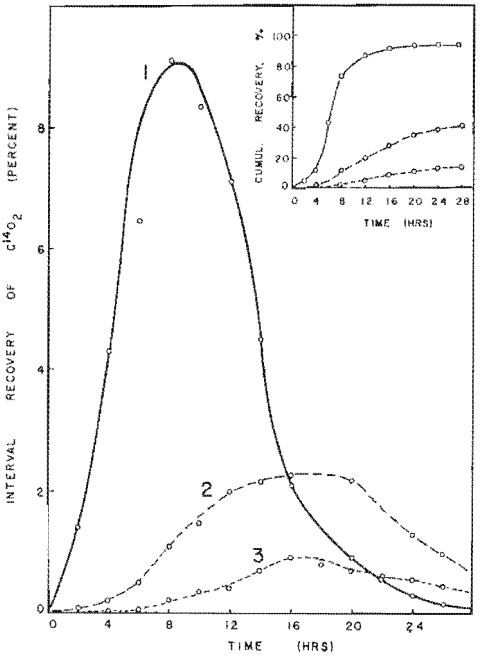

Figure 1.—Radiorespirometric patterns for sugar beets metabolizing C^{14} specifically labeled pyruvate, pyruvate-1-C^{14} _____, pyruvate-2-C^{14} - - - -, pyruvate-3-C^{14} · · · · ·.

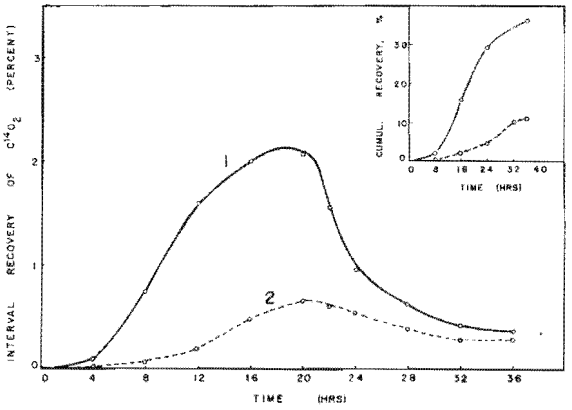

Figure 2.—Radiorespirometric patterns for sugar beets metabolizing C^{14} specifically labeled acetate. Acetate-1-C^{14}_____, acetate-2-C^{14} . .

suggests that the tricarboxylic acid (TCA) cycle may have been the principal pathway. The observed ratio, 3.5, for C-1/C-2 of acetate in CO_2 is somewhat higher than what has been observed with microorganisms (12). This finding can probably be accounted for by considering the effect of isotope dilution upon various labeled TCA cycle intermediates from unlabeled beet constituents (13). In the present case, the effect is presumably more pronounced in view of the amount of carbohydrate reserve present in beet roots.

The radiorespirometric patterns for the utilization of glyoxylate-1 and 2-C^{14} by beet roots are given in Figure 3. It is interesting to note that although preferential conversion of C-1 of

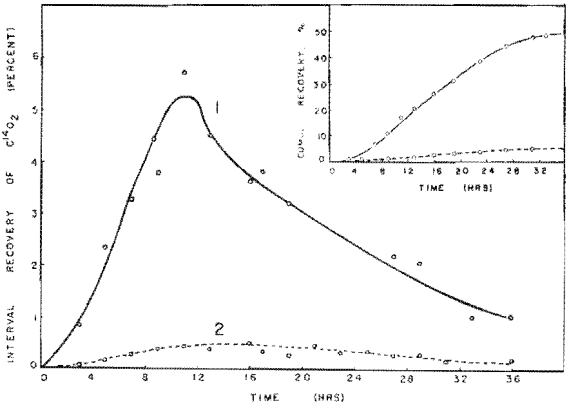

Figure 3.—Radiorespirometric patterns for sugar beets metabolizing C^{14} specifically labeled glyoxylate. Glyoxylate-1-C^{14}_____, Glyoxylate-2-C^{14}

glyoxylate to CO_2 is observed, the ratio of $C^{14}O_2$ recoveries C-1/C-2, for glyoxylate is found to be approximately 10, a value considerably higher than the analogous ratio observed in the acetate experiment. This fact may indicate that in addition to the operation of glyoxylate bypass pathway (9, 10), there may exist other pathways which are responsible for the preferential incorporation of C-2 of glyoxylate into beet constituents hence the preferential combustion of C-1 to CO_2. Possible mechanisms of this type, to cite a few, are the incorporation of C-2 of glyoxylate to a formyl group which is an important intermediate in C_1 metabolism.

With C^{14} specifically labeled glucose as tracing substrate, the radiorespirometric patterns for C-1, C-2, C-3,4 and C-6 of glucose are given in Figure 4. The prompt and extensive recovery of C-3,4 of glucose in respiratory CO_2 suggests strongly that the operation of the Embden-Meyerhof-Parnas (EMP) glycolytic pathway in conjunction with the oxidative decarboxylation of pyruvate is the major initial step for glucose breakdown. Meanwhile, the observed preferential conversion of C-1 of glucose to CO_2 in comparison with C-6 points to the participation of an

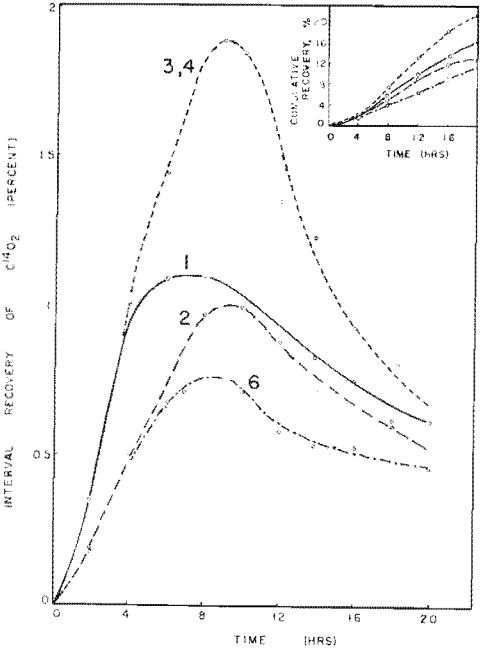

Figure 4.—Radiorespirometric patterns for sugar beets metabolizing C^{14} specifically labeled glucose. Glucose-1-C^{14} _____, Glucose-2-C^{14} - - - - -, Glucose-3,4-C^{14} _ _ _ _ _, Glucose-6-C^{14} ___.___.___.

alternate pathway, since these two carbon atoms should behave metabolically identical in the EMP-TCA scheme. The nature of the alternate pathway can be best analyzed with the discussion of the findings in the gluconate experiment.

It is reasonable to further speculate that the acetate, derived from glucose by way of the EMP-pyruvate decarboxylation mechanism, is catabolized via the TCA cyclic pathway as evidenced by the preferential conversion of C-2 of glucose to CO_2 relative to that of C-6. Conclusion is drawn from the fact that in the classical EMP-TCA scheme, one would expect to find that C-2 and C-6 of glucose corresponding to the carboxyl and methyl carbon atoms of acetate respectively. The possible operation of the TCA cycle in beet roots has been discussed earlier in connection with the acetate experiment.

The nature of the alternative pathway for glucose catabolism in sugar beets was further elucidated by the use of C^{14} specifically labeled gluconates as substrates in radiorespirometric experiments. Although the phosphogluconate decarboxylation pathway is known to occur in plant systems (1) the fate of the pentose phosphate, derived from glucose-6-phosphate, in plant catabolism has yet to be fully clarified. The use of C^{14} labeled gluconate offers the advantage that the catabolic behavior of pentosephosphates can be recognized without interference from the concurrent catabolism of substrate glucose since it is known that 6-phosphogluconate cannot be readily converted to glucose-6-phosphate directly (7).

The $C^{14}O_2$ recovery patterns for C-1, C-2, C3,4 and C-6 of gluconate are presented in Figure 5. The extensive conversion of C-1 of gluconate to CO_2, 90% after 36 hours, substantiates the occurrence of a C_1-C_6 cleavage pathway which is presumably in the nature of phosphogluconate decarboxylation. The catabolic fate of pentose derived from the latter pathway can be elucidated by examining the radiorespirometric patterns for the other carbon atoms of gluconate. The findings that $C^{14}O_2$ recoveries from gluconate carbon atoms are in the order of C-3,4 > C-2 > C-6 indicate that the pentose, presumably in the nature of ribose-5-phosphate, is converted rather extensively to fructose-6-phosphate through the action of transketolase and transaldolase. The conclusion is drawn from the consideration that two-thirds of the C-3,4 of gluconate should remain in C-3,4 position of the re-formed hexose phosphate (4) which can be in turn converted extensively to CO_2 when it is subjected to the glycolytic catabolism (5).

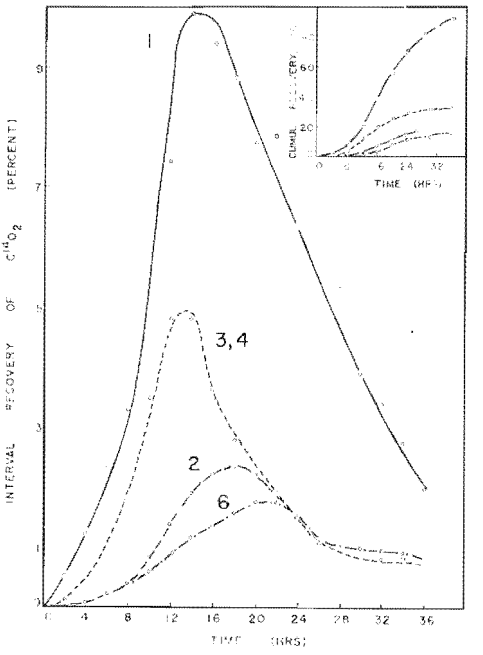

Figure 5.—Radiorespirometric patterns for sugar beets metabolizing C^{14} specifically labeled gluconate. Gluconate-1-C^{14} _____, Gluconate-2-C^{14} _____ _____, Gluconate-3,4-C^{14} , Gluconate-6-C^{14} . . .

The catabolism of glucose via the pentose cycle pathway has been recently examined by Katz and Wood (8, 15). These authors evaluated the effect of recycling of hexose monophosphates upon pathway participation under the assumption that the fructose-6-phosphate formed via the pentose cycle is in perfect equilibrium with glucose-6-phosphate and is hence engaged exclusively in repeated operations of the pentose cycle. The assumption thus implies that the re-formed fructose-6-phosphate would not be catabolized glycolytically by way of fructose-1, 6-diphosphate, a situation which cannot be accepted without experimental evidence particularly with biological systems wherein the EMP pathway is one of the major pathways.

A more realistic approach has been employed by Dawes and Holms (6) for the estimation of pathway participations. In a study with *Sarcina lutea*, these authors evaluated the relative contribution of the EMP pathway and the pentose cyclic pathway on the basis that thee fructose-6-phosphate formed in the latter pathway is in equilibrium with both glucose-6-phosphate and fructose-1,6-diphosphate and hence should display catabolic be-

haviors identical to that of substrate glucose. The treatment, although derived only from theoretical considerations, appears to be plausible when one takes into consideration the respiratory and biosynthetical functions of glucose pathways. The radiorespirometric data presented in Figure 5 renders experimental proof to the assumption made by Dawes and Holms (6).

Estimation of Pathways.

If one may assume, on the basis of the foregoing discussion, that in beet root glucose is catabolized primarily by way of two major pathways in the manner given in Figure 6 (4, 6), it would then be possible to make use of the cumulative $C^{14}O_2$ recovery data from the glucose experiments to estimate the relative participation for each of these two pathways. The principle of the method involved has been presented by Barbour et al. (2) for fruit studies.

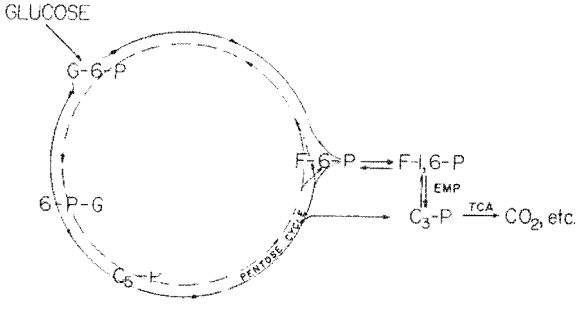

Figure 6.—Fate of glucose in beet catabolism. G-6-p glucose-6-phosphate; F-6-p, fructose-6-phosphate; 6-PG, 6-phospho-gluconate; C_5-P, pentose phosphate; C_3-P, triose phosphate; EMP, the Embden-Meyerhof-Parnas pathway; PC, the pentose cycle pathway; TCA, the tricarboxylic acid cycle pathway. Main pathways _____, Pentose cycle recycling .

The necessary assumptions required for the estimation of pathway participation are essentially the same as that described previously for fruit studies (2). In short, these assumptions can be summarized as follows: (a) The administered glucose is catabolized extensively by way of the EMP glycolysis and the phosphogluconate decarboxylation pathway; (b) The trioses formed in the glycolytic pathway are equivalent to each other at the pyruvate level with respect to further metabolic reactions; (c) The pyruvate derived from the trioses is decarboxylated promptly and extensively giving rise to acetate which is in turn catabolized by way of the TCA processes; (d) The conversion of C-1 of glucose to CO_2 in the phosphogluconate decarboxylation is a prompt and practically quantitative process; (e) The pentose

formed in the phosphogluconate decarboxylation does not contribute promptly to CO_2 production particularly in the earlier stage of the time course experiments. The evidence supporting these assumptions has been presented earlier in the discussion of the nature of catabolic pathways functioning in beet root.

In the estimation of pathway participation in plant systems, it is essential to first estimate the exact amount of the labeled glucose catabolized, since the administered labeled glucose was utilized only in part for catabolic functions. Bearing in mind the foregoing assumptions, one would expect that for each mole of glucose catabolized by way of glycolysis there would be formed one mole of respiratory CO_2 from either C-3 or C-4 of glucose and similarly one mole of CO_2 would be recovered in the respiratory CO_2 from C-1 of glucose catabolized via the phosphogluconate decarboxylation pathway. The latter is superimposed on the recovery of C-1 of glucose in CO_2 via the glycolytic pathway which is identical with that derived from C-6 of glucose. Consequently the percentage fraction of labeled glucose catabolized by beet root can be expressed as in Equation 1:

$$G_t = (G_1 - G_6) + G_{3(4)} \tag{1}$$

where G_t is equal to the percentage fraction of administered labeled glucose catabolized at time t and G_1, $G_{3(4)}$ and G_6 are the percentage cumulative radiochemical recoveries of the substrates in the respired CO_2 at time t from beet roots metabolizing equal amounts of glucose-1-C^{14}, glucose-3,(4)-C^{14} and glucose-6-C^{14} respectively.

The estimation of pathway participation in the catabolism of glucose with respect to the fraction G_t of the administered amount is given in Equations 2 and 3:

$$G_p = \frac{G_1 - G_6}{(G_1 - G_6) + G_{3(4)}} \times 100 \tag{2}$$

$$G_e = 100 - G_p \tag{3}$$

where G_p represents the percentage fraction of administered glucose catabolized via the phosphogluconate decarboxylation and G_e represents the percentage fraction of glucose engaged in the EMP glycolytic pathway.

The values of G_t, G_p and G_e calculated according to these equations along with the radiorespirometric data (average results from three replicate experiments) employed for these calculations are given in Table 2 for experiments extended to a period of 20

Table 2.—Pathway distribution in beet roots.

Time	% Cumulative radiochemical recovery of substrate activity in CO_2				% Glucose-C^{14} catabolized	% Pathway distribution	
Hrs.	G-1-C^{14}	G-2-C^{14}	G-3,4-C^{14}	G-6-C^{14}	G_t (Eq. 1)	G_p (Eq. 2)	G_e (Eq. 3)
2	0.5		0.4	0.3	0.7	36	64
4	2.2	1.7	2.0	1.2	3.0	33	67
6	4.3		4.1	2.4	6.0	32	68
8	6.5	5.6	7.5	4.1	10	24	76
10	8.6		11.2	5.5	14	22	78
12	10.4	8.3	13.7	6.5	18	22	78
14	12.0		16.2	7.9	20	20	80
16	13.5	11.3	18.2	8.9	23	20	80
18	14.8		20.0	10.2	25	19	81
20	16.0	12.9	21.3	10.9	26	19	81

hours. It is noted that the value of G_t rises sharply in the earlier stage of the experiment and levels toward the end of the experiment; meanwhile, the values of G_p decline sharply at the beginning and soon reached a steady value at 10 hours after substrate administration. The trend of diminution in G_p values in fact reflects much to the validity of assumptions set forth for the derivation of equation 1 and 2, particularly the assumption on the fate of pentose (assumption e). It can be readily seen that the terms G_6 and $G_{3(4)}$ in these equations were meant to represent the percentage conversion of C-6 and C-3(4) of glucose respectively in CO_2 via exclusively the EMP-TCA pathway. This is indeed true in the early phase of the radiorespirometric experiment (up to a duration of six hours in Figure 4). However, as catabolism of the administered glucose proceeded, the contribution of CO_2 production via the pentose cycle pathway (Figure 6) to the terms G_6 and $G_{3(4)}$ became more pronounced, as shown in Figure 5, thus resulting in the diminution of the calculated values for G_p. On the basis of the foregoing understanding, it would appear that the values G_p calculated from the data collected in the early phase of the experiment are closer to the actual participation of the individual pathways.

It, therefore, appears that in sugar beet roots over one-half of the glucose engaged in catabolism is routed through the EMP-TCA pathway with the remaining portion degraded via the pentose cycle mechanism. The latter pathway presumably serves as the major avenue for pentose production. The possible involvement of pentose cycle reactions in the catabolism of pentose derived from 6-phosphogluconic acid has been discussed earlier.

Summary

The nature and participation of pathways for the catabolism of acetate, glyoxylate, pyruvate, gluconate and glucose in sugar beet roots has been studied by the radiorespirometric method. It appears that glucose is utilized mainly by way of the EMP glycolytic pathway and to a minor extent via the phosphogluconate decarboxylation route. The bulk of the pentose derived from glucose via the latter pathway is believed to undergo pentose cycle reactions giving rise to the reformation of hexose which is in turn catabolized in a manner identical to that of beet glucose. The pyruvate derived from glucose by way of the EMP mechanism presumably undergoes extensive oxidative decarboxylation which is in turn metabolized via the TCA cyclic pathway and to some extent the glyoxylate bypass pathway.

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