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Respiration of *Bdellovibrio bacteriovorus* Strain 109J and Its Energy Substrates for Intraperiplasmic Growth

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Measurements of oxidation rates, respiratory quotients (RQ), and release of $^{14}CO_2$ from uniformly labeled substrates showed that glutamate, α-ketoglutarate, and synthetic and natural amino acid mixtures are oxidized by suspensions of *Bdellovibrio bacteriovorus* strain 109J. The oxidation of these substrates largely suppress the endogenous respiration of the *Bdellovibrio* cells and may or may not cause a small increase, 20 to 50%, in their rate of oxygen consumption. The failure of respired substrates to increase markedly the respiration rate of the *Bdellovibrio* cells over the endogenous value is discussed. Carbon from these substrates is incorporated into the *Bdellovibrio* cells during oxidation. Acetate is also oxidized, but its oxidation inhibits endogenous respiration by only about 40% and no acetate is assimilated. The RQ of the *Bdellovibrio* cells changes from a value characteristic of endogenous respiration to that characteristic of the oxidation of glutamate or of a balanced amino mixture very shortly after the attack of the *Bdellovibrio* cells on their prey, and the latter RQ is maintained during intraperiplasmic growth. Glutamate, or a mixture of amino acids in the external environment, contributes to the carbon dioxide produced by the *Bdellovibrio* cells growing intraperiplasmically. It is concluded from these data that amino acids, derived from the breakdown of the protein of the prey, serve as a major energy source during intraperiplasmic growth of *B. bacteriovorus* 108J. Insofar as they were tested, *B. bacteriovorus* strains 109D and A.3.12 were similar in respiration to strain 109J.

The bdellovibrios are unique among described bacteria in their potential to attack, penetrate, and grow in the intraperiplasmic space of other bacteria. Shortly after a bdellovibrio attacks its prey and before it initiates growth per se, the respiratory apparatus of the prey organism is rendered nonfunctional (7). It follows, therefore, that bdellovibrios must generate their own energy during intraperiplasmic growth. (The use of the term "prey" here and throughout this paper is deliberate. Published and unpublished data, which will be discussed in detail elsewhere, indicate to us that the relation between a bdellovibrio and the bacterium it grows on is more accurately described as a predator-prey rather than a parasite-host relationship.)

Little is known about the energy metabolism of the bdellovibrios. They are obligately aerobic organisms; oxygen is required for their motility (13), for attachment to their prey (15), for growth of the so-called host-independent (H-I) strains (9), and for intraperiplasmic development. Azide and cyanide inhibit endogenous respiration of *Bdellovibrio bacteriovorus* strain 6-5-S and this strain contains cytochromes, enzymes of the tricarboxylic acid cycle, reduced nicotinamide adenine dinucleotide oxidase, and low levels of some glycolytic enzymes (11). Similarly, cytochromes (9) and enzymes of the tricarboxylic acid cycle (8) were present in all H-I strains tested. Simpson and Robinson (11) suggested that strain 6-5-S obtains energy by oxidative phosphorylation during electron transport and by substrate-level phosphorylations of the glycolytic system.

The identity of the compounds respired, especially during intraperiplasmic development, remains completely obscure. Except for three cultures that were stimulated by gluta-

mate and α-ketoglutarate, no enhancement of growth was observed for any of nine H-I strains when any of 14 compounds were added to 0.2% yeast extract-0.2% peptone medium (9). Since gelatin is hydrolyzed and ammonia is produced from peptone by all of these strains, Seidler and Starr (9) suggested that bdellovibrio "is limited only to the utilization of complex mixtures of proteins, peptides and amino acids." However, since it is not known what was growth-limiting in the unsupplemented medium, the possibility remains that some or all of the compounds tested could be respired. Simpson and Robinson (11) showed that the respiration of suspensions of strain 6-5-S was stimulated by asparagine, glutamate, and "particularly by glutamine." As best one can estimate from the published plots, the latter compound increased respiration by only some 30%.

Rittenberg and Shilo (7) also found a 30% increase in respiration rate of strain 109J in the presence of yeast extract, peptone, or casein hydrolysate. No stimulation of respiration occurred in the presence of glucose, succinate, malate, or acetate, among other compounds tested. It was pointed out, however, that strain 109J has a high rate of endogenous respiration, six to seven times that of *Escherichia coli* on an equivalent protein basis. If bdellovibrio respires endogenous materials at a rate that nearly saturates its respiratory potential, then respiration of exogenously supplied substrates, measured only by oxygen uptake, could be masked by a corresponding decrease in the rate of oxidation of endogenous materials. In fact, as this paper reports, this is indeed the situation, and some of the compounds mentioned above are respired by free bdellovibrios (i.e., bdellovibrios not associated with prey organisms) and also during their intraperiplasmic growth.

MATERIALS AND METHODS

Organisms and growth procedures. *E. coli* strain ML35 (*lac I, lac Y*) served as the substrate organism. Cell suspensions were obtained from nutrient broth cultures grown at 30 C as described previously (7). *B. bacteriovorus* strains 109J, 109D, and A.3.12 were grown on *E. coli* cells growing in dilute nutrient broth (DNB; 10).

Uniformly labeled ^{14}C- *E. coli* cells were obtained from cultures grown on a glucose-salts medium: U-^{14}C-glucose (1 μCi), 0.6 g; $(NH_4)_2SO_4$, 0.3 g; 1.0 M K_2HPO_4, 3 ml; salts solution (3), 0.2 ml; and distilled water to a final volume of 100 ml. Prior to sterilization, the medium was adjusted to pH 7.4 with KOH.

Uniformly labeled ^{14}C-*E. coli* cells, for use in the preparation of uniformly labeled ^{14}C-*Bdellovibrio* cells, were grown as described, harvested by centrifugation, and resuspended in DNB medium at a concentration (10^9 to 2×10^9/ml) which permitted at most one doubling with the available nutrients. The cultures were then inoculated with about 2×10^7 *Bdellovibrio* cells per ml and shaken at 30 C until all *E. coli* cells had lysed. Final bdellovibrio titers were about 3×10^9 to 4×10^9/ml. The specific activity of the *Bdellovibrio* cells was about 70% of that of the starting *E. coli* cells. The small reduction in specific activity is a consequence of the limited growth of the *E. coli* cells in DNB.

All cell suspensions, except where noted, were prepared in 10^{-3} M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (pH 7.6) after washing twice in this buffer. Cell numbers were determined from turbidity measurements by reference to standard curves and also from viable-cell counts performed by direct plating of serially diluted samples on nutrient agar medium for *E. coli* or on double-layer DNB medium for *B. bacteriovorus* (15).

General design of experiments. The experimental protocols were similar to those employed previously (7). *E. coli* ML35 and *B. bacteriovorus* 109J were used, unless indicated otherwise. Suspensions of these organisms in HEPES buffer (10^{-3} M, pH 7.6) were mixed (zero time) to initiate experiments. Initial *E. coli* populations in the cultures ranged from 3×10^8 to 6×10^8 cells/ml. Between 1.4 and 2.0 *Bdellovibrio* cells per *E. coli* cell were used to ensure rapid attack of all *E. coli* cells present. This results in an essentially synchronous culture of intraperiplasmically growing *Bdellovibrio* cells. The cultures (40 to 60 ml) were shaken (230 to 280 rpm) at 30 C in 250-ml Erlenmeyer flasks. At appropriate time intervals, samples were removed for measurements of respiration rates or other parameters.

In experiments in which cultures were supplemented with a single organic compound, the final concentration of the exogenous compound was 5 μmol/ml. The amino acid mixture used as a supplement consisted of the L isomers of the following amino acids (mg/ml): glycine, 0.96; alanine, 1.7; serine, 1.4; threonine, 0.85; proline, 0.56; valine, 3.5; leucine, 2.6; isoleucine, 2.1; phenylalanine, 2.1; tyrosine, 2.16; sodium aspartate, 1.1; sodium glutamate, 1.1; lysine·HCl, 1.4; arginine·HCl, 2.6; and histidine·HCl, 0.16. The amino acids were dissolved in 10^{-3} M HEPES buffer and the pH of the solution was adjusted to 7.5 with KOH. The final concentrations of amino acids in the supplemented cultures were half of those above. In experiments in which ^{14}C-labeled amino acids were used, an identical amino acid mixture was used in which all of the amino acids were uniformly labeled with carbon-14 to yield approximately 0.1 μCi of total ^{14}C per ml of the mixture.

Respiration and manometry. Oxygen consumption by cell suspensions was measured with an oxygen electrode cell (Rank Bros., Cambridge, England) connected to a Sargent model SR recorder so adjusted that full-scale deflection was equal to the

uptake of 0.5 μmol of oxygen from the reaction mixture (2.0 ml). Oxygen uptake rates were measured before (unsupplemented) and after the addition of a substrate such as glucose or succinate (5 μmol/ml final concentration) to the suspension. Measurements were made at 30 C.

Respiration rates of cell suspensions were also measured by conventional Warburg techniques (14). For bdellovibrio suspensions, each Warburg vessel contained, unless indicated otherwise, 2×10^{10} cells, 10 μmol of substrate, 200 μmol of H_2SO_4 (side arm), 0.1 ml of 10% (wt/vol) KOH or 0.2 ml of hydroxide of Hyamine 10X (center well), and 10^{-3} M HEPES buffer (pH 7.6) to a final volume of 2.1 ml. The experiments were run at 30 C and terminated by the addition of the H_2SO_4 to the main flask.

The data obtained from respiratory measurements are expressed as Q_{O_2} (microliters of O_2 consumed per milligram [dry weight] of cells per hour) or respiratory quotient (RQ: moles of CO_2 released/mole of O_2 consumed) values. One milligram of dry weight is equivalent to about 10^{10} *Bdellovibrio* cells.

Radioactivity. All radioactivity measurements were made by scintillation counting. Each sample (0.1 to 1.0 ml) was placed into a vial containing 10 ml of a counting solution: toluene, 1,000 ml; Triton X-100, 500 ml; 2,5-diphenyloxazole, 8.0 g; and 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene, 0.5 g.

CO_2 was collected by two methods. For experiments done in 250-ml Erlenmeyer flasks, a sealed flask was constantly swept with CO_2-free air (60 ml/min), and the exiting air was passed through 10 ml of a trapping solution consisting of ethanol-ethanolamine (1:2, vol/vol). The trapping solution was replaced every 20 or 30 min, and a 1.0-ml sample was removed from each portion for radioactivity measurements. In such experiments, bound CO_2 was not released from the slightly alkaline cultures (pH 7.6), so radioactivity values for the CO_2 are somewhat low and for the supernatant fluid, after removing cells, are high. In Warburg experiments, CO_2 was trapped by hydroxide of Hyamine 10X in the center well, and all bound CO_2 was released from the culture fluid by H_2SO_4 at the end of the experiment. The trapping solution was quantitatively transferred via three 0.3-ml methanol washes to a scintillation vial containing the counting solution.

For determinations of the radioactivity in cellular material, samples (3 to 6 ml) were removed from the cultures. Cells were sedimented by centrifugation for 5 min at 27,000 × *g*, washed once, and resuspended to the original volume in 10^{-3} M HEPES buffer (pH 7.6). A 1.0-ml portion of each sample was added to a vial containing the counting fluid.

Glutamate assay. Glutamate concentrations were determined by the oxidative deamination of L-glutamate of α-ketoglutarate and ammonia by glutamate dehydrogenase (Type II, Sigma Chemical Co.). The reaction was followed spectrophotometrically by measuring the increase in optical density at 340 nm due to the formation of reduced pyridine nucleotide (16).

RESULTS

Effects of chloramphenicol and streptomycin on the respiration of Bdellovibrio growing intraperiplasmically. The effects of two inhibitors of protein synthesis, chloramphenicol and streptomycin, on the respiration rate of a population of bdellovibrio growing essentially synchronously on *E. coli* as their only source of nutrients was tested. Suspensions of these organisms in HEPES buffer were mixed and incubated for 90 min. By this time, all *E. coli* cells contained *Bdellovibrio* cells in their periplasmic space; the potential of the mixture to oxidize succinate, a measure of *E. coli* respiratory potential (7), had declined almost to zero; and the respiration rate of the system had started to increase (Fig. 1). At 90 min, chloramphenicol and streptomycin were added to separate portions of the culture. This prevents further development of the *Bdellovibrio* cells. The additions also inhibited the increase in respiration rate which normally occurs, and the rate declined somewhat with further incubation (Fig. 1). Thus, inhibition of *Bdellovibrio* development by these inhibitors concomitantly eliminates the increase in respiration rate. The results confirm our previous conclusion (7) that the increase normally observed results from bdellovibrio growth. The question remains, what is respired?

Effects of various compounds on the Q_{O_2} and RQ of free bdellovibrio suspensions. The Q_{O_2} of washed *Bdellovibrio* suspensions was measured in the presence of a variety of compounds. Such suspensions have a high endogenous Q_{O_2}, ranging between 14.8 and 17.3 μliters of oxygen used per mg (dry weight) of cells per h, and an RQ ranging between 0.80 and 0.89. None of the individual compounds tested caused large increases in the Q_{O_2} of *Bdellovibrio* suspensions (Table 1). At the most, individual compounds such as glutamate caused a 20 to 30% increase in respiration rate. Complex mixtures of protein hydrolysis products had a somewhat greater effect, stimulating respiration by about 50% on the average. None of the substrates caused the multifold increase in respiration rate typically observed when a respirable substrate is added to suspensions of common aerobic heterotrophic bacteria.

The variations in the endogenous respiration rate of independently prepared cell suspensions and in respiration rates obtained in multiple tests of a single substrate were such that it was difficult to conclude from the small changes in

Q_{O_2} alone which, if any, of the tested compounds were indeed respired. However, certain substrates caused an unequivocal and significant change in the RQ. With glutamate and the amino acid mixtures, for example, the RQ changed from the endogenous value of 0.86 to about 1.04 on the average. This change shows that a new class of compounds was being respired. The data could mean either that the exogenous substrate caused a shift in the type of endogenous reserve being used by the *Bdellovibrio* cells, or, more likely, that the exogenous substrate per se was being metabolized.

To permit unequivocal conclusions, *Bdellovibrio* suspensions were incubated with uniformly labeled ^{14}C compounds for a fixed period of time, and measurements were made of the radioactivity of the respired CO_2 as well as the total oxygen consumed, the CO_2 formed,

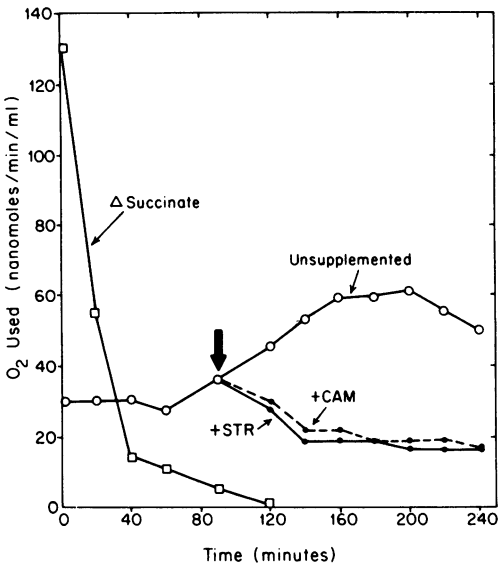

FIG. 1. *Effects of inhibitors of protein synthesis on the respiration of bdellovibrio growing intraperiplasmically. Individual cell suspensions were mixed to yield a culture containing 7.4 × 10⁸ bdellovibrio cells and 5.1 × 10⁸ E. coli cells per ml. At 90 min after mixing, the culture was divided into three portions. Two portions were supplemented (200 μg/ml, final concentration) with either chloramphenicol (CAM) or streptomycin (STR), and the remaining portion received no additions. Samples of the cultures were removed at intervals, and their rates of oxygen utilization before (unsupplemented) and after succinate addition were determined with an oxygen electrode. The Δ succinate plot is the respiration rate in the presence of succinate minus the unsupplemented respiration rate, i.e., the stimulation of respiration rate by succinate.*

TABLE 1. *Respiration of compounds by cell suspensions of B. bacteriovorus 109J*

Compound added	Q_{O_2}	RQ
None	16.8	0.86
Alanine	14.9	0.82
Aspartate	16.1	0.83
Glutamate	18.0	1.03
Glycine	14.6	0.70
Lysine	17.8	0.91
Tryptophan	14.9	0.87
N-acetyl-glucosamine	11.0	0.80
Glucosamine	10.3	0.81
Fructose	16.4	0.80
Glucose	18.2	0.94
Ribose	17.7	0.85
Pyruvate	13.9	0.80
Acetate	16.8	0.89
α-Ketoglutarate	21.4	1.11
Succinate	18.5	0.87
Oxalacetate	18.3	0.94
Adenine	14.1	0.87
Peptone	24.8	1.04
Casein hydrolysate	23.7	1.02

and the radioactivity incorporated into the cells (Table 2). The data show that significant quantities of acetate, glutamate, and the amino acid mixture were oxidized. For these substrates, approximately 15 to 20% of the initial radioactivity of the compounds was released as CO_2. With respect to the amino acid mixture, no single amino acids accounted for more than 5% of the initial radioactivity. Since 16% of that radioactivity was released as CO_2, it must be concluded that several amino acids in the mixture were oxidized. The data also show that appreciable quantities of glutamate carbon and amino acid carbon, but not acetate carbon, were assimilated by the *Bdellovibrio* cells.

From the radioactivity of the CO_2 formed and the nature and quantity of the substrate added, the moles of CO_2 released from each exogenous substrate were calculated (column 4, Table 2). If one assumes that respired substrates are completely oxidized to CO_2, water, and NH_3 (for nitrogen-containing compounds), then the "oxygen equivalents" required for CO_2 formation from substrate (column 5, Table 2) can be obtained by dividing the moles of CO_2 formed from substrate by the theoretical RQ of that compound. For an organism that obtained energy by electron transport to oxygen (see Discussion), the oxygen equivalents are the best measure of the value of a compound as an energy substrate. On this basis, among the individual compounds tested,

TABLE 2. *Utilization of uniformly labeled ^{14}C substrates by cell suspensions of B. bacteriovorus 109J*[a]

Compound added	Radioactivity recovered in		$^{14}CO_2$ formed (μmol)	O_2 equivalents (μmol)	Total O_2 used (μmol)	Total CO_2 formed (μmol)	Percent CO_2 formed from	
	CO_2[b]	Cells[b]					Substrate	*Bdellovibrio*
Acetate	22.0	1.2	4.4	4.4	10.8	10.4	42.4	57.6
Adenine	0.13	0.10	0.02	0.01	7.5	6.5	0.3	99.7
Alanine	1.80	0.54	0.54	0.54	8.0	6.4	8.4	91.6
Glutamate	17.4	28.0	8.8	7.93	9.9	10.3	88.8	11.2
Pyruvate	1.90	0.75	0.57	0.48	7.7	7.0	8.2	91.8
Tryptophan	0.78	0.78	0.86	0.90	8.0	6.8	12.6	87.4
Glucose	1.40	0.16	0.84	0.84	8.5	8.0	10.6	89.4
Amino acid mixture[c]	16.0	10.0			9.4	10.4		

[a] Each Warburg flask contained 2×10^{10} bdellovibrios and 10 μmol of uniformly labeled ^{14}C-substrate (11,000 to 173,000 counts per min per μmol). Incubation was at 30 C for 180 min.

[b] Percentage of initial counts per minute added.

[c] See Materials and Methods.

glutamate was the best substrate, acetate was about 50% as effective, and the other individual compounds were roughly one-fifth or less as effective. Since it is not known precisely what is oxidized in the amino acid mixture, a similar calculation cannot be made for it.

The difference between the total CO_2 produced and the $^{14}CO_2$ derived from the individual substrates shows the contribution of *Bdellovibrio* cell material to respiration in the presence of the exogenous compounds (column 9, Table 2). In the presence of glutamate, respiration of endogenous cell materials was almost completely suppressed. In the presence of acetate, endogenous materials accounted for about 60% of the respiratory CO_2, and in the presence of the other compounds tested, for 90% or more.

These results were confirmed by the reverse type of experiment in which the respiration of uniformly labeled ^{14}C-*Bdellovibrio* cells was studied in the absence and presence of unlabeled exogenous substrates. In the presence of each of the four substrates tested, glutamate, α-ketoglutarate, amino acid mixture, and acetate, oxygen consumption and carbon dioxide evolution increased, and the radioactivity of respired CO_2 decreased, all relative to the endogenous controls (Table 3). The carbon dioxide derived from cell material in the presence of the four substrates amounted to 24, 40, 37, and 49% of the total CO_2 evolved, respectively. The data for acetate and glutamate are in good agreement with the results of the previous experiment.

Respiration during intraperiplasmic growth of bdellovibrio on E. coli. Figure 2 shows cumulative oxygen utilization and carbon dioxide release as measured by the Warburg respirometer technique during growth of bdellovibrio on *E. coli* in buffer. Oxygen consumption by the separate population is also shown. Initially, carbon dioxide production was lower than oxygen utilization, but this relation was reversed as bdellovibrio development proceeded. The overall RQ from the start to the end of the development cycle was 1.03.

From the primary data, the rates of oxygen consumption and the RQ values for each 30-min interval during the bdellovibrio development cycle were calculated. A plot of the rates against time (Fig. 2) shows the typical oxygen utilization pattern already described (Fig. 1; see also 7). These calculations also show that

TABLE 3. *Effects of exogenous substrates on the endogenous respiration of uniformly labeled ^{14}C-B. bacteriovorus 109J*[a]

Experimental condition	Total O_2 used (μmol)	Total CO_2 formed (μmol)	$^{14}CO_2$ formed (μmol)	Percent $^{14}CO_2$ of total CO_2
Bdellovibrio	6.0	5.5	5.5	100
+ Glutamate	8.8	10.4	2.5	24
Bdellovibrio	8.7	7.7	7.7	100
+ α-Ketoglutarate	11.4	12.8	5.2	40
Bdellovibrio	6.4	55.8	5.8	100
+ Amino Acids	9.3	10.7	3.9	37
Bdellovibrio	8.0	7.4	7.4	100
+ Acetate	10.6	9.6	4.7	49

[a] Each Warburg flask contained 2×10^{10} uniformly labeled ^{14}C-bdellovibrios (11,000 counts/min) and 10 μmol of substrate, where indicated. Incubation at 30 C for 180 min.

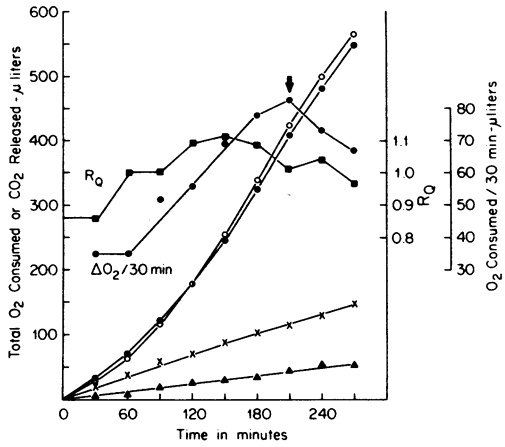

FIG. 2. *Respiratory patterns during intraperiplasmic growth of B. bacterivorus on E. coli. Each Warburg flask contained 10^{10} E. coli cells and 2 × 10^{10} bdellovibrio cells in tris(hydroxymethyl)aminomethane buffer. The curves show: cumulative O_2 uptake (●); cumulative CO_2 release (○); RQ/30-min interval (■); and oxygen consumed (ΔO_2) per 30-min interval (●). O_2 used by an equivalent number of bdellovibrio cells (×) or E. coli cells (▲) in buffer is also shown.*

between 30 and 60 min, even before the rate of oxygen consumption started to increase, the RQ had shifted from 0.86, the value characteristic of the endogenous respiration of free *Bdellovibrio* cells, to 1.0 to 1.1, a value characteristic of free *Bdellovibrio* cells respiring glutamate or amino acid mixtures (Fig. 2).

A similar experiment with uniformly labeled ^{14}C-*Bdellovibrio* cells showed that, during intraperiplasmic growth, respiration of endogenous materials is largely suppressed, as was true during glutamate oxidation by suspensions of free *Bdellovibrio* cells. A culture initially containing 11.4 × 10^9 *Bdellovibrio* cells (103,400 counts per min) and 7.8 × 10^9 *E. coli* cells per ml was incubated for 6.5 h, by which time lysis was complete. The progeny *Bdellovibrio* cells, collected by centrifugation, had 87% of the initial radioactivity and the supernatant fluid had some 7.5%. Thus, only about 5% of the initial bdellovibrio carbon was converted to CO_2 over the 6.5-h period. Some of the CO_2 could have been derived from the endogenous respiration of bdellovibrio cells that did not penetrate the *E. coli*.

In an experiment designed to determine whether exogenous substrates could be respired during intraperiplasmic growth, uniformly labeled ^{14}C-glutamate was added to a population of bdellovibrio and *E. coli* cells at the time of mixing the two suspensions. Radioactive CO_2 was detected at the earliest time sampled, 20 min after mixing, and its rate of release increased until the end of the experiment (Fig. 3). At 240 min, by which time essentially all *E. coli* cells had lysed, the CO_2 contained some 6% (equivalent to about 0.4 μmol of glutamate) of the initial radioactivity. This figure is somewhat low since bound CO_2 in the medium was not released (see Materials and Methods). The free *Bdellovibrio* cells, sedimented by centrifugation at 240 min, contained about 3% of the radioactivity of the initial glutamate (Fig. 3). In concordance with the above results, glutamate disappeared from the medium with time and its decrease paralleled the loss of radioactivity from the medium. Carbon dioxide release and uptake of radioactivity by the *Bdellovibrio* cells continued after 240 min, at which time lysis was complete. Very similar results were obtained when a radioactive amino acid mixture was substituted for glutamate in the same type of experiment.

The effect of exogenous substrates on the yield of *Bdellovibrio* cells growing intraperiplasmically was determined in an experiment in which uniformly labeled ^{14}C-*E. coli* cells were the substrate (Table 4). With glutamate or an amino acid mixture present, the final *Bdellovibrio* populations increased about 10% over the control, in which *E. coli* served as the only substrate. Concomitantly, there was an approximately 10% decrease in the radioactiv-

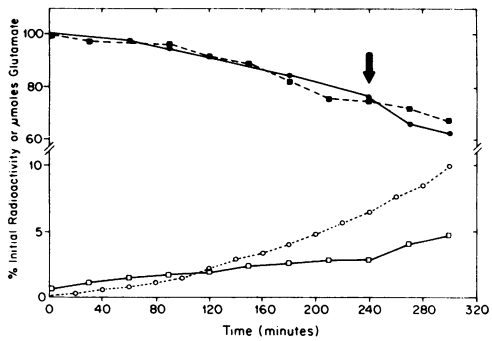

FIG. 3. *Utilization of exogenous glutamate during intraperiplasmic growth of B. bacteriovorus on E. coli. The mixture (80 ml) contained, per milliliter: 6.4 × 10^9 bdellovibrios, 4.2 × 10^9 E. coli cells, and 5 μmol of uniformly labeled ^{14}C-glutamate (74,130 counts/min). Samples (5 ml) were removed at intervals and centrifuged, and the radioactivity in the supernatant fluid, was also determined. Arrow indicated. The radioactivity in the released CO_2 (○), as well as the glutamate concentration (●) in the supernatant fluid was also determined. Arrow indicates time at which lysis of E. coli was complete.*

ity incorporated by the *Bdellovibrio* cells. Thus, the progeny *Bdellovibrio* cells in the presence of the exogenous substrates had a specific activity of approximately 20% less than the control. Since the starting *E. coli* cells were uniformly labeled with ^{14}C, these data show that the glutamate or the amino acid mixture provided some 20% of the total bdellovibrio carbon. This result is in agreement with the finding that 20% of bdellovibrio deoxyribonucleic acid is derived from exogenous substrates during its growth on *E. coli* in a dilute nutrient broth (4).

Effect of exogenous substrates on viability of free Bdellovibrio cells. When *Bdellovibrio* was grown on *E. coli* growing in DNB under conditions described by Varon and Shilo (15), complete lysis of the *E. coli* cells and maximal titers of *Bdellovibrio* were attained in about 20 h. If such cultures were incubated beyond the time of complete lysis, there was a brief stationary period followed by a rapid drop in *Bdellovibrio* titer (15). An almost immediate loss in viability was observed when *Bdellovibrio* suspensions were shaken in the lysate after a single developmental cycle on *E. coli* in buffer. Within 20 h of continued incubation in the lysate, *Bdellovibrio* titers dropped by over 90% (Table 5). Over this period of time, there was a 35% decrease in *Bdellovibrio* cell carbon as determined from the loss in radioactivity from the starting uniformly labeled *Bdellovibrio*.

The addition of glutamate or an amino acid mixture to the culture at the time of *E. coli* lysis preserved, in part, the viability of the *Bdellovibrio* cells with continued incubation (Table 5). The sparing effect was more pronounced with glutamate than with the amino acid mixture. When glutamate and amino acids were added together, essentially all *Bdellovibrio* cells remained viable over the 20-h period. The presence of glutamate or glutamate plus amino acids significantly reduced the amount of *Bdellovibrio* carbon oxidized to CO_2 during the "starvation" period. Direct analyses showed the disappearance of glutamate during incubation of the supplemented cell suspensions.

TABLE 4. *Effects of glutamate and amino acids on cell yields of B. bacteriovorus 109J and on its assimilation of E. coli carbon*[a]

Substrate	Radioactivity of progeny bdellovibrios (counts per min per ml)	Final bdellovibrio population (cells/ml)	Final specific activity (counts per min per 10^9 bdellovibrios)
E. coli	1,345	15×10^9	89.6
+ Glutamate	1,240	17.5×10^9	70.9
+ Amino acids	1,200	16×10^9	75.0
+ Glutamate + amino acids	1,244		

[a] The initial culture contained, per milliliter: 5.4×10^9 bdellovibrios, 3.7×10^9 uniformly labeled ^{14}C-*E. coli* cells (2,066 counts/min), and 5 μmol of glutamate or 0.5 ml of an amino acid mixture as indicated. Cultures were incubated at 30 C until complete lysis of *E. coli* cells.

Strains 109D and A.3.12. Insofar as they were tested, *B. bacteriovorus* strains 109D and A.3.12 were similar in respiration to strain 109J. Glutamate stimulated the respiration rates of these strains by approximately 40%. The RQ during endogenous respiration was 0.83 and 0.89 for strains 109D and A.3.12, respectively. The RQ during intraperiplasmic growth was 1.09 for strain 109D (one experiment) and 1.0 for strain A.3.12 (three experiments).

DISCUSSION

Our results show clearly that amino acids, derived from protein breakdown, serve as a major energy source during intraperiplasmic growth of bdellovibrio on its prey. This conclusion is supported by the following evidence: (i) glutamate and synthetic and natural amino acid mixtures are oxidized by suspensions of free *Bdellovibrio* cells and the oxidation of these compounds largely inhibits the endogenous respiration of *Bdellovibrio* cell material; (ii) the combination of glutamate and a balanced amino acid mixture prolongs the viability of free *Bdellovibrio* cells; (iii) the RQ of the *Bdellovibrio* cells changes from a value characteristic of its endogenous respiration to that characteristic of the oxidation of glutamate or of a balanced amino acid mixture very shortly after bdellovibrio attack on its prey, and the latter RQ is maintained during intraperiplasmic growth; and (iv) glutamate or a mixture of amino acids in the external environment contributes to the carbon dioxide produced by the *Bdellovibrio* cells growing intraperiplasmically.

It is not excluded that other components of the prey's cell material beside proteins also supply respirable substances for the bdellovibrio. It is unlikely that polysaccharides are involved in energy metabolism since *Bdellovibrio* suspensions do not release signifi-

cant quantities of CO_2 from either glucose or ribose. Further, direct analyses for the glycolytic enzymes in our strains (see also 8, 11, 12) show that they are present at very low levels in the *Bdellovibrio* cells. The lipids of the attacked cell could be energy substrates, assuming that their fatty acid components could be oxidized to acetate, since the latter compound is oxidized by bdellovibrio. No information is yet available on this point.

It should be noted that, although acetate is oxidized, it is not used as effectively as glutamate nor is it incorporated by the *Bdellovibrio* cells to any appreciable extent. This suggests that the *Bdellovibrio* cells do not store poly-β-hydroxybutyrate, at least under the conditions we have examined. This is in contrast to other bacteria which convert acetate to poly-β-hydroxybutyrate in environments not permitting growth (2, 5). The rapid loss of viability of free *Bdellovibrio* cells in the absence of an exogenous energy source further suggests that these organisms accumulate very little in the way of special reserve materials during intraperiplasmic growth.

One interesting facet of bdellovibrio respiration is how little a respirable substrate stimulates its Q_{O_2}. This is not only true of free *Bdellovibrio* cells, for which, at the most, we have observed a 50% increase in the rate of oxygen utilization of *Bdellovibrio* suspensions, but also holds for *Bdellovibrio* cells growing intraperiplasmically. At the end of a development cycle, as free *Bdellovibrio* cells are being released and the RQ is changing from that characteristic of growth (1.05) to the endogenous value (0.86), the rate of oxygen uptake drops some 30 to 50% (see Fig. 1 and 2). This change is of about the same magnitude, but in the opposite direction, as that observed when free *Bdellovibrio* cells are provided with glutamate or protein hydrolysis products.

Compounds such as acetate or α-ketoglutarate usually do not increase oxygen uptake by free *Bdellovibrio* cells and on occasion may even depress the Q_{O_2} of a particular *Bdellovibrio* suspension. Yet, studies with radioactive substrates or radioactive cells show unequivocally that these compounds are oxidized. One minor consequence of this situation is that oxygen uptake data alone are not adequate to decide what is or is not oxidized by the *Bdellovibrio* cells, and any conclusions in the literature based on this technique alone must be suspect.

If bdellovibrio oxidizes a compound without an appreciable increase in Q_{O_2}, it must do so by shutting off part or all of its endogenous respiration. As our data show, this indeed occurs. During glutamate oxidation or during intraperiplasmic growth, there is an almost complete inhibition of the oxidation of *Bdellovibrio* cellular material; during acetate oxidation, the endogenous respiration is inhibited about 50%. The effects of exogenous substrates on endogenous metabolism have been extensively studied (1), and it is known (depending on the substrate, the organism, how it was grown, and other factors) that the oxidation of an exogenous compound may completely or partially inhibit or have no effect at all on endogenous respiration. For example, Ribbons and Dawes (6) found that glucose completely inhibited the endogenous utilization of cell carbohydrate in *E. coli* pregrown with glucose-ammonium salts, whereas succinate or acetate had no effect at all on the rate of utilization of this endogenous reserve. *Bdellovibrio*, however, appears unique among bacteria so far studied in showing almost complete suppression of endogenous respiration by an exogenous substrate without a sig-

TABLE 5. *Effects of glutamate and amino acids on "starving" B. bacteriovorous 109J*[a]

Addition	Radioactivity of bdellovibrios at 20 h[b]	Loss of radioactivity (%)	Bdellovibrio titer[c] at 20 h	Survival (%)	Glutamate[d]	
					0 h	20 h
None	47,800	37.6	5×10^8	2.2	0.01	0.002
Glutamate	58,900	23.3	110×10^8	48.0	5.00	2.70
Amino acids	48,900	36.3	24×10^8	10.4	0.80	0.40
Glutamate + amino acids	60,980	20.5	220×10^8	96.0	5.70	3.60

[a] The initial culture contained, per milliliter, 7.4×10^9 bdellovibrios and 5×10^9 uniformly labeled ^{14}C-*E. coli* cells (130,200 counts/min). At complete lysis (0 h), the resulting bdellovibrio culture was divided into four portions and additions of glutamate (5 μmol/ml) or of an amino acid mixture (0.5 ml/ml) were made as indicated. Incubation was continued for 20 h at 30 C.

[b] Expressed as counts per minute per milliliter. The radioactivity at 0 h was 76,600 counts per min per ml.

[c] Cells per milliliter of suspension. The titer at 0 h was 230×10^8 cells/ml.

[d] Expressed as micromoles per milliliter of cell suspension.

nificantly increased Q_{O2}.

Although the data are as yet fragmentary, it appears that *Bdellovibrio* cells possess a complete tricarboxylic acid cycle and an electron-transport chain. Simpson and Robinson (11) have shown that *B. bacteriovorus* strain 6-5-S contains the enzymes of the tricarboxylic acid cycle as well as cytochromes. Seidler, Mandel, and Baptist (8) have also demonstrated tricarboxylic acid cycle enzymes in H-I strains, and we have shown their presence in *B. bacteriovorus* strains 109J and 109D (*unpublished data*). It is therefore a reasonable assumption that *Bdellovibrio* cells generate adenosine triphosphate (ATP) primarily by oxidative phosphorylation during electron transport. Substrate-level phosphorylation would play a minor role in energy generation during intraperiplasmic growth if, as our data indicate, amino acids are the significant energy source. One possible explanation, therefore, for oxidation of an exogenous substrate without change in Q_{O_2} is that the endogenous respiration of *Bdellovibrio* essentially saturates the functional capacity of either its tricarboxylic acid cycle or its electron-transport chain. Under these circumstances, a shift from an endogenous to an exogenous energy substrate would have little or no effect on the Q_{O_2}. Alternatively, the respiration rate could be closely regulated by ATP pool levels, the energy charge, or other precursors or products of energy metabolism. As yet, there are no experimental data to support one of these or possibly other alternatives. Regardless of what limits the respiration rate, the fact that the endogenous rate and the rate during intraperiplasmic growth are very similar suggests that the energy requirements for growth are unusually low.

Since we have examined only *B. bacteriovorus* strain 109J in any detail, our conclusions must be limited to this particular strain. Preliminary experiments with strain 109D (guanine plus cytosine, 50 mol %) and strain A.3.12 (guanine plus cytosine, 42.9 mol %) suggest, however, that these strains are very similar to 109J in their respiration.

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