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Ribonucleic Acid Destruction and Synthesis During Intraperiplasmic Growth of *Bdellovibrio bacteriovorus*

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During growth of *Bdellovibrio bacteriovorus* on [2-^{14}C]uracil-labeled *Escherichia coli* approximately 50% of the radioactivity is incorporated by the bdellovibrio and most of the remainder is released as free nucleic acid bases. Kinetic studies showed that 50 and 30*S* ribosomal particles and 23 and 16*S* ribosomal ribonucleic acid (RNA) of *E. coli* are almost completely degraded by the first 90 min in a 210- to 240-min bdellovibrio developmental cycle. Synthesis of bdellovibrio ribosomal RNA was first detected after 90 min. The specific activity and the ratio of radioactivity in the bases of the synthesized bdellovibrio RNA were essentially the same as those of the substrate *E. coli*. The total radioactivity of the bdellovibrio deoxyribonucleic acid (DNA) exceeded that in the DNA of the substrate *E. coli* cell, and the ratio of radioactivity of cytosine to thymine residues differed. Intraperiplasmic growth of *B. bacteriovorus* in the presence of added nucleoside monophosphates (singly or in combination) significantly decreased the uptake of radioactivity from [2-^{14}C]uracil-labeled *E. coli*; nucleosides or nucleic acid bases did not. It is concluded that the RNA of the substrate cell, in the form of nucleoside monophosphates, is the major or exclusive precursor of the bdellovibrio RNA and also serves as a precursor for some of the bdellovibrio DNA.

Bdellovibrio bacteriovorus is unique among bacteria in having the ability to grow in the periplasmic region of any of a number of diverse gram-negative bacteria (29, 31). The bdellovibrio growing intraperiplasmically needs no additional nutrients beyond those available from the substrate organism (23, 27, 35). These are used in preference to exogenous compounds even in environments containing complex mixtures of organic nutrients (10, 16, 22).

Intraperiplasmic growth is highly efficient. Somewhat over 50% of the cell carbon of the substrate organism is converted into bdellovibrio cell material (21). This conversion proceeds with minimum energy expenditure and has a Y_{ATP} (grams of cell material formed per mole of adenosine 5′-triphosphate produced [1]) of about 26 (21). This value approximates the maximum theoretical value (6, 9, 32) for an organism expending adenosine 5′-triphosphate only for polymerization of monomers into macromolecules.

Studies on specific classes of compounds have shown that fatty acids of the bdellovibrio are derived almost exclusively and with little or no alteration from the lipids of the substrate organism (13). Likewise, much of the deoxyribonucleic acid (DNA) of the bdellovibrio is made from deoxyribonucleoside monophosphates obtained by a regulated breakdown of substrate cell DNA (16, 22). No quantitative data have been reported on the extent of transfer of amino acids from the proteins of the substrate organism to those of the bdellovibrio, although it is known from labeling experiments (10, 21) that such transfer occurs. It has been shown that amino acids of the substrate organism serve as a major energy source for intraperiplasmic growth of the bdellovibrio (10). This paper is concerned with the ribonucleic acid (RNA) of the substrate cells. We will report on its degradation and reuse by the bdellovibrio for nucleic acid synthesis.

MATERIALS AND METHODS

Organisms and growth procedures. *B. bacteriovorus* 109J was the experimental organism and *Escherichia coli* ML 35 (*lac* I, *lac* Y) served as its substrate. Bdellovibrios used as inocula were cultured on glucose-grown *E. coli* cells. To obtain radioactive bdellovibrios, appropriately labeled *E. coli* were used as the substrate.

E. coli was grown at 37 C in shaken glucose salts medium (11). Unlabeled *E. coli* were harvested after

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overnight growth. Labeled *E. coli* were obtained by diluting overnight cultures to a cell concentration of 10^8 to 1.5×10^8/ml. The diluted cultures were incubated for 1 h (about 3×10^8 cells/ml), the appropriate compound for radioactive labeling was added, and incubation was continued for 2 to 2.5 h (10^9 to 1.5×10^9 cells/ml). Either (i) [2-^{14}C]uracil, 0.1 to 0.2 μCi/ml, or (ii) [5-^{3}H]uracil, 0.3 to 0.4 μCi/ml, at a final concentration of 9×10^{-6} M was used for labeling.

All bdellovibrio and *E. coli* cultures were harvested by centrifugation. Cell suspensions were made in 5×10^{-3} M *N*-2-hydroxyethyl-piperazine-*N*′-2′-ethanesulfonic acid buffer, pH 7.8, containing $MgCl_2$ and $CaCl_2$ after two washes in this buffer. The salts were at 10^{-4} M in single cycle cultures and at 10^{-3} M in multicycle cultures. Cell numbers in the suspension were standardized by turbidity measurements and reference to standard curves based on plaque counts or plate counts (23, 34). All cell suspensions were used immediately after preparation.

Growth experiments. *E. coli* cells served as the sole source of all nutrients except in those experiments in which specific RNA precursors were added. Generally, 3×10^9 to 6×10^9 *E. coli* cells/ml of buffer were used with input ratios (*B. bacteriovorus* to *E. coli* cells) of 1.4 to 2.0:1 for single cycle cultures and 2.5×10^{-3} to 5×10^{-3}:1 for multicycle cultures. Relatively synchronous attack on the substrate cells and growth of the bdellovibrios occurred in the single cycle cultures, which were fully developed in 210 to 240 min with shaking at 30 C. An incubation time of 14 to 16 h was required for complete lysis of the *E. coli* cells in multicycle cultures. Bdelloplasts (the *E. coli* cell containing an intraperiplasmic bdellovibrio) or bdellovibrios were separated from the culture fluid by centrifugation during or at the end of growth. Cells were washed twice with buffer before analyses.

Fractionation of cells. Cells were fractionated into their major components by sequential extractions. The washed cell pellet (5×10^9 to 25×10^9 *E. coli* cells or 10^{10} to 3×10^{10} *B. bacteriovorus* cells) was resuspended in 5 ml of cold 0.2 M $HClO_4$. The suspension was held in ice for 20 min and then centrifuged (8,800 $\times$ *g*, 5 min, 0 C). The pellet was resuspended in 4.0 ml of 0.3 M KOH, incubated 6 to 14 h at 37 C, and cooled to 0 C. Then 2.0 ml of cold 2 M trichloroacetic acid was added, and the suspension was held in ice for 30 min and centrifuged (22,000 $\times$ *g*, 20 min, 4 C). The pellet was washed by suspending it in 4 ml of cold 0.2 M trichloroacetic acid and centrifuging as above. The supernatant liquid was combined with the previous one to yield the RNA fraction. The pellet was then resuspended in 2.5 ml of 0.5 M $HClO_4$, incubated at 70 C for 20 min, cooled in ice, and centrifuged as above. The extraction was repeated, and the supernatant fluids were combined to yield the DNA fraction. The residue (protein) was resuspended in 2.5 ml of 0.1 M KOH, incubated at 70 C for 10 min, and cooled to 22 C, and 2.5 ml of distilled water was added.

Preparation of ribosomes. The cell pellet (3.8×10^{10} cells) was suspended in 0.4 ml of 25% (wt/vol) sucrose in 10^{-2} M tris(hydroxymethyl)aminomethane (Tris) buffer (pH 8.1). The cells were lysed by the lysozyme-deoxycholate-Brij 58 method of Godson (8). A 0.3-ml sample of the lysate was layered onto a 12-ml linear sucrose gradient (15 to 30% [wt/vol] sucrose containing 5×10^{-3} M Tris, 10^{-2} M $MgSO_4$, 6×10^{-2} M KCl [pH 7.2]) and centrifuged (Spinco SW41 rotor, 37,000 rpm, 4 h, 4 C). The absorption profile (260 nm) of each gradient was continuously recorded by pumping the solution from the bottom of the gradient through a flow cell mounted in either a Cary or Gilford spectrophotometer. Fractions (0.2 or 0.4 ml) were collected from the cell outflow in scintillation vials for determination of radioactivity and/or chemical analysis.

Analysis of RNA. RNA was purified from cells using modifications of known procedures. The cell pellet (3×10^{11} to 12×10^{11} *B. bacteriovorus* or 10^{11} to 6×10^{11} *E. coli*) was suspended in 2.5 ml of 25% (wt/vol) sucrose in 10^{-2} M Tris-2×10^{-2} M ethylenediaminetetraacetic acid (EDTA) (pH 7.6) and then was subjected to three rapid freeze-thaw treatments. The sample was warmed to 37 C, 0.2 ml of lysozyme solution (10 mg/ml) was added, and, after 10 min of incubation, the sample was subjected to two additional freeze-thaw treatments. Approximately 100 μg of deoxyribonuclease I was added, the sample was incubated (37 C, 10 min), and then chloroform (10 ml) was added and the mixture was swirled for 5 min at room temperature to yield an emulsion. After centrifugation (12,000 $\times$ *g*, 10 min, 10 C), the lower, lipid-containing chloroform layer was removed, 4.0 ml of extraction buffer (0.05 M sodium acetate-10^{-2} M EDTA-1% [wt/vol] sodium dodecyl sulfate [pH 5.1]) and 0.3 ml of 10% (wt/vol) macaloid solution were added. The mixture was swirled for 5 min, and 7.0 ml of phenol saturated with extraction buffer was added. After gentle shaking for 30 min (22 C), the mixture was centrifuged (22,000 $\times$ *g*, 30 min, 20 C), most of the upper aqueous layer was removed, and the extraction was repeated twice more. To the combined upper aqueous layers, one-tenth volume of 2 M sodium acetate (pH 5.1) and 2 volumes of cold (−30 C) ethanol were added, and the samples were stored overnight at −30 C. The precipitated RNA was collected by centrifugation (22,000 $\times$ *g*, 30 min, 0 C), dissolved in 3 ml of 0.2 M sodium acetate-2×10^{-3} M EDTA (pH 5.1), and precipitated with ethanol as before. Contaminating DNA and protein were removed from the RNA by dissolving the RNA precipitate in 3 ml of 5×10^{-2} M Tris-5×10^{-3} M $MgCl_2$ (pH 7.6) and incubating with 50 μg of deoxyribonuclease for 20 min at 37 C. Then, 0.2 ml of 0.5 M EDTA (pH 8.0) was added, and the mixture was extracted and precipitated from ethanol as described above. The purified RNA had a 260–280-nm optical density ratio of 1.90 or greater.

RNA samples were analyzed by sucrose gradient centrifugation. A sample containing 400 to 1,500 μg of RNA was layered onto a 10 to 30% (wt/vol) or 15 to 30% (wt/vol) linear sucrose gradient in 0.2 M sodium acetate-2×10^{-3} M EDTA (pH 5.1) and centrifuged (SW41 rotor, 37,000, 14 or 16 h, 5 C). For denatured RNA, the sample was heated at 80 C for 2.5 min, quickly chilled in ice, and then placed onto the

gradient. The absorption profiles were recorded, and samples were collected as described above for ribosomal gradients.

DNA analysis. The DNA fractions were purified using the same procedures employed for RNA with the following substitutions: ribonuclease (RNase) A for deoxyribonuclease I; 0.1 M Tris-10^{-2} M EDTA-1% (wt/vol) sodium dodecyl sulfate buffer (pH 9.0) for extraction buffer; 2 M Tris-10^{-2} M EDTA (pH 9.0) for 2 M sodium acetate (pH 5.1); and 0.2 M Tris-10^{-3} M EDTA (pH 9.0) for 0.2 M sodium acetate-2 $\times$ 10^{-3} M EDTA (pH 5.1).

Degradation of nucleic acids. The purified nucleic acid was dissolved in 0.05 M Tris-0.01 M NaCl (pH 8.7) to a concentration of 1,200 to 1,350 μg/ml. The following mixture was made: 0.2 ml of nucleic acid sample, 0.05 ml of 1 M $MgCl_2$, 0.10 ml of snake venom phosphodiesterase I (1 U/ml), and 0.55 ml of buffer (0.5 M Tris-0.01 M NaCl, pH 8.7). After 3 h at 37 C, another 0.1 ml of phosphodiesterase solution was added, and incubation was continued for another 12 h. For DNA samples, a pretreatment for 45 min with 15 μg of deoxyribonuclease I was done prior to phosphodiesterase digestion. The final mixtures obtained after phosphodiesterase digestion were lyophilized to dryness and reconstituted with 200 μl of distilled water.

Thin-layer chromatography. The procedures outlined by Randerath and Randerath (20) were used for thin-layer chromatography of nucleic acid components. The deoxyribo- and ribonucleoside monophosphates were separated by two-dimensional chromatography on plates covered with polyethyleneimine-impregnated cellulose. The first dimension developer was 1.0 M acetic acid-3.0 M LiCl (9:1, wt/vol) and the second was borate-ethylene glycol (6 g of $Na_2B_4O_7 \cdot 10H_2O$, 3 g of H_3BO_3, 25 ml of ethylene glycol, and 70 ml of distilled water). Nucleic acid bases and ribonucleosides were separated using thin-layer cellulose plates. The first dimension developer was CH_3OH-HCl-H_2O (70:20:10, vol/vol) and the second was *N*-butanol-CH_3OH-H_2O-NH_3 (60:20:20:1, vol/vol/vol/vol). The separated compounds were located with ultraviolet light. The visualized spots were cut out into square pieces which were placed into vials (cellulose side up), and the radioactivity was determined by scintillation counting.

Polyacrylamide gel electrophoresis. RNA samples were separated into their varying molecular weight components by polyacrylamide gel electrophoresis using slightly modified accepted procedures (14, 17, 18). To determine the distribution of radioactivity in the RNA bands within the gel slabs the desired portion was cut out of the dried gel and placed into a scintillation vial containing 1.0 ml of 10% (wt/vol) piperazine, and the RNA was hydrolyzed by 12-h incubation of the vial at 45 C. The radioactivity was then measured after the addition of the scintillation fluid.

The molecular weights of unknown RNA species appearing as bands in the gels were estimated from their migration distances by using the procedures of Peacock and Dingman (18). Known *E. coli* RNA species were used as standards; the species, molecular weight, and migration distances were, respectively: 23*S*, 1.1 $\times$ 10^6, 1.5 $\pm$ 0.5 mm; 16*S*, 5.6 $\times$ 10^5, 12 $\pm$ 1.0 mm; 5*S*, 3.5 $\times$ 10^4, 56 $\pm$ 1.0 mm; and 4*S*, 2.6 $\times$ 10^4, 62 $\pm$ 1.5 mm.

Chemical assays. RNA was determined by the orcinol method (26) and DNA by the diphenylamine method (2) using yeast RNA and salmon sperm DNA as standards. Orthophosphate-P was determined by direct analysis and total phosphorus after ashing the sample (3), and the difference was taken as organic phosphorus. Protein was determined by the method of Lowry et al. (15).

Radioactivity and miscellany. All radioactivity measurements were made by scintillation counting using PCS solubilizer fluid (Amersham-Searle, Arlington Heights, Ill.) or Triton fluid as described previously (10). Oxygen consumption of cell suspensions was measured using an oxygen electrode (10). All biochemicals and reagents used were reagent or biological grade, and enzymes were of electrophoretically pure grade.

RESULTS

Release of radioactivity during growth of B. bacteriovorus on [2-^{14}C]uracil-labeled E. coli. Figure 1 shows the distribution of radioactivity, initially in the substrate organism, between the culture fluid and sedimentable material (bdelloplasts and cells) during a single cycle culture of *B. bacteriovorus* on *E. coli*. As an internal control, the respiration rate of the

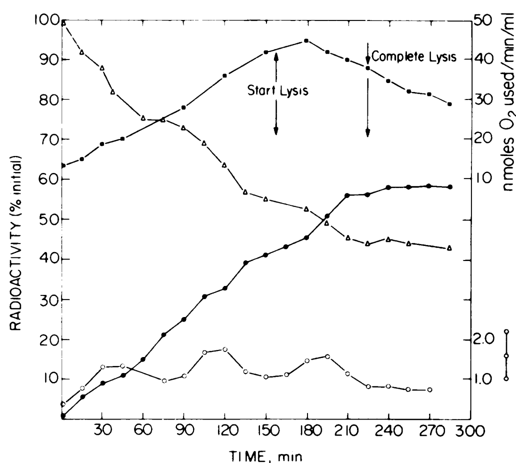

FIG. 1. *Distribution of radioactivity and respiration rate during intraperiplasmic growth of B. bacteriovorus on [2-^{14}C]uracil-labeled E. coli. The initial culture contained 7.6 $\times$ 10^9 B. bacteriovorus and 3.6 $\times$ 10^9 [2-^{14}C]uracil-labeled E. coli (260,000 counts/min per ml) cells/ml. Samples were removed from the culture for measurements of oxygen consumption (■), radioactivity in the cell pellet (Δ), radioactivity in the culture fluid after centrifugation (●), and radioactivity extractable from the pellet by cold perchlorate (O; scale on right).*

culture is also shown. The oxygen uptake data correspond closely to that previously reported (10, 23) and show that a normal, relatively synchronous development had occurred.

A rapid, almost linear, loss of radioactivity from the substrate cells into the culture fluid began almost immediately after starting the culture (Fig. 1). The rate of release decreased somewhat around 135 min and ceased after about 210 min. By this time more than 90% of the bdelloplasts had lysed. At the end of culture development approximately one-half the initial radioactivity was in the progeny bdellovibrios and the remainder was in the culture fluid. Direct measurement on parallel cultures showed that less than 0.2% of the initial radioactivity was released as CO_2. Of the sedimentable radioactivity, less than 2% was soluble in cold trichloroacetic acid or $HClO_4$ at any stage of culture development (Fig. 1).

Bdellovibrios were removed from cultures at the end of growth by centrifugation, and the culture fluids were analyzed. Essentially none of the radioactivity was sedimented when these culture fluids were made 0.5 M in trichloroacetic acid or $HClO_4$, and centrifuged (27,000 × *g*, 20 min, 5 C). About 90 to 95% of the radioactivity was lost when culture fluids were dialyzed against distilled water. No diphenylamine-positive material and only small amounts (ca. 0.2 μmol/ml) of orcinol-positive material (ribose) were detected. Substantial quantities of phosphorous were present (ca. 7.0 μmol/ml), of which only 15% of the total was organically bound. Thin-layer cellulose chromatography showed that most (65 to 75%) of the total radioactivity in the culture fluid migrated as free nucleic acid bases (Table 1). Uracil and uridine contained 90 to 95% of the radioactivity associated with the base and nucleoside spots, respectively. However, cytidine monophosphate accounted for 95% of the radioactivity in the nucleotide spots. The small amount of polynucleotide material detected was that which remained near or at the origin during chromatography, and its nature was not investigated further.

TABLE 1. *Nucleic acid derivatives in culture fluid after growth of B. bacteriovorus on [2-¹⁴C]uracil-labeled E. coli*[a]

Derivative	Radioactivity (counts/min/sample)[b]	
	I	II
Cytosine	225	659
Thymine	1,556	1,989
Uracil	16,441	33,000
Cytidine	65	95
Thymidine	71	93
Uridine	1,335	1,580
Cytidine 5'-monophosphate	3,545	3,998
Uridine 5'-monophosphate	79	205
Polynucleotide material	4,935	6,515

[a] Culture fluids obtained from a single cycle (I) or a multicycle (II) culture at the end of growth were concentrated 15:1, 10-μl samples (28, 970 counts/min, I; 44,980 counts/min II) were chromatographed on cellulose, and the radioactivity of the individual spots was determined. The initial culture contained (cells per milliliter): I, 7 × 10^9 *B. bacteriovorus* and 4 × 10^9 *E. coli* (460,000 counts/min); II, 2 × 10^7 *B. bacteriovorus* and 4 × 10^9 *E. coli* (600,000 counts/min).

[b] Only radioactivity levels slightly above background levels were detected in the vicinity of purine derivatives on the chromatograms.

Ribosome degradation. Figure 2 shows a series of 260-nm absorption profiles of ribosomal particles sedimented in sucrose gradients. The samples were removed over the first 60 min of a typical single cycle *B. bacteriovorus* culture growing on *E. coli*. By 20 min the 70*S* peak had decreased by about 50%, and by 60 min only a small quantity of these particles remained. The 50 and 30*S* peaks decreased more slowly and at 60 min were at about one-third of their initial values. The 60-min profile resembled qualitatively and quantitatively the profile obtained from an equivalent-sized sample of the *B. bacteriovorus* suspension used as the inoculum. Although the ratio of 70*S* to 50*S* or 30*S* material in the 60-min profile is low, we have consistently obtained such profiles from *B. bacteriovorus* in these and previous studies (11). It may be that bdellovibrio 70*S* ribosomes are less stable than *E. coli* 70*S* ribosomes under our preparative conditions, but we have not investigated this phenomenon. Profiles prepared from samples taken between 15 and 90 min frequently showed shoulders on the major peaks and were slightly skewed toward a lighter density, indicating the presence of partially degraded ribosomes. The data show that a rapid degradation of the ribosomes of the substrate cells occurs.

To confirm this interpretation, bdellovibrios were labeled by growth on [5-³H]uracil-labeled *E. coli*, and these bdellovibrios were then grown for a single cycle on [2-¹⁴C]uracil-labeled *E. coli*. The ¹⁴C and ³H radioactivities were distributed throughout the 0-min sucrose gradient in parallel with 260-nm absorption. With time there was a loss of ¹⁴C from the ribosomal preparations, and by 80 to 90 min less than 10% of the initial ¹⁴C radioactivity was present in the ribosomal bands. In contrast, the total ³H

radioactivity in the ribosomal bands remained fairly constant throughout the development cycle. Thus, it was clear that the ribosomes of substrate cells were degraded, whereas the bdellovibrio ribosomal particles remained intact. After 100 to 120 min, ^{14}C radioactivity began to reappear in the ribosomes concomitantly with increases in the absorption of the 50 and 30*S* peaks.

When chloramphenicol (200 μg/ml) or rifampin (10 to 30 μg/ml) was added to cultures at 0 min, no observable degradation of ribosomes occurred. The addition of these compounds at 20 to 30 min, when most of the substrate cells were bdelloplasts, significantly reduced further ribosome degradation.

Degradation and synthesis of ribosomal RNA. RNA was isolated from serial samples taken from a single cycle *B. bacteriovorus* culture growing on [2-^{14}C]uracil-labeled *E. coli* and centrifuged on sucrose gradients. The profiles (Fig. 3A-E) of 260-nm absorption and radioactivity of the gradients changed with time in a manner consistent with the changes in ribosomal profiles already described. The kinetics of the changes are shown in Fig. 3F as a plot of areas under the major peaks versus time. At 0 min the absorption profile showed three distinct peaks characteristic of 23*S*, 16*S*, and 4 to 5*S* RNA, and the radioactivity was distributed correspondingly (Fig. 3A). The 45-min profiles (Fig. 3B) showed four significant changes which were detectable in samples taken as early as 15 to 20 min: (i) a substantial decrease in absorption and radioactivity of the 32 *S* peak; (ii) the development of a shoulder and broadening of the base area of the 16*S* peak; (iii) a shifting of 23 and 16*S* regions toward lighter densities; and (iv) an increase in absorption and radioactivity in the 4 to 5*S* region. These changes were magnified with time, and maximum effects were observed between 75 and 90 min (Fig. 3C, F), by which time the 23 and 16*S* regions retained only about 10% of their initial radioactivity.

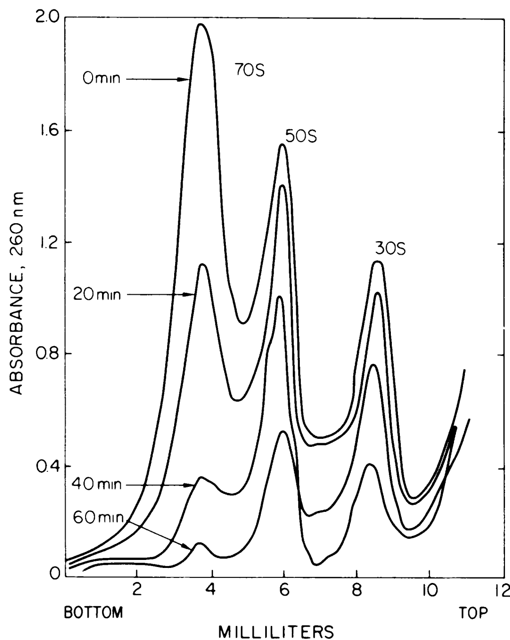

FIG. 2. *Degradation of ribosomal particles during intraperiplasmic growth of B. bacteriovorus on E. coli. The initial culture contained 6 × 10⁹ B. bacteriovorus and 4 × 10⁹ E. coli cells/ml. At the indicated time, cells from a 5-ml sample of the culture were harvested by centrifugation and lysed, and the ribosomal particles were separated by sucrose gradient centrifugation.*

After 90 min the patterns of change were reversed (Fig. 3D–F). The quantity and radioactivity of the slow sedimenting (4 to 5*S*) material decreased with concomitant increases in the 16 and 32*S* components. The peaks sharpened and narrowed, and the maxima shifted back toward higher densities. At 210 min when most bdelloplasts had lysed and bdellovibrio development was complete, the absorption profile and radioactivity distribution were similar to what was observed in the 0-time sample.

The above results show that the bulk of the *E. coli* ribosomal RNA was degraded to at least 4*S*-RNA-sized fragments during the first 90 min of the developmental cycle. Most synthesis of bdellovibrio ribosomal RNA followed over the next 90 min or so. The flow of radioactivity showed that the products of degradation of the RNA of the substrate cells were incorporated into newly synthesized bdellovibrio RNA.

Characterization of degraded RNA. The appearance of shoulders and broadening of the peak bases in the sucrose gradient absorption profiles of native RNA (Fig. 3) suggested the production of partially degraded RNA products of varying sizes. These products could include fragments produced by single-strand breaks in the double-stranded helical region of the RNA, which sedimented as double-stranded forms due to the remaining base pairing. To detect such products, RNA obtained at various times during a single cycle culture was converted to single-stranded forms by heat denaturation and centrifuged in sucrose gradients. The 260-nm absorption profiles (Fig. 4) obtained by this procedure, besides showing the degradation and resynthesis of ribosomal RNA, also revealed a substantially greater amount of RNA sedimented in the 4 to 5*S* region at all times within

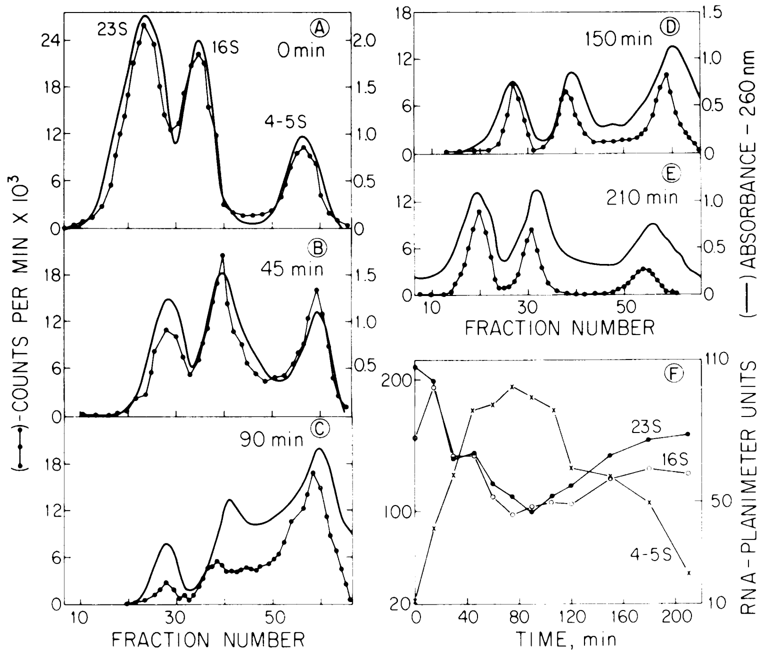

FIG. 3. *Changes in native ribosomal RNA during intraperiplasmic growth of B. bacteriovorus on [2-^{14}C]uracil-labeled E. coli. Single cycle culture initially contained 5 × 10^{9} B. bacteriovorus and 3 × 10^{9} E. coli (150,000 counts/min) cells/ml. RNA was isolated at intervals from 5-ml samples of the culture and centrifuged on sucrose gradients. Profiles of 260-nm absorption (——) and radioactivity (●) of the gradients. (F) Kinetics of change of the RNA species as areas under the peaks in arbitrary planimeter units; symbols: ●, 23S; O, 16S; ×, (scale on left) 4 to 5S.*

the first 90 min in comparison to that observed with native RNA. The 10- to 20-min profiles showed that the 16*S* region was skewed towards the 5*S* region, and by 40 min the profiles revealed the formation of fairly discrete degradation fragments of about 8 and 10*S* sizes (Fig. 4).

A more detailed picture of native normal and degraded RNA species present at stages during the bdellovibrio's intraperiplasmic development was obtained by polyacrylamide gel electrophoresis. The RNA band patterns increased in complexity with time up to 90 min and then simplified with continuing development of the bdellovibrio. The RNA isolated at 0 time generally gave four major gel bands corresponding to normal 23, 16, 5, and 4*S* RNAs, as well as some indistinct minor ones. By 30 min numerous additional intense bands appeared corresponding to low- (ca. $< 1.7 \times 10^{4}$) and high-(ca. $<10^{5}$) -molecular-weight fragments. After 90 min, less remained of the large fragments, and with time increasing amounts of 23 and 16*S* RNAs and decreasing amounts of low-molecular-weight material were observed. Table 2 gives an analysis of a 90-min band pattern from an experiment in which [^{3}H]uracil-labeled bdellovibrios were grown on [2-^{14}C]uracil-labeled *E. coli*. Some 19 discrete bands were apparent, and in other experiments as many as 25 were seen. The ^{14}C radioactivity was fairly uniformly distributed among these bands, with less than 15% in the 23 and 16*S* bands. In contrast, about 60% of the ^{3}H radioactivity was in these bands, and little more than background levels were found in the other bands. The differences in the distribution of ^{3}H and ^{14}C radioactivity not only show the stability of the bdellovibrio ribosomal RNA but also show that the various ^{14}C-containing bands apart from the 23 and 16*S* were in fact in vivo RNA degradation products and not fragments created during isolation and manipulation of the RNA.

Use of E. coli RNA during intraperiplasmic growth of B. bacteriovorus. Table 3 shows the distribution of radioactivity in *E. coli* grown in the presence of [2-^{14}C]uracil and in *B. bacteriovorus* after growth on these *E. coli*. Over 90% of the initial radioactivity in the *E. coli* was in the RNA. As previously noted (Fig. 1), about 50% of the *E. coli* radioactivity was incorporated by the bdellovibrios. Of this radioactivity, 66% was in the RNA and 30% was in the DNA. The total radioactivity in the DNA was about three

times that initially present in the *E. coli* DNA. The data show that *E. coli* RNA not only served as a precursor of bdellovibrio RNA but also was converted to bdellovibrio DNA.

The distribution of radioactivity among the individual nucleotides was determined for the nucleic acids isolated from the starting *E. coli* and the progeny bdellovibrios. More than 95% of the radioactivity from both sources was in the pyrimidine nucleotides (Table 4). The ratio of radioactivity in cytidine 5'-monophosphate to that in uridine 5'-monophosphate was similar in *E. coli* and *B. bacteriovorus* RNA for both single cycle and multicycle cultures. In contrast, the ratios of deoxycytidine 5'-monophosphate to thymidine 5'-monophosphate differed greatly: 0.216 for *E. coli* DNA versus 1.19 for bdellovibrio DNA. The data, combined with that in Table 3, indicate that *E. coli* RNA contributes more to *B. bacteriovorus* deoxycytidine 5'-monophosphate than to thymidine 5'-monophosphate.

A comparison was made of the specific activity of the RNA in the substrate organism with that of the progeny bdellovibrios. The experiment was done with a multicycle culture in which the quantity of RNA in the bdellovibrio inoculum was negligible and did not influence the results. The bdellovibrio RNA specific activity was approximately the same (13% higher) as the initial specific activity of the *E. coli* RNA (Table 5). Similar results were obtained in single cycle experiments after correcting the data for the input bdellovibrio RNA. The results indicate that essentially all the bdellovibrio RNA was derived directly from precursors in the *E. coli* RNA.

Effects of exogenous RNA precursors. The

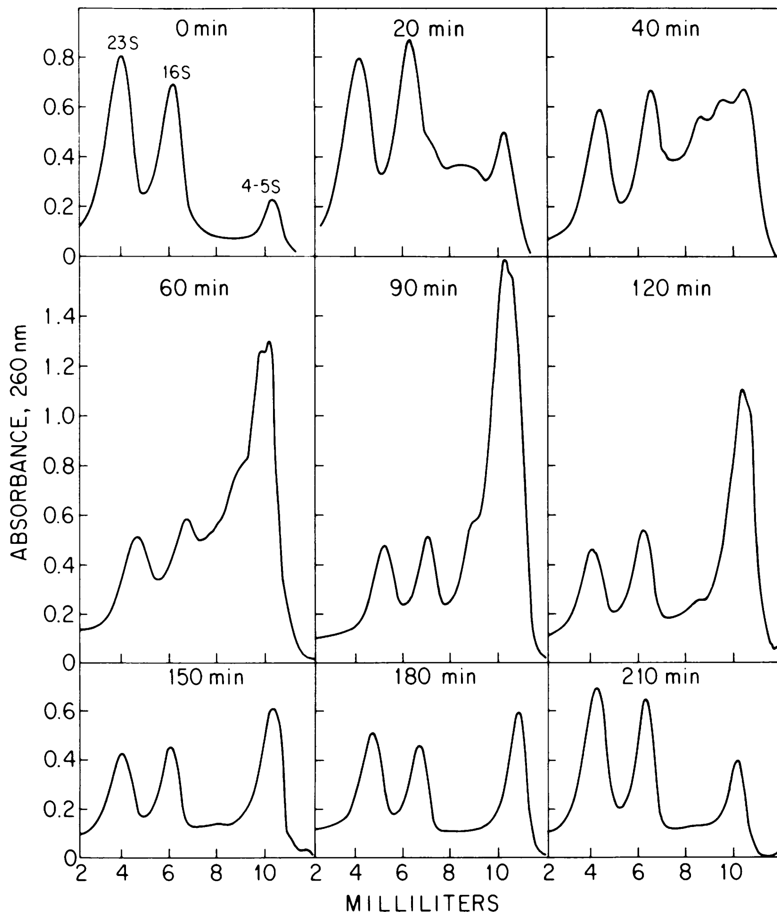

FIG. 4. *Changes in 260-nm absorption profiles of sucrose gradients of denatured RNA isolated during intraperiplasmic growth of B. bacteriovorus on E. coli. The experimental conditions were similar to those given for Fig. 3, except that the purified RNA was heated at 80 C for 2.5 min and rapidly cooled in ice before centrifugation.*

TABLE 2. *Distribution of ^{3}H and ^{14}C radioactivities after gel electrophoresis of RNA present at 90 min during intraperiplasmic growth of ^{3}H-labeled B. bacteriovorus on ^{14}C-labeled E. coli*[a]

Band no.	Distance migrated (mm)	Estimated mol wt (× 10^3)	Radioactivity	
			^{3}H (counts/ min/band)	^{14}C (counts/ min/band)
1 (23*S*)[b]	2.0	1,100	1,345	1,405
2 (16*S*)[b]	11.0	560	864	1,713
3	20.0	360	66	1,476
4	25.6	250	45	1,018
5	27.5	225	79	1,262
6	32.0	168	97	1,469
7	35.0	138	62	1,221
8	37.5	115	84	1,064
9	39.5	105	41	673
10	41.0	96	53	1,064
11	45.0	73	74	1,918
12 (5*S*)[b]	54.5	40	85	1,212
13	58.5	31	284	1,287
14	62.0	25	50	822
15 (4*S*)[b]	68.5	17	182	1,860
16	76.0	13	134	1,590
17	81.0	11	110	684
18	85.0	<10	83	543
19	88.0	<10	56	607

[a] Single cycle culture initially containing 8 × 10^9 ^{3}H-labeled *B. bacteriovorus* (376,000 counts/min) and 5 × 10^9 [2-^{14}C]labeled *E. coli* (204,000 counts/min) per ml. A 10-μl sample of RNA (37 μg; 2,670 ^{3}H counts/min and 29,380 ^{14}C counts/min) isolated from the culture at 90 min was subjected to electrophoresis on a 10% polyacrylamide gel, and the band patterns and radioactivity were determined.

[b] Migration distances and molecular weights of *E. coli* RNA species.

TABLE 3. *Distribution of radioactivity in cell fractions before and after intraperiplasmic growth of B. bacteriovorus on [2-^{14}C]uracil-labeled E. coli*[a]

Fraction	% Initial radioactivity in	
	Initial *E. coli*	Final *B. bacteriovorus*[b]
Whole cells	100.0	48.5 (100.0)
Cold trichloroacetic acid soluble	0.3	1.1 (2.3)
RNA	91.0	32.2 (66.4)
DNA	5.7	14.8 (30.4)
Residue (protein)	3.2	2.8 (5.8)

[a] Data are the average of three separate single cycle cultures initially containing 5 × 10^9 to 7 × 10^9 *B. bacteriovorus* and 3.6 × 10^9 to 4.5 × 10^9 [2-^{14}C]uracil-labeled *E. coli* (260,000 to 456,000 counts/min/per ml) cells/ml.

[b] The numbers in parentheses are percentages of total radioactivity in *B. bacteriovorus*.

effects of added nucleic acid components on the incorporation of radioactivity into *B. bacteriovorus* growing on [2-^{14}C]uracil-labeled *E. coli* were examined. Mixtures of nucleic acid bases or ribonucleosides had little effect on the amount of radioactivity taken up (Table 6). In contrast, the addition of the four ribo- or deoxyribonucleoside monophosphates to the cultures caused a 25 to 35% decrease in incorporated radioactivity. Cytidine 5′-monophosphate or uridine 5′-monophosphate added individually caused about a 20% decrease as did deoxycytidine 5′-monophosphate, thymidine 5′-monophosphate, or deoxyuridine 5′-monophosphate. Individual purine ribonucleotides had essentially no effect (Table 6), as would be expected since essentially all of the *E. coli* radioactivity was in the pyrimidines (Table 4). The addition of the four ribo- or deoxyribonucleotides to a culture resulted in a 10 to 15% increase in RNA and DNA synthesis. No increase in the quantity of nucleic acids formed was caused by the addition of individual nucleotides or by mixtures of the four bases or nucleosides.

DISCUSSION

The developmental cycle of *B. bacteriovorus* growing intraperiplasmically is conceptually divisible into temporally distinct phases: attachment, penetration, preparation for growth, growth per se, and lysis of the substrate cell and release of progeny (23, 27). A single cycle is completed in about 3 to 3.5 h under the conditions used in the experiments reported. Penetration follows attachment by about 10 min (34). During the next 30 to 50 min, the substrate cell is altered without detectable growth of the bdellovibrio. Growth then ensues, and release of bdellovibrio progeny follows after an additional 2 to 2.5 h. After bdellovibrio attack, a substrate organism loses control over permeability of small molecules (23) and cannot respire (23) nor synthesize RNA and protein (33). The processes leading to these effects are initiated after irreversible attachment and during penetration into the periplasmic space. Degradation of the DNA of the substrate organism is also initiated early (16). It is a controlled process and is complete before bdellovibrio DNA synthesis is initiated (16). The data presented in this paper show that ribosome and ribosomal RNA degradation and synthesis follow a similar pattern as that shown for DNA. Changes in ribosome or ribosomal RNA profiles are well advanced in samples from single cycle cultures taken as early as 15 to 20 min after bdellovibrio attack.

TABLE 4. *Distribution of radioactivity in nucleic acid nucleotides before and after intraperiplasmic growth of B. bacteriovorus on [2-¹⁴C]uracil-labeled E. coli*[a]

Nucleotide	Radioactivity (counts/min)					
	Single cycle culture				Multiple cycle culture	
	RNA		DNA		(RNA)	
	E. coli	*B. bacteriovorus*	*E. coli*	*B. bacteriovorus*	*E. coli*	*B. bacteriovorus*
Total sample	14,033	8,031	6,445	4,944	10,109	10,238
Total recovered	14,235	7,245	6,645	4,667	8,880	8,933
AMP or dAMP	65	116	232	132	142	77
CMP or dCMP	8,841	4,665	1,107	2,409	5,619	6,079
GMP or dGMP	197	105	121	105	157	101
UMP	5,132	2,359	–	–	2,962	2,676
dTMP	–	–	5,185	2,021	–	–
CMP/UMP or dCMP/dTMP	1.72	1.97	0.216	1.19	1.89	2.27

[a] The nucleic acids were isolated as described from the initial [2-^{14}C]uracil-labeled *E. coli* cells and from the *B. bacteriovorus* cells after growth on these cells. Each sample (10 to 13 μg of nucleic acid) was treated with phosphodiesterase, and the resulting products were separated by two-dimensional polyethyleneimine-cellulose chromatography. The spots were located with ultraviolet light and cut out, and their radioactivity was determined by scintillation counting. CMP, Cytidine 5'-monophosphate; UMP, uridine 5'-monophosphate; dCMP, deoxycytidine 5'-monophosphate; dTMP, thymidine 5'-monophosphate; AMP, adenosine 5'-monophosphate; dAMP, deoxyadenosine 5'-monophosphate; GMP, guanosine 5'-monophosphate; dGMP, deoxyguanosine 5'-monophosphate.

TABLE 5. *Initial and final specific activity of RNA after intraperiplasmic growth of B. bacteriovorus on [2-¹⁴C]uracil-labeled E. coli*[a]

Sample	RNA		
	Amt (μg/ml)	Radioactivity 10^3 × (counts/min/ml)	Sp act (counts/min/μg)
Starting *E. coli*	350	462	1,320
Harvested *B. bacteriovorus*	124	185	1,490

[a] Multicycle culture initially containing 4 × 10^9 [2-^{14}C]uracil-labeled *E. coli* (502,000 dpm) and 10^7 *B. bacteriovorus* cells/ml.

Degradation is essentially complete by 90 min under the experimental conditions used, whereas formation of bdellovibrio ribosomes and ribosomal RNA is first detectable at about 100 min. Since *E. coli* protein is used as an energy source for growth of *B. bacteriovorus* (10), it is possible that ribosomal proteins are also degraded in concert with the ribosomal RNA. This aspect has not been investigated.

Little is known about the source and nature of the enzymes involved in the degradative process. Since chloramphenicol or rifampin added to cultures shortly after bdelloplast formation inhibits ribosome breakdown, it can be inferred that enzymes synthesized by the bdellovibrio are responsible. These data are, however, fragmentary and do not completely exclude the involvement of nucleases and proteases of the substrate cell in the degradation. The bdellovibrio degradative enzymes could be the same as those detected extracellularly during growth on autoclaved cells (4, 12) or during axenic growth of certain bdellovibrio strains (5, 7, 28, 30). However, no evidence is available to relate these activities directly to changes occurring during intraperiplasmic development.

Regardless of the source of enzymes, the data do suggest that the initial RNase attack on the *E. coli* RNA might consist of only a few single-strand nicks. The skewness of both the ribosome and RNA sucrose gradient profiles toward a lighter density in early samples, the increased degradation made evident by denatured RNA profiles, and the early detection of discrete bands of large-molecular-weight RNA fragments by polyacrylamide gel electrophoresis support this suggestion. It is not known whether this RNase or other RNases are responsible for the small-molecular-weight (ca. $<1.1 \times 10^4$) fragments that predominate later on in the developmental cycle. Accurate determinations of the molecular weights of the degraded RNA

TABLE 6. *Effects of exogenous nucleic acid components on incorporation of radioactivity during intraperiplasmic growth of B. bacteriovorus on [2-^{14}C]uracil-labeled E. coli*[a]

Addition	Incorporated radioactivity	
	Counts/ min/ml	% Control
None (control)	77,700	100
Nucleic acid bases	70,500	91
Ribonucleosides	71,000	91
Ribonucleotides	58,900	76
Deoxyribonucleotides	50,400	65
AMP	78,200	101
GMP	74,500	96
CMP	62,700	81
UMP	64,900	83
dCMP	62,300	80
dUMP	61,300	79
dTMP	61,200	79

[a] The cultures contained 6×10^9 *B. bacteriovorus* and 4×10^9 [2-^{14}C]uracil-labeled *E. coli* (156,000 counts/min) cells/ml. The cultures were supplemented as indicated with a mixture of adenosine 5'-monophosphate (AMP), cytidine 5'-monophosphate (CMP), guanosine 5'-monophosphate (GMP), uridine 5'-monophosphate (UMP) or the four corresponding ribosides, or the four ribo- or deoxyriboside monophosphates, or individual compounds (1 mM final concentration of each). The additions were made at 45 min, at which time all the *E. coli* were bdelloplasts. dCMP, Deoxycytidine 5'-monophosphate; dUMP, deoxyuridine 5'-monophosphate; dTMP, thymidine 5'-monophosphate.

species and/or purification of the RNase(s) should indicate whether the RNase(s) involved are similar to other RNases which degrade *E. coli* 30*S* ribosomes, for example, RNase T (24, 25).

The degraded RNA of the substrate organism is the major, if not exclusive, source of precursors for the synthesis of bdellovibrio RNA. The close similarity in specific activity of the starting and final RNAs and the distribution of radioactivity in the cytosine and uracil moieties of the two RNAs provide the strongest support for this conclusion. We have previously shown (22) that the bdellovibrio incorporates nucleoside monophosphates per se. The data presented in this paper showing that exogenous nucleoside monophosphates, but not nucleosides or free bases, compete with RNA precursors from the substrate cell support our previous finding. Thus, the evidence indicates that the pyrimidine nucleotides of the bdellovibrio are derived as a unit primarily, if not exclusively, from the RNA of the substrate cell. We have not examined the origin of purine nucleotides, but there is no reason to suspect that it will differ.

The radioactivity in *B. bacteriovorus* DNA after its growth on [2-^{14}C]uracil-labeled *E. coli* was two- to threefold greater and equally divided between the thymine and cytosine moieties, as compared to that in the *E. coli* DNA in which thymine had five times the radioactivity of cytosine. It can be concluded that a portion of the ribonucleotides derived from RNA degradation is utilized by the bdellovibrio for DNA synthesis. Presumably *B. bacteriovorus* possesses a ribonucleotide reductase system for converting ribonucleotides to their homologous deoxyribonucleotide. Results of previous studies suggest that about 20% of the *B. bacteriovorus* DNA is derived from RNA degradation products when *E. coli* cells of normal composition are the substrate (16, 22) and as much as 50% when *B. bacteriovorus* is grown on *E. coli* cells having an abnormally high protein-to-DNA ratio (19). The component(s) of the substrate cell which limits overall bdellovibrio growth is not known, but the above data show that DNA is not limiting as long as substantial levels of substrate cell RNA are available.

One other aspect of the nucleic acid metabolism of *B. bacteriovorus* merits mention. Despite the fact that about 50% of the radioactivity in uracil-labeled and 35 to 50% in the thymidine-labeled *E. coli* (16) are released into the culture fluid during bdellovibrio growth, the culture fluid at the end of growth contained no detectable deoxyribose and only small amounts of ribose. Some 60 to 80% of released radioactivity is in free nucleic acid bases (Table 1). Similarly, there is a large release of inorganic phosphorous. The ribose found, however, is not at a commensurate level. The data suggest that ribose and deoxyribose derived from the nucleic acids of the substrate organism are extensively metabolized by the bdellovibrio.

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