A COMPARATIVE STUDY OF THE CARBOHYDRATE CATABOLISM IN PSEUDOMONADACEAE MICROORGANISMS

by

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TO MY HUSBAND

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF FIGURES - Cont.

LIST OF TABLES

A COMPARATIVE STUDY OF THE CARBOHYDRATE CATABOLISM IN PSEUDOMONADACEAE MICROORGANISMS

INTRODUCTION

Catabolism of carbohydrate in living cells produces energy and intermediates for biosynthetic functions. A good understanding of the degradation mechanism of carbohydrates in organisms should be very helpful in elucidating the complete organization of life processes.

To study the nature of catabolic pathways, several valuable tools have been developed in recent years. Among them are modern techniques for enzyme purification, ion-exchange resin, chromatography, and radioactive tracer methods, which are instrumental in the identification and estimation of many heretofore unknown pathways for cellular metabolism.

Glucose often is considered one of the most important carbon sources, and other carbohydrates are often metabolized by way of glucose. The present work is centered on the study of glucose catabolism in microbial cells, and literature on the degradation of glucose in microbial cells is reviewed in the following sections.

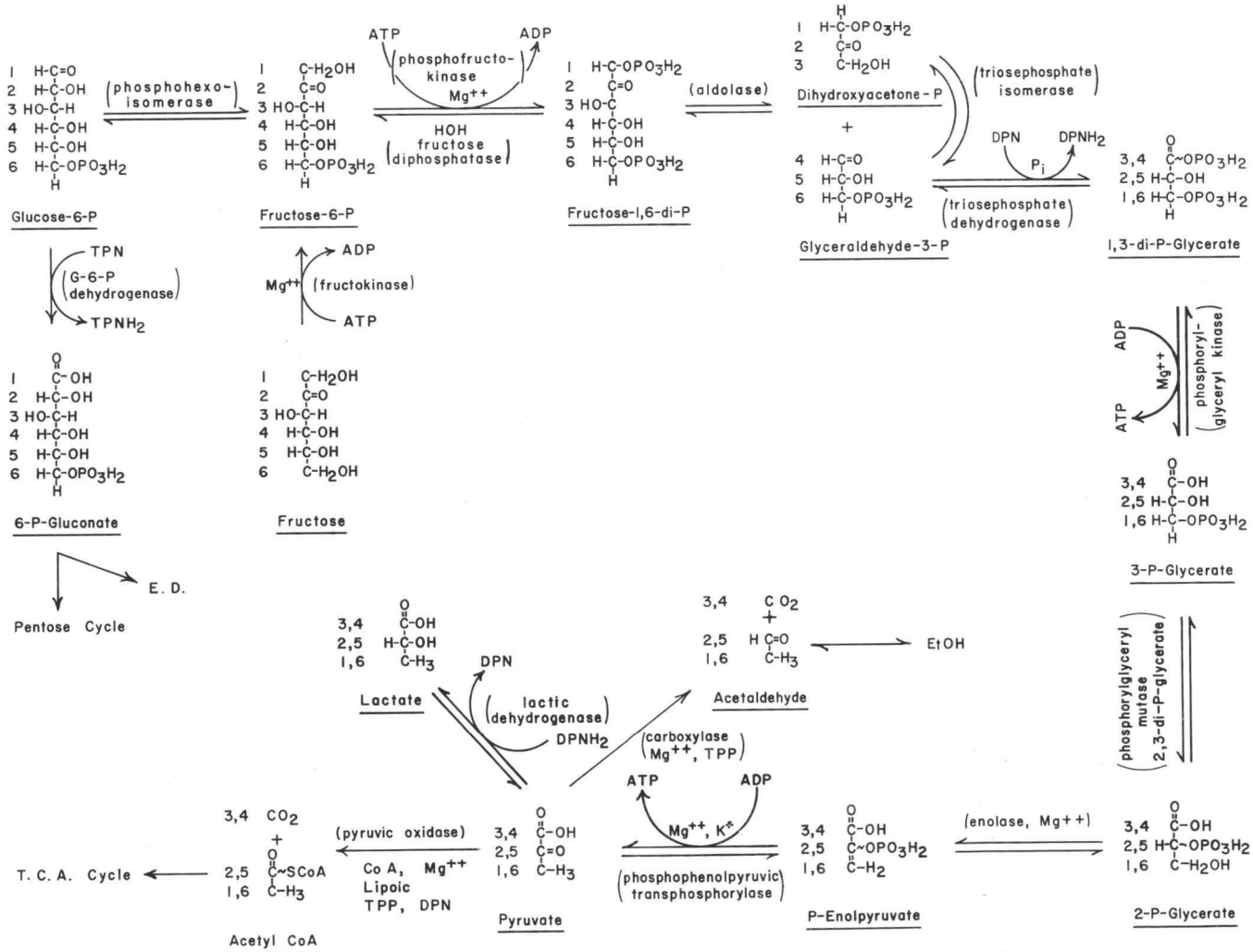
Pathways of Glucose Dissimilation

The systematic study of glucose catabolism in microorganisms can be traced back to 1897, when Büchner demonstrated that cell-free yeast extract was able to ferment sugar (38). During the next 50 years many workers were active participants in elucidating the exact nature of this metabolic route, and as a result of their work the glycolytic scheme, or Embden-Meyerhof-Parnas (EMP) pathway was proposed. The EMP pathway plays an important role in biological systems, such as microorganisms, plants, and animal cells. The scheme as it is known today is shown in Figure 1.

Another pathway, which has been demonstrated in some microorganisms (35, 98, 99, 107), involves the conversion of 6-phosphogluconate to 2-keto-3-deoxy-6-phosphogluconate by an enzyme system discovered by Entner and Doudoroff (27, 75). The hexose chain is cleaved to form pyruvate and glyceraldehyde-3-phosphate by an aldolase-like reaction. Since the triose-phosphate can be readily converted to pyruvate, the end product of the Entner-Doudoroff (ED) pathway is the same as that in the EMP pathway, although the mechanism is entirely different. Whereas the carboxyl groups of pyruvate formed by the

THE EMBDEN - MEYERHOF - PARNAS (EMP) PATHWAY

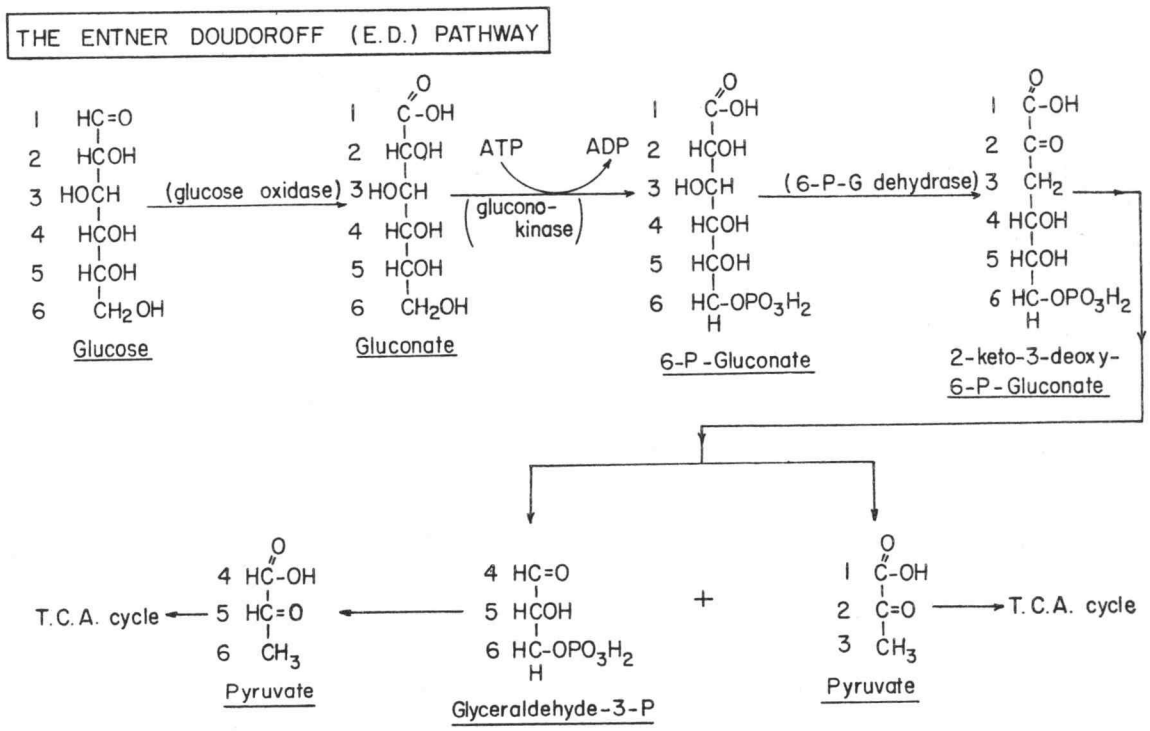
FIGURE 1



EMP pathway are derived from carbons 3 and 4 of glucose, the carboxyl carbon atoms of the pyruvate formed in the ED pathway are derived from C-1 and C-4 of glucose (27, 31). The ED pathway is depicted in Figure 2. In Pseudomonas fluorescens, 2-ketogluconate has also been shown to enter the ED scheme after phosphorylation by a kinase (29).

A direct oxidative pathway for glucose catabolism which does not involve a cleavage of hexoses into two triose molecules is also operative in many microorganisms, plants, and a variety of animal tissues. The occurrence of this alternative pathway was first demonstrated by inhibition studies. Compounds such as iodoacetate and fluoride, which are potent inhibitors of the glycolytic scheme, do not inhibit glucose utilization in some biological systems. The "alternate" pathway does not require inorganic phosphate, an essential component in the glycolytic scheme. The first reaction of this pathway, the direct oxidation of glucose-6-phosphate was discovered by Warburg, Christian, and Griese in 1935 (108). Efforts by investigators such as Dickens, Lipman, Dische, and more recent work by Scott and Cohen (92, 93), Horecker (43, 44), and Racker (84) have made it possible to establish a detailed scheme of the intermediary stages

FIGURE 2


THE ENTNER DOUDOROFF (E.D.) PATHWAY
Glucose
(glucose oxidase)
Gluconate
ATP
ADP
(glucono-kinase)
6-P-Gluconate
(6-P-G dehydrase)
2-keto-3-deoxy-6-P-Gluconate
Pyruvate
T.C.A. cycle
Glyceraldehyde-3-P
+
Pyruvate
T.C.A. cycle

of the oxidation of glucose-6-phosphate. The sequence, named the pentose phosphate cyclic (PC) pathway, is shown in Figure 3. According to this scheme, six moles of glucose-6-phosphate are converted to four moles of hexose-6-phosphate, six moles of CO_2 and two moles of glyceraldehyde phosphate. The accumulation of glyceraldehyde-3-phosphate may not be realized if the enzymes triose phosphate isomerase, aldolase, fructose 1,6-diphosphatase and hexose phosphate isomerase are present. The trioses are then converted through the action of the latter enzymes to glucose-6-phosphate, as shown in Figure 3. Alternatively, glyceraldehyde-3-phosphate can be metabolized via phosphoglycerate and phosphoenol pyruvate to pyruvate as depicted in Figure 1.

Some other pathways related to the pentose phosphate cyclic pathway have been found in biological systems. Studies with a mutant of Acetobacter xylinum (91) grown on glucose have shown the presence of an enzyme which, under anaerobic conditions, carried out a phosphorylytic cleavage of fructose-6-phosphate, as follows:

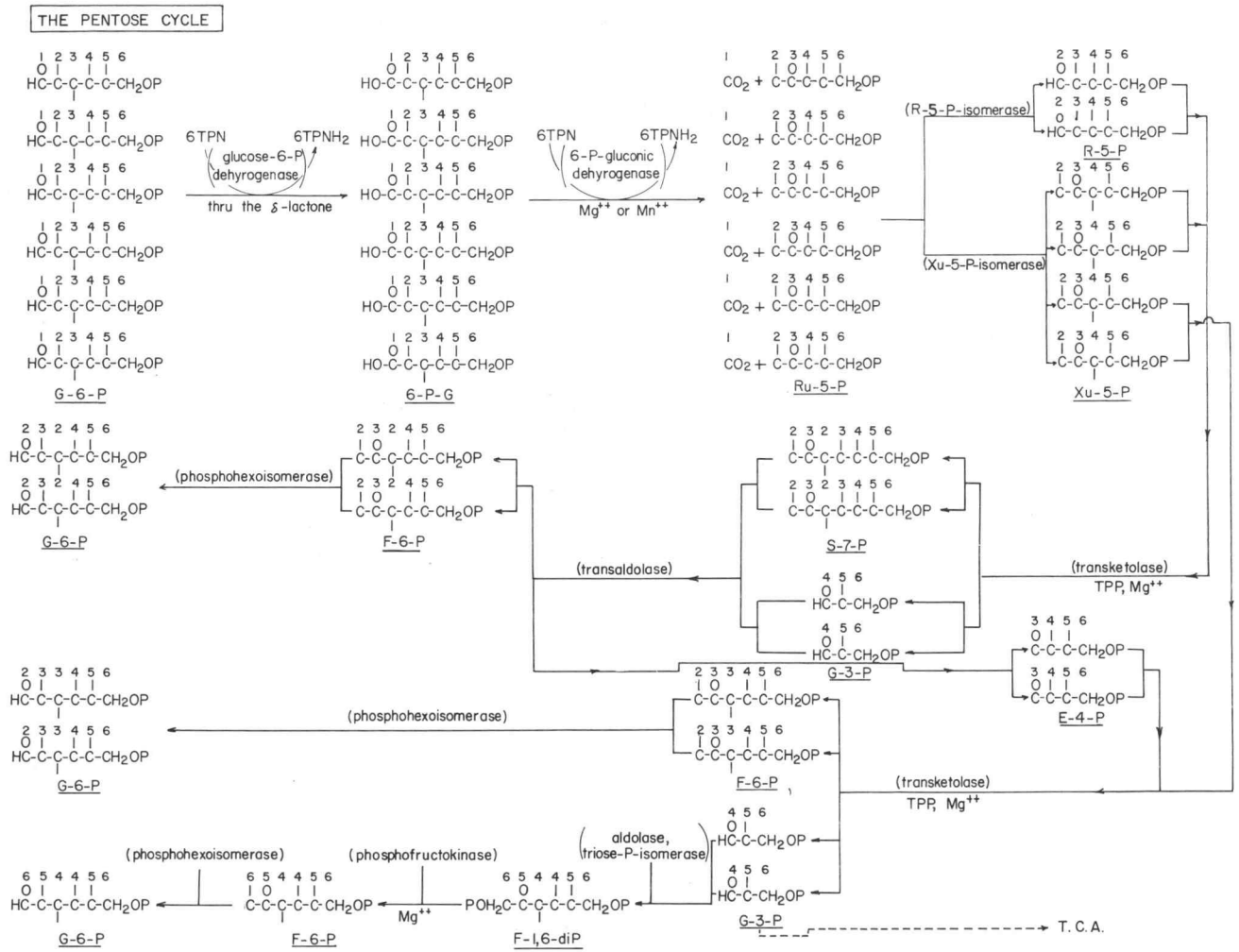

THE PENTOSE CYCLE
G-6-P
6TPN
6TPNH2
glucose-6-P dehyrogenase
thru the δ-lactone
6-P-G
6-P-gluconic dehyrogenase
Mg++ or Mn++
Ru-5-P
(R-5-P-isomerase)
(Xu-5-P-isomerase)
R-5-P
Xu-5-P
(phosphohexoisomerase)
F-6-P
(transaldolase)
S-7-P
(transketolase)
TPP, Mg++
G-3-P
E-4-P
aldolase, triose-P-isomerase
(phosphofructokinase)
F-1,6-diP
T. C. A.

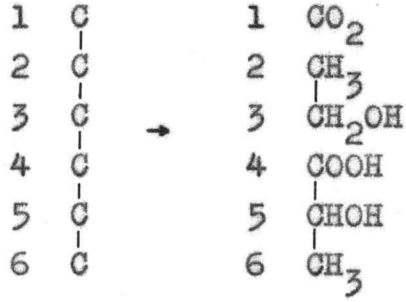
FIGURE 3

fructose-6-phosphate + inorganic phosphate →

acetyl phosphate + erythrose-4-phosphate + H_2O.

This enzyme also splits xylulose-5-phosphate to acetyl-phosphate and glyceraldehyde-3-phosphate, a reaction also carried out by an enzyme from Lactobacillus pentosus (42).

Leuconostoc mesenteriodes converts one mole of glucose to one mole each of CO_2, ethanol, and lactic acid, according to the following scheme:



The glucose degradation appears to proceed via the PC pathway to CO_2 and xylulose-5-phosphate, which is in turn cleaved to give rise to C_2 and C_3 fragments (35).

Recent work on mammalian tissues has revealed a novel metabolic cyclic pathway, subsequently named the glucuronic acid cyclic pathway, after one of its intermediates. Little information is yet available as to the quantitative significance of this pathway, though it has been suggested that this cyclic pathway may be

operative in plants, animals, and microorganisms (102, p. 252-257).

Pathways of Terminal Oxidation

The tricarboxylic acid (TCA) cyclic pathway, as shown in Figure 4, has been established as a major oxidation route in animal tissues, plants, and in a number of species of microorganisms. One revolution along the TCA cyclic pathway results in the oxidation of one mole of acetyl-CoA to two moles of CO_2 with the regeneration of oxaloacetate. It is also recognized that in growing cells the reactions of the TCA cyclic pathway, in addition to the production of energy by acetate oxidation, may also provide carbon skeletons for cellular materials (106, 82, 67, 99, 68, 88). Aspartic acid and glutamic acid, which can be derived from intermediates of the TCA cyclic pathway by amination, can give rise to a series of other amino acids (88, 20). The synthesis of protein during cellular growth thus always results in a drainage of the TCA intermediates from the cyclic processes. It should be realized, though, that the TCA cyclic pathway does not provide a net synthesis of any of its intermediates. To maintain a continuous operation of the cyclic processes, some mechanism must

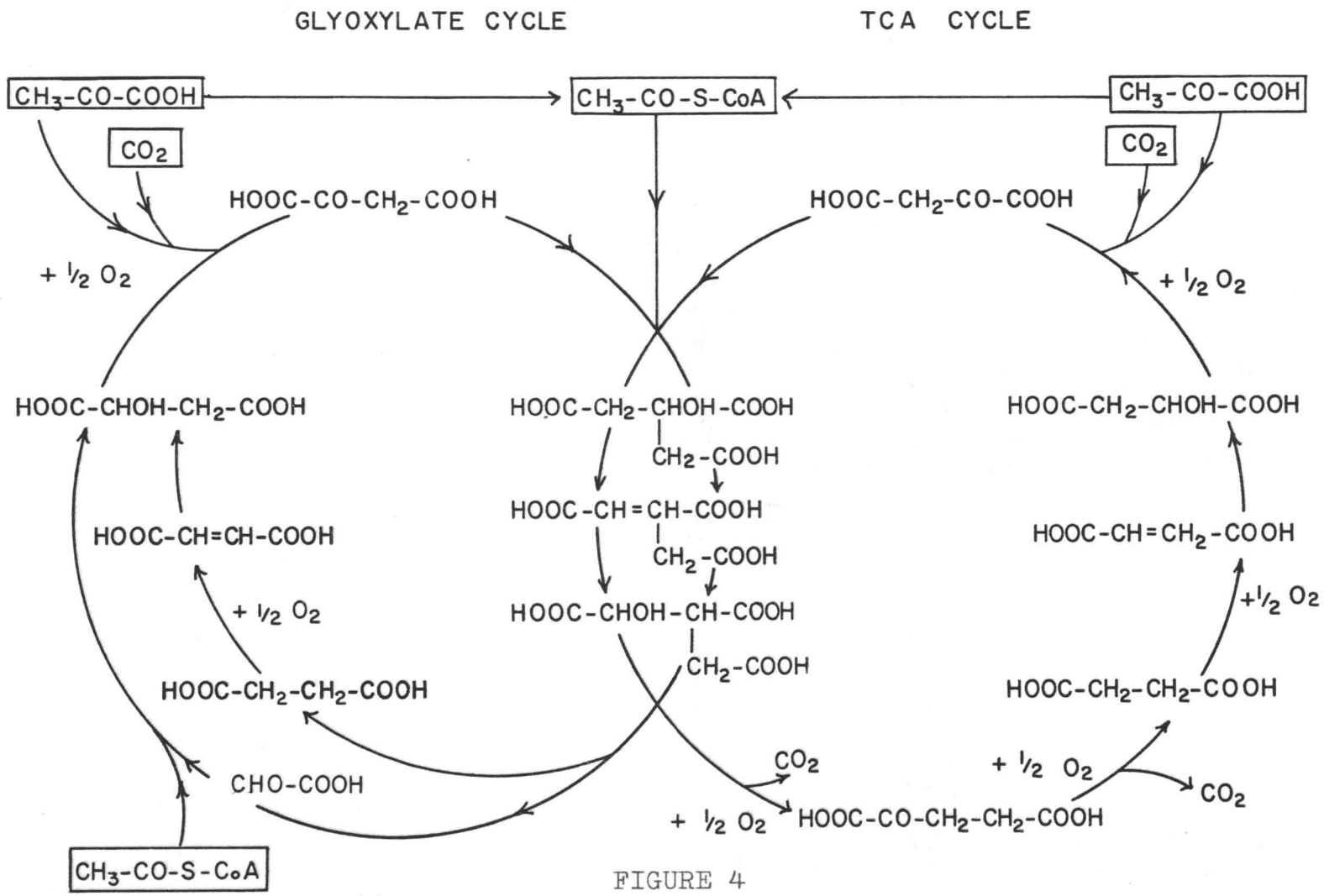

GLYOXYLATE CYCLE
TCA CYCLE
CH3-CO-COOH
CH3-CO-S-CoA
CH3-CO-COOH
CO2
CO2
HOOC-CO-CH2-COOH
HOOC-CH2-CO-COOH
+ 1/2 O2
+ 1/2 O2
HOOC-CHOH-CH2-COOH
HOOC-CH2-CHOH-COOH
HOOC-CH2-CHOH-COOH
CH2-COOH
HOOC-CH=CH-COOH
HOOC-CH=CH-COOH
CH2-COOH
+ 1/2 O2
+1/2 O2
HOOC-CHOH-CH-COOH
CH2-COOH
HOOC-CH2-CH2-COOH
HOOC-CH2-CH2-COOH
CO2
CHO-COOH
+ 1/2 O2
+ 1/2 O2
CO2
HOOC-CO-CH2-CH2-COOH
CH3-CO-S-CoA

FIGURE 4

operate to provide a continuous feed of intermediates. The replacement of these carbon skeletons, particularly that of C_4 compounds, is made by CO_2-fixation reactions (21, 82, 45, 76, 16, 111) and from compounds derived via the glyoxylate cyclic pathway (61, 59).

The glyoxylate cyclic pathway has been demonstrated in plants and Pseudomonas species. The metabolism of glyoxylate has also been a subject of study in animals, where glyoxylate plays a role in glycine metabolism. The glyoxylate cyclic pathway depicted in Figure 4 differs from the TCA cyclic pathway in that the oxidative steps from isocitrate to succinate are bypassed, and instead isocitrate is cleaved into succinate and glyoxylate. The operation of this cyclic pathway does not involve CO_2 production, and the net formation of a C_4 acid through the action of the malate synthetase is realized. Detection of the operation of the glyoxylate cyclic pathway has mainly been based on the presence of the key enzymes isocitrase, which splits isocitrate into succinic acid and glyoxylic acid, and malate synthetase, which effects the condensation of acetate with glyoxylic acid to form malic acid (61, 65). More recently, the operation of the glyoxylate cycle has been established in microorganisms from information on the

labeling patterns of intermediates formed from certain isotopic substrates (65).

The Classification and Metabolism of *Acetobacter* and *Pseudomonas* Species

The present work is devoted to the study of carbohydrate catabolism in *Acetobacter* and *Pseudomonas* species. The genera *Acetobacter* and *Pseudomonas* belong to the family *Pseudomonadaceae*. Species of this family are usually found in soil and water, and their shapes are elongate, straight rods, occasionally coccoid. Many of the species are motile by means of polar flagella (10, p. 88). They are usually Gram negative and aerobic. Frequently they develop pigments, and while many are plant pathogens, very few are animal pathogens.

Species of *Acetobacter* and *Pseudomonas* have marked morphological and physiological similarities, and they are noted for their ability to oxidize a variety of substances as the sources of carbon and energy. These organisms are well known for their unique pathways of carbohydrate metabolism. One can, however, distinguish between the species of *Pseudomonas* and *Acetobacter* by the fact that all species of *Acetobacter* can oxidize ethanol under extremely acid conditions, while

Pseudomonas species require a slightly alkaline or neutral medium for the same oxidation (96). Species of *Acetobacter* vary in their ability to oxidize various compounds. Some oxidize a substance to a high degree, often to a complete oxidation of the substrate, giving off CO_2 and water. Other species oxidize the substrate to a limited extent. Products of these incomplete oxidations are of considerable commercial consequence, particularly as pharmaceuticals (97, 12). The variation in the oxidative ability of *Acetobacter* has been used to classify the *Acetobacter* genus into groups of species as shown in Tables I and II. Vaughn (103) has reduced the number of species of *Acetobacter* to seven, considering all other *Acetobacter* species to be varieties of these few species. He considered *Acetobacter industrium* to be a variety of *Acetobacter oxydans*. Rainbow *et al.* (85, 11) divided the species into two groups, the lactophilic group, which can grow on lactate, and the glycogenic group, which can grow on glucose. Leifson (70) proposed a scheme for classification based on the behaviour in connection with flagellation and acetate oxidation. He suggested that the species that are petrichously flagellated or non-flagellated and capable of oxidizing acetate be classified as *Acetobacter* and

TABLE I

CLASSIFICATIONS OF *ACETOBACTER* SPECIES BY DIFFERENT INVESTIGATORS

Vaughn 1942	Frateur 1950	Rainbow and Mitson 1953 Rainbow and Brown 1956
Oxidize Acetate to CO_2 and H_2O	Oxidize Acetate and Lactate	Lactophilic Group
A. aceti	1. Group Peroxydans	*A. acidum-mucosum*
(*A. ascendens*)[1]	*A. peroxydans*	*A. mobile*
A. xylinum	*A. paradoxum* nov. sp.	*A. suboxydans*
(*A. xylinoides*)	2. Group oxydans	*A. aceti*
(*A. acetigenum*)	*A. lovaniense* nov. sp.	*A. acetosum*
A. rancens	*A. ascendens*	*A. ascendens*
(*A. pasteurianum*)	*A. rancens*	*A. orleanse*
(*A. kützingianum*)	3. Group Mesoxydans	*A. oxydans*
(*A. zeidleri*)	*A. aceti*	*A. rancens*
	A. xylinum	
	A. mesoxydans	

TABLE I - Cont.

Vaughn 1942	Frateur 1950	Rainbow and Mitson 1953 Rainbow and Brown 1956
Do Not Oxidize Acetate	Do Not Oxidize Acetate or Lactate	Glycophilic Group
A. melanogenum	A. suboxydans	A. capsulatum
A. roseum	A. melanogenum	A. gluconicum
A. suboxydans		A. turbidans
A. oxydans		A. viscosum
(A. industrium)		

[1]Bracketed organisms were classified as varieties of the organism they are listed under.

TABLE II

CLASSIFICATIONS OF *ACETOBACTER* SPECIES BY DIFFERENT INVESTIGATORS

Leifson 1954	Bhat and Rijhsinghani 1955	Kondô and Ameyama 1957
Oxidize Acetate or Lactate. Petrichous or no Flagella (Acetobacter)	Oxidize Acetate to CO_2 and H_2O	Oxidize Acetate to CO_2 and H_2O
A. aceti	*A. aceti*	*A. aceti*
A. orleanense	*A. acetigenum*	*A. ascendens*
A. acetosum-mucosum	*A. pasteurianum*	*A. pasteurianum*
A. rancens	*A. xylinum*	*A. rancens*
A. oxydans	*A. rancens*	*A. kützingianum*
A. turbidans	*A. viniaceti*	
A. xylinum		
A. mesoxydans		

TABLE II - Cont.

Leifson 1954	Bhat and Rijhsinghani 1955	Kondô and Ameyama 1957
Do not Oxidize Acetate or Lactate. Polar or no Flagella (Acetomonas gen. nov.)	Do not Oxidize Acetate	No not Oxidize Acetate
A. suboxydans	A. oxydans	A. rubiginosus
A. capsulatum	A. melanogenum	A. melanogenum
A. viscosum	A. viscosum	A. aurantium
A. melanogenum	A. roseum	A. acetosum
	A. industrium	A. dioxyacetonicus
		A. orleanens
		A. cerinus
		A. gluconicum
		A. roseus
		A. xylinum
		A. industrium
		A. oxydans
		A. albidus
		A. suboxydans

species that are polarly flagellated or non-flagellated and incapable of oxidizing acetate be classified as Acetomonas. Frateur (30) derived his classification of four groups of Acetobacter on the basis of catalase activity and oxidative ability. Bath and Rijhsinghani (5) in 1955 and Kondô and Ameyama (57) in 1957 classified main groups of Acetobacter based on the ability to oxidize acetate. As shown in Tables I and II, the classification of several organisms on the basis of the foregoing principles resulted in conflicting views. Thus, A. oxydans was classified by three workers in the group that is capable of oxidizing acetate, and by three other workers in the group that is incapable of oxidizing acetate.

The nature of the carbon sources plays an important role in the proliferation of Acetobacter species (87). It was noted that A. aceti can grow in Hoyer's medium (3), which is a mineral salts-ammonium medium with ethanol and acetate as the sources of carbon. Kaushal and Walker (51) observed growth of A. pasteurianum, A. kützingianum and A. acetigenum with ammonium in a medium containing both ethanol and glucose. It was also reported (37) that A. xylinum can be grown in a simple medium with glucose as the carbon source. Meanwhile,

other reports indicated that the growth of A. xylinum requires essential growth factors and amino acids such as isoleucine, valine and alanine (101). Foda and Vaughn (28) found that A. melanogenum requires growth factors, but not amino acids in contrast to A. oxydans and A. rancens which can grow on valine, isoleucine, alanine, cystine, histidine and proline. Acetobacter rancens was reported to need also aspartic acid or glutamic acid for best growth. Rao and Stokes (87) demonstrated that A. gluconicum, A. rancens, some strains of A. suboxydans, and some strains of A. melanogenum required some yet unidentified factors, which occur in yeast autolysate, for proliferation.

The catabolism of glucose by Acetobacter species has been studied by several workers. Although many oxidation products have been identified, little attempt was made to elucidate the mechanism responsible for the formation of these products (12, 69, 4). It has been reported that species of Acetobacter can convert glucose to gluconic acid and further to 2- and 5-ketogluconic acids (3, 57, 69). Metabolic products such as D-aldehydogluconic acid were also reported with some species (12, 4, 69). Katznelson, Tanenbaum and Tatum (50) found that A. melanogenum accumulated 2,5-diketogluconic acid

from substrate glucose. No CO_2 was liberated by aged cells, while with young cells a more complete oxidation of glucose, with evolution of CO_2, was observed. Kondô and Ameyama (57) have studied the ketogenic activity of the Acetobacter organisms and proposed a scheme for classification. They found that A. rubiginosus nov. sp., A. melanogenum and A. aurantium nov. sp. are capable of oxidizing gluconate with formation of pigments in glucose media. On the other hand, A. acetosum, A. dioxyacetonicus and A. orleanens produced mainly 2-ketogluconate from glucose. Acetobacter gluconicum, A. cerinus, A. roseus, A. xylinum, A. oxydans and A. industrium oxidized glucose to 2- and 5-ketogluconates. Acetobacter albidus nov. sp., A. suboxydans and A. suboxydans var. 5-ketogluconicum nov. sp. produced chiefly 5-ketogluconate.

The enzymatic conversion of glucose via gluconate to 2- and 5-ketogluconate has been studied (57, 26). Several workers have reported alterations due to growth conditions in the ratio of 2- and 5-ketogluconate formed (57, 69). Thus Kondô and Ameyama (57) investigated the optimal pH for the enzyme system in question for oxidation of glucose, gluconate and 2- and 5-ketogluconates, using A. dioxyacetonicus which produces 2-ketogluconate and

A. suboxydans var. 5-ketogluconicum nov. sp. which produces 5-ketogluconate. They found that the optimal pH for oxidation of glucose and 2-ketogluconate was between pH 6 and 7 for organisms producing either 2- or 5-ketogluconate. The oxidation of 5-ketogluconate is favored in more acidic medium, pH 4-5, for both types. However, a difference in optimal pH for oxidation of gluconate by the two types was noted, with a pH of 6.2 optimal for 2-ketogluconate producer and a pH of 4.2 optimal for 5-ketogluconate producer.

The exact pathway of 5-ketogluconate catabolism in bacteria is yet to be elucidated. However, metabolic intermediates such as α-ketoglutaric, succinic and pyruvic acids have been detected (57). Jackson et al. (46) reported that the degradation products may include d-tartaric and oxalic acids. Thus, Pseudomonas fluorescens is capable of oxidizing 5-ketogluconate to d-tartaric acid, glycolic acid, formic acid and CO_2 (46). De Ley and Stouthamer (26) reported that 2- and 5-ketogluconates are not intermediates of the main pathway of glucose catabolism in Acetobacter species. The oxidation of glucose presumably takes place via reduction of 2- and 5-ketogluconates by a reductase to gluconate, which is then phosphorylated by gluconokinase. The

gluconate-6-phosphate is further catabolized via the pentose cycle.

Investigations of the carbohydrate metabolism in A. suboxydans (39, 40, 41, 54) have shown that glucose is degraded mainly via the PC pathway. It has also been indicated (66), however, that the ED pathway is operating in A. suboxydans and A. melanogenum, since the key enzymes, 6-phosphogluconate dehydrase and 2-keto-3-deoxy-6-phosphogluconate aldolase were detected in cell-free extracts of these two organisms.

DeLey and Schell (25) reported that lactate grown cells of A. aceti oxidize glucose via a dual pathway, 80% to gluconic acid and 15% to CO_2, while galactose, mannose, L-arabinose, D-ribose and L-xylose are oxidized to the corresponding acids.

Further degradation of glucose via 2,5-diketo-gluconate by A. melanogenum has been assumed (1) to yield D-lyxuronic acid, with 4-ketoarabinose and 4-ketoarabonic acid as intermediates. Datta et al. (19, 18) have purified an enzyme from A. melanogenum that decarboxylates 2,5-diketogluconate to a C_5-compound which is converted to α-ketoglutaric acid.

Katznelson (49) found that a cell-free preparation of A. melanogenum is capable of oxidizing glucose-6-phosphate, glucose-1-phosphate, fructose-6-phosphate,

6-phosphogluconate and ribose-5-phosphate, while fructose-1,6-diphosphate was oxidized only very slightly. The following enzymes were detected in the foregoing extracts: triphosphopyridine nucleotide linked glucose-6-phosphate-dehydrogenase, phosphohexose isomerase, phosphoglucomutase, glyceraldehyde-3-phosphate-dehydrogenase, hexokinase, glucokinase, 2-ketogluconokinase and aldolase.

Pyruvate and Acetate Metabolism in Acetobacter Species

Acetate can be oxidized to CO_2 by many species of Acetobacter (103, 85, 11, 70, 30). Rao (86) has studied the metabolism of pyruvate and acetate in A. aceti and A. suboxydans. He found that A. aceti oxidized pyruvate to acetate by a pathway not involving acetaldehyde as an intermediate nor lipoic acid as a cofacter. However, pyruvate in A. suboxydans (86, 62) is first decarboxylated to acetaldehyde, and further oxidized to acetate. The TCA cyclic pathway has been found to be operative in A. aceti (86) and A. pasteurianum (53), but not in A. suboxydans (86, 53). In the latter case, only aconitase and fumarase were detected among the TCA cycle enzymes. When A. aceti is grown on acetate, isocitrase is formed, which splits isocitrate into glyoxylate and

succinate (95).

DeLey (23) has investigated the formation of acetoin with 44 strains from Acetobacter genus. DL-Lactate was used as the main carbon source. Most of the strains produced small amounts of acetoin, but A. rancens, A. pasteurianum, and A. ascendens converted most of the substrate into acetoin.

Anaerobic Dissimilation of Carbohydrates in Acetobacter Species

Neuberg and Simon (81) demonstrated in 1928 that A. ascendens and A. pasteurianum utilized glucose under anaerobic conditions, with the formation of appreciable amounts of alcohol and CO_2. Later, it was shown by Simon (94) that A. ascendens, A. pasteurianum and A. suboxydans dissimilated glucose under anaerobic condition into ethanol, acetic acid and CO_2. Schramm et al. (91) reported in 1958 that A. xylinum converted glucose into acetate anaerobically. The transformation was effected by the enzyme fructose-6-phosphate-phosphoketolase.

Cellulose Synthesis by Acetobacter Species

Cellulose is synthesized by A. acetigenum and A. xylinum. Extensive studies have been made of cellulose

transformations is not clear. The ability to form cellulose does, however, depend on the growth conditions (90). Cells growing on glucose medium yield cellulose under aerobic static conditions. The production of cellulose formation is said to be decreased in yield when the culture is agitated. Enzyme studies in cell-free extracts of A. xylinum showed the presence of glucokinase, gluconokinase, phosphoglucomutase, the enzymes of the pentose cyclic pathway, 1,6-diphosphatase and fructose-6-phosphate-phosphoketolase (34, 91).

Carbohydrate Catabolism in Pseudomonas Species

Pseudomonas and Acetobacter species can convert glucose, without previous phosphorylation, to gluconic acid and further to 2-ketogluconate (74). The ability to form ketogluconate has been used as a distinguishing characteristic between Pseudomonas and Acetobacter (10, p. 184). The formation of 5-ketogluconate was considered to be peculiar to the Acetobacter group, but it has recently been demonstrated to occur also in some species of Pseudomonas (100). The further breakdown of 2-ketogluconate by growing cultures of P. fluorescens (73, 56, 55) and P. aeruginosa (109, 14) gave various intermediates, including acetate, pyruvate and

and P. aeruginosa (109, 14) gave various intermediates, including acetate, pyruvate and α-ketoglutarate. Since the oxidation of glucose could proceed in certain pseudomonads in the presence of fluoride, it indicated that the glycolytic pathway did not occur to a significant extent (109, 2).

Considerable knowledge about glucose catabolism in Pseudomonas species was gained when Entner and Doudoroff (27) identified the pathway known today as the Entner-Doudoroff pathway in Pseudomonas saccharophila. The identification was done by means of inhibitors and radiotracer studies. Shortly afterward, Wood et al. (113) demonstrated the ED pathway in P. fluorescens. Additional information concerning glucose oxidation in this organism has been provided by the identification of gluconokinase and 2-ketogluconokinase (80). Gibbs and DeMoss (31), with the use of radiotracers, found the ED pathway to occur in P. lindneri. It was shown that both fructose and glucose were metabolized via the same pathway (ED) to CO_2 and ethanol. Work by Lewis et al. has indicated that P. fluorescens catabolized glucose mainly via the PC and the ED pathways, and to a very small extent, if any, via the EMP route. A comparative and a quantitative study of the participation of pathways in glucose degradation in five pseudomonads has been carried out by

Stern, Wang and Gilmour (98, 99, 107), using the radiorespirometric method of Wang et al. (105). Four different patterns of glucose degradation were obtained. Zymonas motilis (formerly P. lindneri) degraded glucose solely via the ED pathway. In P. saccharophila the ED pathway is operative together with the TCA cyclic pathway. The ED pathway and the TCA cyclic pathway were operative concurrently with a pentose involving pathway in Pseudomonas reptilivora (formerly P. fluorescens), P. aeruginosa and P. fluorescens KB1. The extent of participation of these pathways in P. reptilivora was estimated to be 72 percent of the substrate glucose via the ED and 28 percent via the pentose phosphate pathway. In P. aeruginosa and P. fluorescens KB1, the ED pathway participation was 71 percent and 87 percent of the substrate glucose, respectively, and the participation of the pentose phosphate pathway was 29 percent in P. aeruginosa and 13 percent in P. fluorescens KB1. Studies by Wang, Stern and Gilmour (107) indicated that while the glucose was degraded by both the ED pathway and the pentose phosphate pathway in P. aeruginosa, P. reptilivora and P. fluorescens KB1, gluconate was degraded only via the ED route.

The terminal oxidation, by which acetate or the

product of pyruvate decarboxylation, is converted into CO_2 and cellular material proceeds in several Pseudomonas species via the TCA cyclic pathway and the glyoxylate cyclic pathway (98, 99, 59). Campbell et al. (15) first reported the formation of glyoxylate and succinate from tricarboxylic acids in extracts of P. aeruginosa. Kornberg and Madsen (62, 63) demonstrated that acetate grown cells of P. fluorescens KB1 contained the key enzymes of the glyoxylate cycle, malate synthetase and isocitrase, responsible for the formation of C_4 acids from acetate (62). Ikeda (45) found, however, that in P. fluorescens KB1, pyruvate played an important role in the biosynthesis of C_4 acids by means of CO_2 fixation. The operation of glyoxylate cycle which took place in acetate grown cells could not be demonstrated to any significant extent in cells grown with glucose as the sole carbon source.

Doudoroff et al. (24, 83) found that P. saccharophila metabolizes D-galactose via 2-keto-3-deoxy-D-galactonic acid, and D-arabinose via 2-keto-3-deoxy-D-arabonic acid. The former intermediate is phosphorylated and the product is converted to pyruvate and glyceraldehyde-3-phosphate. The latter is cleaved without prior phosphorylation to yield pyruvate and glycolate.

However, in P. saccharophila α-ketoglutaric acid is produced by oxidation of L-arabinose via the intermediates 2-keto-3-deoxy-L-arabonic acid and L-2-keto-4,5-dikydroxyvaleric acid (110).

With the finding of unique pathways of glucose degradation in certain species of Pseudomonadaceae, and because of comparatively little information about the bulk of species in this group, interest has been centered on the identification and estimation of the catabolic pathways of glucose in members of this family. Therefore, in the present work the catabolism of carbohydrates in three Acetobacter species and three Pseudomonas species have been studied by means of the radiorespirometric method.

EXPERIMENTAL METHODS

Cultures and Cultural Conditions

The origin and cultural conditions for each of the microorganisms used in the present study are given in Table III. The medium employed in each tracer experiment was identical to that used in obtaining the cell crop with the exception that C^{14}-labeled substrates were used to replace the non-isotopic carbon source. In all experiments, conditions were used which were optimum for growth in the hope that the catabolic rates thus observed might represent more closely those prevailing in normal cells.

C^{14}-labeled Substrates

Glucose-1-, -2-, -3- and -6-C^{14} were obtained from Dr. H. S. Isbell, National Bureau of Standards. Glucose-3,4-C^{14} was prepared from rat liver glycogen according to the method of Wood, Lifson and Lorber (114). Potassium-D-gluconate-1-, -2-, -3-, -3,4- and -6-C^{14} were prepared in this laboratory from the correspondingly labeled glucose compounds according to the method of Moore and Link (79). Pyruvate-1-, -2-, -3-C^{14} and acetate-1- and -2-C^{14} were obtained from Tracerlab, Inc.

TABLE III

ORGANISMS AND CULTURAL CONDITIONS

Organism	Source and/or Strain	Basal Medium
Pseudomonas viridilivida	ATCC 512 Brown	$(NH_4)_2SO_4$, phosphate buffer, salts, trace yeast extract
Pseudomonas iodinum	ATCC 9897 Davis	yeast extract 0.5% KH_2PO_4 0.05% K_2HPO_4 0.05% Glucose 0.01%
Pseudomonas reptilivora	Dr. W. C. Haynes, NURB of USDA, strain NRRLB-6bs	Same as that used for *P. viridilivida*
Acetobacter xylinum	ATCC 10821 strain NCIB 1375	Peptone 0.5% yeast extract 0.5% KH_2PO_4 0.1% Glucose 2.0%
Acetobacter industrium	ATCC 12302 strain IFO 3260	Yeast extract 1.0% KH_2PO_4 0.5% Glucose 2.0%
Acetobacter melanogenum	ATCC 9937 strain MA 61	Same as that used for *A. industrium*

Radiorespirometric Experiments

The radiorespirometric studies on the utilization of carbohydrates were carried out according to the method of Wang et al. (105), except that the CO_2 trap was modified to include two identical CO_2 traps connected to the incubation flask by means of a three-way stopcock as shown in Figure 5. The double CO_2 trap permits instantaneous change of trap solution without interrupting aeration of the culture.

In an experiment of this type, the labeled substrates, diluted with non-isotopic substrates to a prescribed level, were added to the sidearm. The harvested cells, after being washed with carbohydrate-free medium, were suspended in medium and a given amount of cell suspension was introduced to the main compartment of each incubation flask. The flasks were then shaken in a waterbath at the desired temperature under aeration. After a depletion phase of one-half hour, the substrates were tipped individually into the cultures. The radiochemical recovery of the substrate activity in respiratory CO_2 from cells metabolizing specifically labeled substrates was followed in prescribed time intervals. The trapping reagent for CO_2 employed in the present experiment was 0.25M methanolic hyamine solution. The

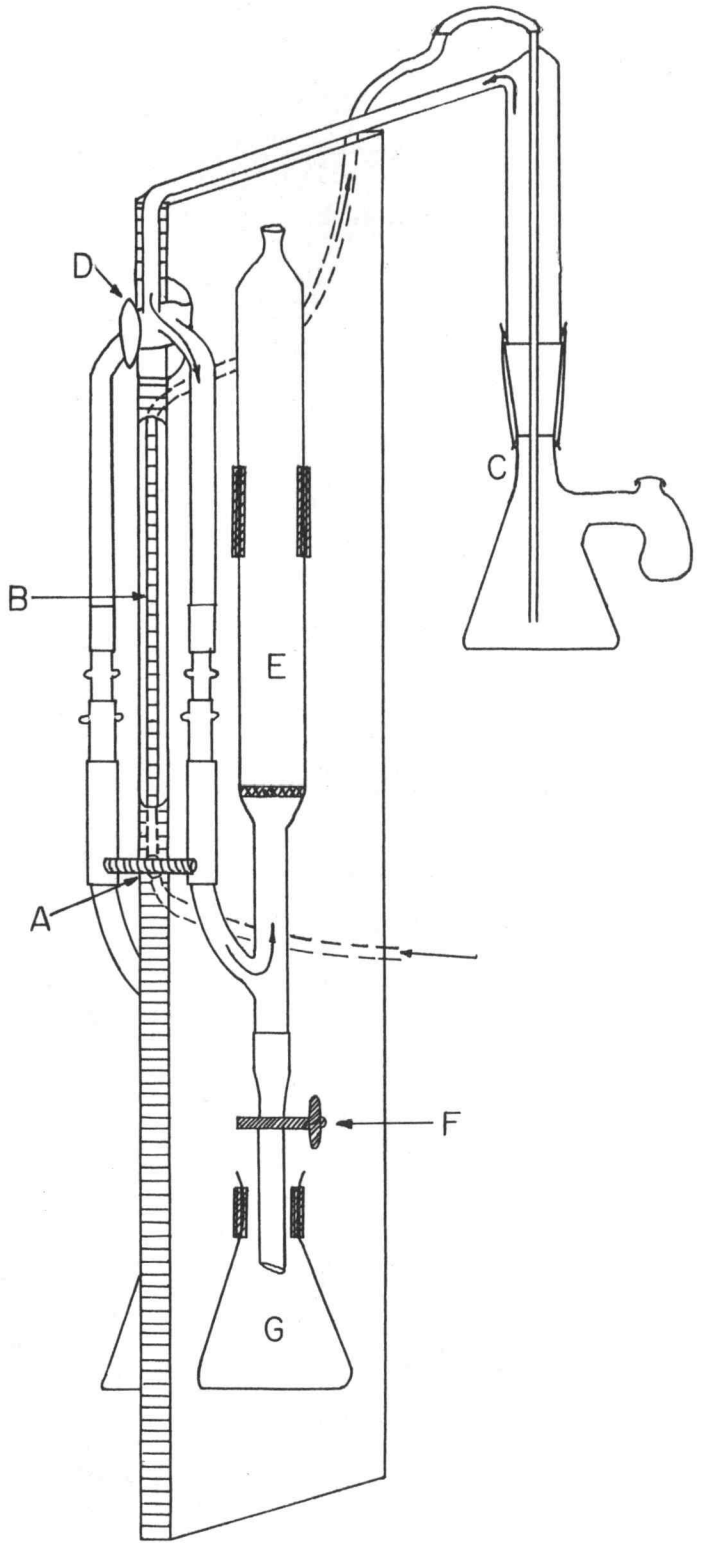


FIGURE 5. Incubation Flask and CO_2-trap Assembly for Radiorespirometric Experiment

efficiency of CO_2 absorption was established to be satisfactory by means of preliminary experiments. Incubations were generally carried out over a period of six to eight hours, at which time, cells were separated from the incubation medium by centrifugation and both fractions were processed for radioactivity determination.

Radioactivity Measurements

The radioactivity of the respiratory CO_2 was determined in the following manner: the trap solution was diluted to 15 ml with methanol, 5 ml of which were transferred to a counting vial containing 10 ml of a phosphor solution consisting of 0.3 mg of POPOP and 30 mg of terphenyl in toluene. The activities of substrates were determined by diluting 1 ml to 10 ml with hyamine-methanol solution. One ml of this mixture was added to 4 ml of hyamine-methanol solution and processed for radioactivity determination.

The cells were diluted to 10 ml, and a 1 ml aliquot of the cell suspension or the medium was added to 14 ml of a scintillating gel prepared by mixing 18.75 g thixotropic gel powder, 6 ml of a 1:9 mixture of Tween-80 and Span-80, 7.5 ml glycerol, 25 mg POPOP, 3 g

terphenyl and 750 ml toluene in a Waring blendor. The counting samples were shaken vigorously prior to counting. Counting efficiency of the latter system was determined by diluting 1 ml of the labeled substrate, calibrated previously, to 10 ml with non-isotopic cell suspension or non-isotopic medium and counting a 1 ml aliquot in the gel, as described above.

All measurements of radioactivity were determined in a Packard TriCarb scintillation spectrometer to a standard deviation of no greater than 1%. Counting data were corrected for counting efficiency variations and background in the conventional manner.

RESULTS

The radiorespirometric pattern for glucose catabolism in P. reptilivora is presented in Figure 6. All of the patterns to be presented here have been plotted on the basis of time in hours against percent interval recovery of substrate radioactivity in CO_2. The radiochemical inventories of substrate activity in CO_2, cells and media at the end of each experiment are shown in Table IV.

The radiorespirometric patterns for P. viridilividа metabolizing labeled acetate, pyruvate, glucose and gluconate, respectively, are shown in Figures 7, 8, 9 and 10. The radiochemical inventories of substrate activity in CO_2, cells and media at the end of each experiment are shown in Table V.

Preliminary experiments with P. iodinum indicated that glucose and gluconate were not utilized, while pyruvate and acetate were readily metabolized by this organism (Figures 11 and 12). The radiochemical recovery of substrate activities in CO_2 and the conditions under which the radiorespirometric experiments were performed are shown in Table VI.

The radiorespirometric patterns obtained from

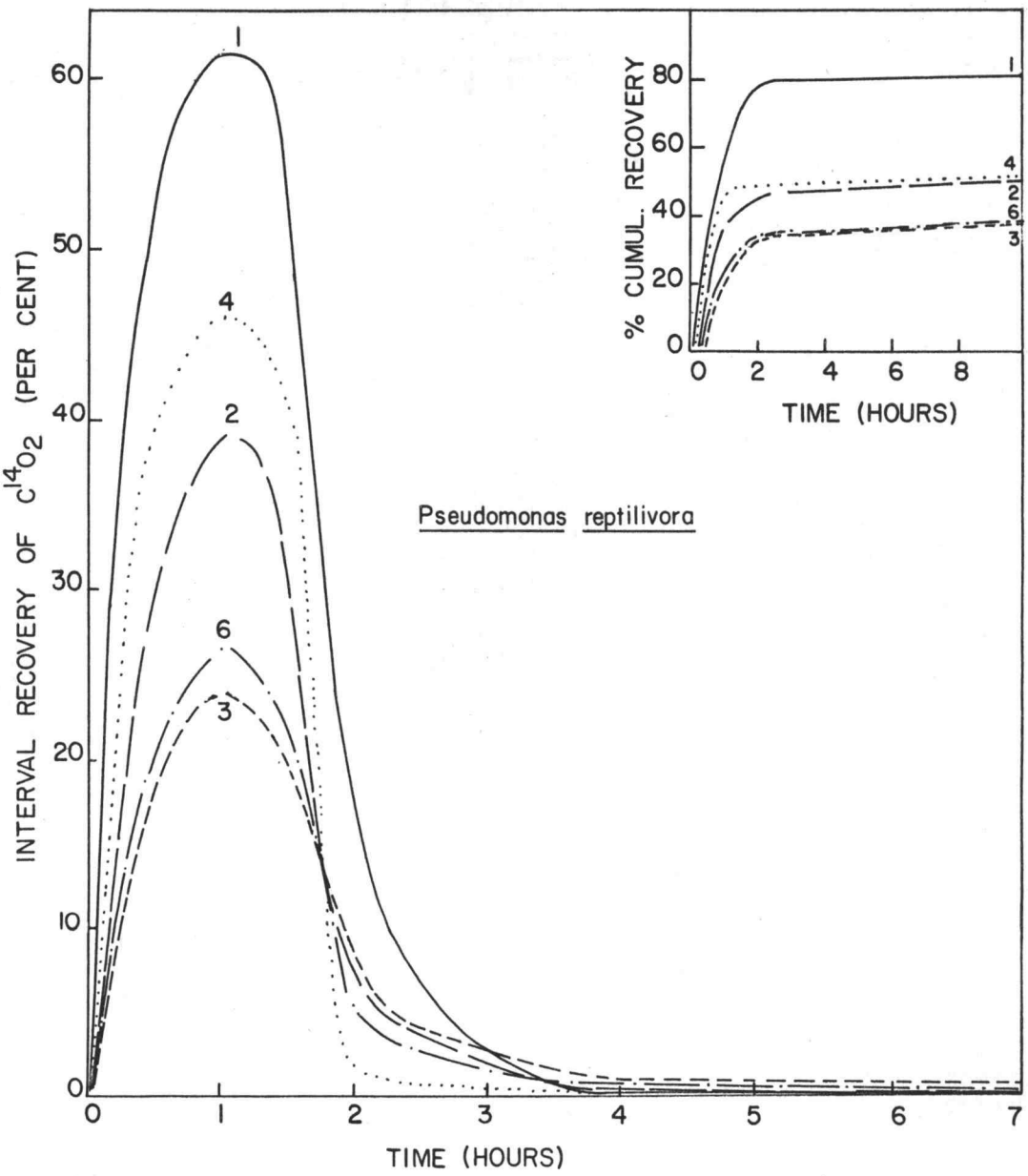


FIGURE 6. Radiorespirometric Pattern. Pseudomonas reptilivora metabolizing specifically labeled glucose.
Legend: Glucose-1-C^{14} ——, -2-C^{14} — —, -3-C^{14} ----, -4-C^{14} ...., -6-C^{14} —.—.

TABLE IV

DISSIMILATION OF GLUCOSE IN
PSEUDOMONAS REPTILIVORA

Substrate	Level		Radiochemical Recovery of Substrates, Percent			
	uc	mg	CO_2	Cells**	Medium	Total**
Glucose-1-C^{14}	0.22	10	81	6	3	90
Glucose-2-C^{14}	0.24	10	51	24	6	81
Glucose-3-C^{14}	0.06	10	38	33	7	78
Glucose-4-C^{14}*	-	-	51	11	11	73
Glucose-3,4-C^{14}	0.10	10	44	22	9	75
Glucose-6-C^{14}	0.44	10	37	28	9	74

* Calculated values

** Low recovery due to incomplete collection of cells

Experimental Conditions - incubation temperature, 28°C; cell age, 14 hours; acidity of growth medium, initial pH 6.7, final pH 6.2; acidity of medium for radiorespirometry, pH 6.7; cell suspension, 16 mg (dry weight) in 15 ml medium; aeration rate, 41 ml per min.; experimental duration, 8 hours.

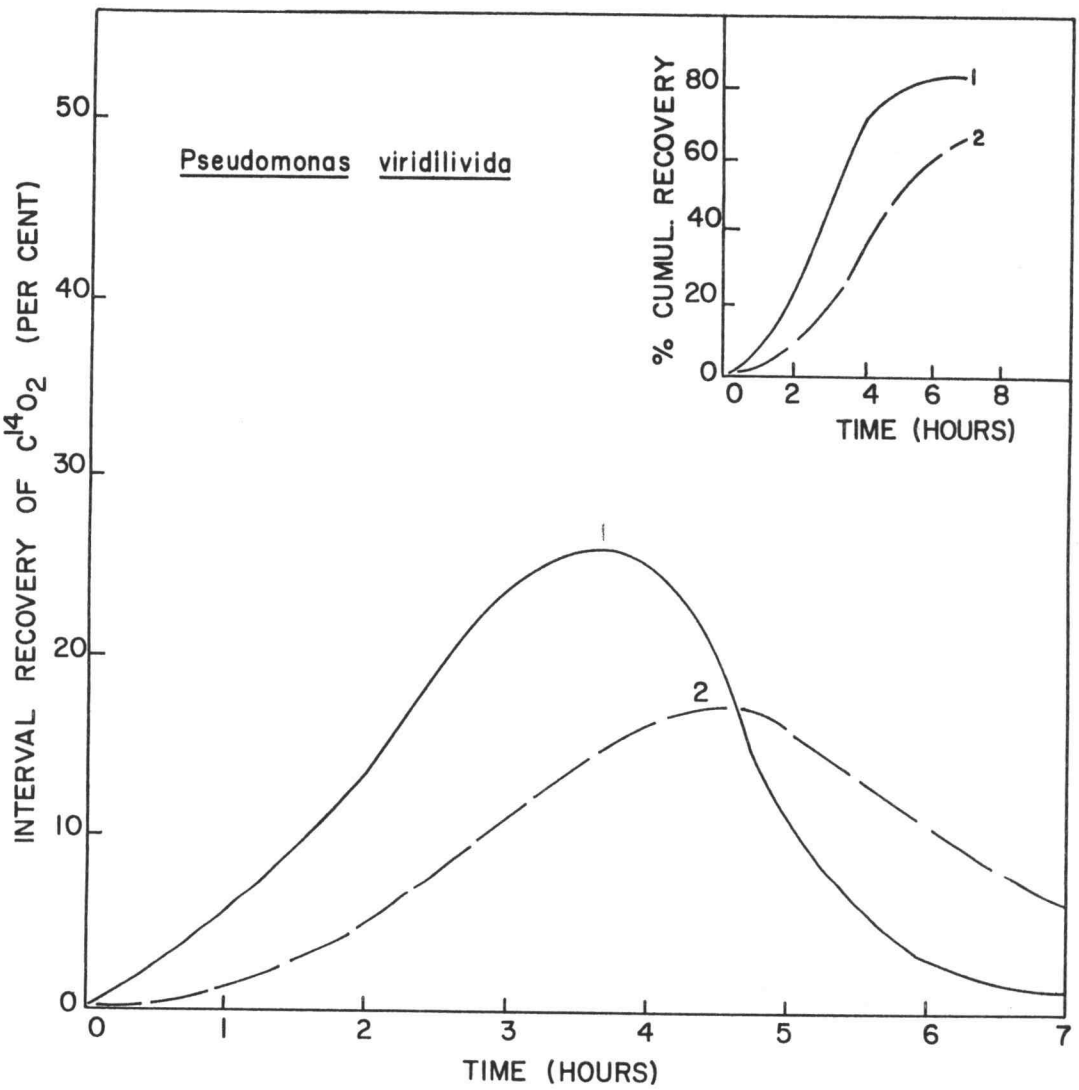


FIGURE 7. Radiorespirometric Pattern.
Pseudomonas viridilivida metabolizing specifically labeled acetate.
Legend: Acetate-1-C^{14} ——, -2-C^{14} — —.

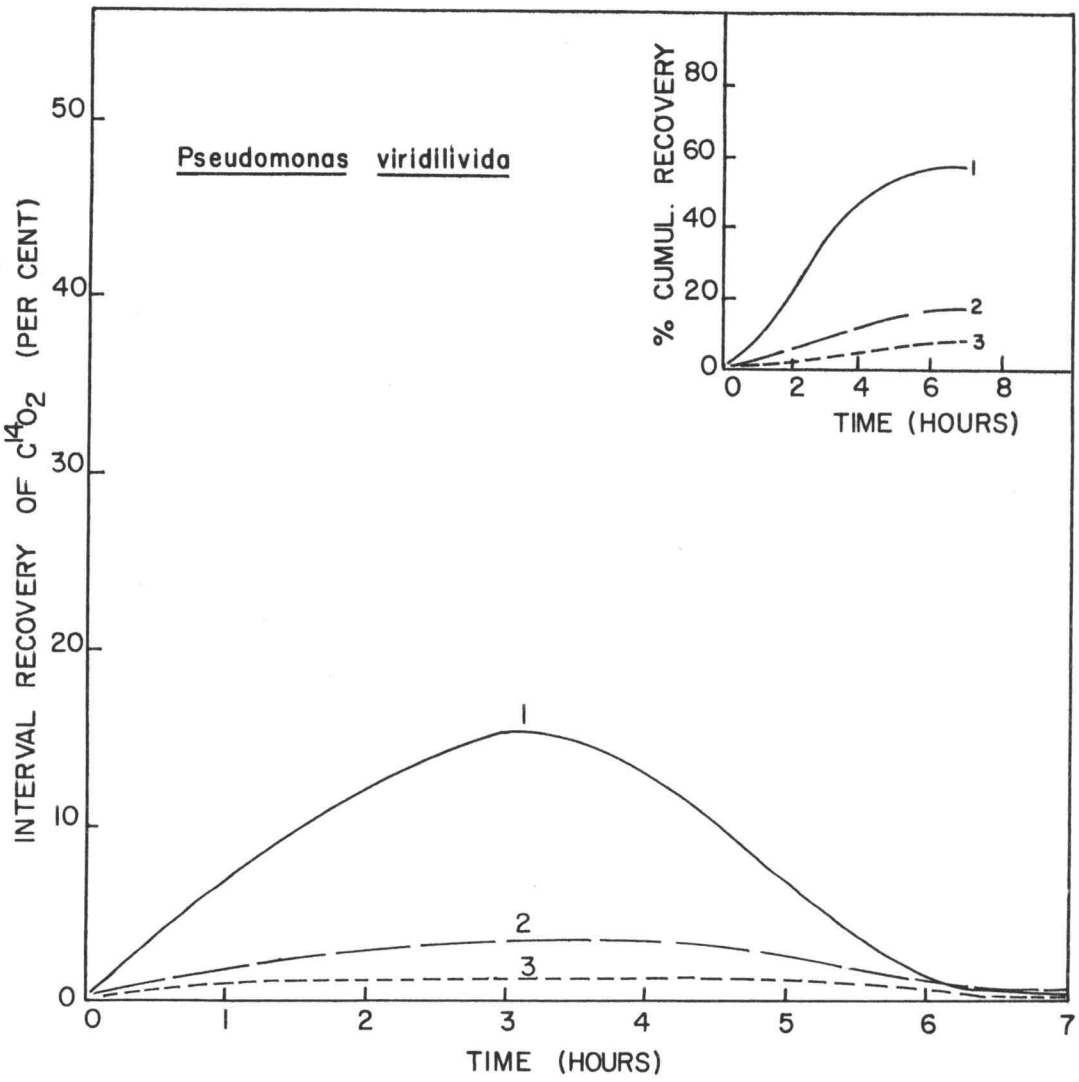


FIGURE 8. Radiorespirometric Pattern. Pseudomonas viridilivida metabolizing specifically labeled pyruvate. Legend: Pyruvate-1-C^{14} ——, -2-C^{14} — —, -3-C^{14} ---.

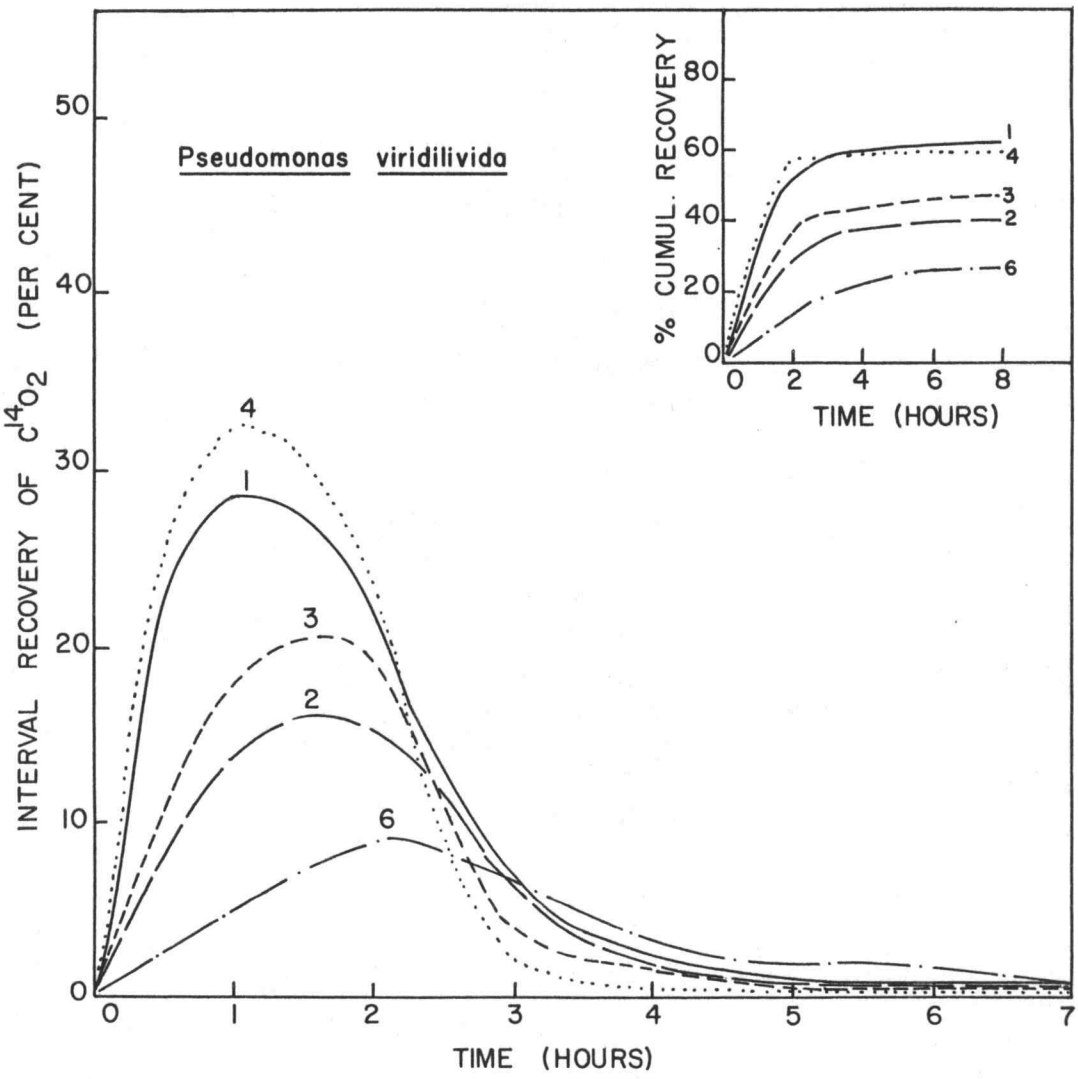


FIGURE 9. Radiorespirometric Pattern. Pseudomonas viridilivida metabolizing specifically labeled glucose. Legend: Glucose-1-C^{14} ——, -2-C^{14} — —, -3-C^{14} ---, -4-C^{14} ...., -6-C^{14} —.—.

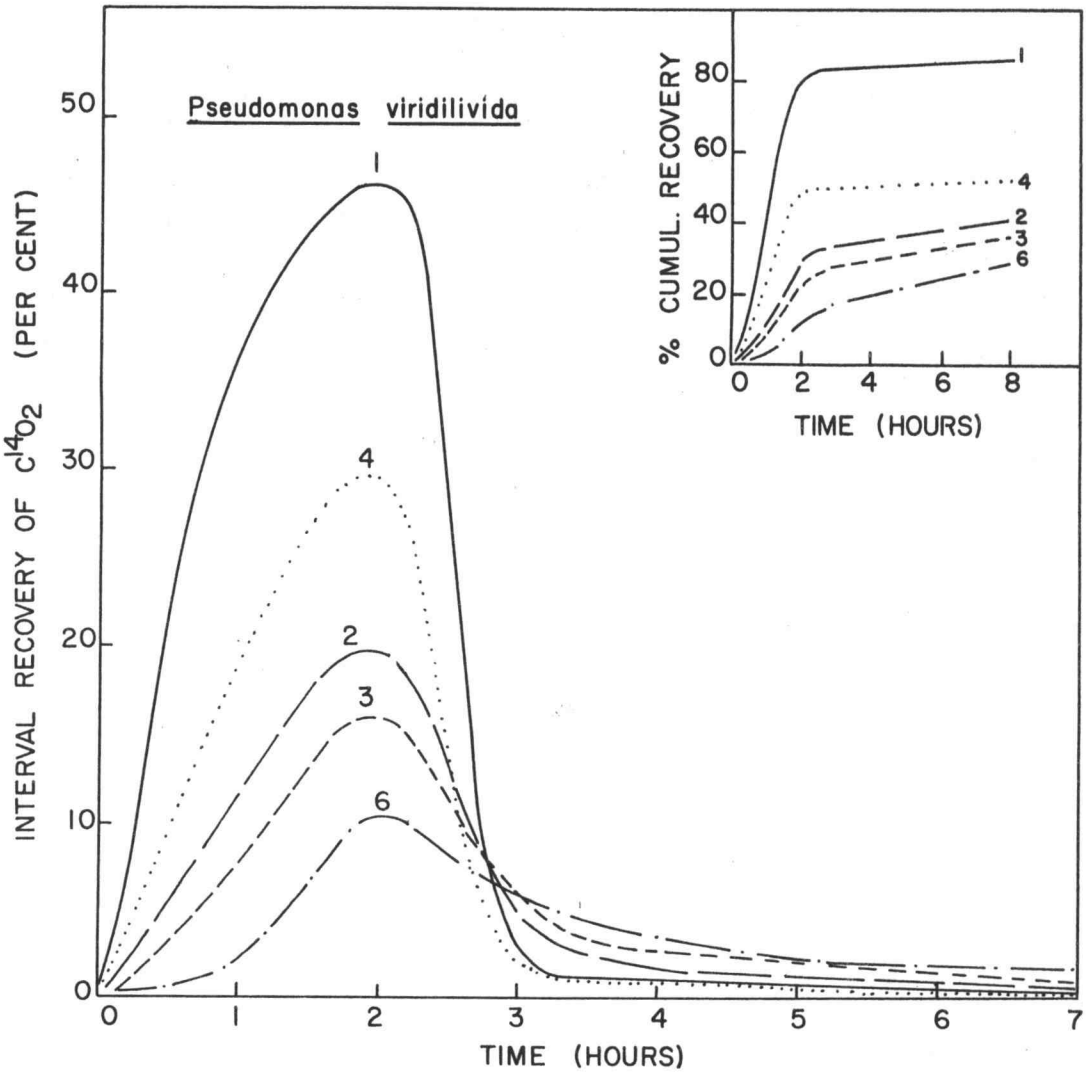


FIGURE 10. Radiorespirometric Pattern. Pseudomonas viridilivida metabolizing specifically labeled gluconate. Legend: Gluconate-1-C^{14} ——, -2-C^{14} — —, -3-C^{14} ----, -4-C^{14} ...., -6-C^{14} —.—.

TABLE V

DISSIMILATION OF GLUCOSE, GLUCONATE, PYRUVATE AND ACETATE IN PSEUDOMONAS VIRIDILIVIDA

Substrate	Level		Radiochemical Recovery of Substrates, Percent			
	uc	mg	CO_2	Cells	Medium	Total
Glucose-1-C^{14}	0.22	10.0	62	31	6	99
Glucose-2-C^{14}	0.24	10.0	41	53	6	100
Glucose-3-C^{14}	0.10	10.0	46	46	4	96
Glucose-4-C^{14}*	--	--	59	43	5	107
Glucose-3,4-C^{14}	0.10	10.0	53	44	5	102
Glucose-6-C^{14}	0.44	5.0	28	60	7	95
Glucose-6-C^{14}	0.44	10.0	27	49	11	87
Gluconate-1-C^{14}	0.20	13.0	87	5	12	104
Gluconate-2-C^{14}	0.30	13.0	42	49	9	100
Gluconate-3-C^{14}	0.08	13.0	37	60	6	103
Gluconate-4-C^{14}*	--	--	53	43	6	102
Gluconate-3,4-C^{14}	0.10	13.0	45	51	6	102
Gluconate-6-C^{14}	0.24	13.0	30	59	8	97
Pyruvate-1-C^{14}**	0.20	12.2	57			
Pyruvate-2-C^{14}	0.20	12.2	17			
Pyruvate-3-C^{14}	0.19	12.2	7			

TABLE V - Cont.

Substrate	Level		Radiochemical Recovery of Substrates, Percent			
	uc	mg	CO_2	Cells	Medium	Total
Acetate-1-C^{14}**	0.16	10.9	82			
Acetate-2-C^{14}	0.26	10.9	66			

*Calculated values

**Radiochemical recoveries in cells and medium were not determined

Experimental Conditions - incubation temperature, 28°C; cell age, 17 hours; acidity of growth medium, initial pH 6.7, final pH 6.4; acidity of medium for radiorespirometry, pH 6.7; cell suspension for glucose and gluconate, 14 mg (dry weight) in 20 ml medium; cell suspension for pyruvate and acetate, 16 mg (dry weight) in 20 ml medium; aeration rate, 41 ml per min.; experimental duration for glucose and gluconate, 8 hours; experimental duration for pyruvate and acetate, 7 hours

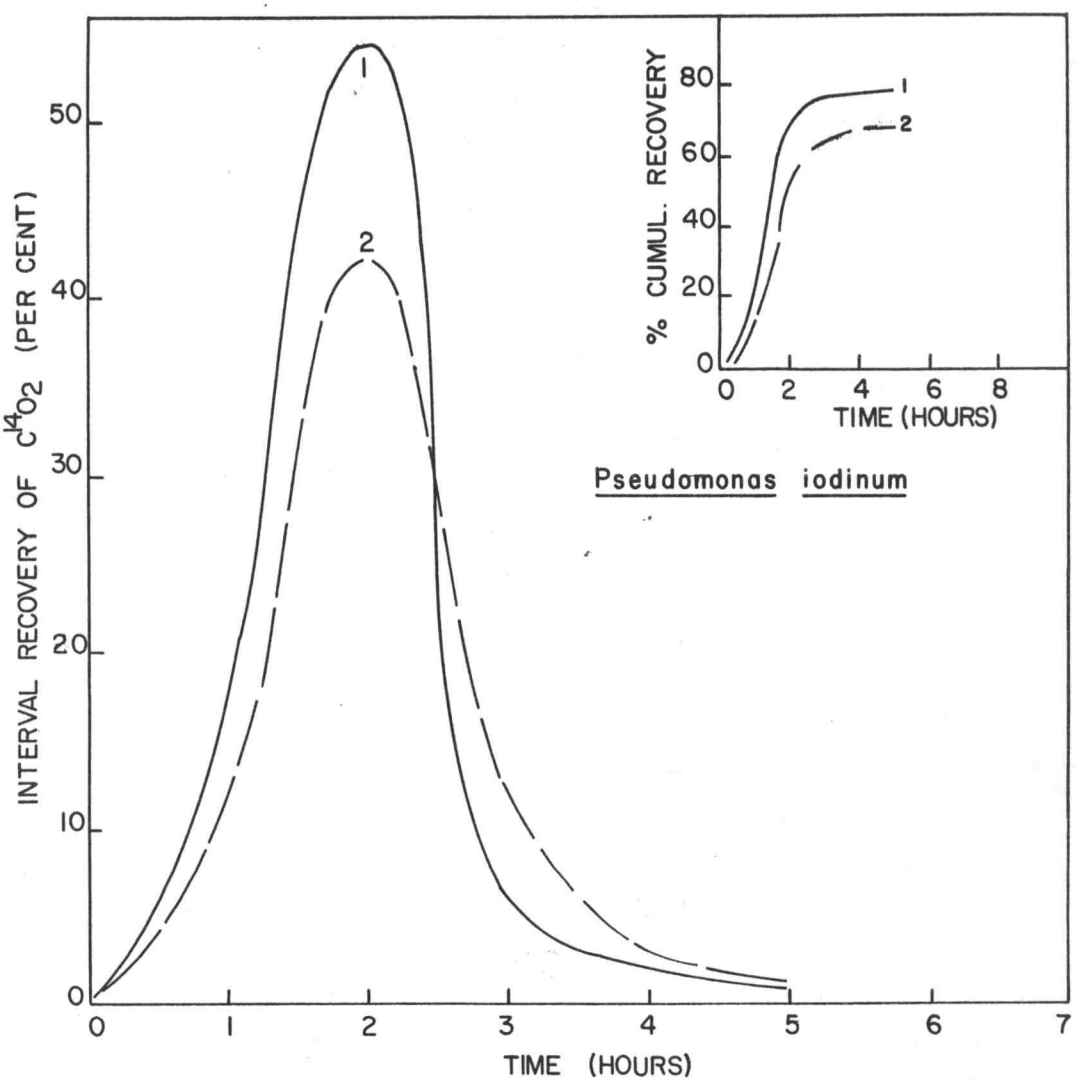


FIGURE 11. Radiorespirometric Pattern. Pseudomonas iodinum metabolizing specifically labeled acetate. Legend: Acetate-1-C^{14} ——, -2-C^{14} — —.

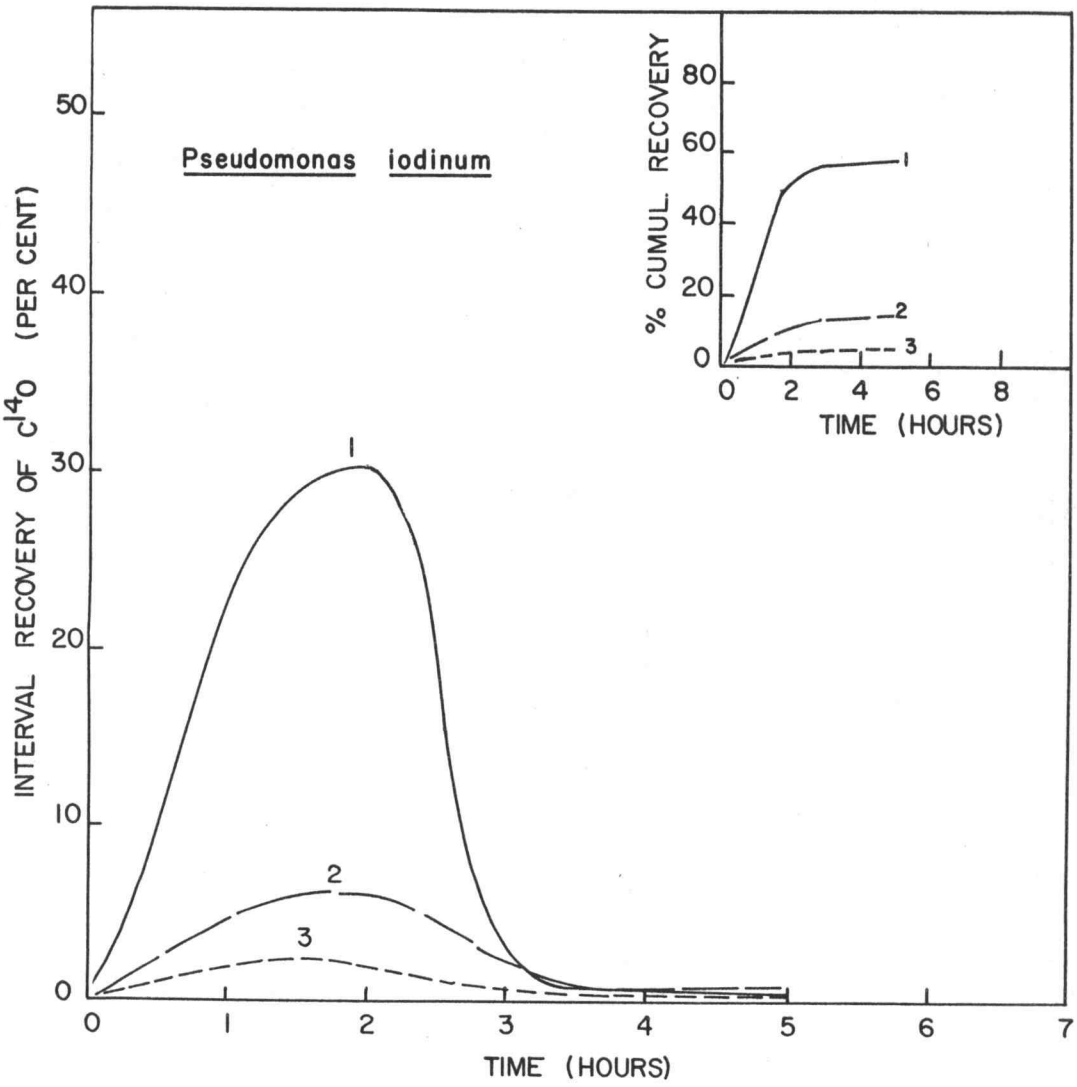


FIGURE 12. Radiorespirometric Pattern.
Pseudomonas iodinum metabolizing specifically labeled pyruvate.
Legend: Pyruvate-1-C^{14} ——, -2-C^{14} — —, -3-C^{14} ----.

TABLE VI

DISSIMILATION OF PYRUVATE AND ACETATE IN PSEUDOMONAS IODINUM

Substrate	Level		Radiochemical Recovery of Substrates, Percent
	uc	mg	CO_2
Pyruvate-1-C^{14}	0.24	6.1	57
Pyruvate-2-C^{14}	0.20	6.1	14
Pyruvate-3-C^{14}	0.20	6.1	5
Acetate-1-C^{14}	0.18	5.5	80
Acetate-2-C^{14}	0.22	5.5	69

Experimental Conditions - incubation temperature, 29°C; cell age, 18 hours; acidity of growth medium, initial pH 6.8, final pH 7.6; acidity of medium for radiorespirometry, pH 6.8; cell suspension of pyruvate and acetate, 12 mg (dry weight) in 20 ml medium; aeration rate for pyruvate and acetate, 41 ml per min.; experimental duration, 5 hours.

Acetobacter industrium metabolizing labeled glucose and gluconate are shown in Figures 13 and 14. This organism was demonstrated in preliminary experiments to be incapable of oxidizing acetate. Preliminary experiments indicated that A. industrium utilized glucose faster than gluconate. The radiorespirometric experiment for gluconate catabolism was, therefore, carried out by using twice the amount of cells and half the amount of substrate gluconate as compared to that in the glucose experiments. The radiochemical inventories of substrate activity in CO_2, cells and media at the end of each experiment are shown in Table VII.

Preliminary experiments with A. melanogenum indicated that it does not utilize acetate and that the CO_2 recovery from substrate glucose and from substrate gluconate is low. The yield of CO_2 from substrate gluconate was to a high degree dependent on the pH of the medium as shown in Table VIII. The optimal pH of the medium was found to be 5.5 for the oxidation of substrate glucose and substrate gluconate. The radiorespirometric patterns of glucose catabolism and gluconate catabolism obtained with a medium of pH 5.5 are shown in Figures 15 and 16. Preliminary experiments showed that glucose is converted to CO_2 faster than substrate gluconate.

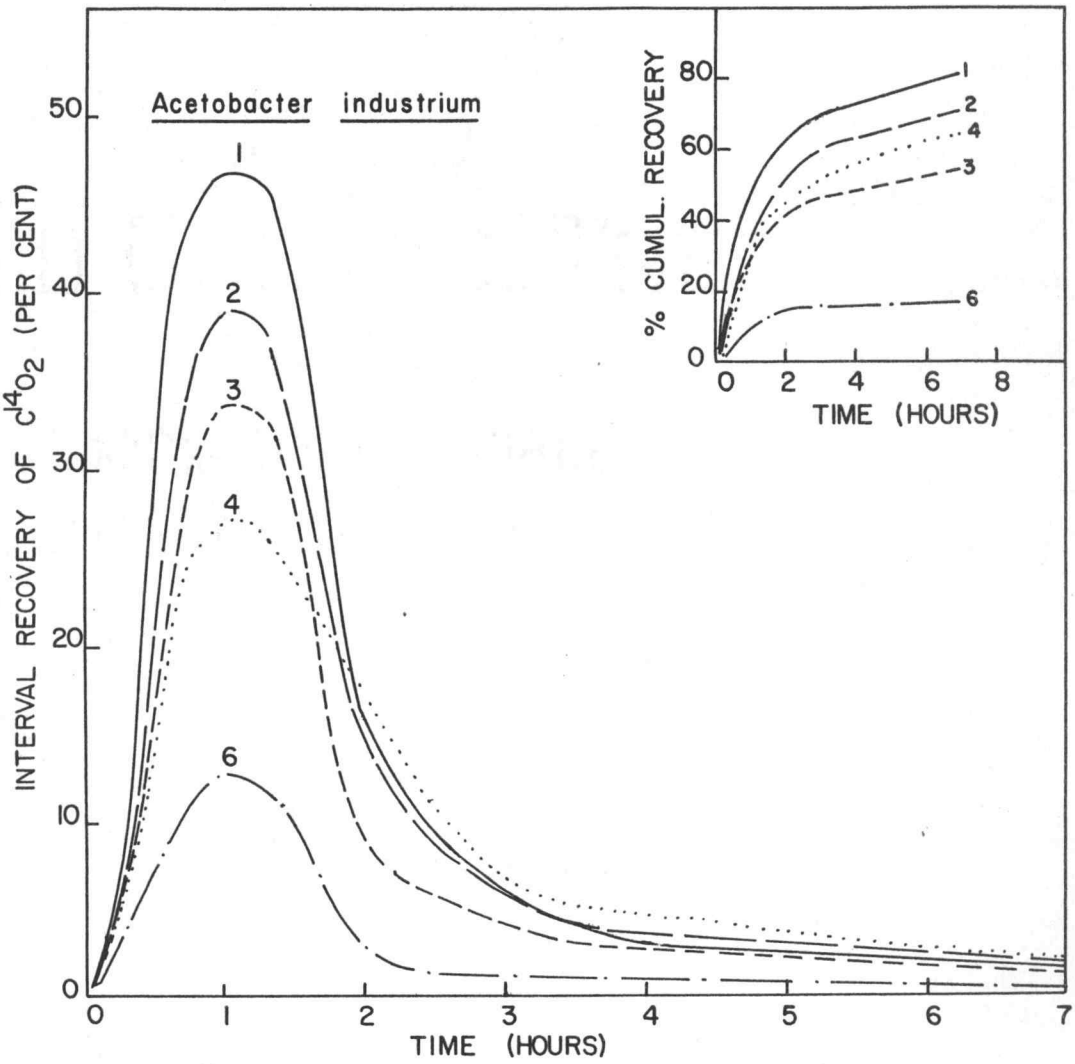


FIGURE 13. Radiorespirometric Pattern.
Acetobacter industrium metabolizing specifically labeled glucose.
Legend: Glucose-1-C^{14} ——, -2-C^{14} — —, -3-C^{14} ----, -4-C^{14} ...., -6-C^{14} —.—.

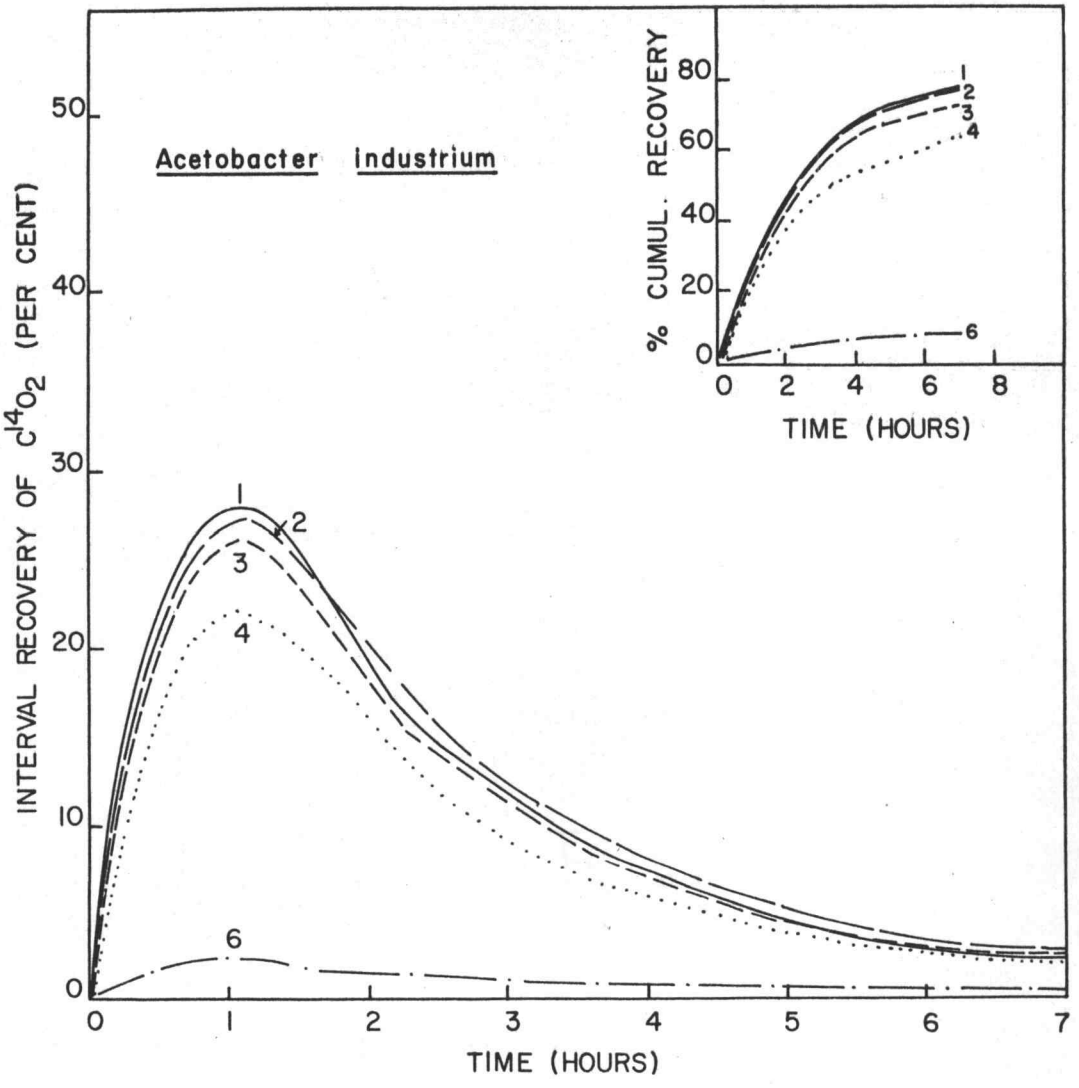


FIGURE 14. Radiorespirometric pattern. Acetobacter industrium metabolizing specifically labeled gluconate.

Legend: Gluconate-1-C^{14} ——, -2-C^{14} — —, -3-C^{14} ----, -4-C^{14} ...., -6-C^{14} —.—.

TABLE VII

DISSIMILATION OF GLUCOSE AND GLUCONATE IN ACETOBACTER INDUSTRIUM

Substrate	Level		Radiochemical Recovery of Substrates, Percent			
	uc	mg	CO_2	Cells	Medium	Total
Glucose-1-C^{14}	0.25	5.0	81	1	18	100
Glucose-2-C^{14}	0.24	5.0	70	7	26	103
Glucose-3-C^{14}	0.03	5.0	54	14	29	97
Glucose-4-C^{14}*	-	-	66	14	29	109
Glucose-3,4-C^{14}	0.06	5.0	60	14	29	103
Glucose-6-C^{14}	0.26	5.0	18	27	60	105
Gluconate-1-C^{14}	0.23	3.25	77	1	31	109
Gluconate-2-C^{14}	0.20	3.25	77	4	22	103
Gluconate-3-C^{14}	0.02	3.25	72	10	20	102
Gluconate-4-C^{14}*	-	-	63	9	23	95
Gluconate-3,4-C^{14}	0.08	3.25	67	10	22	99
Gluconate-6-C^{14}	0.08	3.25	6	18	77	101

*Calculated values

Experimental Conditions - incubation temperature, 28°C; cell age, 17 hours; acidity of growth medium, initial pH 7.6, final pH 4.0; acidity of medium for radiorespirometry, pH 6.5; cell suspension for glucose, 6.5 mg (dry weight) in 20 ml medium; cell suspension for gluconate, 13 mg (dry weight) in 20 ml medium; aeration rate for glucose and gluconate, 61 ml per min.; experimental duration, 7 hours.

TABLE VIII

DISSIMILATION OF GLUCOSE AND GLUCONATE
IN *ACETOBACTER MELANOGENUM*
AT DIFFERENT MEDIUM pH

Substrate, pH of medium	Percent recovery of C^{14}			
	CO_2	Cells	Medium	Total
Glucose-U-C^{14} pH 4.5	25	5	60	90
Glucose-U-C^{14} pH 5.5	28	8	56	92
Glucose-U-C^{14} pH 6.5	25	7	63	95
Gluconate-1-C^{14} pH 4.5	8	0	88	96
Gluconate-1-C^{14} pH 5.5	41	0	56	97
Gluconate-1-C^{14} pH 6.5	26	0	76	102

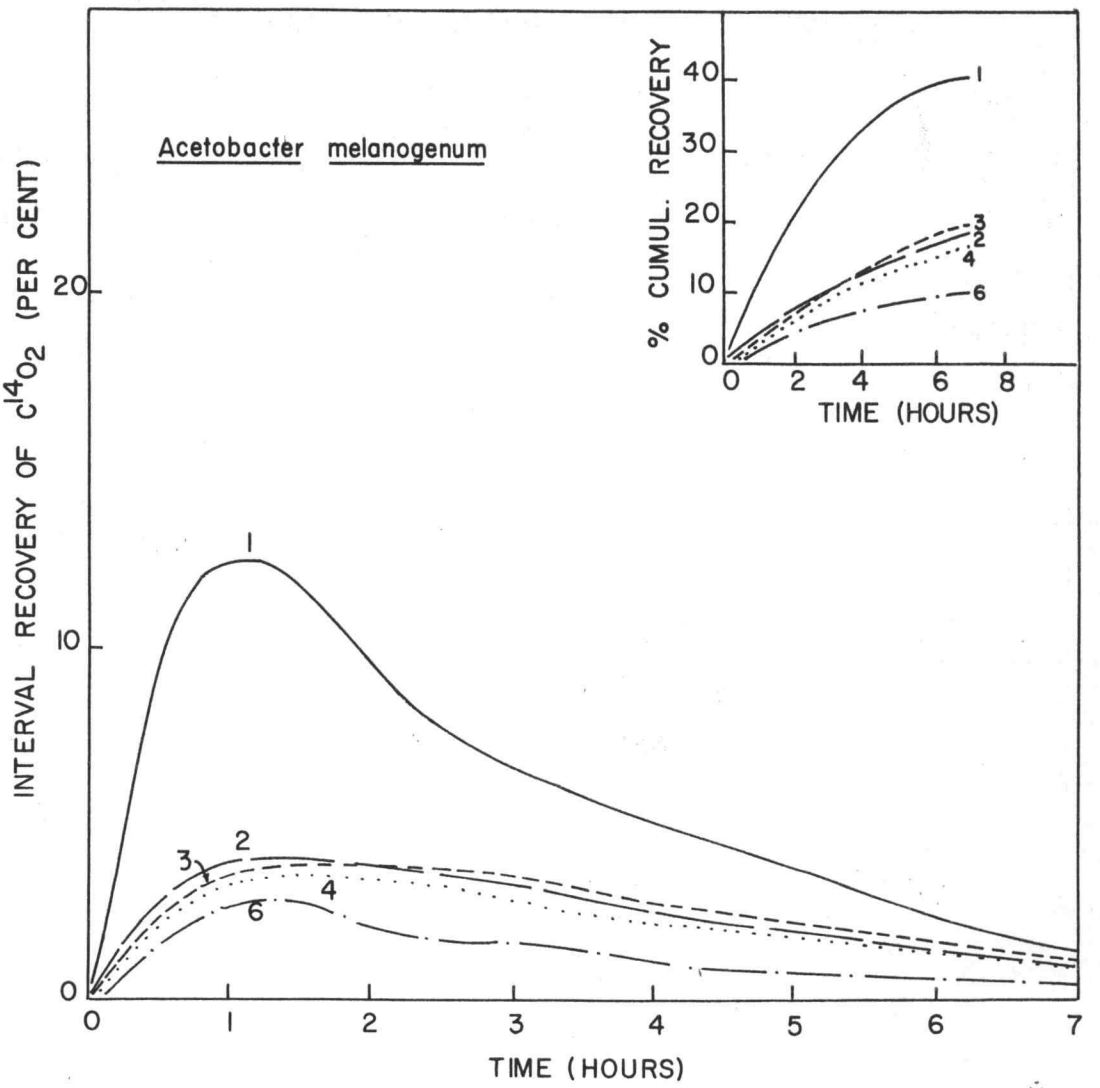


FIGURE 15. Radiorespirometric Pattern.
Acetobacter melanogenum metabolizing specifically labeled glucose.
Legend: Glucose-1-C^{14} ——, -2-C^{14} — —, -3-C^{14} ----, -4-C^{14} ...., -6-C^{14} —.—.

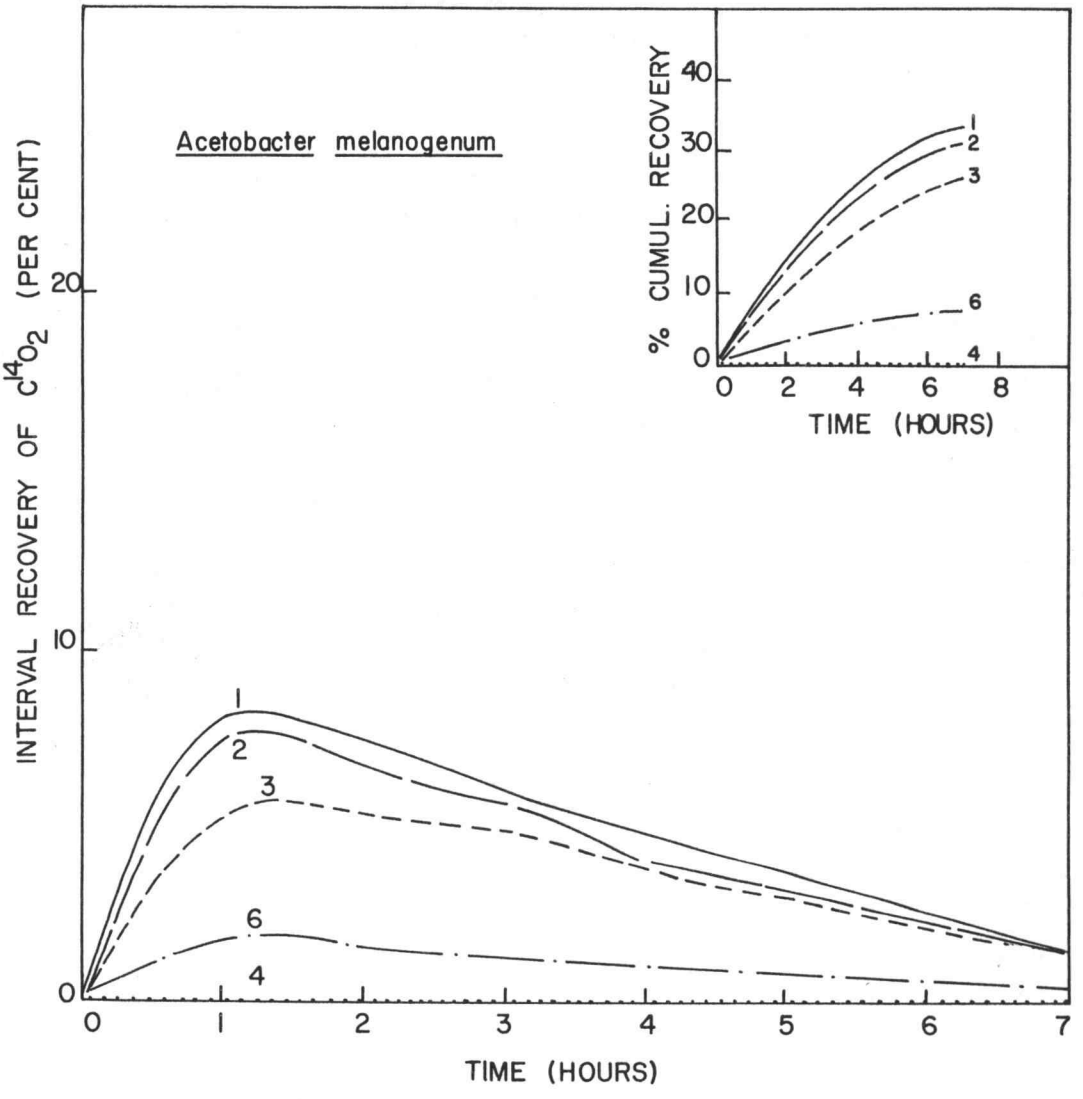


FIGURE 16. Radiorespirometric Pattern. Acetobacter melanogenum metabolizing specifically labeled gluconate.
Legend: Gluconate-1-C^{14} ——, -2-C^{14} — —, -3-C^{14} ----, -4-C^{14} ...., -6-C^{14} —.—.

The radiorespirometric experiment was, therefore, performed using half the amount of substrate gluconate that is equivalent to the amount of substrate glucose used. The experiment on A. melanogenum has not been repeated. The radiochemical inventories of substrate activity in CO_2, cells and media at the end of each experiment are shown in Table IX.

The radiorespirometric data from A. xylinum metabolizing acetate, glucose and gluconate are represented in Figures 17, 18 and 19. The radiochemical inventories of substrate activity in CO_2, cells and media at the end of each experiment are shown in Table X.

TABLE IX

DISSIMILATION OF GLUCOSE AND GLUCONATE IN ACETOBACTER MELANOGENUM

Substrate	Level		Radiochemical Recovery of Substrates, Percent			
	uc	mg	CO_2	Cells	Medium	Total
Glucose-1-C^{14}	0.25	5.0	41	1	56	98
Glucose-2-C^{14}	0.24	5.0	18	2	81	101
Glucose-3-C^{14}	0.02	5.0	19	2	80	101
Glucose-4-C^{14}*	-	-	17	5	83	105
Glucose-3,4-C^{14}	0.05	5.0	18	3	81	102
Glucose-6-C^{14}	0.26	5.0	9	3	84	96
Gluconate-1-C^{14}	0.25	3.25	33	1	67	101
Gluconate-2-C^{14}	0.08	3.25	31	2	65	98
Gluconate-3-C^{14}	0.02	3.25	26	2	73	101
Gluconate-4-C^{14}*	-	-	0	3	90	93
Gluconate-3,4-C^{14}	0.08	3.25	12	2	81	95
Gluconate-6-C^{14}	0.06	3.25	7	2	86	95

*Calculated Values

Experimental Conditions - incubation temperature, 28°C; cell age, 18 hours; acidity of growth medium, initial pH 7.6, final pH 4.0; acidity of medium for radiorespirometry, pH 5.5; cell suspension for glucose and gluconate, 7 mg (dry weight) in 15 ml medium; aeration rate for glucose and gluconate, 61 ml per min.; experimental duration, 7 hours.

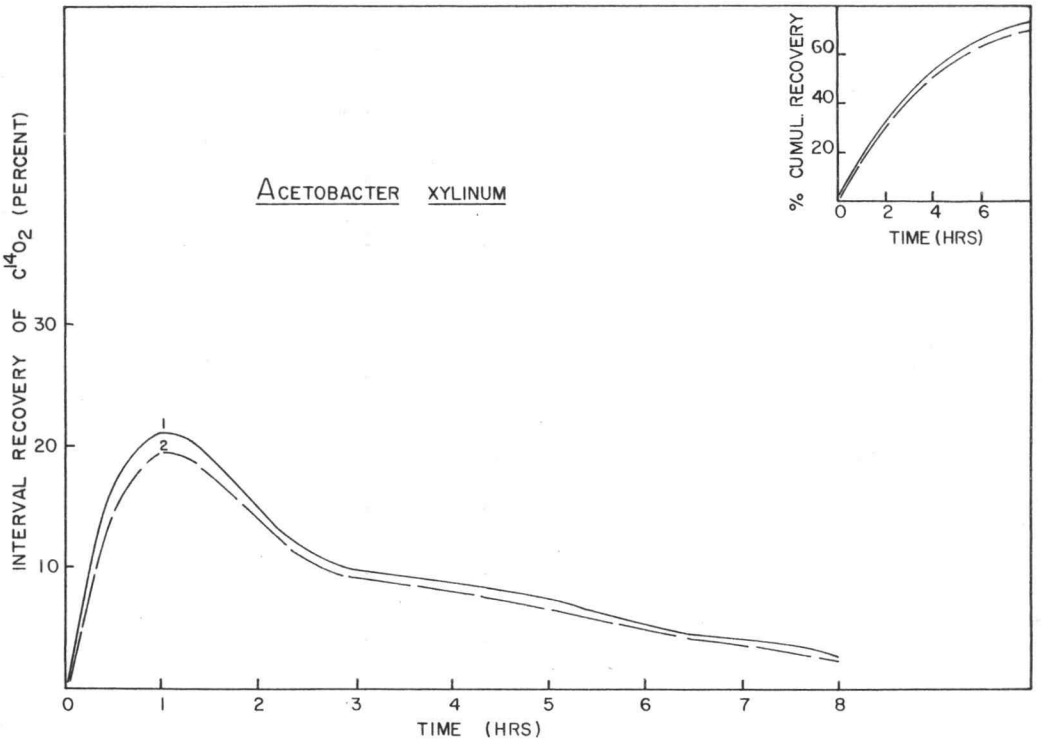


FIGURE 17. Radiorespirometric Pattern. Acetobacter xylinum metabolizing specifically labeled acetate.
Legend: Acetate-1-C^{14} ——, -2-C^{14} — —.

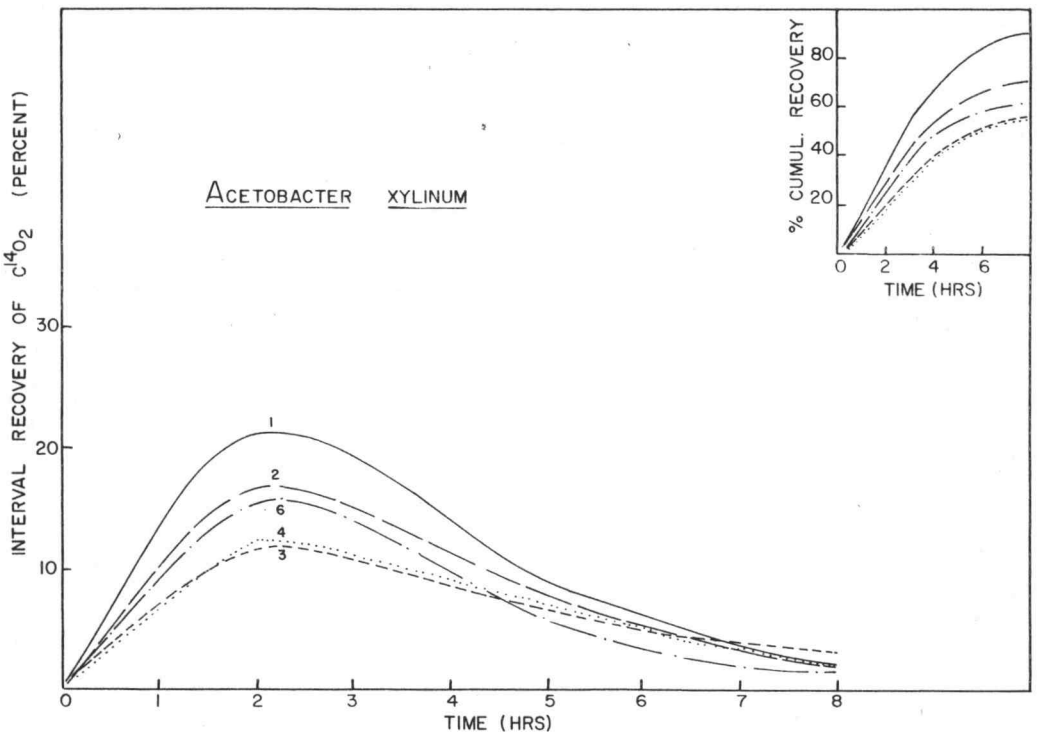


FIGURE 18. Radiorespirometric Pattern. Acetobacter xylinum metabolizing specifically labeled glucose.
Legend: Glucose-1-C^{14} ——, -2-C^{14} — —, -3-C^{14} ----, -4-C^{14} ...., -6-C^{14} —.—.

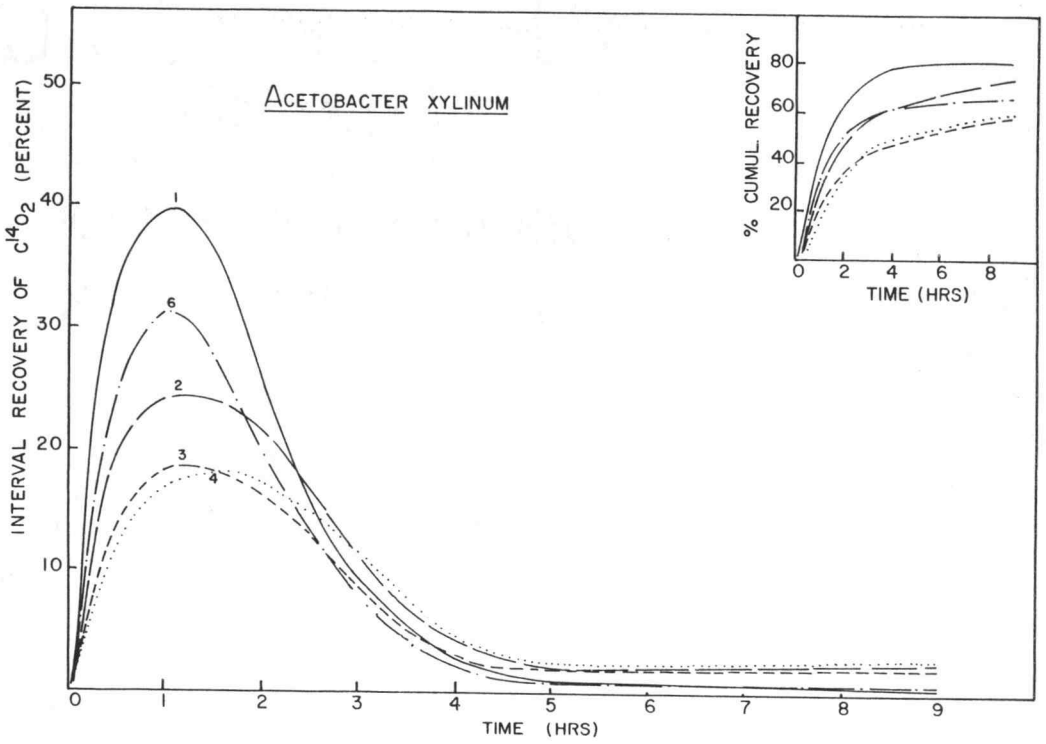


FIGURE 19. Radiorespirometric Pattern. Acetobacter xylinum metabolizing specifically labeled gluconate.
Legend: Gluconate-1-C^{14} ——, -2-C^{14} — —, -3-C^{14} ----, -4-C^{14} ...., -6-C^{14} —.—.

TABLE X

DISSIMILATION OF GLUCOSE, GLUCONATE AND ACETATE IN *ACETOBACTER XYLINUM*

Substrate	Level		Radiochemical Recovery of Substrates, Percent			
	uc	mg	CO_2	Cells	Medium	Total
Glucose-1-C^{14}	0.22	5.0	90	1	6	97
Glucose-2-C^{14}	0.20	5.0	72	8	19	99
Glucose-3-C^{14}	0.10	5.0	56	10	28	94
Glucose-4-C^{14}*	-	-	55	14	30	99
Glucose-3,4-C^{14}	0.10	5.0	56	12	29	97
Glucose-6-C^{14}	0.44	5.0	62	12	22	96
Gluconate-1-C^{14}	0.20	6.5	80	2	22	104
Gluconate-2-C^{14}	0.19	6.5	74	10	21	105
Gluconate-3-C^{14}	0.06	6.5	58	18	22	98
Gluconate-4-C^{14}*	-	-	58	18	32	108
Gluconate-3,4-C^{14}	0.09	6.5	58	18	27	103

TABLE X - Cont.

Substrate	Level		Radiochemical Recovery of Substrates, Percent			
	uc	mg	CO_2	Cells	Medium	Total
Gluconate-6-C^{14}	0.20	6.5	66	12	22	100
Acetate-1-C^{14}	0.20	5.5	74	2	15	91
Acetate-2-C^{14}	0.17	5.5	70	3	16	89

*Calculated values

Experimental Conditions - incubation temperature, 28°C; cell age, 20 hours; acidity of growth medium, initial pH 5,5, final pH 5.1; acidity of medium for radiorespirometry, 5.5; cell suspension for glucose and gluconate, 9 mg (dry weight) in 15 ml medium; cell suspension for acetate, 11 mg (dry weight) in 20 ml medium; aeration rate for glucose and gluconate, 41 ml per min; aeration rate for acetate, 61 ml per min; experimental duration for glucose and gluconate, 9 hours; experimental duration for acetate, 8 hours.

DISCUSSION

The problem of taxonomy in the field of microbiology has been well recognized as a difficult task by workers in the field. The existing system, although useful and in most cases logical, suffers from the fact that morphological manifestations and the ability to carry out individual chemical reactions are subject to the physiological states and cultural conditions associated with the microorganism in question. In recent years the availability of several research methods such as radiotracer techniques and an ever mounting knowledge in enzymology have unveiled the nature of many biochemical sequences functioning in microorganisms for respiration and biosynthesis. This fact has thus prompted many laboratories to attempt classifying microorganisms on a purely biochemical basis. It is obvious that an understanding in this regard, in addition to the morphological taxonomy, should eventually lead to a satisfactory system for the classification of microorganisms.

A review of the modern literature reveals that considerable effort has been devoted by workers to elucidating the nature and the participation of catabolic

pathways for carbohydrate catabolism in microorganisms. Existing methods of estimating catabolic pathways of glucose metabolism may be classified into the following three groups:

1. Methods based on specific activity of $C^{14}O_2$, produced by metabolizing C^{14}-labeled substrates (8, 6, 48).

2. Methods based on specific activity of intermediates produced by C^{14}-labeled substrates (7, 9, 71, 22). This includes estimation of specific activity of intermediates isolated with or without the presence of an inhibitor (72). Thus an inhibitor such as arsenite will prevent the decarboxylation of α-ketoacids (47) and result in the accumulation of pyruvate from glucose when the latter is degraded via the EMP pathway and the ED pathway. This approach has been applied by Lewis et al. (72) to estimate the participation of the ED pathway and the pentose phosphate pathway in Pseudomonas fluorescens.

3. Methods based on the kinetics of $C^{14}O_2$ production from organisms metabolizing C^{14}-labeled substrates (104, 105). The method has been named the radiorespirometric method (105).

Some criticisms of these methods have been raised by Korkes (58) and Wood (112). They pointed out that

the true pathways of glucose catabolism do not need to be the same as estimated by oxidative respiration, because the relative role of the pathways in synthesis is not determined. Also, randomization of the labeling in the labeled substrate prior to catabolic reactions may give rise to misleading information.

In this laboratory, effort has been directed mainly toward the study of respiratory mechanisms of microorganisms by means of the radiorespirometric (105) method. With this method the utilization of individual carbon atoms in a given carbon source can be traced by following the time course of $C^{14}O_2$ production from the specifically C^{14}-labeled carbon atoms. The rates of $C^{14}O_2$ production and the overall radiochemical recoveries from the labeled carbon atoms in CO_2 enable one to identify the nature of the metabolic sequence operative in the respiratory function and to estimate the participation of the identified pathways. Moreover, the use of C^{14} specifically labeled substrate makes it possible to trace the utilization of the carbon source in question with a given microorganism cultured under proliferating conditions. The information so obtained should, therefore, be very close to that prevailing in normal growing cells.

It should also be emphasized that radiorespirometric experiments carried out previously on a score of microorganisms have indicated that the carbon sources employed were oxidized to respiratory CO_2 to an extent no less than 30% and as high as 100% with some readily oxidizable carbon atoms in some substrates. This fact indicates that under normal growing conditions, the fate of a readily utilizable carbon source in a given microorganism is by and large unidirectional leading to the production of terminal metabolic end products. Consequently, little confusion is expected in the interpretation of radiorespirometric data insofar as the randomization of labeling in a given substrate is concerned. The latter point, as well as the abnormal physiological conditions encountered in some other types of experiments such as inhibition studies, etc., constitutes the major criticism to the common radiotracer methods employed in the studies of pathway participation described previously.

The present work is confined to the study of catabolism of simple carbohydrates by microorganisms classified in the family Pseudomonadaceae. Attention has been particularly focused on species of Pseudomonas and Acetobacter.

In the case of pseudomonads, previous work done by Doudoroff et al. (27) and in this laboratory (105, 98, 99, 107) has revealed that glucose and gluconate are catabolized mainly by way of the ED pathway. In some species the TCA cyclic pathway is also operative as a secondary pathway for the catabolism of glucose degradation products. With Acetobacter species the pentose phosphate pathway appears to be the most important pathway for glucose and gluconate catabolism (54). The TCA cyclic pathway may or may not play a role in the terminal oxidation of glucose intermediates.

In the present work, three pseudomonads and three species of Acetobacter have been examined radiorespirometrically with respect to the utilization of C^{14} specifically labeled acetate, pyruvate, glucose and gluconate. The microorganisms included in this study are: Pseudomonas reptilivora, Pseudomonas viridilivida, Pseudomonas iodinum, Acetobacter industrium, Acetobacter melanogenum and Acetobacter xylinum. Considerable information with regard to respiratory and biosynthetic pathways in these microorganisms can be obtained by analyzing the data on $C^{14}O_2$ production, the formation of fermentation products and cellular incorporation.

To facilitate ready comparison, the catabolic

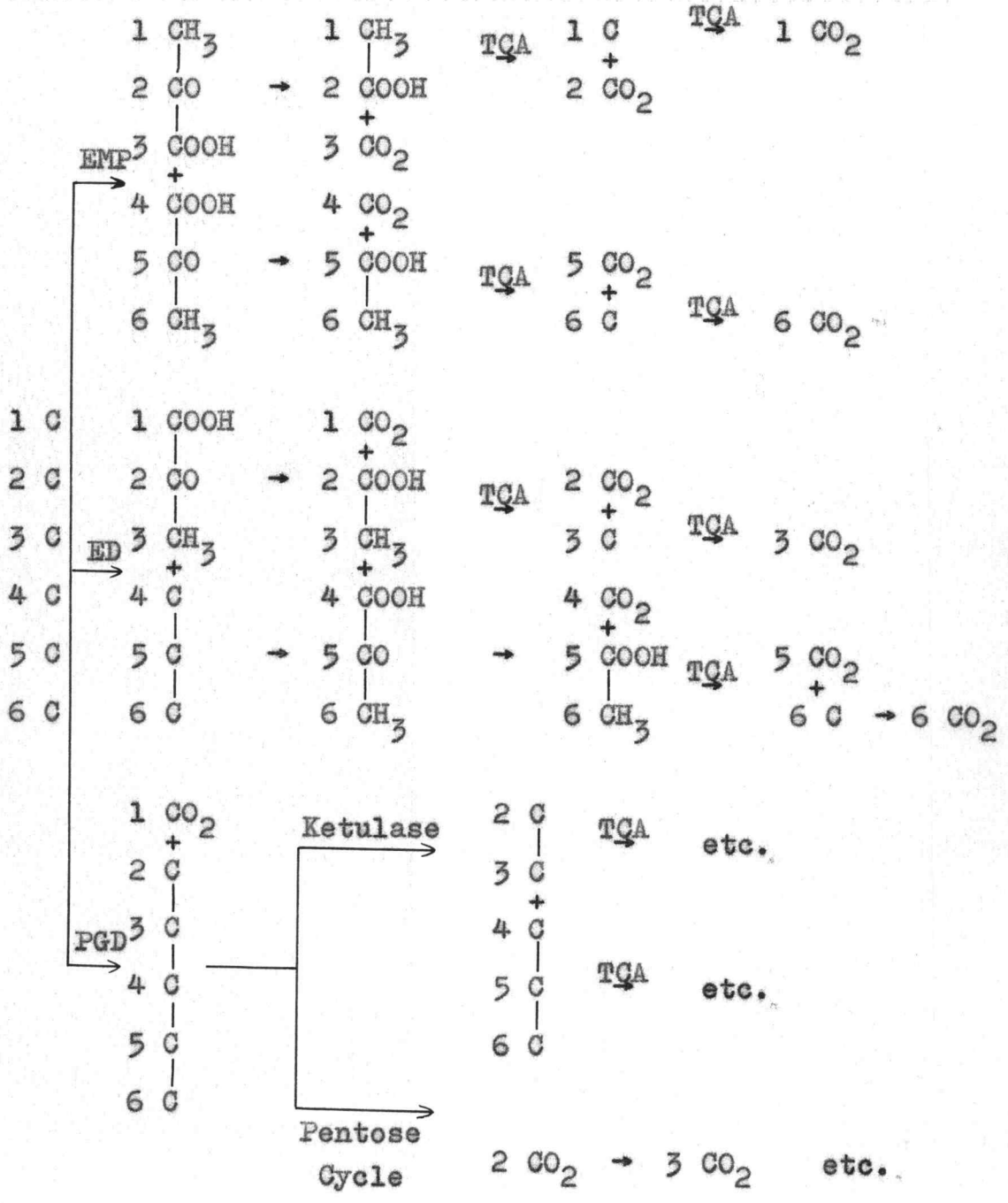
sequences for glucose degradation described previously are presented in the condensed form shown in Figure 20.

In the case of P. reptilivora the radiorespirometric experiments on the utilization of glucose-1-, -2-, -3,4- and -6-C^{14} have been previously carried out in this laboratory (98, 99, 105). The findings indicate that approximately 28% of the substrate glucose is catabolized by the pentose phosphate pathway with the remaining portion presumably routed through the ED pathway. The latter estimation was made on a set of assumptions (104, 105), including one which calls for the complete metabolic equivalence of C-3 and C-6 of glucose. The assumption in question is now verified unequivocally in the present study of the use of glucose-3-C^{14} as an additional substrate, a compound recently made available through the efforts of the National Bureau of Standards.

As shown in Figure 6, the radiorespirometric pattern is essentially the same as that reported earlier (98, 105). The fact that in the present study the radiochemical recovery of C-3 of glucose in CO_2 is essentially the same as that of C-6 indicates that the portion of glucose catabolized via the pentose phosphate pathway was not utilized extensively beyond the pentose stage. This

FIGURE 20

PATHWAYS OF GLUCOSE CATABOLISM IN MICROORGANISMS

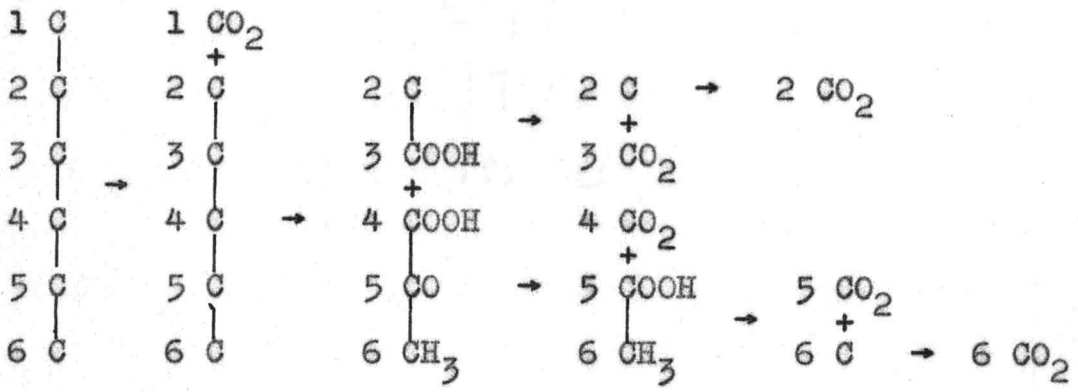


is reasonable in view of the important role played by intact pentose units in cellular biosynthesis.

Pathway estimation according to the method of Wang et al. (105) revealed that in P. reptilivora 69 percent and 31 percent of the substrate glucose was catabolized by way of the ED pathway and the pentose phosphate pathway, respectively. The findings are in good agreement with that reported earlier by Stern et al. (98, 99, 105).

The aerobic organism P. viridilivida displayed some rather unique metabolic characteristics, as evidenced by the radiorespirometric patterns given in Figures 7, 8, 9 and 10. The operation of the TCA cyclic pathway is evidenced by the radiorespirometric patterns for the utilization of acetate and pyruvate (Figures 7 and 8), which demonstrate the preferential conversion of the acetate carboxyl carbon to CO_2. In the early phase of the experiment (5 hours), one finds that the C-1/C-2 ratio for the conversion of acetate carbon atoms to CO_2 is 1.58 in contrast to the analogous ratio of 1.23 observed at the end of the experiment. This fact is in line with the sequential oxidation of the acetate carbon atoms via the TCA cyclic processes. Similar findings are also observed with pyruvate as the carbon source.

With C^{14} specifically labeled glucose as substrate one finds the radiorespirometric pattern (Figure 9) is difficult to interpret. Identical $C^{14}O_2$ recoveries, with respect to both rate and amount, from C-4 and C-1 of glucose were observed, a fact in accordance with the operation of the ED pathway. This is further supported by the preferential oxidation of C-2 to CO_2 over that of C-6 of glucose. However, the unexpected high recovery of C-3 of glucose in CO_2, much greater in magnitude than C-6 and significantly greater than C-2, may have unveiled a heretofore unknown pathway for degradation of glucose or its metabolic intermediates. This contention is derived from the consideration that one would expect the operation of the ED pathway to result in a metabolic equivalence of C-3 and C-6, and the operation of the pentose cyclic pathway to result in the preferential oxidation of C-2 over that of C-3. It is possible that a major portion of the substrate glucose may have been degraded in this organism in the manner described in the following speculative scheme, shown on the following page. Further work is needed to elucidate in full the nature of the glucose catabolism in this organism.



The utilization of C^{14} specifically labeled gluconate by P. viridilivida is presumably by way of the combined operation of the pentose cyclic and the ED pathways as evidenced by the radiorespirometric pattern given in Figure 10. In the present case it appears that the pentose phosphate, derived from glucose, may have engaged quite extensively in catabolic functions as indicated by the significantly greater recovery of C-3 over that of C-6 in CO_2. It should be noted that with gluconate as substrate the recovery of C-3 in CO_2 is less than that of C-2 in contrast to the findings in the glucose experiment.

Pathway estimation, according to the method of Wang et al. (105) and under the assumption that the ED pathway and pentose phosphate pathway are the only pathways concerned, revealed that in this organism 66% and 34% of the substrate gluconate are catabolized

by the ED and the pentose phosphate pathways, respectively. These findings are in contrast to an earlier report in which the ED pathway was found to be the exclusive mechanism for gluconate catabolism in three species of pseudomonads examined.

The organism P. iodinum was demonstrated in preliminary experiments to be capable of proliferation solely on amino acids and yeast extract. This has been verified by the failure to detect $C^{14}O_2$ production from substrate glucose-U-C^{14}. Meanwhile, as shown in Figures 11 and 12, the radiorespirometric patterns observed for the utilization of C^{14} specifically labeled acetate and pyruvate provide evidence that the TCA cyclic pathway is probably operative in this organism. Thus, the C-1/C-2 ratio for the conversion of acetate carbon atoms to CO_2 was observed to be 1.17 at the end of experiment, a value in good agreement with that observed with P. viridilivida. Evidently a complete mechanism for glucose degradation is absent in this organism and carbon skeletons for cellular biosynthesis are derived exclusively from substrate amino acids. This fact implies further that some mechanism must be operative for the biosynthesis of hexoses, pentoses, and other carbohydrates needed for cellular construction. It, therefore,

appears that P. iodinum could serve as a good test system for the biosynthesis of carbohydrates from small fragments.

The unique metabolic behaviours of the Acetobacter species are well recognized by workers in the field. In fact, reports on the enzymatic studies of glucose catabolism in A. suboxydans by Hauge, King and Cheldelin (39, 40, 41) constituted the first demonstration of exclusive operation of the pentose cyclic pathway in a microbial system. More recently Kondô and Ameyama (57) have studied 20 species of Acetobacter with respect to their metabolic behaviours and a classification system was proposed on these findings. In the present work, three species of Acetobacter, namely, A. melanogenum, A. industrium and A. xylinum were employed as the test organisms to study the catabolism of simple carbohydrates in Acetobacter by means of the radiorespirometric method. These organisms were chosen on the grounds that they were demonstrated by Kondô and Ameyama to be incapable (57) of oxidizing acetate but capable of oxidizing glucose and gluconate under resting conditions. Furthermore, A. melanogenum differs from the other two Acetobacter species and A. suboxydans in its ability to oxidize 2-ketogluconate and 5-ketogluconate. Inasmuch as the

radiorespirometric patterns for the oxidation of glucose and gluconate by A. suboxydans are available (54), a comparable examination of the latter with the patterns observed in the present study should shed more light on the basic catabolic behaviours of Acetobacter species.

The catabolism of glucose and gluconate in A. industrium, as depicted by the radiorespirometric patterns shown in Figures 13 and 14, are equally interesting. The respective substrates are converted to metabolic CO_2 rather extensively with only a small portion being converted to fermentation products in the medium or cellular constituents (Table VII). The predominance of pentose cyclic pathway activity is evidenced by the observed extensive conversion to CO_2 from the top three carbon atoms of glucose or gluconate in the order C-1 > C-2 > C-3. Carbon-6 of glucose, and particularly that of gluconate, is converted to CO_2 to a very limited extent, a fact understandable if one considers the possibility that intermediates such as triose can be drained out to prevent an extensive reformation of hexose from trioses in the metabolic sequence of the pentose cyclic pathway (Figure 3). The latter contention is further supported by the finding that a considerable amount of C-4 activity from either glucose or gluconate was

detected in CO_2, evidently reflecting the operation of the sequence: triose phosphate → pyruvate → acetate + CO_2.

In the case of A. melanogenum, it was discovered by preliminary experimentation that the conversion of glucose and particularly gluconate carbon atoms to CO_2 is subject significantly to the variation in pH of the cultural medium. As shown in Table VIII, the oxidation of C-1 of gluconate to CO_2 is very limited at pH 4.5, extensive at pH 5.5 and declines again at a less acidic pH of 6.5.

An analysis of the radiorespirometric patterns for the utilization of glucose and gluconate by proliferating cells of A. melanogenum (Figures 15 and 16) revealed that the pentose cyclic pathway probably plays an important, if not exclusive, role in the catabolism of these substrates, particularly that of gluconate (Figure 16). This conclusion is drawn from the order of $C^{14}O_2$ recovery from substrate carbon atoms, i.e., C-1 > C-2 > C-3 > C-6 > C-4 in the gluconate experiment, a pattern similar to that reported for A. suboxydans (54).

It is noted, however, that the $C^{14}O_2$ recovery from C-4 of gluconate is practically nil. At the present

time no information is available to explain this observation other than the possibility that the conversion of C-4 of gluconate to CO_2 calls for the extensive operation of recycling along the pentose cyclic pathway, a situation which may not be readily realized in this organism.

With glucose as substrate, although the general radiorespirometric pattern is similar to that observed in the gluconate experiment, there is a striking difference in the order of $C^{14}O_2$ production from C-4 and C-6 of glucose. As shown in Figure 15, the $C^{14}O_2$ recovery from C-4 is significantly greater than that of C-6 of glucose. This fact may indicate to some extent that the triose formed in the pentose cyclic pathway has been converted in part to pyruvate which is in turn decarboxylated oxidatively giving rise to the production of acetate as the end product.

It is also interesting to note, however, that the $C^{14}O_2$ recovery of C-1 is much greater than that of the other carbon atoms in the glucose experiment, a fact which led us to believe that a C_5 compound may have been excreted into the medium as a fermentation product. The exact nature of this process is unknown at the present time.

With C^{14} specifically labeled glucose as substrate, as shown in Table IX, one finds that the bulk

of the substrate was converted to fermentation products in the medium with only a minor portion of the substrate activity being converted to $C^{14}O_2$. Little substrate activity is detected in the cells. Similar findings were also observed in labeled gluconate experiments (Table IX).

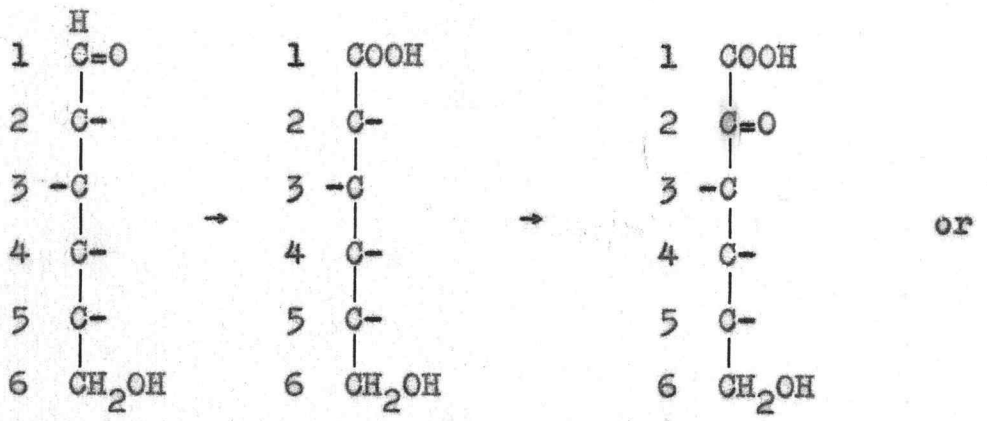
In accordance with the production of ketogluconates from glucose or gluconate, described by Kondô and Ameyama (57), one finds 67 percent of substrate gluconate and 59 percent of the substrate glucose were converted to intact C_6 compounds, presumably in the nature of ketogluconate, in the present experiment.

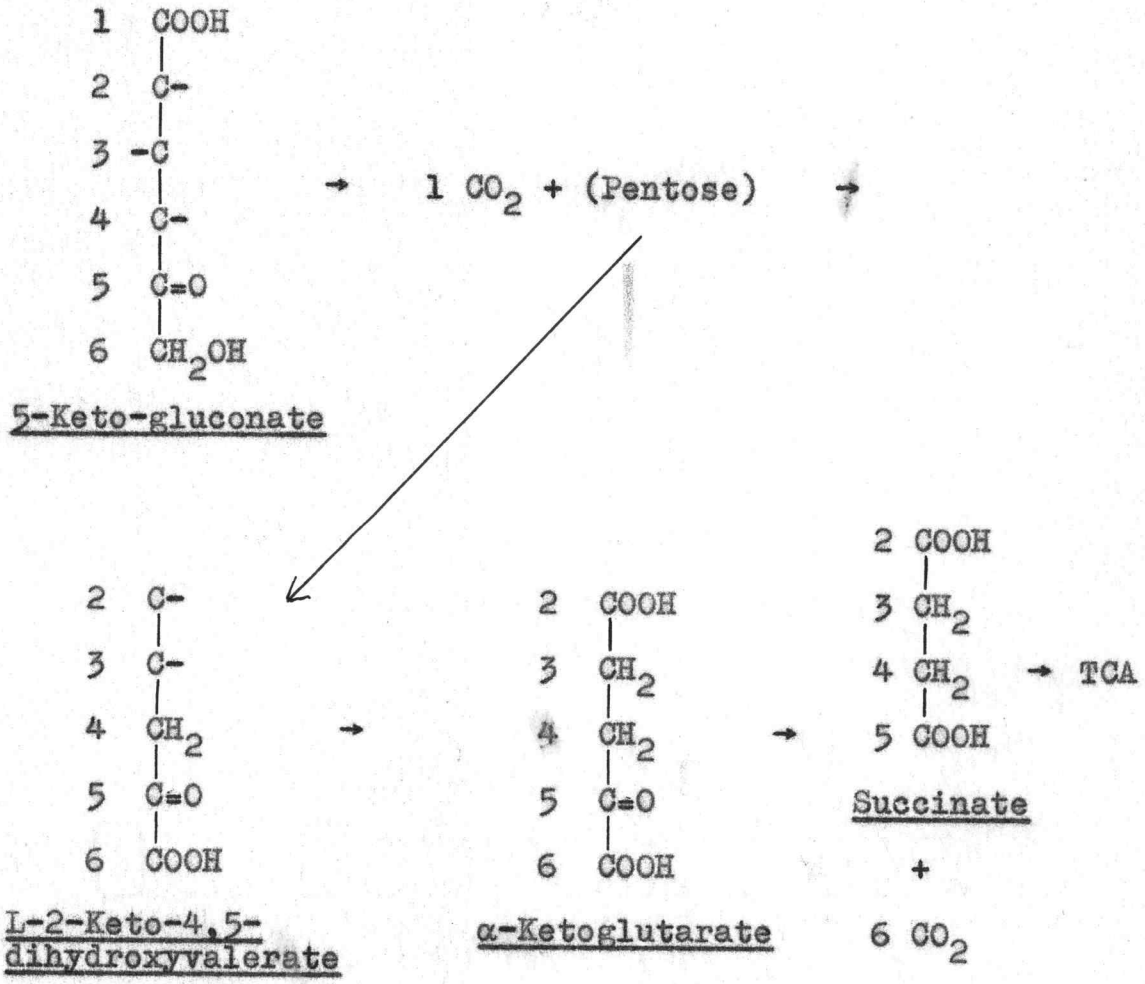
Several important metabolic behaviours have been observed in the present study with A. xylinum. The radiorespirometric pattern for acetate as shown in Figure 17 revealed that this substrate can be readily oxidized by A. xylinum. This finding is in direct contrast to that reported by Kondô and Ameyama (57) who indicated that this organism is incapable of oxidizing acetate and TCA cycle intermediates. On the other hand, the observation in the present study agrees with that of a number of other investigators (5, 70, 30, 103).

The reason for this discrepancy remains to be elucidated. It is noted that the C-1/C-2 ratio for the

conversion of acetate carbon atoms to CO_2 approaches unity, a fact illustrating well the extreme oxidative nature of cellular catabolism in this organism. Although the presence of the TCA cyclic mechanism cannot be definitely established in the present work, it is not unreasonable to speculate that the TCA cyclic pathway plays a role in this organism.

With labeled glucose and gluconate samples as substrates, the radiorespirometric patterns shown in Figures 18 and 19 revealed that the basic nature of the catabolic mechanism is of a type similar to that of the pentose cyclic pathway. Some unique catabolic behaviours are noted. This includes the unexpected high recovery of C-6 of glucose and gluconate in CO_2 and the complete metabolic equivalence of C-3 and C-4 of either glucose or gluconate, both being low in their conversion to CO_2. These findings, together with known metabolic behaviours of Acetobacter, lead one to speculate that glucose may have been degraded according to the scheme as shown on the following page. According to this scheme one would expect to have C-3 and C-4 of glucose or gluconate converted to the middle carbon atoms of succinate, which can be converted further to CO_2 via the TCA cyclic processes at a slower rate as compared


1 C=O
2 C-
3 -C
4 C-
5 C-
6 CH2OH
Glucose
1 COOH
2 C-
3 -C
4 C-
5 C-
6 CH2OH
Gluconate
1 COOH
2 C=O
3 -C
4 C-
5 C-
6 CH2OH
2-Keto-gluconate
or


1 COOH
2 C-
3 -C
4 C-
5 C=O
6 CH2OH
5-Keto-gluconate
1 CO2 + (Pentose)
2 C-
3 C-
4 CH2
5 C=O
6 COOH
L-2-Keto-4,5-dihydroxyvalerate
2 COOH
3 CH2
4 CH2
5 COOH
α-Ketoglutarate
2 COOH
3 CH2
4 CH2
5 COOH
Succinate
+
6 CO2
TCA

to other carbon atoms. The scheme is further supported by the data on cellular incorporation (Table X) which revealed that C-2, C-3, C-4 and C-6 carbons of the glucose or gluconate are incorporated into cellular constituents to a much greater extent than is C-1. The extent of formation of fermentation products in the nature of C_6 or C_5 compounds are evidenced by detection of signficant amounts of substrate activity in the incubation media.

A comprehensive examination of the radiorespirometric data obtained in the present work together with that reported earlier for A. suboxydans (54) and the Pseudomonas species (98, 99, 107) makes it possible to summarize the various fates of glucose carbon in species of the family Pseudomonadaceae as shown in Table XI.

TABLE XI

CATABOLIC BEHAVIOURS OF SPECIES IN THE FAMILY PSEUDOMONADACEAE

Microorganisms	The Fate of Glucose				
	ED Pathway	Pentose Phosphate Pathway	PC with Intermediate Drainage		TCA Cyclic Pathway
			C_3	C_5	
Zymomonas motilis	+				-
Pseudomonas saccharophila	+				+
Pseudomonas fluorescens KB1	+	+		+	+
Pseudomonas aeruginosa	+	+		+	+
Pseudomonas reptilivora	+	+		+	+
Pseudomonas viridilivida	+	+		++	+
Pseudomonas iodinum	-	-			+
Acetobacter suboxydans	-	+		+	-
Acetobacter industrium	-	+	+++		-
Acetobacter melanogenum	-	+	+	++	-
Acetobacter xylinum	-	+		++	+

SUMMARY

The mechanisms of carbohydrate catabolism in six species of the family Pseudomonadacea have been examined by means of the radiorespirometric method, employing C^{14} specifically labeled acetate, pyruvate, glucose and gluconate as tracing substrates. The microorganisms included in these studies are: Pseudomonas reptilivora, Pseudomonas viridilivida, Pseudomonas iodinum, Acetobacter industrium, Acetobacter melanogenum and Acetobacter xylinum.

The findings indicate that in P. reptilivora 69 percent of the substrate glucose is catabolized via the ED pathway and 31 percent via the pentose phosphate pathway. This result is in good agreement with earlier findings (98, 105). With P. viridilivida the findings in the glucose experiments are difficult to interpret. However, it appears that glucose may have been metabolized by way of the ED route concurrently with the operation of a pentose phosphate involving pathway, the latter pathway including a pentose cleavage reaction giving rise to C_2 and C_3 monobasic acids, with the carboxyl groups of these acids corresponding to glucose carbon atoms C-3 and C-4. These acids are believed to be further catabolized by way of the TCA cyclic pathway.

The gluconate catabolism in P. viridilivida occurs by way of combined operation of the ED pathway (66 percent) and the pentose phosphate pathway (34 percent). In P. iodinum a complete mechanism for glucose degradation is evidently absent; however, the radiorespirometric patterns for pyruvate and acetate provide evidence for the operation of the TCA cyclic pathway. In A. industrium the main pathway of glucose and gluconate catabolism seems to be the pentose phosphate cyclic pathway. A portion of the triose formed in the cyclic pathway is believed to be routed through the sequence:

triose phosphate → pyruvate → acetate + CO_2.

In A. melanogenum, 67 percent of substrate gluconate and 59 percent of substrate glucose were converted respectively to intact C_6 compounds, presumably to the ketogluconates (57). The portion of glucose and gluconate that is catabolized appears to be mainly by way of the pentose cyclic pathway. From the radiorespirometric pattern for substrate glucose and gluconate in A. xylinum it appears that the principal pathway for glucose catabolism in this organism involves the following sequence:

glucose → gluconate → 5-ketogluconate → CO_2 + ketopentose → α-ketoglutarate → TCA intermediates.

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