COMPARATIVE CATABOLISM OF CARBOHYDRATES IN *PSEUDOMONAS* SPECIES[1]

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Carbohydrate metabolism of the Pseudomonadaceae is known to involve unique catabolic pathways. Lockwood *et al.* (1941) reported the possible use of *Pseudomonas* and *Phytomonas* species for commercial production of gluconate and 2-ketogluconate. Since these compounds are not involved in glycolysis it was apparent that a pathway for glucose degradation other than glycolysis is present in the pseudomonads. Investigations of glucose catabolism by Campbell *et al.* (1954) and Koepsell (1950) revealed that gluconate, 2-ketogluconate, α-ketoglutarate, and pyruvate are important intermediates of glucose breakdown in *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. Further insight into the metabolism of this family was gained when Entner and Doudoroff (1952) identified the pathway of glucose catabolism in *Pseudomonas saccharophila* by means of inhibition and radiotracer studies. Wood (1955) described a similar pathway, which functions concurrently with the pentose phosphate pathway in *P. fluorescens*. Participation of Entner-Doudoroff and pentose phosphate pathways in pseudomonads were also evaluated by Lewis *et al.* (1955) and Gibbs and DeMoss (1954).

With the establishment of the pathways of glucose catabolism in pseudomonads, it is therefore of interest to compare the relative participation of individual pathways in the over-all catabolism of various pseudomonads. In the present work radiorespirometric experiments described by Wang *et al.* (1958) were carried out with *Zymomonas mobilis*, *P. saccharophila*, and *Pseudomonas reptilivora* (formerly *P. fluorescens*) (Haynes, 1957, *personal communication*) as test systems to obtain reference radiorespirometric patterns for organisms in which glucose is known to be catabolized exclusively via the Entner-Doudoroff pathway; the Entner-Doudoroff pathway in conjunction with the tricarboxylic acid cycle pathway; or the concurrent operation of the Entner-Doudoroff pathway and the pentose phosphate pathway, in conjunction with the tricarboxylic acid cycle pathway, respectively. In addition to the above organisms, radiorespirometric studies were also carried out on the utilization of glucose, acetate, and pyruvate by *P. aeruginosa*. In this organism Campbell *et al.* (1954) demonstrated the occurrence of nonglycolytic pathways, but the exact nature and extent of participation of the alternate catabolic routes is still uncertain.

EXPERIMENTAL METHODS

Radiorespirometric experiment. The origin and cultural conditions for each organism used in this study are given in table 1. The medium employed in each tracer experiment was identical to that used in obtaining the cell crop except that C^{14}-labeled substrates were used to replace the unlabeled carbohydrate. In all experiments, cultural conditions optimal for growth were used in the hope that the catabolic rates observed in the radiorespirometric experiments might correspond closely with those prevailing in normal cells.

For the radiorespirometric experiments described, cells in the logarithmic stage of growth were washed once and resuspended in carbohydrate-free medium at pH 6.8. Ten-ml samples

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[2] A portion of the work described herein is taken from the thesis presented by Ivan J. Stern for the Ph.D. degree at Oregon State College, 1958. Present address, Eaton Laboratories, Norwich, New York.

TABLE 1

Organisms and cultural conditions used in this study

Organisms	Strain	Basal Medium
Zymomonas mobilis	NRRL B-804	Tryptone, yeast extract, 0.03 M phosphate, pH 6.7
Pseudomonas saccharophila	G*	NH_4Cl, salts, 0.03 M phosphate, $MgSO_4$ traces, $FeCl_3$ and $CaCl_2$
Pseudomonas reptilivora	NRRL B-6bs	$(NH_4)_2SO_4$, 0.03 M phosphate, KCl, $MgSO_4$, trace $MnSO_4$, $FeSO_4$, yeast extract
Pseudomonas aeruginosa	ATCC 9027	As for *P. reptilivora*, but without yeast extract

* Obtained from the wild strain by streaking on glucose agar.

of these suspensions (usually containing 10 mg of cells) were added to the main compartment of each flask and a fixed amount (usually 50 to 100 μmoles) of specifically C^{14}-labeled substrate in 0.5 ml of aqueous solution was introduced into the flask from the side arm. Samples of respiratory CO_2 were collected periodically, precipitated as $BaCO_3$, and assayed for radioactivity as described in a previous report (Wang *et al.*, 1958). The radioactivities of substrates, cells, and incubation media were determined by wet combustion of the carbonaceous constituents to CO_2 (Chen and Lauer, 1957) and counted as $BaCO_3$.

C^{14}-labeled substrates. Glucose-1-, -2-, and -6-C^{14} were obtained from the National Bureau of Standards through the kind cooperation of Dr. H. S. Isbell. Glucose-3,4-C^{14} was prepared from rat liver glycogen according to the method of Wood *et al.* (1945). Acetate-1- and -2-C^{14} and pyruvate-1-, -2-, and -3-C^{14} were purchased from Nuclear-Chicago Corporation. All labeled substrates were adjusted to a prescribed specific activity before administration.

RESULTS AND DISCUSSION

In four of the five organisms studied, the catabolism of glucose can be visualized as proceeding sequentially through primary and secondary pathways. By "primary" pathways are meant those catabolic routes for which glucose is the initial substrate; by "secondary" pathways are meant those for which the immediate degradation products of glucose such as pyruvate or acetate are serving as the substrate. It is evident that the radiorespirometric data obtained with organisms employing both classes of pathways can be better understood if the patterns inherent to either the primary or secondary pathways of catabolism can be examined separately. For this reason, radiorespirometric experiments were first carried out employing C^{14} specifically labeled acetate and pyruvate, two key intermediates of glucose degradation, as substrates for three pseudomonads capable of oxidizing these compounds. Previous reports (Gibbs and DeMoss, 1954) have indicated that acetate cannot be utilized by *Z. mobilis*, evidently due to the absence of the tricarboxylic acid cycle mechanisms in this organism. The results of these experiments are given graphically in figures 1, 2, and 3 with the over-all radioactivity inventories summarized in table 2. Preferential oxidation of C-1 pyruvate to CO_2, presumably oxidative decarboxylation, occurs with all organisms tested. In fact, the ratio of C-1 to C-2 for pyruvate (designated as P_1/P_2) has a relatively constant value of 1.8, which reflects directly the extent of the decarboxylation process. Similarly, CO_2 formation is more extensive from C-2 of pyruvate than from C-3, and from C-1 of acetate than from C-2, as shown by the values of P_2/P_3 and A_1/A_2, respectively, given in table 2. It is evident that C-3 of pyruvate and C-2 of acetate, which are metabolically identical if acetate is the product of pyruvate decarboxylation, are conserved in the oxidation process and are preferentially used in cellular biosynthesis. Presumably various intermediates of the tricarboxylic acid cycle were drained out for biosynthetic purposes and the methyl carbon of acetate is involved to a much greater extent than the carboxyl carbon in these reactions. Similar observations have been made with yeast (Forbusch, 1958) in which it has been established that the tricarboxylic acid cycle plays an important role in both respiratory and biosynthetic functions. The close resemblances of the $C^{14}O_2$ production pattern for the catabolism of acetate or pyruvate by *P. saccharophila*, *P. reptilivora*, and *P.*

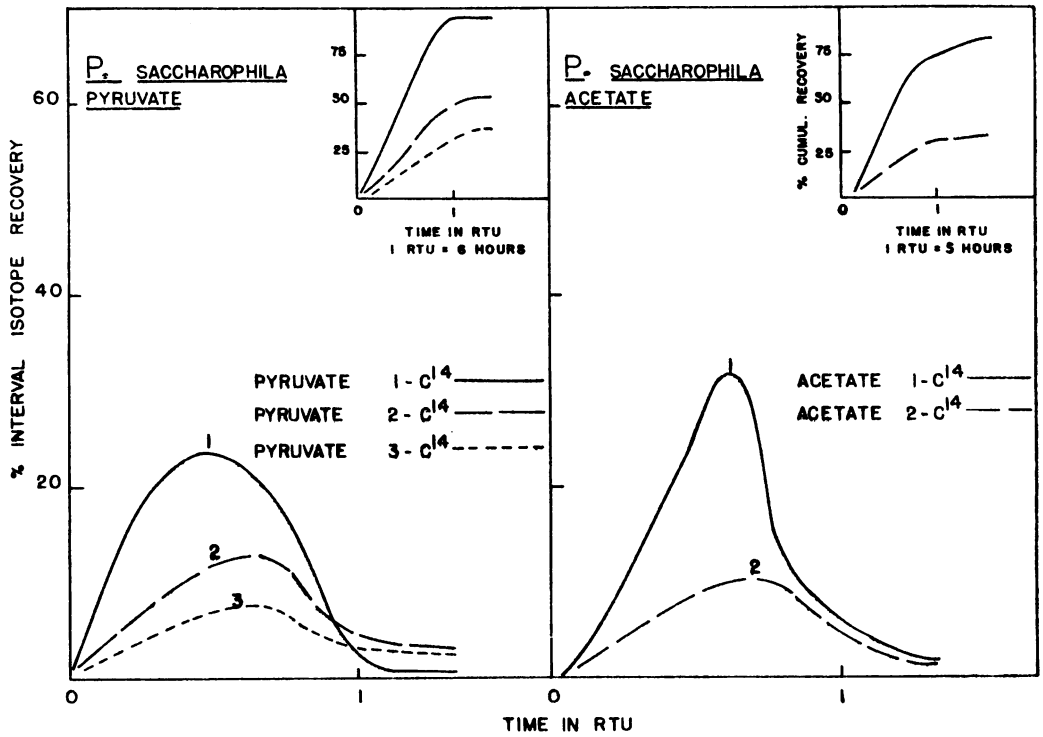

Figure 1. The recoveries of $C^{14}O_2$ from *Pseudomonas saccharophila* metabolizing acetate or pyruvate. *RTU* = relative time units.

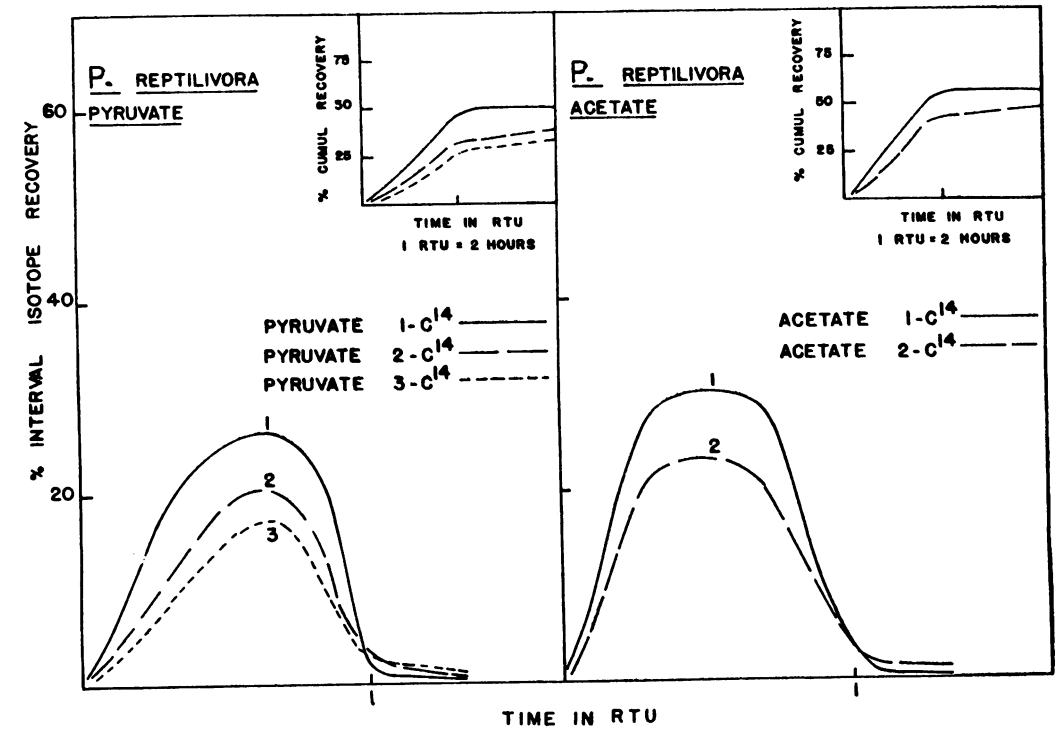

Figure 2. The recoveries of $C^{14}O_2$ from *Pseudomonas reptilivora* metabolizing acetate or pyruvate. *RTU* = relative time units.

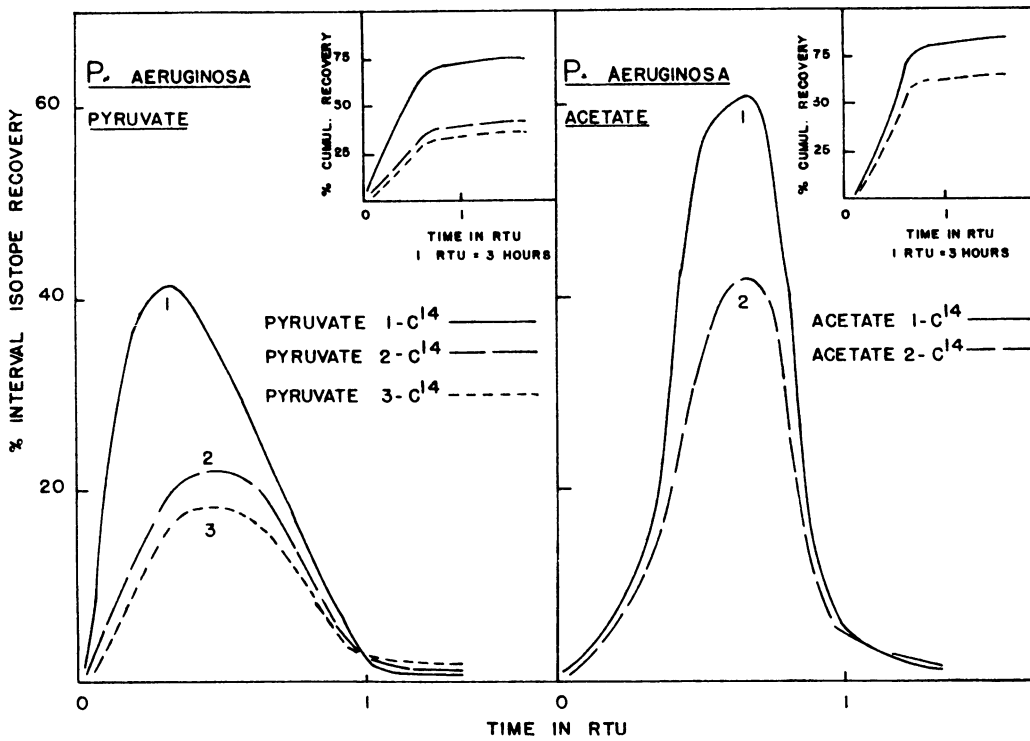

Figure 3. The recoveries of $C^{14}O_2$ from *Pseudomonas aeruginosa* metabolizing acetate or pyruvate. *RTU* = relative time units.

TABLE 2

Utilization of C^{14}-labeled acetate or pyruvate by the pseudomonads

Organism	Substrate	Radioactivity Distribution*				Labeling Ratio†		
		Respiratory CO_2	Cells	Medium	Total	P_1/P_2	P_2/P_3	A_1/A_2
		%	%	%	%			
Pseudomonas saccharophila	Pyruvate-1-C^{14}	95	3	10	108	1.76		
	Pyruvate-2-C^{14}	54	10	32	96		1.38	
	Pyruvate-3-C^{14}	39	35	27	101			
	Acetate-1-C^{14}	79	14	2	95			2.13
	Acetate-2-C^{14}	37	58	3	88			
Pseudomonas reptilivora	Pyruvate-1-C^{14}	70	8	10	88	1.84		
	Pyruvate-2-C^{14}	38	28	37	103		1.19	
	Pyruvate-3-C^{14}	32	30	45	107			
	Acetate-1-C^{14}	79	12	3	94			1.27
	Acetate-2-C^{14}	62	29	8	99			
Pseudomonas aeruginosa	Pyruvate-1-C^{14}	70	10	17	97	1.75		
	Pyruvate-2-C^{14}	40	26	16	82		1.08	
	Pyruvate-3-C^{14}	37	32	22	91			
	Acetate-1-C^{14}	85	10	1	96			1.29
	Acetate-2-C^{14}	66	36	3	105			

* Data collected at the end of experiment.

† P_1: $C^{14}O_2$ recovery from carboxyl carbon atom of pyruvate; P_2: $C^{14}O_2$ recovery from carbonyl carbon atom of pyruvate; P_3: $C^{14}O_2$ recovery from methyl carbon atom of pyruvate; A_1: $C^{14}O_2$ recovery from carboxyl carbon atom of acetate; A_2: $C^{14}O_2$ recovery from methyl carbon atom of acetate.

aeruginosa suggest that a common secondary pathway is operative in these pseudomonads. Although the exact nature of this pathway cannot be defined in the present study, it is likely that the tricarboxylic acid cycle with or without the concurrent operation of the glyoxylate bypass (Kornberg and Medsen, 1957) plays an important role.

In the case of glucose catabolism, it appears useful to first examine the radiorespirometric pattern for a pseudomonad in which a single primary mechanism for glucose breakdown is functioning. *Z. mobilis*, previously reported as using solely the Entner-Doudoroff pathway, consequently serves as the choice for obtaining a reference radiorespirometric pattern.

The radiorespirometric patterns for glucose catabolism in *Z. mobilis* are presented in figure 4. Gibbs and DeMoss (1954) showed that glucose dissimilation in this organism proceeds exclusively via the Entner-Doudoroff sequence to yield CO_2, ethanol, and small amounts of lactic acid. To facilitate comparison, all of the radiorespirometric patterns presented here have been plotted on the basis of relative time units against per cent interval recovery. One relative time unit is defined as the time required for all of the administered substrate to be consumed by the cells (Wang *et al.*, 1958). The rapid and nearly complete conversion of C-1 to CO_2 shown in figure 4 undoubtedly reflects the degree to which pyruvate, arising from glucose, is decarboxylated, whereas the negligible recovery of C-2 and C-6 as CO_2 are indications that, in *Z. mobilis*, the products of pyruvate decarboxylation are not further oxidized.

The data obtained with glucose-3,4-C^{14} (figure 4 and table 3) represent the average recovery of C-3 and C-4 in the CO_2, and hence cannot be compared directly with data obtained from the singly labeled substrates. However, it should be noted that with *Z. mobilis*, C-3 of

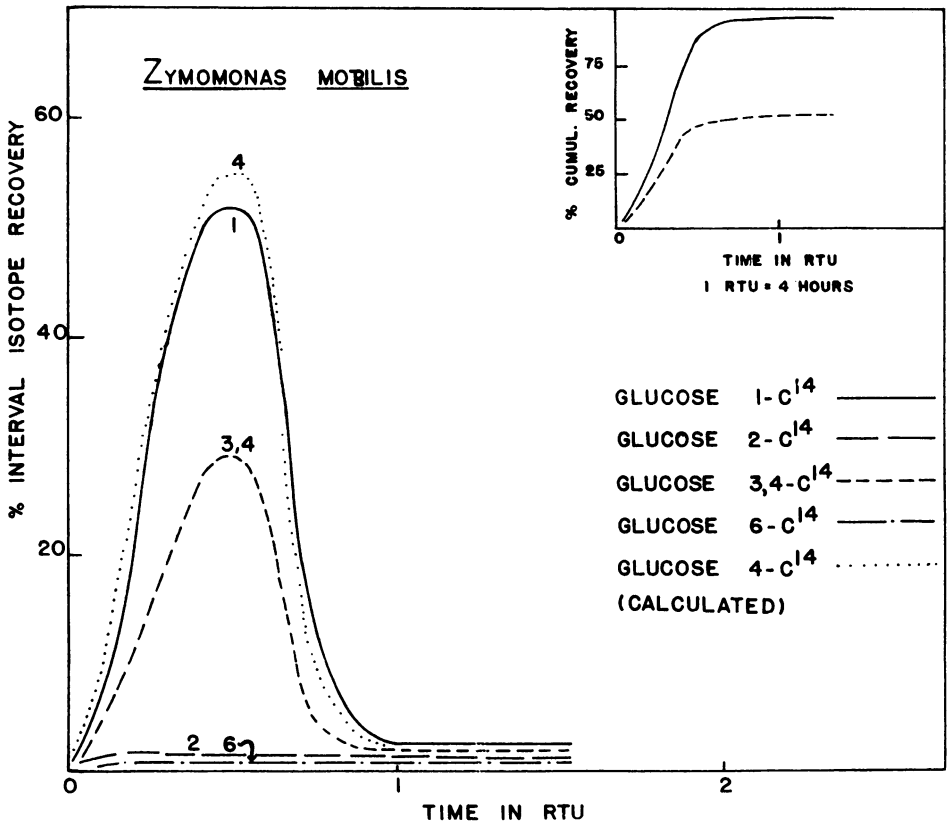

Figure 4. The recoveries of $C^{14}O_2$ from *Zymomonas mobilis* metabolizing glucose. *RTU* = relative time units.

TABLE 3

Utilization of C^{14}-labeled glucose by the pseudomonads

Organism	Substrate	Radioactivity Distribution*				Pathway Participation†	
		Respiratory CO_2	Cells	Medium	Total	ED	PP
		%	%	%	%	%	%
Zymomonas mobilis	Glucose-1-C^{14}	87	1	4	92	100	
	Glucose-2-C^{14}	0	1	61	62‡		
	Glucose-3,4-C^{14}	50	2	30	82		
	Glucose-6-C^{14}	0	3	47	50‡		
Pseudomonas saccharophila	Glucose-1-C^{14}	91	8	4	103	100	
	Glucose-2-C^{14}	43	50	7	100		
	Glucose-3,4-C^{14}	63	39	7	109		
	Glucose-6-C^{14}	28	57	18	103		
Pseudomonas reptilivora	Glucose-1-C^{14}	87	10	10	107	72	28
	Glucose-2-C^{14}	61	32	13	106		
	Glucose-3,4-C^{14}	56	36	11	103		
	Glucose-6-C^{14}	49	40	10	99		
Pseudomonas aeruginosa	Glucose-1-C^{14}	88	9	1	98	71	29
	Glucose-2-C^{14}	48	38	3	89		
	Glucose-3,4-C^{14}	46	34	6	86		
	Glucose-6-C^{14}	33	43	6	82		

* Data collected at end of each experiment.

† ED = the Entner-Doudoroff pathway; PP = the pentose phosphate pathway.

‡ Low recovery due to loss of volatile fermentation products.

glucose is converted exclusively to the methyl carbon atom of pyruvate which is in turn transformed to the methyl carbon atom of ethanol, the terminal product. It therefore follows that the recoveries of glucose-3,4-C^{14} represent the conversion of C-4 of glucose to CO_2. Inasmuch as equal amounts of radioactivity were used for each labeled substrate employed, the value for the $C^{14}O_2$ recovery from C-4 can be obtained by doubling the interval isotope recoveries in the CO_2 from glucose-3,4-C^{14}. The calculated interval CO_2 recoveries from C-4, given as a dotted line in figure 4, are in good agreement with those observed for C-1 of glucose, which substantiates the assumption that C-1 and C-4 of glucose are converted to CO_2 at equal rates and to an equal extent via the Entner-Doudoroff mechanisms.

Similarly, the radiorespirometric pattern for the utilization of glucose by *P. saccharophila* (figure 5) can be used as the reference pattern for microorganisms that rely on the Entner-Doudoroff pathway exclusively as the primary mechanism and the tricarboxylic acid cycle as the secondary mechanism. The operation of the tricarboxylic acid cycle in this organism has been discussed previously under acetate experiments. In the present combination of catabolic mechanisms, one would expect that C-1 and C-4 of glucose, both being equivalent to carboxyl carbon atoms of pyruvate in the Entner-Doudoroff schema, can be readily converted to CO_2 at equal rates when pyruvate is the subject of oxidative decarboxylation. That this is indeed the case is evidenced by the close agreement between the observed recovery of C-1 in CO_2 and the calculated recovery of C-4 in CO_2 (dotted line) shown in figure 5. It is understood that the calculation of C-4 recovery in CO_2 requires the assumption that complete metabolic equivalence is realized between C-3 and C-6 of glucose, or C-2 and C-5 of glucose. The former two carbon atoms correspond to methyl carbon atoms of pyruvate and the latter correspond to carbonyl carbon atoms of pyruvate in the Entner-Doudoroff schema. The observed pref-

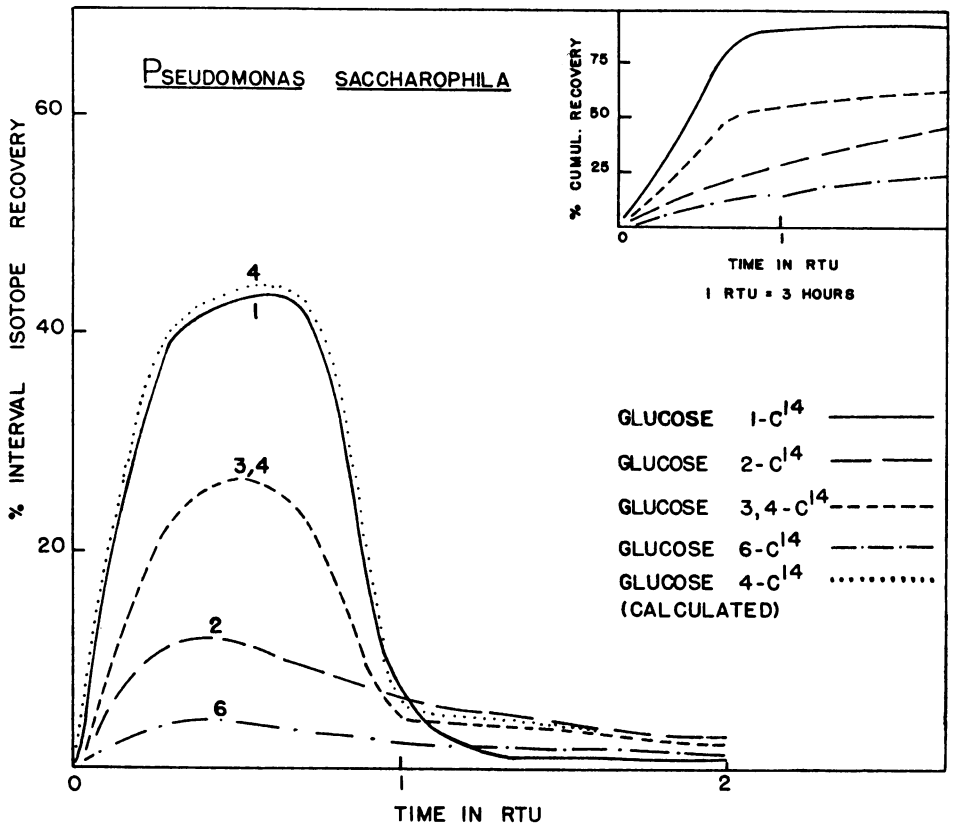

Figure 5. The recoveries of $C^{14}O_2$ from *Pseudomonas saccharophila* metabolizing glucose. *RTU*= relative time units.

erential oxidation of C-2 in comparison to C-6 of glucose can be accounted for by the preferential oxidation of the carboxyl carbon atom of acetate when the latter is catabolized via the tricarboxylic acid cycle.

With the foregoing reference pattern in hand, it is then possible to employ the radiorespirometric method to examine the nature of pathways for glucose catabolism in pseudomonads having multiple primary pathways in conjunction with a secondary mechanism. The radiorespirometric data for such an organism is shown in figure 6, which represents the utilization of C^{14} specifically labeled glucose by *P. reptilivora*. Previously, the participation of the Entner-Doudoroff, the pentose cycle, and the glycolytic pathways have been examined by Lewis *et al.* (1955). These authors reported that one third to one half of the catabolized glucose is routed through the Entner-Doudoroff mechanism and the remaining portion is presumably catabolized via the pentose pathway. The contribution of the Embden-Meyerhof-Parnas pathway to the over-all glucose catabolism is reported to be insignificant. In the present work, the radiorespirometric pattern shown in figure 6 for the catabolism of glucose by *P. reptilivora* is notably different from that observed with *Z. mobilis* and *P. saccharophila*, evidently due to the operation of an additional primary mechanism in this organism, presumably the pentose phosphate cycle. Thus, the $C^{14}O_2$ recoveries of C-4 of glucose calculated from the data of glucose-3,4-C^{14} and glucose-6-C^{14} experiments are much lower than that of C-1. In fact, the magnitude of the difference between the observed $C^{14}O_2$ recoveries of C-1 and the calculated $C^{14}O_2$ recoveries of C-4 of glucose reflected in part the extent of operation of the pentose phosphate pathway, since these two carbon atoms are presumably identical in their met-

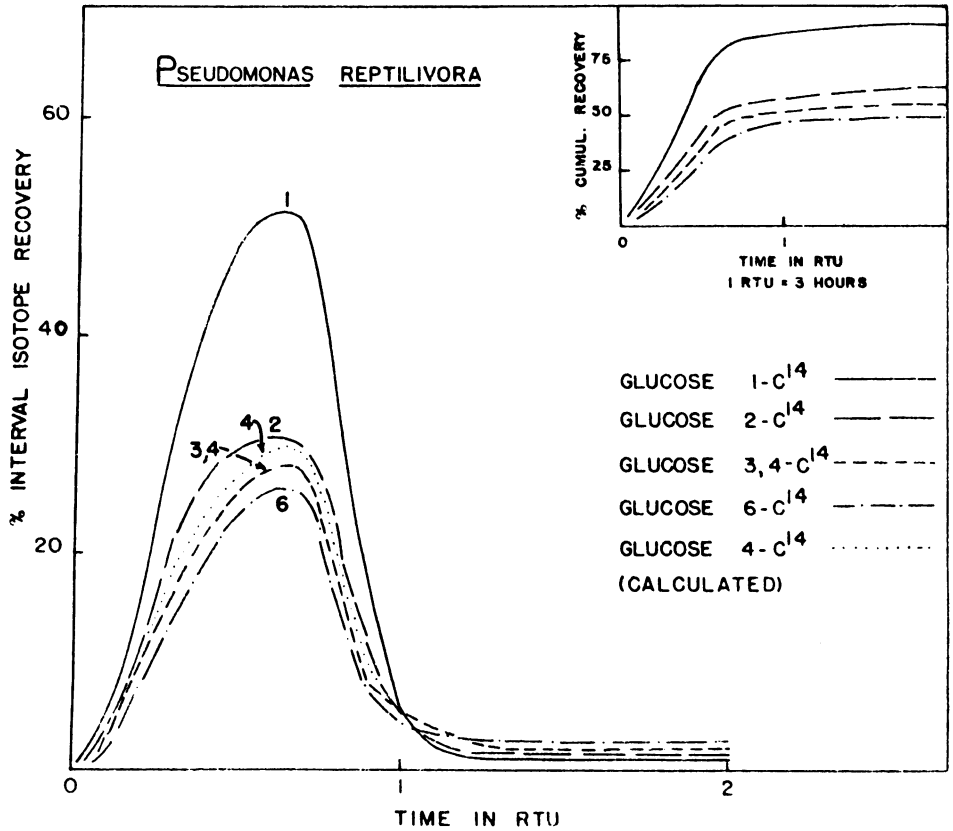

Figure 6. The recoveries of $C^{14}O_2$ from *Pseudomonas reptilivora* metabolizing glucose. *RTU* = relative time units.

abolic behavior when glucose is degraded exclusively by the Entner-Doudoroff pathway.

The equation for the estimation of pathway participation in an organism relying on the Entner-Doudoroff and the pentose phosphate pathways can be derived from the following considerations:

1. The administered glucose is metabolized quantitatively by the organism in question to various degradation products.

2. In the Entner-Doudoroff pathway, the two C_3 degradation products (pyruvate and triose) are equivalent to each other at the pyruvate level.

3. The pyruvate derived from glucose is decarboxylated promptly and extensively.

4. The decarboxylation of gluconate, as derived from glucose, is extensive and rapid.

5. C-6 of the pentose derived from glucose via the pentose phosphate pathway does not contribute significantly to the production of respiratory CO_2.

On the basis of these assumptions, for each mole of glucose catabolized via the pentose phosphate pathway, 1 mole of CO_2 in the respiratory product should be recovered from C-1 of glucose. Similarily, 2 moles of respiratory CO_2 would be recovered from C-1 and C-4 when 1 mole of glucose is catabolized via the Entner-Doudoroff pathway. It therefore follows that the percentage fraction of the administered glucose catabolized via the pentose phosphate pathway (essentially the decarboxylation of phosphogluconic acid or gluconic acid), designated as G_p, should then be:

$$G_p = G_1 - G_4 \quad (1)$$

whereas,

$$G_4 = 2G_{3,4} - G_6 \quad (2)$$

G_1, $G_{3,4}$ or G_6 = per cent cumulative radiochemical recovery in respiratory CO_2 at the time when the organism has completely metabolized a given amount of substrate glucose in the nature of glucose-1, -3,4-, or -6-C^{14}, respectively.

Inasmuch as it has been assumed that there are only two principal primary pathways functioning in the organisms in question, the per cent fraction of the administered glucose catabolized via the Entner-Doudoroff pathway, designated as G_{ED}, is

$$G_{ED} = 1 - G_p \qquad (3)$$

On the basis of these equations, it was estimated that in *P. reptilivora*, 72 per cent of the administered glucose is catabolized via the Entner-Doudoroff pathway with the remaining 28 per cent routed through the pentose phosphate route.

It should be emphasized that the principal pathways in the pseudomonads under examination are recognized as (a) the C_1—C_5 cleavage type which involves the oxidative decarboxylation of C-1 of glucose and (b) the C_3—C_3 cleavage of glucose described by Entner and Doudoroff (1952) for *P. saccharophila*, by way of either 6-phosphogluconate or gluconate. Any amount of glucose that is incorporated or metabolized by the organism without producing CO_2 will cause inaccuracy in the estimation made according to equation (1) and (3). The magnitude of this inaccuracy is indicated by the combined amount of glucose-1-C^{14} activity detected in cells and medium; i. e., 20 per cent of the administered glucose in *P. reptilivora*, since C-1 of glucose should be readily converted to CO_2 by either of the pathways in question. However, it is equally possible that this fraction of activity from glucose-1-C^{14} may have been

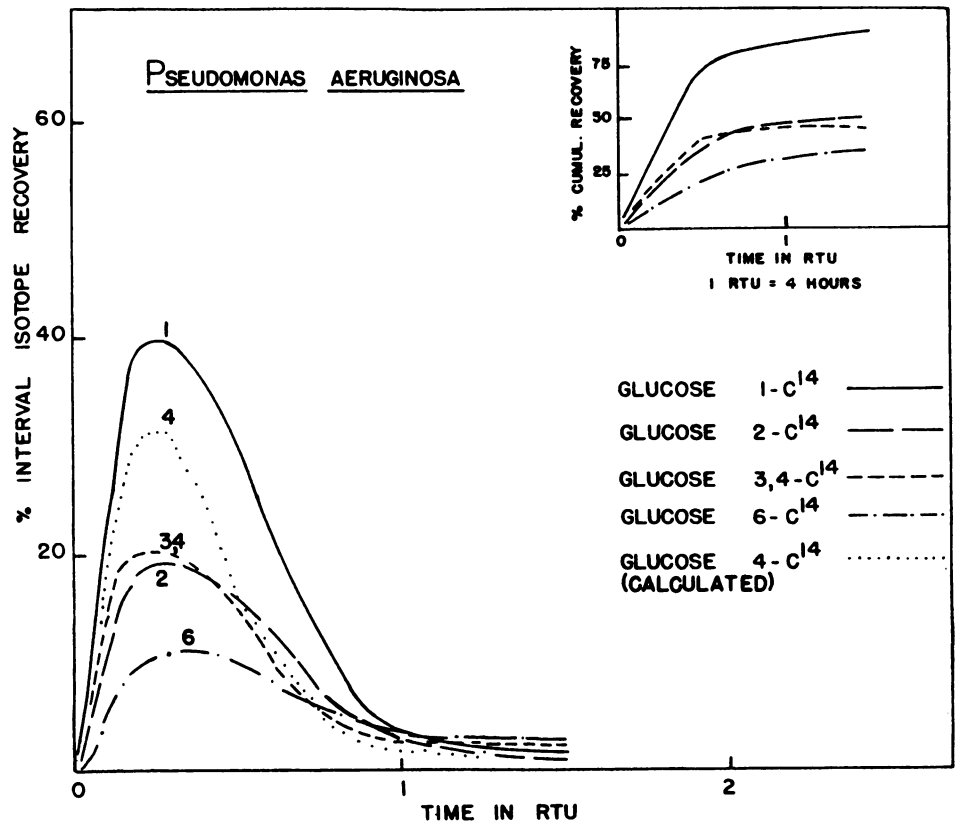

Figure 7. The recoveries of $C^{14}O_2$ from *Pseudomonas aeruginosa* metabolizing glucose. *RTU* = relative time units.

incorporated into the cells or excreted into the incubation medium by way of pyruvate, in view of the fact that similar amounts of activity were detected in the cells and medium respectively in the pyruvate-1-C^{14} experiments. If this were the case, equations (1) and (3) should present a true estimation of the participation of the different pathways in the organism.

Having obtained reference radiorespirometric patterns for pseudomonads in which glucose was catabolized via the Entner-Doudoroff pathway alone (*Z. mobilis*), the Entner-Doudoroff pathway in conjunction with the tricarboxylic acid cycle (*P. saccharophila*), and the Entner-Doudoroff pathway and the tricarboxylic acid cycle in conjunction with the pentose pathway (*P. reptilivora*), similar experiments were carried out with *P. aeruginosa* as the test system. Although the catabolism of glucose and allied compounds has been examined (Campbell *et al.*, 1954), the relative participation of the different pathways for the catabolism of glucose in this pseudomonad has not been thoroughly studied. The radiorespirometric patterns for *P. aeruginosa* metabolizing pyruvate or acetate (figure 3) are similar to those of *P. saccharophila* and *P. reptilivora* metabolizing acetate or pyruvate as sole carbon source. Similar to the findings with *P. saccharophila* and *P. reptilivora*, the rapid conversion of pyruvate C-1 to CO_2 and the preferential incorporation of C-3 over C-2 of pyruvate into cellular material (table 2), indicate that pyruvate is a major intermediate in glucose catabolism.

With glucose as the sole substrate, the radiorespirometric patterns obtained with *P. aeruginosa* (figure 7) resemble closely those with *P. reptilivora* (figure 6). The high rate of recovery of C-1 over the calculated C-4 recoveries probably reflects, as with *P. reptilivora*, the extent of pentose pathway participation in glucose catabolism. Calculation of the relative participation of the pathways indicated that in *P. aeruginosa*, 71 per cent of the administered glucose was catabolized via the Entner-Doudoroff pathway.

The radiochemical recoveries of substrate activity in respiratory CO_2, cells and incubation media (analyzed at the end of each experiment) as well as the relative pathway participation calculated according to equation (1) and (3) are given in table 3 for each of the pseudomonads studied in the present work. The heavy incorporation of glucose carbon atoms into *P. saccharophila*, *P. reptilivora*, and *P. aeruginosa* undoubtedly reflects the important role played by the secondary pathway(s) such as the tricarboxylic acid cycle in cellular biosynthesis. This is also supported by the case of *Z. mobilis* in which the lack of the tricarboxylic acid cycle mechanisms has resulted in practically no cellular incorporation.

SUMMARY

The catabolism of acetate, pyruvate, and glucose in four pseudomonads (*Pseudomonas aeruginosa*, *Pseudomonas reptilivora*, *Pseudomonas saccharophila*, *Zymomonas mobilis*) have been examined by the radiorespirometric method. It appears that the Entner-Doudoroff pathway plays a predominant role in the glucose catabolism of these pseudomonads.

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