COMPARATIVE METABOLISM OF CARBOHYDRATES
IN THE PSEUDOMONADS

by

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Typed by Margaret Barber

The understanding and love of my
wife has made this thesis possible.

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INTRODUCTION

An understanding of the processes whereby carbohy-
drates are utilized for biosynthesis and energy produc-
tion in living cells is a prerequisite to the understand-
ing and control of life processes. The marked increases
in knowledge in this field gained over recent years have
been due largely to the development of new physical and
chemical techniques and to the advent of radioactive
tracers. With these advances in methodology it has become
more feasible to explore metabolic patterns in the intact
cell and thus avoid the possibility of overlooking systems
which may be destroyed when the cell is disrupted.

Many attempts to clarify the processes of assimila-
tion and dissimilation, or metabolism, of carbohydrates
were made during the nineteenth century. However, it was
not until, at the turn of the century, Eduard Buchner
demonstrated fermentation in cell-free yeast preparations
that a practical approach to the study of carbohydrate
metabolism was made available (25, p.180). The isolation
of hexose-diphosphate by Harden and Young was the begin-
ning of work by Embden, Meyerhof, Parnas, Warburg, and
others which eventually led to the description of glyco-
lysis, the pathway by which glucose is degraded to lactate
in muscle or to CO_2 and ethanol in yeast. Although for

many years glycolysis was considered to be the major pathway for glucose catabolism in living things, the finding by Harrison of glucose dehydrogenase in liver and by Warburg and Christian (2, p.203) of a glucose 6-phosphate dehydrogenase in yeast suggested that glycolysis was not the sole mechanism for glucose dissimilation. Since that time other pathways for glucose degradation have been found in the presence or absence of the glycolytic sequence. The findings of Horecker and others (31, pp.141-178) indicate that glucose may be degraded via a cyclic mechanism involving the phosphates of hexose, pentose, sedoheptulose, triose, and tetrose. Observations by De-Moss, Bard, and Gunsalus, and Gunsalus and Gibbs (17, pp. 98-99) suggest that glucose degradation in Leuconostoc mesenteroides proceeds via 6-phosphogluconate to CO_2, ethanol, and lactate by mechanisms entirely different from those of glycolysis. Finally, studies by Entner and Doudoroff (15, pp.853-862) indicate that in Pseudomonas saccharophila, glucose may be degraded via 2-keto-3-deoxy-6-phosphogluconate in another non-glycolytic pathway. The possibility that glucose may be dissimilated via other unknown pathways is still relatively unexplored.

The reaction sequences mentioned above are all pathways for which glucose is the substrate. As such, these pathways may be referred to as primary pathways of glucose

breakdown. Although primary pathways function in provid-
ing energy, they do not provide a great variety of the
intermediates for the biosynthesis of cellular material
(25, p.182). It has been generally observed, however,
that in non-fastidious organisms other sequences of reac-
tions may provide intermediates for synthesis of amino
acids and produce more energy from the products of primary
glucose dissimilation (24, pp.988-995). These reaction
sequences usually have as their initial substrate acetate,
which arises from the decarboxylation of pyruvate formed
via primary degradation pathways. Thus, the acetate-
utilizing pathways have been termed "secondary" pathways
of glucose catabolism. Three general mechanisms have been
proposed for such secondary pathways. The Knoop-Thunberg
condensation (2, pp.489-490) is thought to involve the
union of two moles of acetate to yield succinate, fumarate,
malate, and oxalacetate successively. Decarboxylation of
oxalacetate and the resulting pyruvate produces a single
mole of acetate which may then combine with another mole
of acetate and go around the cycle again. The tricarbox-
ylic acid cycle (TCA cycle) involves the condensation of
acetate and oxalacetate to form citrate which enters a
cyclic series of reactions to reform oxalacetate. Recent
reports indicate that variations may occur in the TCA
cycle, however, when organisms are grown on acetate

(23, pp.988-995). As outlined for <u>Pseudomonas</u> <u>aeruginosa</u>
by Campbell, Smith, and Eagles (10, p.594), and for <u>Ps</u>.
KB1 by Kornberg and Madsen (22, pp.651-653), iso-citrate
may be split by iso-citratase to form succinate and gly-
oxylate. The significance of this reaction appears to be
twofold. Glyoxylate thus produced may serve as a glycine
precursor or source of C_1 fragments; or, according to Wong
and Ajl (36, pp.1013-1014) the combination of glyoxylate
and acetate to form malate may serve as an additional
source of C_4 units to replace those drained off for bio-
synthesis. Finally, acetate may also enter a non-cyclic
pathway which involves its conversion to glycolate, gly-
oxylate, formyl, and eventually CO_2. The function of this
pathway is thought to be to provide a source of C_1 and C_2
fragments for the synthesis of glycine, purines, and
pyrimidines (7, pp.372-376). In addition, reactions which
involve condensation of C_3 and C_1 units, generalized under
the heading of CO_2 fixation, may play an important role in
providing C_4 compounds for biosynthesis (2, p.450).

Although the study of carbohydrate metabolism in ani-
mal or plant tissues has been productive, the use of bac-
teria for this purpose has several advantages. The ease
of obtaining large quantities of cells is, of course, ob-
vious. The fact that bacteria may be studied under what

might be considered normal conditions is in distinct con-
trast to the drastic changes in environment to which high-
er plant or animal tissues must be subjected for study.
In general, higher reaction rates are observed with bac-
teria, probably reflecting in part the relatively unham-
pered diffusion of substances to and away from the cell.
Finally, bacteria display a diversity of metabolic reac-
tions hitherto not found in other forms of life.

Carbohydrate metabolism in the organisms grouped
under the family Pseudomonadaceae has been demonstrated to
be comprised of unique catabolic pathways. These organ-
isms, usually found in soil or in fresh or sea water, have
been noted for the variety of materials they may use as
carbon and energy sources. Pseudomonads are generally
gram negative, straight rods which are motile by virtue of
single or tufted polar flagella (8, p.88). Usually they
are aerobic and many of them produce fluorescent, water-
soluble pigments.

As early as 1941, Lockwood, Tabenkin, and Ward (27,
pp.51-61) had reported on the possible use of Pseudomonas
and Phytomonas species for commercial production of glu-
conate and 2-ketogluconate. Since these compounds are not
involved in glycolysis it was apparent that in these
organisms a pathway for glucose degradation other than
glycolysis was present. Further investigations of glucose

catabolism in Ps. aeruginosa (12, pp.49-50) and Ps. flu-
orescens (20, pp.743-751) indicated that glucose dissimi-
lation in these organisms proceeded through gluconate, 2-
ketogluconate, alphaketoglutarate, and pyruvate. The con-
tinued oxidation of glucose and the formation and utiliza-
tion of pyruvate in the presence of fluoride provided evi-
dence against the participation of the glycolytic pathway
in the pseudomonads (39, p.222). Considerable insight
into the metabolism of Pseudomonas was gained when Entner
and Doudoroff (15, pp.853-862) identified the pathway of
glucose catabolism in Ps. saccharophila with the aid of
arsenite, azide, and iodoacetate blocking techniques and
radioactive tracers. In this pathway, glucose is degraded
via 6-phosphogluconate and 2-keto-3-deoxy-6-phosphoglu-
conate to pyruvate, the carboxyl group of which is derived
from C-1 and C-4, and the methyl group of which is derived
from C-3 and C-6 of glucose. Shortly thereafter, Wood and
his co-workers (39, pp.222-233) described a similar path-
way in Ps. fluorescens which functioned concurrently with
a pentose cycle pathway. Further indications of Entner-
Doudoroff (E-D) and pentose-involving pathways in pseu-
domonads were found by Lewis et al. (26, pp.273-286) and
Gibbs and DeMoss (16, pp.689-694). In the organism stud-
ied by the latter two authors, Zymomonas motilis (formerly
Ps. lindneri), although the E-D sequence apparently was

functioning, the products of pyruvate decarboxylation were reduced to ethanol. This is in contrast to Ps. saccharophila wherein secondary glucose degradation pathways are present by which acetate or the products of pyruvate decarboxylation are oxidized to CO_2 or converted to cell material (36, pp.187-193). That acetate oxidation probably proceeds via the TCA or similar cycle in other pseudomonads is indicated by the findings of Campbell and Stokes (10, pp.853-858), Barret and Kallio (3, pp.517-525), and Kornberg and Krebs (24, pp.988-995).

With the finding of unique pathways of glucose catabolism in certain pseudomonads, interest has become focused on the occurrence and relative importance of these pathways in other members of this group. Existing methods of estimating the participation of pathways in glucose catabolism in microbial systems may be classified into three categories. They are (1) those based on the relative specific activities of fermentation products produced by organisms utilizing specific C^{14}-labeled glucose substrates (7, pp.6093-6097), (2) those based on the specific activity of CO_2 produced during metabolism of specific C^{14}-labeled substrates (22, pp.703-713), and (3) those based on the per cent radiochemical recovery in the respiratory CO_2 of organisms growing on specific C^{14}-labeled glucose substrates (32, pp.1869-1874). The first of these

methods requires that fermentation products be produced in quantities large enough to be collected and analyzed, a requisite which is not fulfilled by many organisms. The second method is useful in determining the relative contributions of labeled substrate carbons to the respiratory CO_2; however, computation of pathways participation by this method requires information which is often not readily available. The last method which gives the pathways participation directly, based on the amount of glucose utilized, requires only the accurate recovery of respiratory CO_2, and is thus well suited for application to a large number of organisms. The application of this method has been reviewed in the recent publication by Wang et al. (33). The approach consists essentially of determining the respiratory patterns of glucose catabolism in an organism by determining the relative rates at which various C^{14}-labeled carbon atoms of glucose are converted to CO_2. The results so obtained provide information which is sufficient to identify and estimate the relative importance of pathways of glucose catabolism functioning in the organism under study.

In the present study the basic catabolism of five pseudomonads will be covered. Radiorespirometric data obtained from Z. motilis, Ps. saccharophila, and Ps. reptilivora (formerly Ps. fluorescens, 18) provided

reference patterns for organisms in which glucose was
known to be catabolized via the E-D pathway alone, the E-D
pathway followed by the TCA cycle, or the E-D pathway and
TCA cycle operating in conjunction with a pentose-involving
pathway, respectively. In addition to the above organisms,
Ps. aeruginosa and Ps. KB1 were chosen for study because
of interesting findings in previous investigations concern-
ing their intermediary metabolism which had not revealed
their overall picture of glucose catabolism (12, pp.49-50;
23, pp.651-653). By comparing the reference patterns men-
tioned above with those obtained from Ps. aeruginosa and
Ps. KB1, insight was gained into the mechanisms of glucose
catabolism in these two organisms and, on the basis of
assumptions which will be discussed later, the proportion
of glucose catabolized via each pathway found to be opera-
tive was estimated.

EXPERIMENTAL METHODS

Cultures and Cultural Conditions

The origin and cultural conditions for each organism used in this study are given in Table I. 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-, and 6-C^{14} were obtained from the National Bureau of Standards through the kind cooperation of Dr. H. Isbell. Glucose 3,4-C^{14} was prepared from rat liver glycogen according to the method of Wood, Lifson, and Lorber (38, pp.475-489). Gluconic acid 1-, 2-, 3,4-, and 6-C^{14}, prepared from the correspondingly labeled glucose compounds according to the method of Moore and Link (30, pp.293-311), were generously and kindly supplied by Mr. Dallas E. Jones. All labeled substrates were adjusted to approximately equal specific activity before use.

Table I

ORGANISMS AND CULTURAL CONDITIONS USED IN THIS STUDY

Organism	Strain	Basal medium	Reference
Zymomonas motilis	NRRL B-806	Tryptone, yeast extract, 0.03 M phosphate, pH 6.7	16, p.689
Pseudomonas saccharophila	G*	NH_4Cl, salts, 0.03 M phosphate, $MgSO_4$, traces $FeCl_3$ and $CaCl_2$	35, p.178
Pseudomonas reptilivora	NRRL B-6bs	$(NH_4)_2SO_4$, 0.05 M phosphate, KCl, $MgSO_4$, trace $MnSO_4$, $FeSO_4$, yeast extract	21, p.5142
Pseudomonas aeruginosa	ATCC 9027	As for Ps. reptil-ivora, but without yeast extract	9, p.166
Pseudomonas KB1	--	As for Ps. aeruginosa	23, p.651

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

Time Course Experiments

The radiorespirometric experiments were carried out in apparatus designed for micro scale operation. The advantages of performing experiments of this type on a micro scale include ease of operation as well as economy in cells and substrate requirements. In addition, the use of small numbers of cells results in cell constituents of sufficient specific activity to permit radioautographic analyses after the utilization of as little as 0.5 microcurie of labeled substrate. The apparatus used for the experiments described herein is shown in Figure 1a. It consists of a flow control system (Figure 1b) and a number of incubation flask-CO_2 trap assemblies (Figure 1c) mounted on Warburg manometer holders. The gas flow control system is made up of two semicircular bypass manifolds each of which is connected to a separate flowmeter (The Manostat Corporation no. 9142). Sweeping gas may be routed directly through the manifold or indirectly via the flowmeter into the flasks via polyethylene connectors (A). This arrangement provides for uniform gas flow rates through each individual flask during the experiment, and also permits the use of different incubation atmospheres in each of the two 7-flask sets.

As is shown in Figure 1c, the incubation flask-CO_2 trap assemblies are mounted on Warburg manometer holders
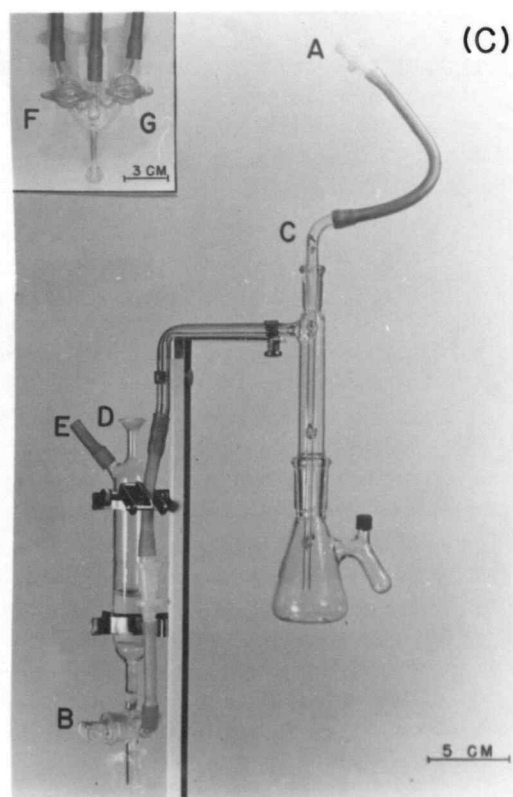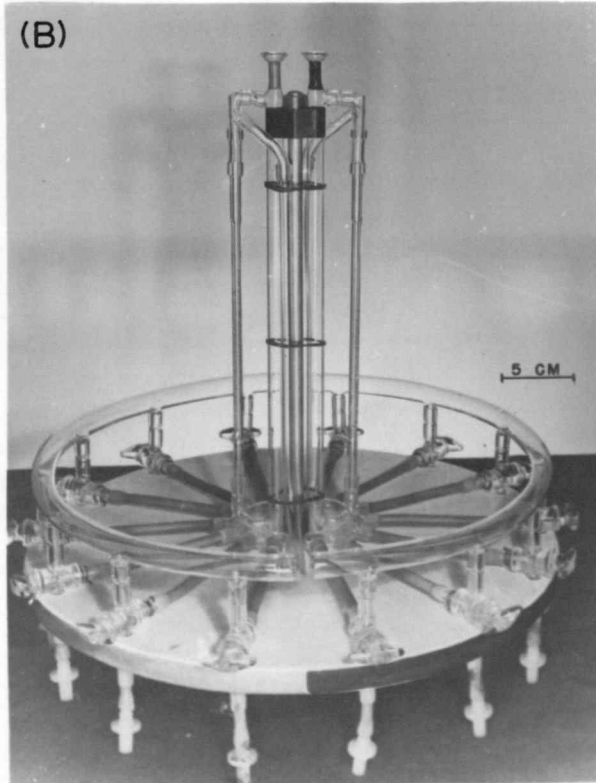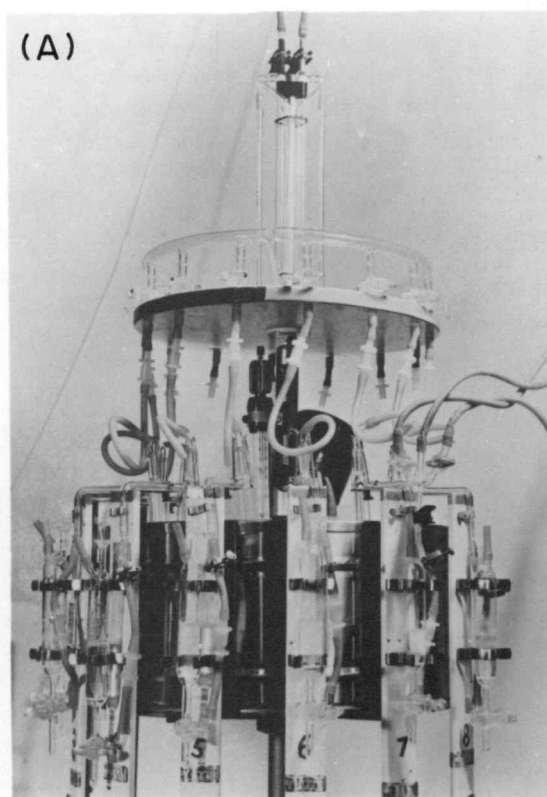
Figure 1. Radiorespiro-
metry apparatus.

(a) Whole assembly.
(b) Gassing manifold.
(c) Incubation flask and
    CO_2-trap assembly.

to enable shaking of the incubation flasks at controlled temperatures. Each assembly consists of an incubation flask (50 ml.) with a tip-in sidearm, a connecting head (Standard Taper 19/38), a sparging tube (Standard Taper 10/30) and a CO_2 trap, which is essentially a sintered glass filter equipped with a three way stopcock (B). The incubation flask is connected to the CO_2 trap through two pieces of gum rubber tubing joined by a polyethylene connector for ease in dissembling. The gas flow rate may be adjusted by means of a screw clamp or needle valve mounted appropriately on the rubber tubing.

During an experiment, the sparging tube C is joined to the gassing manifold with a polyethylene connector. Usually 10 ml. of 0.5 N CO_2-free NaOH are placed in the trap. Experiments designed to test the absorption efficiency of the trapping system have indicated that the CO_2 produced by organisms metabolizing glucose while suspended in a buffered medium (pH 6.7) was quantitatively trapped at an air flow rate of 41 ml. per minute. As an experiment progresses, the trap solution is replaced periodically by the following procedure. A dispensing head (insert, Figure 1c) is connected to the ball joint D, stopcock B is turned to disconnect the incubation flask and connect the trap to the delivery arm, and the trap contents are allowed to flow into an Erlenmeyer flask.

A pinchclamp is applied to position E and the trap solution is forced out into the sample container by applying pressure from a hand pump connected to the center of the dispensing head. Stopcock B is now turned diagonally to block all of its openings, the pinchclamp is removed from E, and 5-10 ml. of CO_2-free water are introduced into the trap through stopcock F. The rinsing water is forced out of the trap through stopcock B as before, stopcock B is closed, and 10 ml. of fresh NaOH are introduced into the trap by opening stopcock G. Stopcock B is now turned to reconnect the incubation flask to the CO_2 trap. The entire sampling operation requires about one minute per flask.

In a typical radiorespirometric experiment using the apparatus just described, identical quantities of specifically labeled substrates are added separately to the incubation flask sidearms. Ten ml. aliquots of an appropriate cell suspension are added to the main compartments of the incubation flasks and the flasks are connected to the CO_2 trap assemblies. The assemblies are placed on the Warburg bath at the desired temperature and the sparging tube inlets are connected to the gassing manifold. Upon adjustment of the gas flow rate to 41 ml. per minute, the CO_2 traps are charged with NaOH and the substrates tipped into the culture compartments. For aerobic experiments air is

used as the sweeping gas to avoid artifacts which might arise with the use of CO_2-free air or oxygen (35, p.184). For anaerobic experiments a mixture of 0.5 per cent CO_2 in nitrogen is usually employed. At hourly intervals, the trap solutions are replaced and the carbonate therein is precipitated as $BaCO_3$ by the addition of 1 N $BaCl_2$-NH_4Cl solution. By following the radiochemical recovery in the CO_2, it is possible to determine the point of substrate exhaustion and from this to estimate the termination point of the experiment. Culture purity tests revealed that no contamination occurred in experiments continued for six to 12 hours. Upon termination of the experiment, the cells and medium are separated by centrifugation and subsequently processed for radioactivity assay.

Radioactivity Measurements

The respiratory CO_2 samples obtained during an experiment in the form of barium carbonate are mounted onto aluminum planchets by centrifugation and assayed for radioactivity with a thin window Geiger-Meuller counter. Determinations are made to a standard deviation of two per cent and corrected for background and self-absorption in the conventional manner. The activities of substrates, cells, and media are determined by wet combustion to CO_2 (13, pp. 1225-1226; 19, pp.1503-1504) and counted as $BaCO_3$ as above.

RESULTS AND DISCUSSION

In four of the five organisms with which the present
study is concerned, the catabolism of glucose is complexed
by the concurrent operation of both primary and secondary
pathways. The interpretation of radiorespirometric data
obtained from such organisms would be facilitated if the
patterns produced either by the primary or secondary path-
ways of catabolism could be examined separately. Conse-
quently, in developing an approach to the study of carbo-
hydrate metabolism with the radiorespirometric method it
appeared reasonable first to examine systems wherein only
a single catabolic route was functioning before attempting
to interpret patterns resulting from combinations of both
primary and secondary glucose degradation routes. This
may be achieved in two ways. The first of these is the
selection of an organism which is known to have only a
single stage of glucose utilization, and the second is
supplying a key intermediate such as acetate, which may be
catabolized only by secondary utilization pathways. Data
obtained in this manner may then serve as reference pat-
terns for determining pathways in organisms whose cata-
bolic pathways are unknown. The findings to be presented
here are the results of at least three identical radio-
respirometric experiments for each organism and are repro-
ducible to a maximum deviation of 10 per cent.

The radiorespirometric patterns for glucose cata-
bolism in Z. motilis are presented in Figure 2. Studies
by other workers (16, pp.689-694) indicate that glucose
dissimilation in this organism proceeds via the E-D se-
quence alone to yield CO_2, ethanol, and small amounts of
lactic acid. To facilitate comparison, all of the pat-
terns to be presented here have been plotted on the basis
of relative time units against per cent interval recovery.
One relative time unit (RTU) is defined as the time re-
quired for all of the administered substrate to be con-
sumed. The rapid and nearly complete conversion of glu-
cose C-1 to CO_2 shown in Figure 2 undoubtedly reflects the
degree to which pyruvate, arising from glucose, is de-
carboxylated, while the negligible recovery of C-2 and C-6
in CO_2 are indications that, in Z. motilis, the products
of pyruvate decarboxylation are not oxidized. Evidently,
Z. motilis, requiring preformed amino acids for growth,
does not possess a secondary catabolic pathway, and the
products of primary glucose breakdown are reduced to
ethanol.

The data obtained from glucose 3,4-C^{14} (Figure 2,
Table II) represent the average recovery of C-3 and C-4
in the CO_2. As such, one cannot compare them directly to
data obtained from the singly labeled substrates. It is
possible, however, to calculate the contribution of either
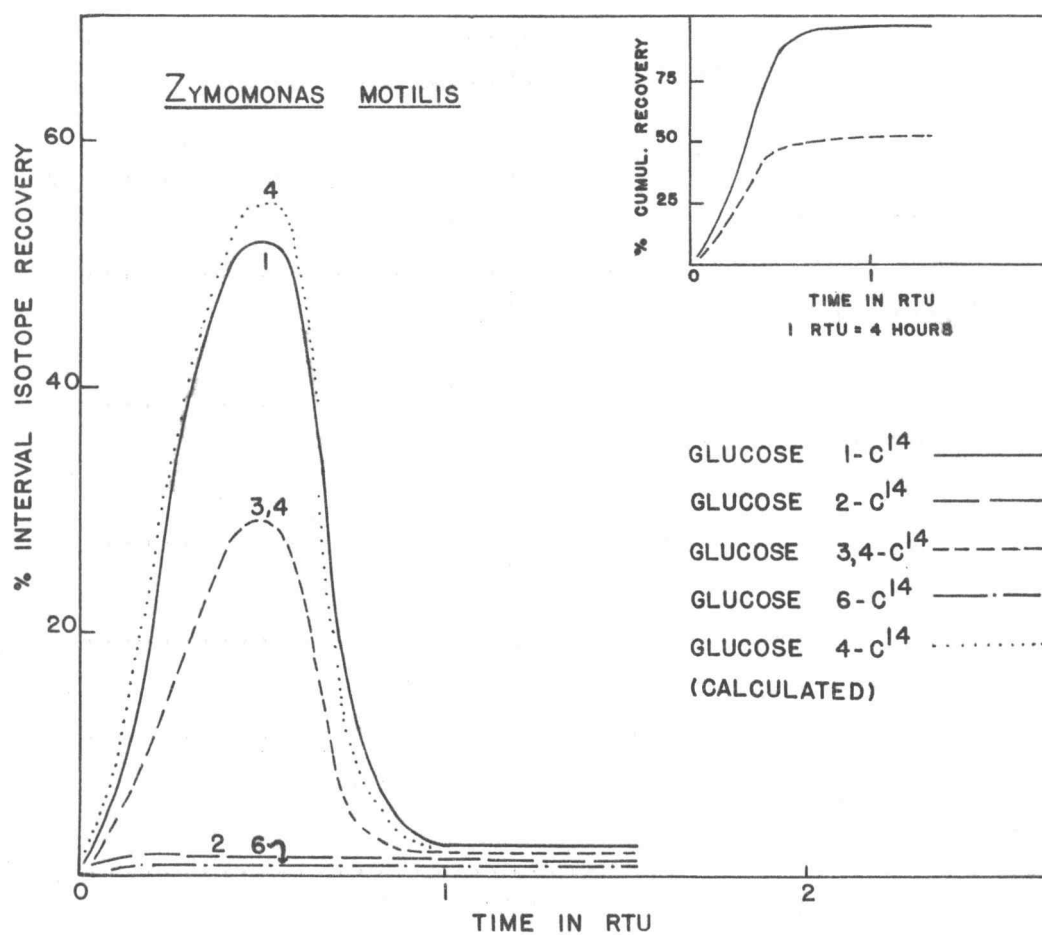
Figure 2. Radiorespirometric pattern. Zymomonas motilis growing on glucose.

Table II

CUMULATIVE HOURLY $C^{14}O_2$ RECOVERIES

C^{14}-LABELED GLUCOSE SUBSTRATES

Hour	Zymomonas motilis					Pseudomonas saccharophila				
	1	4*	2	3,4	6	1	4*	2	3,4	6
1	34	34	0	17	0	40	40	12	23	6
2	86	94	0	47	0	83	85	21	48	11
3**	99	102	0	51	0	90	91	26	53	15
4	99	102	0	51	0	91	95	36	57	19
5	--	--	--	--	--	91	100	39	61	22

*Calculated. See equation (1).

**Point taken as 1 RTU.

C-3 or C-4 alone in this case, since it is known that, in line with the fact that no secondary pathway of glucose oxidation is present, C-3 of glucose does not appear in the CO_2 but is recovered almost quantitatively in the methyl carbon of ethanol (16, p.693). Under these considerations, it is apparent that the recoveries of glucose $3,4-C^{14}$ represent only the conversion of C-4 of glucose to CO_2; hence, multiplying the interval isotope recoveries in the CO_2 from glucose $3,4-C^{14}$ by a factor of two should indicate the $C^{14}O_2$ yield from C-4. The calculated interval CO_2 recoveries from this carbon are indicated by the dotted line in Figure 2, and are in good agreement with those observed for C-1 of glucose. It is thus apparent that the assumption that C-1 and C-4 of glucose are converted to CO_2 at equal rates and to equal extents via the E-D or similar sequence is well supported.

The interpretation of radiorespirometric data from Ps. saccharophila (Figures 3 and 4) is complicated by the fact that glucose is degraded both via both primary and secondary pathways in this organism. The nature of the secondary degradation pathways alone is revealed by the radiorespirometric patterns obtained with specific C^{14}-labeled pyruvate and acetate as substrates. Studies by Baddiley et al. (1, pp.771-776) indicate that, with growing organisms in which the TCA cycle is present, C-2 of
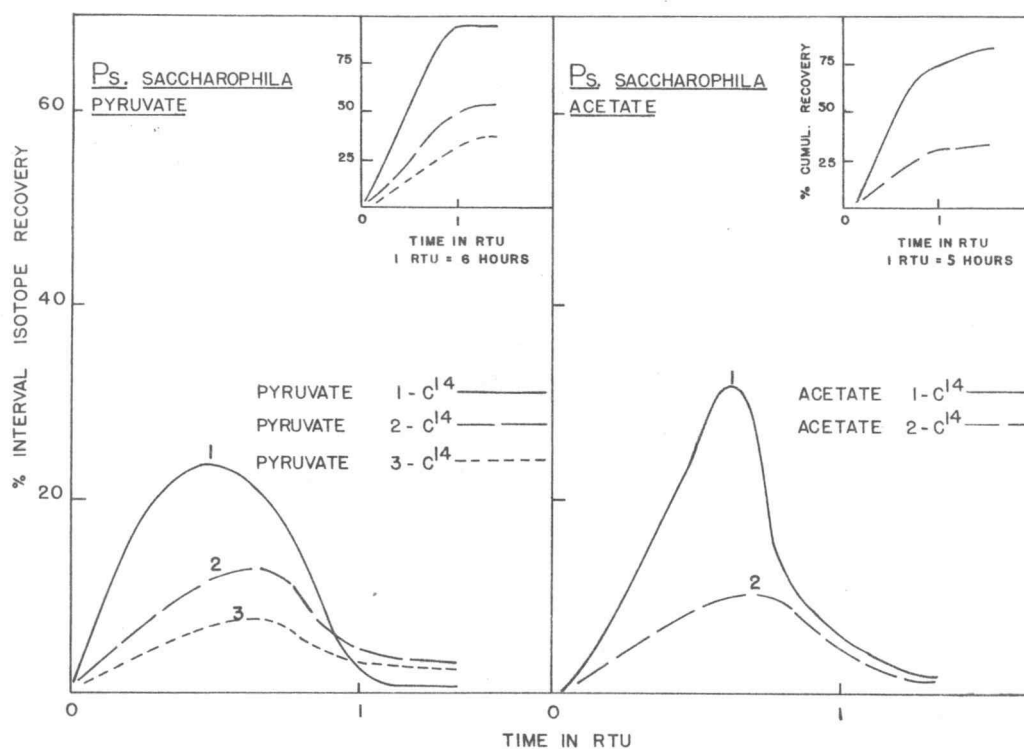
Figure 3. Radiorespirometric patterns. Pseudomonas saccharophila growing on pyruvate or acetate.
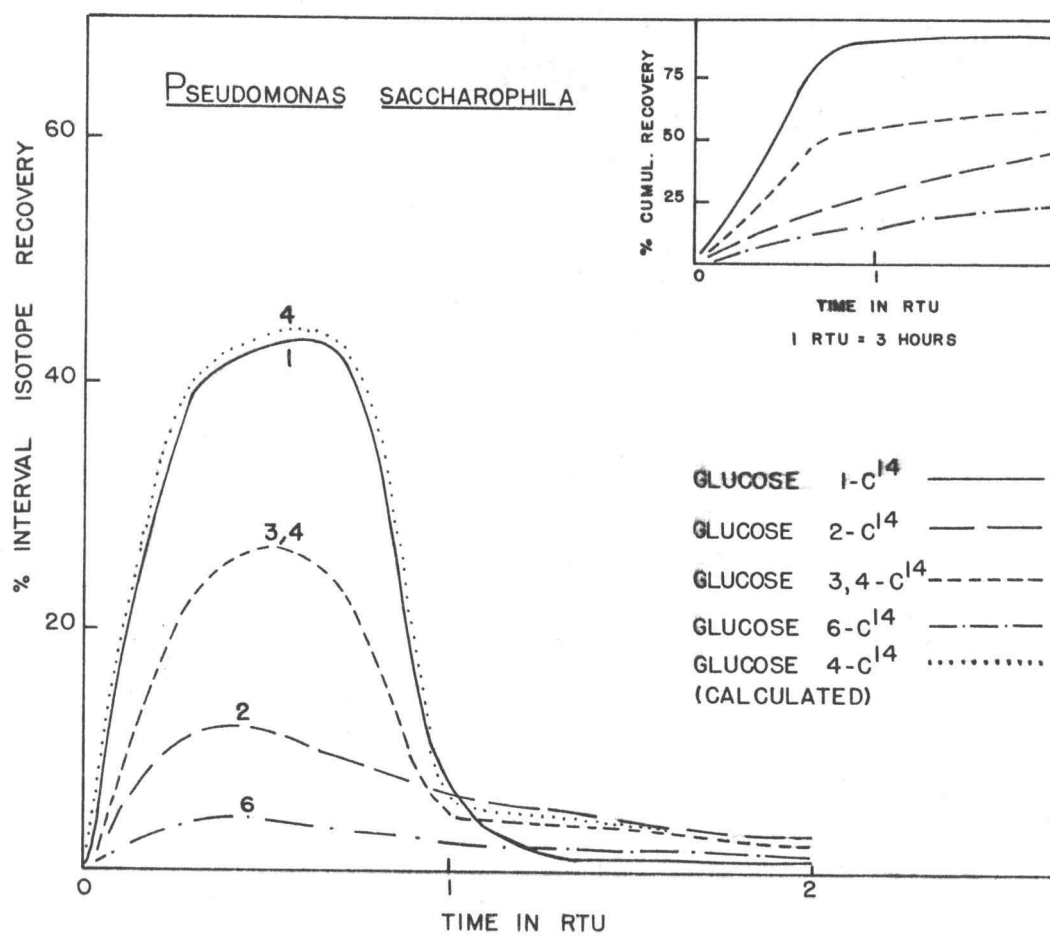
Figure 4. Radiorespirometric pattern. Pseudomonas saccharophila growing on glucose.

acetate is conserved for cellular biosynthesis in contrast to C-1 which appears in the CO_2 in relatively large quantities. The ratio of acetate C-1 to acetate C-2 in CO_2 generally approaches a value of 2. Therefore, the high ratio of acetate C-1 to acetate C-2 observed from Ps. saccharophila is taken as an indication that TCA cycle mechanisms are probably involved in acetate oxidation, and that acetate is utilized in biosynthetic functions. The actual extent of growth of Ps. saccharophila and the other organisms used in this study on glucose, pyruvate, and acetate may be seen in Table III. The higher recoveries of acetate C-2 over C-1 in the cells of Ps. saccharophila also support the above conclusions (Table IV).

The high rate of conversion of C-1 of pyruvate to CO_2 as compared to the successively lower rates of conversion of C-2 and C-3 are most likely the result of extensive pyruvate decarboxylation giving rise to acetate or its derivatives. Reference to Table V indicates that not all of pyruvate C-1 appears in CO_2, and this fact suggests that some pyruvate may have undergone transamination or entered into CO_2 fixation reactions. With over 90 per cent of pyruvate C-1 rapidly appearing as CO_2, it follows that most of C-2 and C-3 of pyruvate are converted to C-1 and C-2 of acetate, respectively. This presumption is supported by the finding that C-2 of pyruvate is recovered

Table III

INITIAL AND FINAL CELL WEIGHTS

Organism	Initial weight (mg.)	Final cell weight (mg. ± average deviation)		
		Glucose*	Pyruvate*	Acetate*
Zymomonas motilis	1.9	2.9 ± 0.1	--	--
Pseudomonas saccharophila	11.2	15.3 ± 0.8	14.5 ± 0.2	13.9 ± 0.3
Pseudomonas reptilivora	6.0	10.3**	6.7 ± 0.4	4.6**
Pseudomonas aeruginosa	10.9	14.5 ± 0.5	11.5 ± 0.0	8.8 ± 0.7
Pseudomonas KB1	6.0	13.1 ± 1.0	--	--

*100 micromoles

**Single flask

Table IV

FINAL PER CENT ISOTOPE RECOVERIES

Organism	Substrate	Per cent recovery*			
		CO_2	Cells	Medium	Total**
Zymomonas motilis	Glucose 1-C^{14}	99	1	4	92
	Glucose 2-C^{14}	0	1	61	62
	Glucose 3,4-C^{14}	51	2	30	82
	Glucose 6-C^{14}	0	3	47	50
	Glucose U-C^{14}	27	0	41	68
Pseudomonas saccharophila	Glucose 1-C^{14}	91	8	4	103
	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
	Glucose U-C^{14}	30	50	8	88
	Pyruvate 1-C^{14}	95	3	10	108
	Pyruvate 2-C^{14}	54	10	32	96
	Pyruvate 3-C^{14}	39	35	27	101
	Acetate 1-C^{14}	79	14	95	95
	Acetate 2-C^{14}	37	58	88	88

*At termination of experiment (5 hours).
**No attempt made to recover volatiles.

Table V

CUMULATIVE HOURLY $C^{14}O_2$ RECOVERIES

C^{14}-LABELED GLUCOSE SUBSTRATES

Hour	Pseudomonas reptilivora					Pseudomonas aeruginosa					Pseudomonas KB1				
	1	4*	2	3,4	6	1	4*	2	3,4	6	1	4*	2	3,4	6
1	33	14	23	18	17	40	31	19	21	11	27	26	15	19	11
2	82	49	53	46	43	70	55	36	34	21	60	55	34	40	25
3**	85	57	57	52	47	83	58	43	41	28	69	57	38	44	31
4**	86	60	59	54	48	86	57	46	44	31	70	58	39	45	32
5	86	61	61	55	49	88	59	48	46	33	71	59	40	46	33
6	87	63	61	56	49	--	--	--	--	--	71	59	40	46	33

*Calculated. See equation (1).

**Point taken as 1 RTU in column where underlining occurs.
  Underlined values used for estimation of pathways.

in the CO_2 to a greater extent than C-3, a relationship which is expected if acetate arising from pyruvate were oxidized via the TCA cycle (32, p.1870). Also in line with this concept is the greater recovery of C-3 over C-2 of pyruvate in the cells (Table IV).

From the foregoing discussion it might be predicted that the ratio of pyruvate C-2 to C-3 in the CO_2 should be comparable to that of acetate C-1 to C-2. Such is not the case, however, an observation which may be partially rationalized by the suggestion that, in the case of pyruvate, energy is obtained both from the decarboxylation step and from subsequent TCA cycle oxidation of acetate, resulting in more C_2 units being incorporated into cellular material. With acetate, however, both energy-producing and biosynthetic reactions are competing for substrate. Consequently, C-1 of acetate is combusted to CO_2 by extensive operation of TCA cycle processes and a greater ratio of C-1 to C-2 of acetate appears than that observed for pyruvate C-2 to C-3.

The establishment of patterns to be expected from the secondary catabolic pathways in Ps. saccharophila facilitates the interpretation of radiorespirometric data obtained from this organism on glucose (Figure 3). Since, in Ps. saccharophila, glucose is known to be catabolized via the same primary pathway as found in Z. motilis

(17, p.99), some resemblance between the two patterns is expected. Thus, in both cases, rapid and nearly complete conversion of C-1 to CO_2 is observed. In the E-D sequence, pyruvate formed from glucose derives its carboxyl carbon from C-1 and C-4, its carbonyl carbon from C-2 and C-5, and its methyl carbon from C-3 and C-6. In Ps. saccharophila the carboxyl and methyl carbons of acetate arising from the decarboxylation of pyruvate should thus correspond to C-2 and C-6 of glucose. The radiorespirometric pattern from these carbon atoms (Figure 4) resembles that given in Figure 3 for C-2 and C-3 of pyruvate and thus is in support of this contention. The recovery of glucose 3,4-C^{14} in the CO_2 is higher, however, in relation to that from glucose 1-C^{14} than was observed in the case of Z. motilis. This results from the fact that both C-3 and C-4 of glucose are contributing to the CO_2 in the case of Ps. saccharophila (although by different pathways), while in Z. motilis, only C-4 contributes. To confirm the prediction that C-1 and C-4 should appear equally in the CO_2 from Ps. saccharophila, the contribution from C-4 alone must be calculated. If the scheme proposed by Entner and Doudoroff (15, p.851) is accepted, C-3 and C-6 should be equivalent in metabolic behavior at the level of pyruvate. Thus, the recovery of C-3 in the CO_2 can be represented by that of C-6, and

$$(1) \qquad G_4 = 2\, G_{3,4} - G_6$$

where G represents the per cent recovery of isotope from glucose labeled as indicated by the subscripts. As is shown in Figure 4 and Table II, the calculated C-4 recoveries in the CO_2 agree with those from C-1 and so tend to bear out the above assumptions.

After establishing the type of patterns to be expected from microorganisms in which the E-D sequence alone, or the E-D sequence in conjunction with the TCA cycle were functioning, the patterns given by an organism in which a pentose pathway was present in addition to the E-D sequence and TCA cycle were examined. The radio-respirometric data for such an organism, Ps. reptilivora, are shown in Figures 5 and 6 and in Tables V and VI. The patterns for pyruvate and acetate resemble closely those obtained from Ps. saccharophila, and thus support the contention that acetate or its derivatives is metabolized via the TCA cycle in this organism (3, pp.517-525). It may be noted that growth did not occur in Ps. reptilivora on acetate, a finding which was of interest in view of the fact that Lewis et al. (26, pp.273-286) trapped this substance to identify and estimate the importance of pathways involved in glucose catabolism in this organism. This lack of growth in the acetate experiment is probably the cause of the relatively low ratio of acetate C-1 to C-2 found in
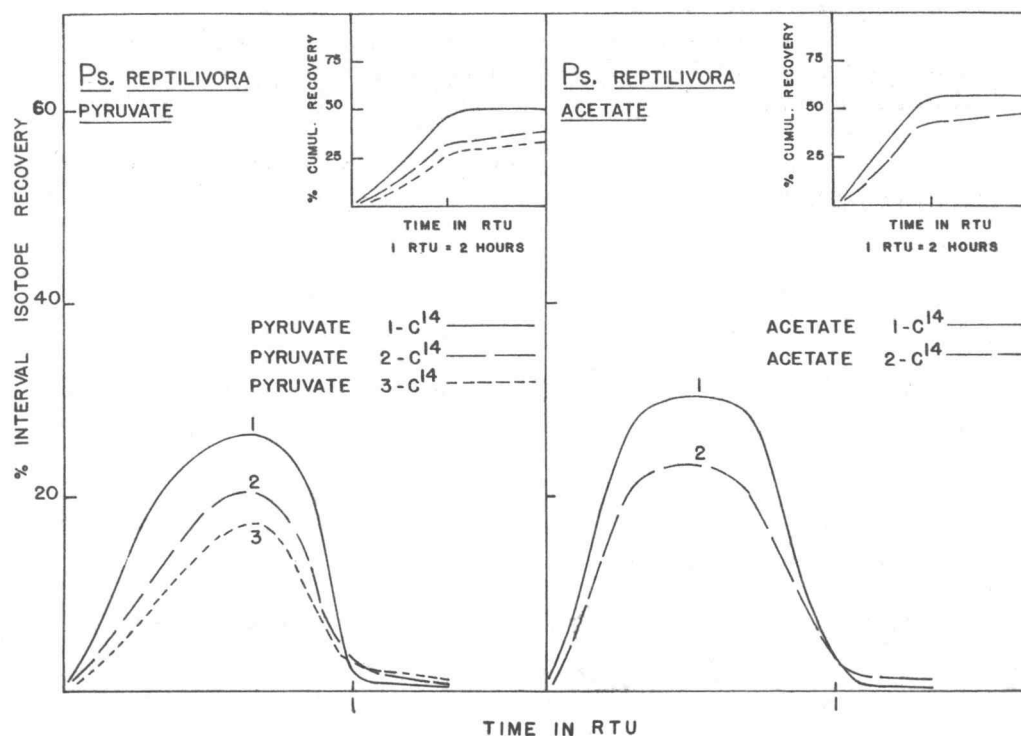
Figure 5. Radiorespirometric pattern. Pseudomonas reptilivora utilizing pyruvate or acetate.
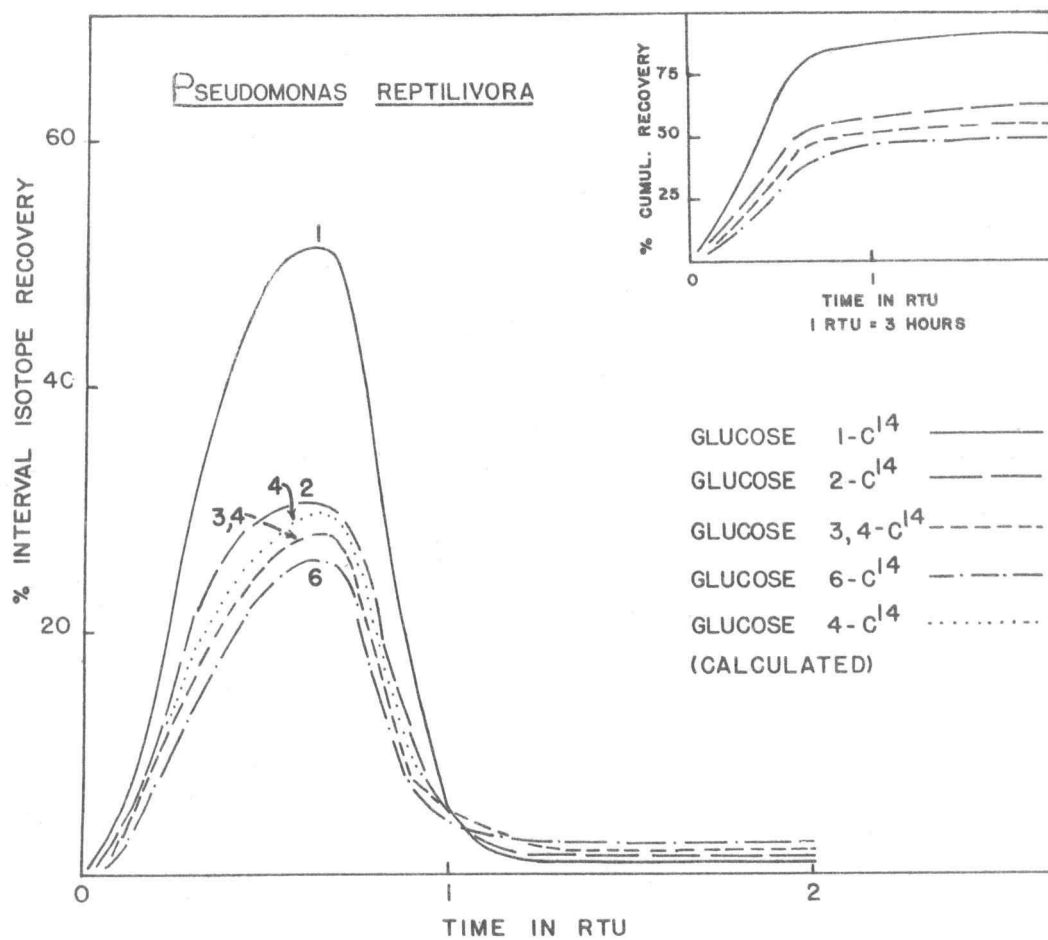
Figure 6. Radiorespirometric pattern. Pseudomonas reptilivora growing on glucose.

Table VI

FINAL PER CENT ISOTOPE RECOVERIES

Pseudomonas reptilivora

Substrate	Per cent recovery*			
	CO_2	Cells	Medium	Total**
Glucose 1-C^{14}	87	10	10	107
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
Glucose U-C^{14}	63	34	9	105
Pyruvate 1-C^{14}	70	8	10	88
Pyruvate 2-C^{14}	38	28	37	103
Pyruvate 3-C^{14}	32	30	45	107
Acetate 1-C^{14}	79	12	3	94
Acetate 2-C^{14}	62	29	8	99

*At termination of experiment (6 hours).

**No attempt made to recover volatiles.

the respiratory CO_2. The acetate activity found in the cells (Table VI) probably indicates that partial oxidative assimilation and exchange occurred under these conditions.

It is interesting to speculate on the failure of Ps. reptilivora to grow on acetate even though this compound is apparently an intermediate in glucose oxidation. The recent review by Kornberg and Krebs (24, pp.988-995) and the paper by Wong and Ajl (37, pp.1013-1014) indicates that the ability of an organism to utilize acetate as a sole source of carbon and energy may be related to its content of malate synthetase. This enzyme is presumably essential for the production of C_4 units to be channeled into biosynthesis as supplements for those oxidized in energy production via the TCA cycle. Since malate synthetase is absent in organisms not grown on acetate, it appears likely that the failure of glucose-grown Ps. reptilivora to grow on acetate may be due to a lack of this enzyme.

The radiorespirometric patterns obtained from Ps. reptilivora growing on labeled glucose are somewhat different from those given by organisms displaying only the E-D pathway and the TCA cycle. Although comparable rates of conversion of C-1 and C-3,4 to CO_2 are evident in both Ps. reptilivora and Ps. saccharophila, marked increases may be seen in the recovery of C-2 and C-6 in the former

organism. The data from pyruvate and acetate experiments have indicated that TCA cycle sequences are operative in Ps. reptilivora. Inasmuch as C-2 and C-6 of glucose are equivalent to the carboxyl and methyl of acetate, respectively, (Figure 13) in the E-D sequence, it is likely that part of the recovery in the CO_2 from C-2 and C-6 is the result of acetate oxidation via the TCA cycle. This presumption is supported by the higher recovery of glucose C-2 over C-6 in the CO_2 and the corollary of glucose C-6 recoveries over C-2 in the cells (Table VI).

The findings of Lewis and her collaborators (26, p.285) indicate that in Ps. reptilivora E-D and TCA cycle processes operate concurrently with a pentose-involving pathway, and it will be of value to illustrate how radio-respirometric data may be interpreted to illustrate this point.

In assessing the contribution of the pentose pathway in Ps. reptilivora it is necessary first to estimate the contribution of C-4 of glucose to the CO_2. This may be done using equation (1) under the following assumptions:

(a) Only the E-D pathway, in conjunction with the TCA cycle, and the pentose pathway participate in glucose catabolism.

(b) In the E-D sequence followed by the TCA cycle, C-1, C-2, and C-3 of glucose are equivalent to C-4, C-5, and C-6 at the level of pyruvate.

(c) The conversion of C-3, C-4, C-5, and
     C-6 to CO_2 via extensive pentose cyclic
     processes is not significant.

The values calculated for C-4 recovery in the CO_2 from Ps.
reptilivora are presented in Table VI and plotted in Fig-
ure 6. The relative height of the C-1 curve over the cal-
culated C-4 curve indicates the extent to which pentose-
involving processes are contributing to glucose catabolism
in addition to the E-D pathway. The recoveries of C-4 in
the CO_2 may be taken as indices of the decarboxylation of
pyruvate arising via the E-D pathway and, as such, should
represent the limiting values for recovery of C-2 of glu-
cose via the E-D and TCA cycle route. Since the conver-
sion of C-2 of glucose proceeded at a higher rate than
C-4, it is probable that pentose cycle mechanisms were
also contributing to the conversion of this carbon to CO_2.
If it is assumed that the ratios of CO_2 recoveries from
C-1 to C-2 of pyruvate, where pyruvate is the sole sub-
strate, may be used to estimate the same ratio for pyruvate
derived from glucose via the E-D pathway, the maximum
fraction of C-2 of glucose appearing in the CO_2 via pen-
tose cycle mechanisms may be estimated. Thus:

    Let P = the per cent recovery in the CO_2 of
            the pyruvate carbon atom indicated by
            the subscript.

Let G = the per cent recovery in the CO_2
of the glucose carbon atom in-
dicated by the subscript.

Then, assuming for glucose catabolized via the
E-D pathway and TCA cycle

(2) $$\frac{G_4}{G_2} = \frac{P_1}{P_2}$$

(3) G_2 via pentose $= G_2$ observed $- (G_4)\dfrac{P_2}{P_1}$

The value for C-2 of glucose converted to CO_2 via the
pentose pathway is 26 per cent when estimated by equation
(3) above.

The radiorespirometric data may also be used to
estimate the relative importance of each pathway assumed
to be present in Ps. reptilivora. From the assumptions
upon which equation (1) is based, it follows that for
every mole of glucose catabolized via the pentose pathway,
a mole of C-1 is converted to CO_2. For every mole of glu-
cose catabolized by way of the E-D pathway, a mole of C-1
and C-4 are converted to pyruvate carboxyl. Since C-1 and
C-4 presumably behave identically after the level of
pyruvate in the E-D pathway,

(4) Per cent of administered
     glucose catabolized via $= G_1 - G_4$
     the pentose pathway

where G is the cumulative per cent recovery in the CO_2 of
the carbon indicated by the subscript. It is important to

note here, that unless the quantity of glucose actually
utilized is known, the amount metabolized via the E-D
pathway cannot be estimated. This results from the fact
that not all of the pyruvate arising from glucose via this
pathway is decarboxylated, and hence the recovery of C-4
of glucose is not stoichiometrically related to the glu-
cose consumed via the E-D pathway. If, however, the cumu-
lative recovery data used in equation (4) are taken at 1
RTU (point of glucose exhaustion as indicated by low
interval recovery in the CO_2 from C-1 of glucose), then
equation (4) becomes

(5)   Per cent of utilized
      glucose catabolized via $= G_1 - G_4$
      the pentose pathway

By the use of recoveries at 1 RTU, the need for determin-
ing glucose chemically among a number of other reducing
substances is eliminated. The validity of equation (5)
is, of course, limited by the amount of intact hexonic
acid derived from glucose which remains in the medium.
This source of error is indicated by the recovery of C-1
of glucose in the medium, and amounts to a maximum of 10
per cent, as shown in Tables VI and VII. By taking into
account assumption (a) on page 35, wherein

Total glucose $=$ Glucose via $+$ Glucose via
catabolized   E-D pathway   pentose pathway

Table VII

FINAL PER CENT ISOTOPE RECOVERIES

Organism	Substrate	Per cent recovery*			
		CO_2	Cells	Medium	Total**
Pseudomonas aeruginosa	Glucose 1-C^{14}	88	9	1	98
	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
	Glucose U-C^{14}	47	38	5	90
	Pyruvate 1-C^{14}	70	10	17	97
	Pyruvate 2-C^{14}	40	26	16	82
	Pyruvate 3-C^{14}	37	32	22	91
	Acetate 1-C^{14}	85	10	1	96
	Acetate 2-C^{14}	63	36	3	102
Pseudomonas KB1	Glucose 1-C^{14}	71	9	2	82
	Glucose 2-C^{14}	40	40	2	83
	Glucose 3,4-C^{14}	46	39	5	89
	Glucose 6-C^{14}	33	47	3	83
	Glucose U-C^{14}	43	31	3	77

*Duration of experiments 6 hours.

**No attempt to recover volatiles.

then

(6) Per cent of utilized   Per cent of uti-
     glucose catabolized = 100 - lized glucose catab-
     via the E-D pathway   olized via the
                                  pentose pathway

Calculated on this basis, 72 per cent of the glucose uti-
lized by Ps. reptilivora was catabolized via the E-D path-
way with the remainder going via the pentose route.

Having obtained reference patterns for organisms in
which glucose is catabolized via the E-D pathway alone
(Z. motilis), the E-D pathway in conjunction with the TCA
cycle (Ps. saccharophila), and the E-D pathway and TCA
cycle plus a pentose-involving pathway (Ps. reptilivora),
attention was focused on Ps. aeruginosa and Ps. KB1,
organisms whose primary pathways of glucose catabolism
were unknown. The radiorespirometric patterns for Ps.
aeruginosa growing on acetate and pyruvate (Figure 7) are
similar to those of Ps. saccharophila and Ps. reptilivora.
Findings by Campbell and others (11, p.594) indicate that
Ps. aeruginosa catabolizes acetate via a modified TCA
cycle in which glyoxylate and succinate are produced by
the action of "aconitase" on cis-aconitate. It is to be
noted here that no growth occurred on acetate, possibly
reflecting, as discussed earlier, a lack of enzymes re-
quired for biosynthesis of C_4 from C_2 units. As with Ps.
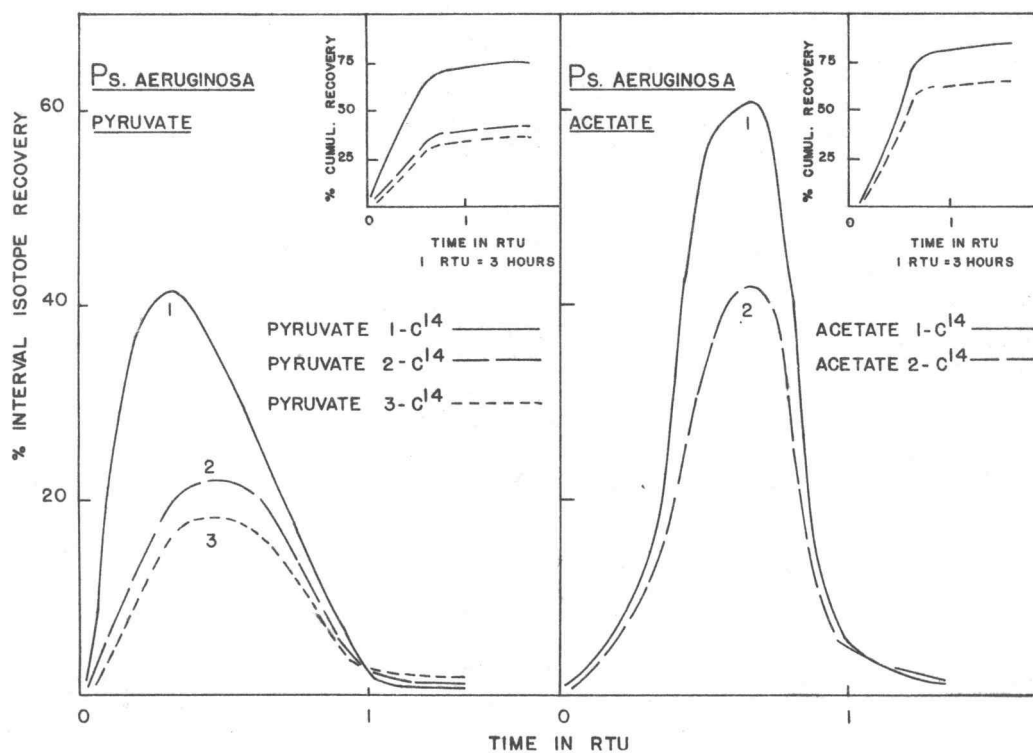saccharophila and Ps. reptilivora, the rapid conversion

Figure 7. Radiorespirometric patterns. Pseudomonas aeruginosa utilizing pyruvate or acetate.

of pyruvate C-1 to CO_2 and the conservation of C-3 over
C-2 for cellular material, as indicated by the cell recoveries (Table VII), indicates that pyruvate is probably a
major intermediate in glucose catabolism.

With glucose as the sole substrate (Figure 8), both
Ps. aeruginosa and Ps. KB1 (Figure 9) produce radiorespirometric patterns similar to those from Ps. reptilivora
(Figure 6). The high rates of C-1 over calculated C-4 recoveries probably reflect, as with Ps. reptilivora, the
extent of pentose pathway participation in glucose catabolism. The conversions of C-2 of glucose to CO_2 via the
pentose pathway, as estimated by equation (3), are for Ps.
aeruginosa, 13 per cent, and for Ps KB1, assuming the same
ratio for pyruvate C-1 to C-2 as in Ps. aeruginosa, five
per cent. Calculation of the pathways participation
(Table IX) indicated that in Ps. aeruginosa, 71 per cent
of the administered glucose was catabolized via the E-D
pathway, whereas in Ps. KB1, 87 per cent of the glucose
was catabolized via the E-D pathway. It is of interest to
note that, although the extent of pentose pathway participation for both Ps. aeruginosa and Ps. reptilivora is
essentially the same, the proportion of C-2 of glucose
converted to CO_2 via the pentose pathway is higher for Ps.
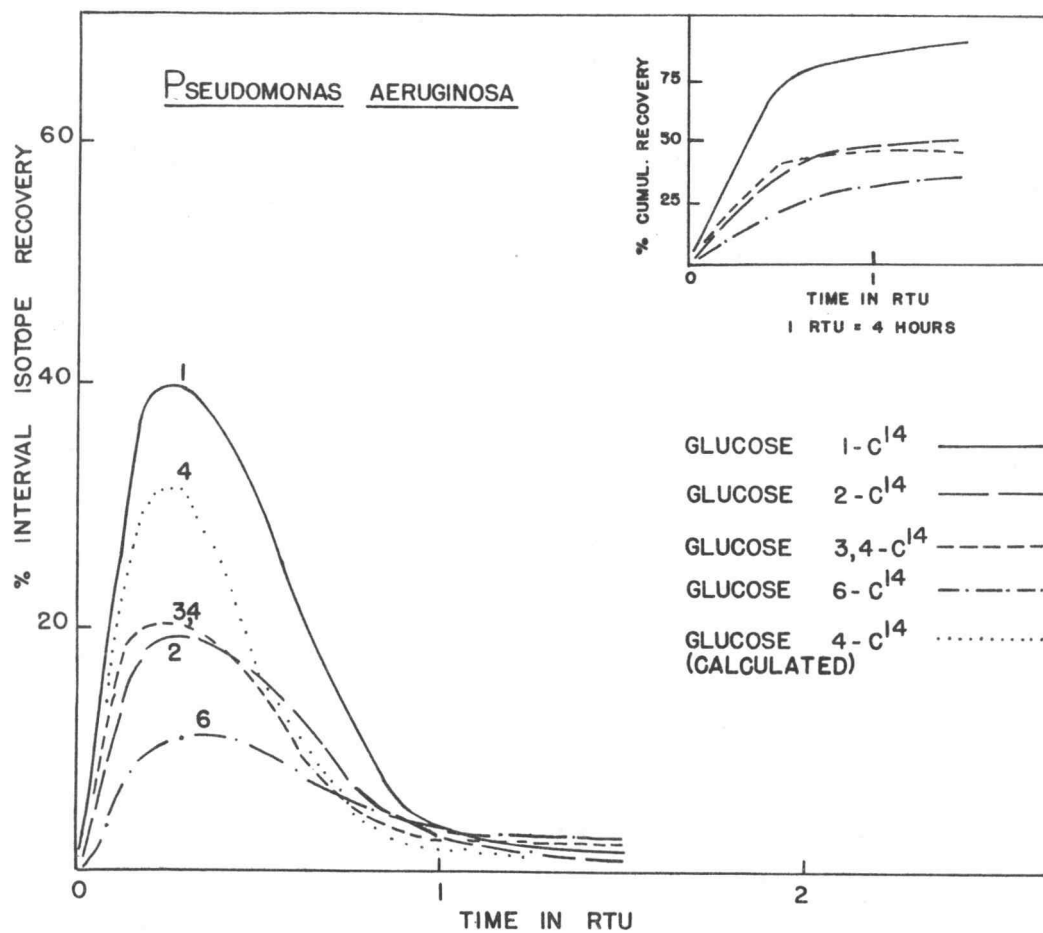reptilivora. It therefore appears that in Ps. reptilivora,

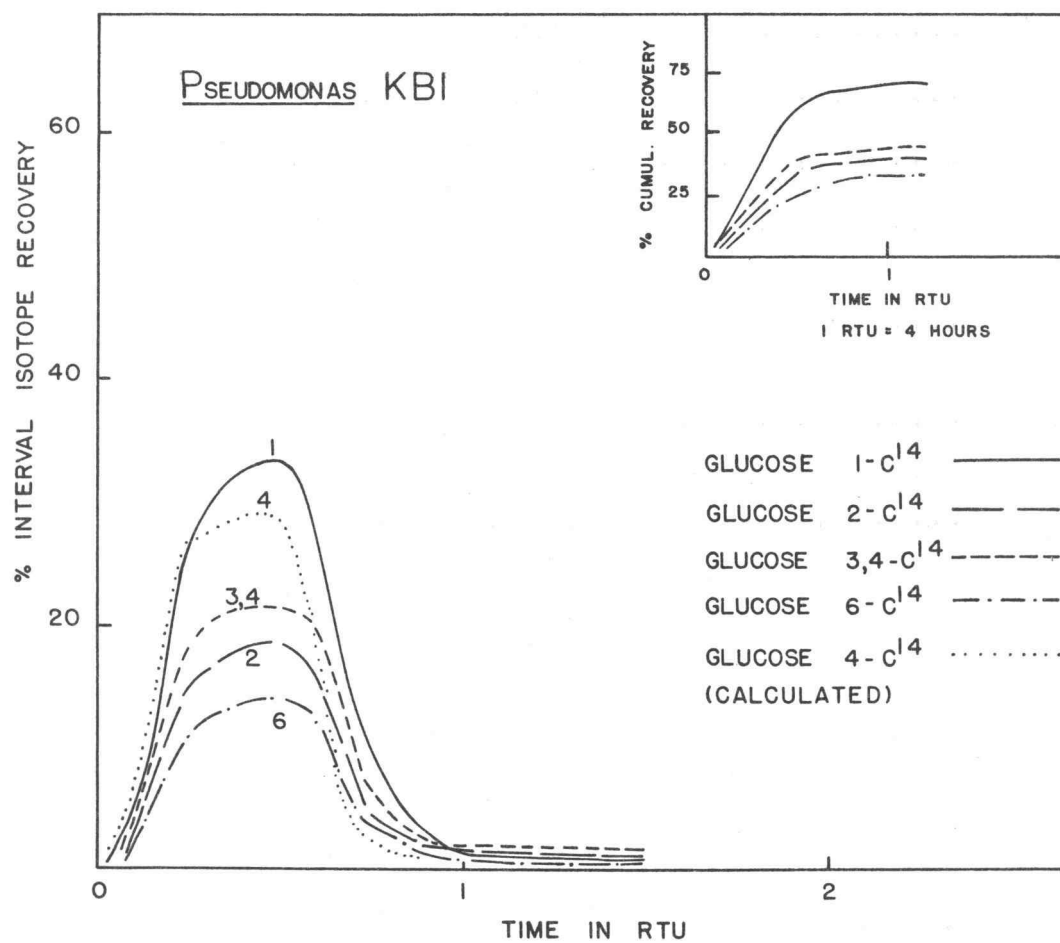Figure 8. Radiorespirometric pattern. Pseudomonas aeruginosa growing on glucose.

Figure 9. Radiorespirometric pattern. Pseudomonas KB1 growing on glucose.

more glucose is diverted into pentose cycle pathways than
in the other organisms studied.

In attempting to gain further insight into the car-
bohydrate metabolism of the pseudomonads, radiorespiro-
metric patterns were obtained for Ps. reptilivora, Ps.
aeruginosa, and Ps. KB1 growing on C^{14}-labeled gluconate
substrates. By the use of these substrates it was hoped
to determine whether gluconate was actually equivalent to
glucose, as suggested by Wood (39, p.232) for a pseu-
domonad having two primary pathways of glucose degrada-
tion. The patterns obtained with gluconate are shown in
Figures 10, 11, and 12. In comparison to the patterns
from glucose, those from gluconate all showed essentially
the same extent of conversion of C-1 to CO_2. The recovery
of C-2 and C-6 of gluconate, however, was lower than that
observed for glucose in Ps. reptilivora, equal to that
observed for glucose in Ps. aeruginosa, and higher than
that observed for glucose in Ps. KB1. Moreover, in all
three organisms, the recoveries of C-3,4 of gluconate in
the CO_2 were higher than recoveries of the corresponding
carbons of glucose. In view of the finding by Campbell,
Linnes, and Eagles that equal growth of Pseudomonas
aeruginosa occurs on glucose and gluconate, the signifi-
cance of this change in radiorespirometric pattern appar-
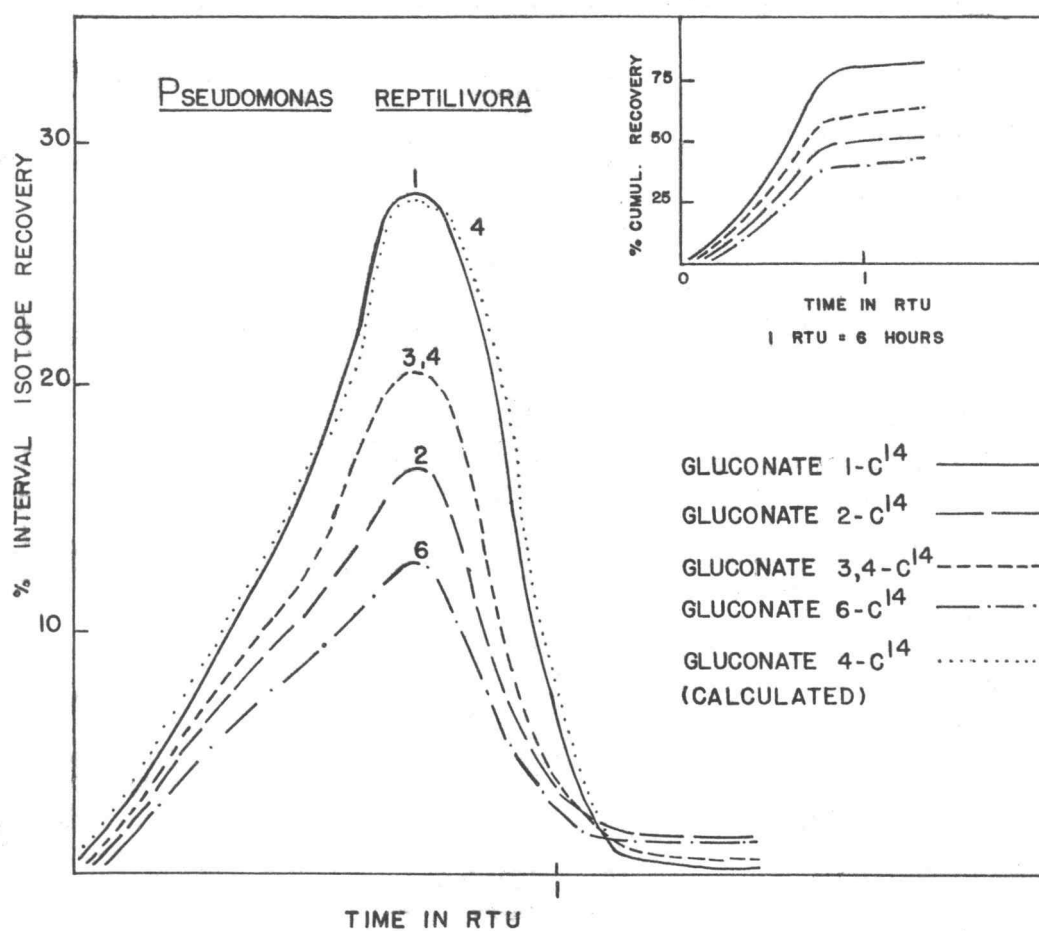ently does not lie in the differences in energy producing

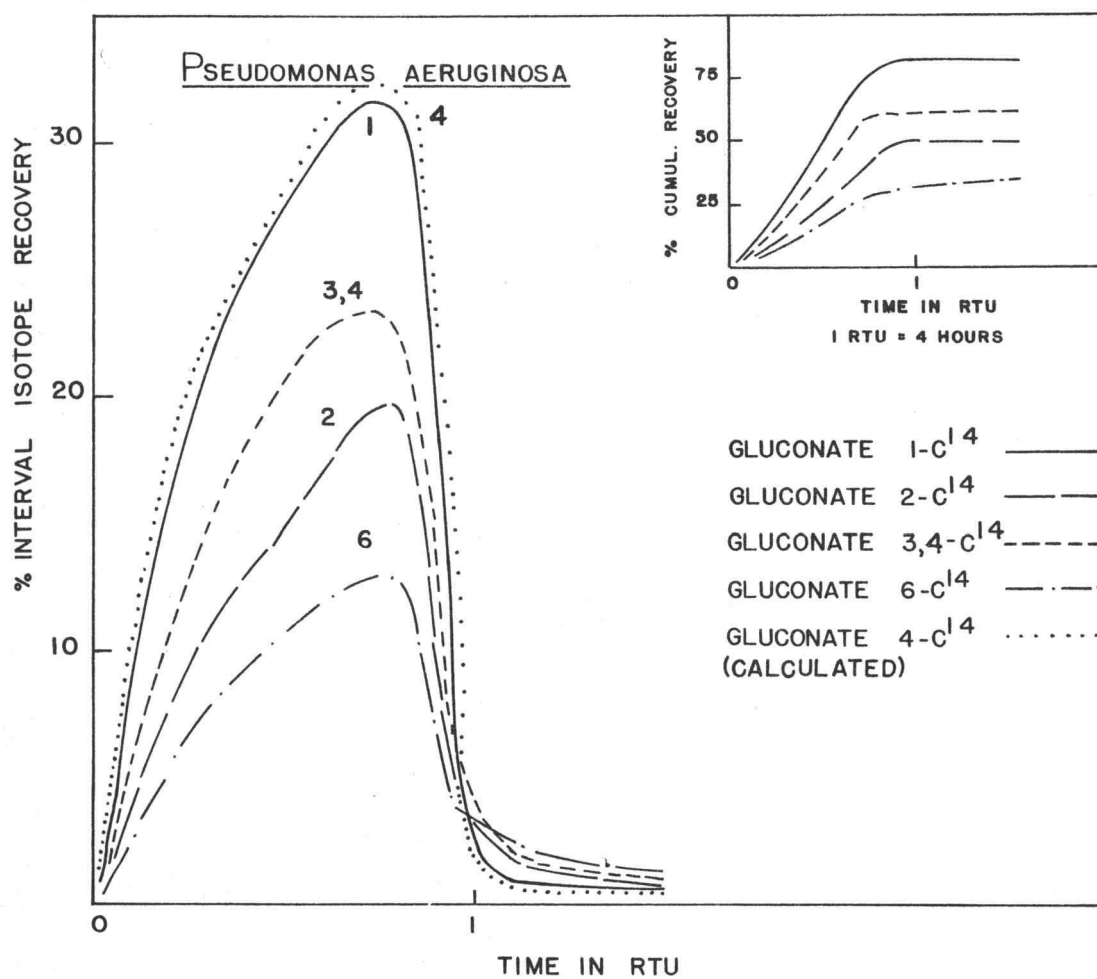Figure 10. Radiorespirometric pattern. Pseudomonas reptilivora growing on gluconate.

Figure 11. Radiorespirometric pattern. Pseudomonas aeruginosa growing on glucose.
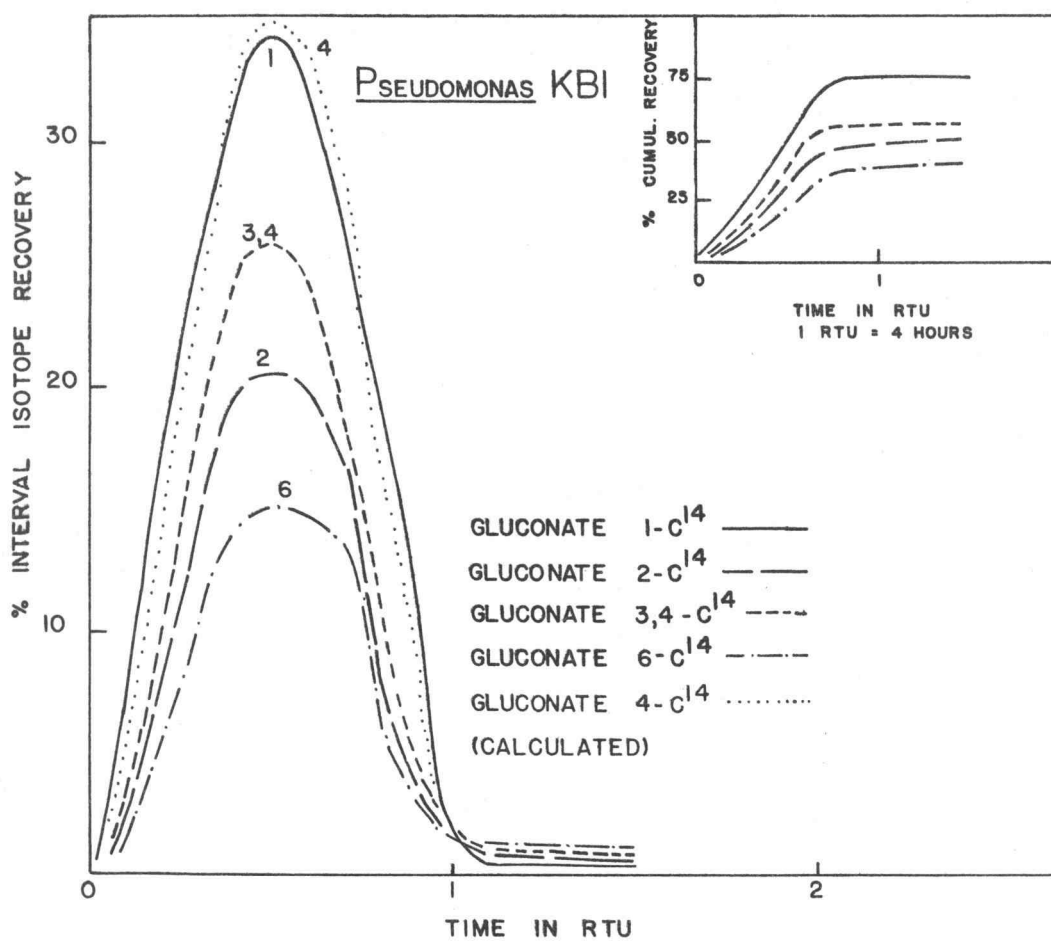
Figure 12. Radiorespirometric pattern. Pseudomonas KB1 growing on gluconate.

potentiality of the two substrates. Moreover, Wood's scheme for the pathways of carbohydrate catabolism in Pseudomonas fluorescens indicates that the patterns obtained from gluconate should be identical to those from glucose. The trend for the radiorespirometric patterns observed with gluconate to resemble those of Ps. saccharophila on glucose, however, suggests that gluconate is catabolized via the E-D route in preference to the pentose pathway. If this implication were true, then the gluconate C-4 recoveries in the CO_2 estimated by equation (1) should agree with the observed recoveries of gluconate C-1. The closeness of the observed values for C-4 in the CO_2 are indicated in Figures 10, 11, and 12 and in Table VIII, and thus lend credence to the hypothesis that gluconate is catabolized in these organisms mainly via the E-D sequence.

In summary, a schematic diagram of the pathways of carbohydrate catabolism in pseudomonads, modified from the scheme presented by Wood (39, loc. cit.) to account for our findings, is presented in Figure 13. The reactions which are germain to each organism studied in the present work are coded as indicated in the legend. The route of glucose catabolism for Z. motilis and Ps. saccharophila proceeds through the E-D pathway alone up through the stage of pyruvate decarboxylation. In Z. motilis, the

Table VIII

CUMULATIVE HOURLY $C^{14}O_2$ RECOVERIES

C^{14}-LABELED GLUCONATE SUBSTRATES

Hour	Pseudomonas reptilivora					Pseudomonas aeruginosa					Pseudomonas KB1				
	1	4*	2	3,4	6	1	4*	2	3,4	6	1	4*	2	3,4	6
1	6	8	5	6	4	22	21	10	14	7	21	16	10	13	8
2	17	18	13	14	10	50	55	25	36	17	50	56	31	39	22
3**	35	36	25	28	20	84	90	45	60	30	74	73	46	54	35
4**	63	64	42	49	33	86	91	48	62	33	75	73	48	55	37
5	80	79	48	59	39	87	91	48	62	33	75	74	49	56	38
6**	82	82	50	61	41	--	--	--	--	--	--	--	--	--	--
7	83	82	50	61	47	--	--	--	--	--	--	--	--	--	--

*Calculated. See equation (1).

**Point taken as 1 RTU in column where underlining occurs.
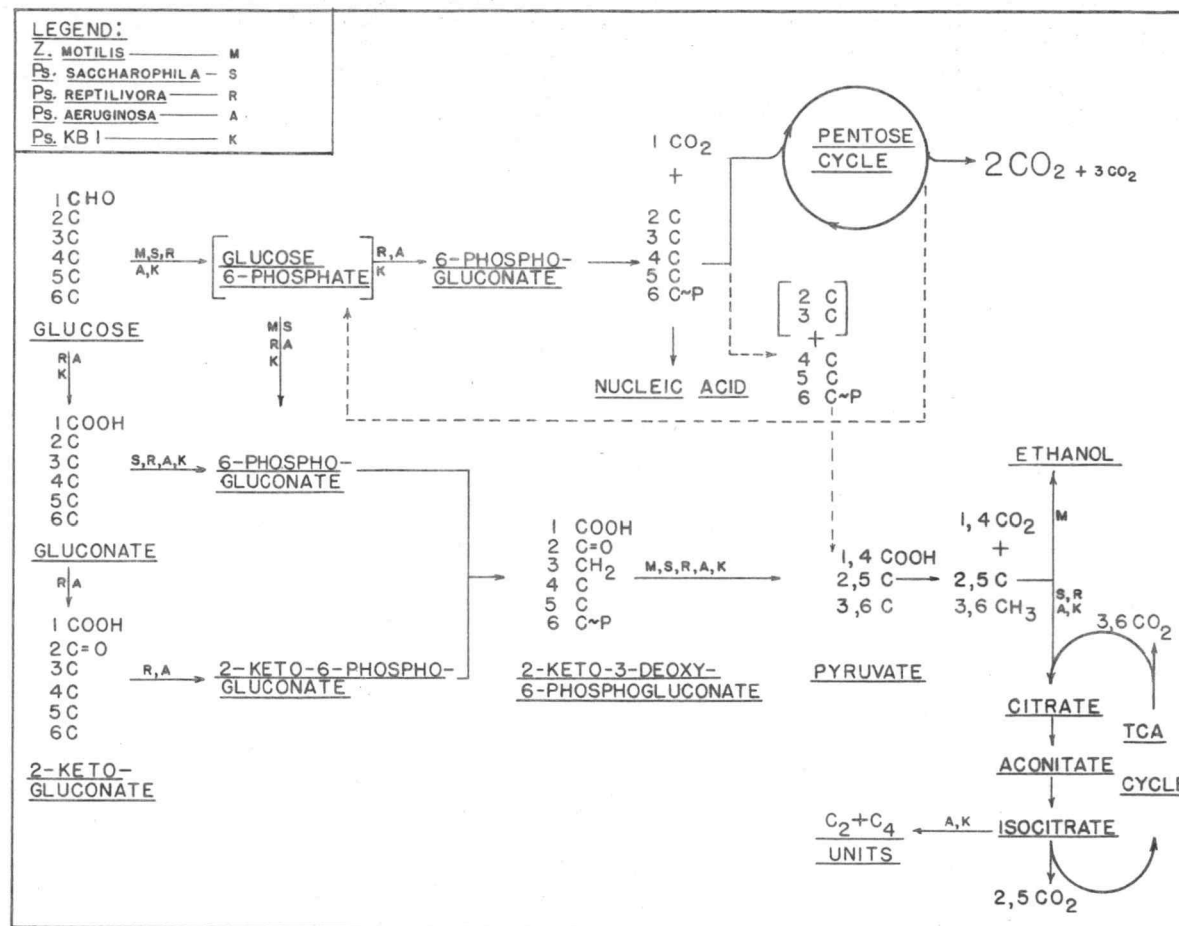
FIGURE 13. PATHWAYS OF CARBOHYDRATE CATABOLISM IN THE PSEUDOMONADS.

products of this step are reduced to ethanol since no
mechanism is available for oxidizing them further, whereas
in Ps. saccharophila, the acetate arising from pyruvate is
converted to CO_2 and cell material probably via the TCA
cycle. In Ps. aeruginosa and Ps. KB1, the data indicate
that primary degradation of glucose proceeds via a combi-
nation of E-D or E-D like routes as well as pentose-
involving pathways, as has been indicated for Ps. reptili-
vora by Lewis et al. (26, p.234). The undefined fate of
pentose carbon is indicated by the dashed arrows. Reports
by Bernstein and Sweet (4, p.153; 5, p.190) show that the
pentose of nucleic acid may be derived directly from
glucose-derived pentose as indicated, while the scheme
presented by Lewis et al. (26, p.277) suggests that keto-
lase cleavage may result in C_2 and C_3 units from pentose.
Acetate arising via this latter route would have as its
carboxyl carbon C-3 of glucose and as its methyl carbon,
C-2. If the C_3 unit representing C-4, C-5, and C-6 of
glucose were in equilibrium with triose arising by way of
the E-D pathway, the net result in the radiorespirometric
pattern would be similar to that from the E-D pathway,
with the exception that C-4, as calculated from equation
(1) would be somewhat higher than C-1 because of the
higher conversion of C-3 of glucose to CO_2 as compared to
C-6. The latter effect would result, of course, from the

identity of C-3 of glucose with acetate carboxyl instead of acetate methyl as is the case with glucose C-6. The type of pattern described above was found with Ps. aeruginosa growing on gluconate and the above sequence is an alternate possibility to explain the pattern obtained on that substrate.

Secondary pathways of carbohydrate catabolism in Ps. saccharophila, Ps. reptilivora, and Ps. KB1 all proceed through acetate or its derivative through the TCA cycle. The routes for additional C_2 and C_4 unit synthesis in Ps. aeruginosa and Ps. KB1 differ, as indicated by the findings of Campbell, Smith, and Eagles (11, p.594) and Kornberg and Madsen (13, p.653) and are portrayed in Figure 13 as indicated by these authors.

A summary of the various fates of glucose carbon in the organisms studied in the present work is shown in Table IX. Equations used for the estimation of glucose utilized via the pentose pathway and for glucose entering the pentose cycle are discussed on pages 35-40. The estimations of the amount of glucose converted to acetate via the E-D pathway are based on the premise that C-1 of glucose is converted to CO_2 by Z. motilis and Ps. saccharophila only via pyruvate decarboxylation, and that this holds true for C-4 of glucose in Ps. reptilivora, Ps. aeruginosa, and Ps. KB1. The amount of acetate thus

Table IX

THE FATE OF GLUCOSE IN THE PSEUDOMONADS

Fate of catabolized glucose	Zymomonas motilis	Pseudomonas saccharophila	Pseudomonas reptilivora	Pseudomonas aeruginosa	Pseudomonas KB1
Per cent catabolized via pentose pathway	0	0	28	29	13
Per cent catabolized via E-D pathway	100	100	72	71	87
Per cent catabolized via pentose cycle (maximum)	--	--	26	13	5
Per cent converted to acetate via E-D pathway*	0	90	57	57	57
Per cent acetate derived via E-D pathway combusted to CO_2**	--	40	82	69	54

*Indicated as minimum per cent by glucose C-4 recovery.
**Computed by dividing per cent glucose converted to acetate by recovery in CO_2 of C-6 of glucose.

indicated represents a minimum value and is low to the
extent of the CO_2 fixation that occurs in these organisms.
Values obtained for the per cent of acetate combusted to
CO_2 are based on the premise that C-6 of glucose is con-
verted to CO_2 only via the TCA cycle in all of the organ-
isms studied. The relatively high combustion efficiencies
found with acetate in the four pseudomonads having a TCA
cycle serve to support the contention that much of the
substrate carbon diverted into the pentose pathway is in-
corporated into cell material. The differences and simi-
larities between pseudomonads made evident in Table IX
indicate that radiorespirometry is a useful tool for de-
tecting differences between organisms as closely related
as Ps. reptilivora and Ps. aeruginosa (8, p.90) on a more
fundamental basis than those heretofore used in classifi-
cation.

SUMMARY

Mechanisms of carbohydrate catabolism in five pseudo-
monads have been investigated by determining the relative
rates at which specific C^{14}-labeled substrate carbon atoms
were converted to CO_2 in growing cultures. Insofar as
this newly devised method involves studying respiratory
characteristics by the use of radioactive tracers, it has
been termed the "radiorespirometric" method. Identifica-
tion of pathways by means of radiorespirometry lies in the
fact that, in growing organisms, the rate of conversion to
CO_2 of various glucose carbon atoms is determined by the
nature and relative importance of the catabolic pathways
operative in a given organism (32, pp.1869-1874). In the
present work, reference patterns were obtained for the
Entner-Doudoroff (E-D) pathway alone from Zymomonas
motilis (16, pp.689-694) for the E-D pathway and tricar-
boxylic acid (TCA) cycle from Pseudomonas saccharophila
(15, pp.853-862), and for the E-D pathway and TCA cycle
operating concurrently with a pentose-involving pathway
from Pseudomonas reptilivora (26, pp.273-286). The degra-
dation of glucose via the E-D pathway alone or in conjunc-
tion with the TCA cycle was found to produce equal rates
of conversion of C-1 and C-4 of glucose to CO_2. Glucose
degradation via a combination of the E-D pathway, TCA

cycle, and pentose pathway, however, resulted in the conversion of C-1 of glucose to CO_2 at a higher rate than C-4. Upon comparing the above patterns with those from two pseudomonads whose pathways of glucose catabolism were unknown, Pseudomonas aeruginosa and Pseudomonas KB1, it was concluded that glucose was catabolized via a combination of the E-D, pentose, and TCA cycle sequences in the latter two organisms. The use of C^{14}-specifically labeled gluconate as substrate to determine the equivalence of glucose and gluconate in organisms possessing a pentose-involving pathway resulted in patterns that resembled those obtained from Pseudomonas saccharophila growing on glucose. Thus gluconate appeared to be catabolized only via the E-D route in organisms in which glucose was degraded by both E-D and pentose pathways. Estimations of the participation of pentose-involving and E-D pathways based on cumulative $C^{14}O_2$ recoveries indicates that the E-D pathway was the major route of glucose catabolism in all of the pseudomonads examined.

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