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The genus Bdellovibrio is characterized by the unique ability to parasitize and enter gram-negative host bacteria. This interaction leads to the consumption and death of the host cell as the Bdellovibrio grows and divides into progeny. Little is known about the metabolism of host cell components by Bdellovibrio. The possibility that Bdellovibrio utilizes host cell RNA, DNA, and protein was investigated.

Initial studies involved the growth of host-independent (H-I) bdellovibrios in a growth limiting medium supplemented with a single potential metabolite. Protein, host extracts, and autoclaved host cells stimulated growth. Purified RNA or DNA in concentrations up to 0.5 mg/ml did not stimulate H-I Bdellovibrio growth as measured by increase in viable count. Subsequent studies with labeled amino acids and nucleosides indicated that Bdellovibrio does utilize these substances. An amino acid mixture was incorporated into Bdellovibrio macromolecules; from 1 to 4% of the added label was taken up and

used anabolically. The amino acids glycine and aspartate are not used for the synthesis of nucleic acid precursors. Although from 1% to 10% of these added amino acids was incorporated into cold TCA precipitable material, none of the label was hot TCA soluble. The nucleosides, adenosine and guanosine, were incorporated into hot TCA soluble material; from 8.5% to 42.7% of these nucleosides were incorporated from the culture medium.

Exocellular enzymes of Bdellovibrio were briefly examined. Exoproteases are well documented (7), but nuclease activity had not been previously shown to exist. These enzymes were used to prepare hydrolysates of host RNA, DNA, and protein.

Labeled RNA, DNA, and protein, prepared from host cells and hydrolyzed by Bdellovibrio enzymes, were incorporated into Bdellovibrio macromolecules. From 4% to 6% of the added protein hydrolysate was incorporated into cold TCA precipitable material. From 8% to 18% of the hydrolyzed RNA was utilized anabolically by Bdellovibrio. Of the hydrolyzed DNA, 10% to 14% was incorporated by Bdellovibrio.

DNA-RNA hybridization studies have shown that during the infectious life cycle prelabeled host cell RNA breaks down and Bdellovibrio incorporates the products into its RNA. The specific activity of the labeled RNA generally decreases throughout the life cycle. At the beginning of the experiment no radioactive RNA binds to the Bdellovibrio filters. At the completion of the burst (5 hours)

almost as much RNA is homologous to Bdellovibrio DNA as had been homologous to E. coli DNA at the start of infection. Concomitant with this is the reduction of homologous RNA to E. coli DNA to about 1% of the preinfection level.

Bdellovibrio Metabolism of Host Macromolecules

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INTRODUCTION

The genus Bdellovibrio contains a group of unique bacteria which parasitize upon gram-negative bacteria (30, 31). Bdellovibrio undergo a life cycle of several distinct stages. The parasite first attaches to its host, then enters the area between the cell wall and membrane. It grows as it consumes the protoplasm, and finally divides and releases its progeny. Studies have shown that the external menstruum has little influence on this life cycle. Bdellovibrio will grow in a buffer solution containing only cations and hosts (20). Thus the host must provide all the carbon and energy sources essential for growth and multiplication of Bdellovibrio (2, 3). The question remains, what host material is used and how is it obtained? One study addressing itself to this question describes the incorporation of labeled thymidine from the DNA of the host into Bdellovibrio DNA (16).

Several studies have demonstrated that amino acids, especially glutamate and alanine, are important in the energy metabolism of Bdellovibrio (8, 24, 27). Since the host cell is nearly two-thirds protein, efforts were made to study the possible utilization of host protein as a source of preformed amino acids for Bdellovibrio biosynthetic purposes. RNA and DNA, which comprise about 25% to 30% of the host cell, are potentially important sources of purines and

pyrimidines for Bdellovibrio nucleic acids. Because of the lack of information concerning Bdellovibrio utilization of its prey, the enzymatic degradation and subsequent use of these host macromolecules was studied by isotope tracer methods.

Host independent (H-I) Bdellovibrio strains were utilized in the nutritional studies to avert the problems of a two-member culture and allow a more definitive interpretation of the data. H-I bdellovibrios are non-host requiring mutants of the predatory host-dependent (H-D) strains (21). Except for the loss of motility by some strains, these cultures appear biochemically identical to the wild-type parasites (22, 24).

An investigation of the kinetics of host RNA destruction and the assimilation of the products by a H-D Bdellovibrio strain growing in a host cell containing prelabeled RNA was conducted. DNA-RNA hybridization techniques allowed the kinetics of host RNA turnover to be followed.

REVIEW OF LITERATURE

The bdellovibrios have the unique capacity to parasitize other gram-negative bacteria. The host bacterium is attacked and entered by the Bdellovibrio, which grows in the interintegumental space between the host cell wall and cytoplasmic membrane (30, 31). Soon after the attack, the host becomes metabolically incompetent and its respiration ceases (19). Thus, during the period of growth within the host cell, the bdellovibrios must be metabolically active and independent of the energy producing systems of the host cell.

Little is known about the metabolism of Bdellovibrio. Research in this area is complicated, because bdellovibrios cannot be grown axenically, but require host cells. Host bacteria may be necessary to provide a growth factor not present in complex media. The host-independent (H-I) mutants of host-dependent (H-D) bdellovibrios were discovered by isolation of colonies that appeared on host free media inoculated with H-D bdellovibrios. These host-independent (H-I) bdellovibrios appear to be single-step derivatives of the parasitic parent (21), but unlike it do not require host bacteria. The nature of the host-independent mutation(s) is unknown. Several methods (12, 21) have been developed to obtain H-I derivatives and these mutants have been characterized. The H-I mutants seem physiologically identical to the H-D parents except in the loss of motility by some strains and

the independence from the host (21). Thus, complex media fulfills the metabolic requirements of Bdellovibrio, but does not support growth of H-D bdellovibrios, because a growth initiating factor(s) from the host must be provided.

Growth Factor

A dozen isolates are described in the first English paper concerning bdellovibrios (32). From two strains, 321 and A3. 12, saprophytic mutants were obtained. The H-I 321 strain was characterized by various biochemical tests. It was shown to be proteolytic, hydrolyzing both casein and gelatin, gram-negative, aerobic, and unable to grow on a variety of carbon sources, other than complex media. The H-I mutants indicated that the requirement for the host could be overcome.

The metabolism of H-I derivatives of strain A3. 12 was examined in another early study (25). The vitamin requirements were partially assessed in a medium containing 1% peptone. It was found that thiamine alone could partially replace the yeast extract requirement. H-I A3. 12 could only grow when inoculated in concentrations of greater than 10^4 organisms per plate or when a 1% (v/v) addition of autoclaved supernatant from a culture of the H-I A3. 12 was added to the nutrient broth agar. This indicated that H-I A3. 12 has some growth factor requirement. Bdellovibrio must be able to produce the factor under some conditions but not others, since it is present in its culture medium.

Reiner and Shilo (18) devoted a study to the nature of the growth initiation factor supplied by the host to H-D bdellovibrios. A cell-free extract of host cells was prepared by sonication and centrifugation at 20,000 x g, 20 min. The supernatant was sterilized by removing intact cells by filtration. Finally the extract was dialyzed against an amino acid buffer. Cell free extracts from gram-negative or gram-positive bacteria and yeast allowed the elongation of about 5% of the H-D 109 inoculum in broth containing 5 mg/ml vitamin free casamino acids and 1 mg/ml extract protein. The elongation of 5% of the population was correlated to the incorporation of (^{3}H methyl)thymidine into the larger growing cells. The amount of cell multiplication, if any, was not reported although the label did appear to be associated with smaller cells with time, indicating division. The growth factor from Pseudomonas aeruginosa was biochemically characterized. The extract was further centrifuged at 120,000 x g for 2 hours. Then by column chromatography on G-150 Sephadex, the extracted factor in the supernatant exhibited a molecular weight of 50,000. The study also indicated the stability of the factor to heat, RNase, DNase, and pronase. These authors postulated the factor to be a regulator of Bdellovibrio division, either by controlling DNA synthesis or the physical division of the cells.

H-D bdellovibrios have been shown to grow without living host cells if provided with a heat stable host factor(s). H-D 6-5-S could

multiply in a buffer solution containing cations and washed, autoclaved host cells (2). Washed, autoclaved cells (5 minutes/121 C) of Sprillum serpens VHL, Escherichia coli ML35, and Bacillus megaterium in concentrations of 10^9/ml could serve as the sole nutrient for Bdellovibrio. The predator inoculum was 10^4/ml and increased to 5×10^7 to 10^8 PFU/ml. The growth was abnormal since it occurred externally to the host cell not within it. B. megaterium, a gram-positive bacterium, is not a host for Bdellovibrio probably because it cannot be penetrated; autoclaving must release the factor and allow growth on this "unnatural" host. The factor is present in a wide variety of organisms.

Ishiguro (12), while attempting to isolate H-I mutants from H-D 109D, found that H-D cells washed free of spent medium would not give rise to H-I derivatives. Spent medium contains a growth initiating factor (GIF), because when it was added to isolation medium, H-I mutants appear. The GIF was also found in cell-free extracts of a variety of microorganisms: yeast, gram-positive and gram-negative bacteria, and also H-I and H-D Bdellovibrio. The extracts were prepared from sonicated cell suspensions. The extract was clarified by centrifugation (9500 x g, 15 minutes) and the supernatant again centrifuged in the same manner. This extract was dialyzed against 0.025 M Tris buffer, pH 7.4 and was filter sterilized. Heating at 70 C or 100 C leads to increased GIF activity (measured arbitrarily as the

H-I CFU formed/10^9 PFU). Additional investigation indicated that the increase in activity is due to the loss of a heat-labile inhibitor. It appears that Bdellovibrio extracts do not contain inhibitors.

Growth of H-D 109D bdellovibrios was followed in complex medium (PYE, 10 mg/ml peptone, 3 mg/ml yeast extract), PYE with unheated extract, and PYE with heated extract. The inoculum of washed cells gave a final concentration of 5×10^8 PFU/ml. The PFU dropped to about 10^4 PFU/ml in all flasks after six days. The CFU (initially 10/ml) increased to 10^2/ml with no extract, to 10^3/ml with unheated extract, and to 10^9/ml with heated extract in 10 days. The PFU rose after six days in the flask with heated extract. The author suggests these are faculative parasites and the rise is from the increased H-I population. The GIF stimulates the growth of H-I derivatives, but not the H-D forms. It appears that H-D Bdellovibrio growth does not occur in the rich medium. This may indicate that the "growth" reported by Reiner (18) was due to H-I not H-D bdellovibrios.

The physiological state of the H-I Bdellovibrio determines its GIF requirement. Exponential cells regardless of inoculum size do not require the GIF for growth. Stationary cells require GIF for growth unless inoculated in excess of 10^7 CFU/ml. The GIF appears to be necessary for metabolically quiescent H-I cells to initiate some important growth function (12).

H-D Bdellovibrio 109J has been grown for several generations in a host-free Escherichia coli extract system (9). E. coli cells were frozen, washed with 0.01 M Tris buffer containing 0.2 μg/ml DNase, resuspended in Tris buffer and washed again. The cell pellet was suspended in Tris buffer and sonicated. The cell suspension was purified by three centrifugations of the supernatants (5,000 x g, 5 min; 20,000 x g, 20 min; 140,000 x g, 2 hr). The final supernatant was clarified through a series of filtrations with a final one through a 0.22 μm filter. The filtrate was dialyzed against 0.01 M Tris buffer and filter sterilized. The basic difference in preparation from the extract prepared by Reiner is the third and final high speed centrifugation which was not done in the earlier study. Ishiguro (12) also did not prepare his extract by high speed centrifugation.

Growth was followed in a casamino acid (3.75 mg/ml) Tris buffer solution containing varying amounts of host extract (based on protein content). Addition of 2 mg/ml host extract protein allowed the optimum of about 50% of the H-D Bdellovibrio to multiply as determined by microscopic observation. Cell counts (PFU) showed only a 4- to 8-fold increase instead of an expected 10- to 20-fold increase expected by microscopic cell counts of the number of cells multiplying. Cell elongation directly correlated with ^{3}H methyl thymidine uptake. The cells divided only once into 20-50 progeny; the single division appears to be the result of inhibitors in the medium.

Removing the cells to fresh medium allowed continued growth if the factor was included. The extract was inactivated by pronase or ribonuclease, but was heat and deoxyribonuclease insensitive.

The authors postulate that the factor initiates DNA synthesis and the depletion of a host factor triggers division. They suggest that the factor interacts with a repressor present in Bdellovibrio cells, which inhibits DNA synthesis. H-I mutants accordingly would be repressor mutants, which become constitutive for DNA synthesis. Since the extract was inactivated by both pronase and RNase and total cell RNA could not replace the extract, two separate factors may exist.

It is difficult to compare these three studies because of the different techniques used in extract preparation and the variation in growth conditions. The similarity of the extract of Reiner to that of Ishiguro suggests that the elongation observed by Reiner may have been due to H-I forms. This cannot be resolved since neither colony counts nor plaque counts were reported. The rich medium used by Ishiguro selects for H-I Bdellovibrio, although no increase in H-D forms occurred with the extract, in dilute medium an increase may be observed. The study by Horowitz (9) indicates that H-D forms do grow in dilute medium containing extract. Colony counts were not performed which would have indicated if this extract would stimulate H-I forms. All studies have shown the factor(s) to be heat and

DNase stable. The factor appears to be macromolecular since it is non-dialyzable and possibly consists of RNA and/or protein. In order to understand the differences, if any, among the factors, they will have to be tested in similar growth conditions and growth assayed both for CFU and PFU.

Nutrition of Bdellovibrios

The host cell supplies all the carbon and energy material required by Bdellovibrio for growth and multiplication. This has been shown by growth of H-D Bdellovibrio in buffer solutions containing only host cells, or only certain heat killed or ultraviolet light killed hosts (2, 31). It seems likely then that Bdellovibrio has evolved a metabolism specialized to the utilization of host compounds. Studies of the metabolic capabilities of Bdellovibrio have shown it to contain a complete cytochrome system, a functional Kreb's cycle, and to be able to utilize amino acids, protein, and DNA for anabolic and catabolic purposes (8, 27, 30).

Bdellovibrio is independent of the ATP generating mechanisms of the host. Bdellovibrio 6-5-S appears to contain a complete cytochrome system and forms high energy phosphate bonds by oxidative phosphorylation. Bdellovibrio grows only aerobically and the respiration of strain 6-5-S is inhibited by potassium cyanide and sodium azide, but not carbon monoxide (27). The total respiration of a two

member culture of host and Bdellovibrio increases greatly when lysis begins and continues for some time after the host has disintegrated (19).

Host enzymatic systems are not necessary to Bdellovibrio growth. A functional Kreb's cycle has been found in all bdellovibrios surveyed (21, 27). The glyoxylate and possibly the pentose pathways are absent. According to Simpson and Robinson, some glycolytic enzymes are present in low levels (2-6% of Escherichia coli controls) (27). Alanine and glutamate dehydrogenases are present. These enzyme studies indicate Bdellovibrio dependence on proteins, peptides, amino acids, and nucleic acids as carbon and energy sources. Carbohydrates and similar simple substrates may not be utilized by bdellovibrios (21).

Rittenberg and colleagues have extended earlier studies which indicated the importance of amino acids to Bdellovibrio metabolism and identified several amino acids as being respired during intraperiplasmic growth of H-D B. bacteriovorus 109J (8). Seidler and Starr (21) previously had shown stimulation of growth of three of nine H-I strains only by glutamate or alpha-ketoglutarate in a growth limiting broth of 0.2% yeast extract and 0.2% peptone broth. Rittenberg and Shilo showed by the consumption of oxygen that peptone, yeast extract, or casein hydrolysate stimulated respiration of H-D Bdellovibrio cultures (19). Amino acids, especially glutamine, stimulated the respiration of strain 6-5-S (27).

By the use of uniformly labeled ^{14}C compounds it has been shown that acetate, glutamate, and amino acid mixtures were oxidized and released as $^{14}CO_2$ (8). Glutamate and amino acids were used anabolically but acetate was not. Uniformly labeled Escherichia coli, when used as the host for Bdellovibrio, are consumed and the label is incorporated into the parasite. Thirty percent of the total ^{14}C labeled exogenous glutamate supplied during interintegumental growth is taken up by Bdellovibrio; another 6% is respired as CO_2. Bdellovibrio viable counts fall rapidly after lysis of the host in dilute nutrient medium. If the medium is supplemented with glutamate and/or an amino acid mixture, the culture remains viable over a much longer period of time. These amino acids reduce the amount of endogenous substrates that are respired during starvation (8). It is evident that Bdellovibrio uses amino acids of its host as a major carbon and energy source.

This amino acid dependent metabolism has indicated to some workers that the exoproteases may be involved in the metabolism of host proteins and not bacteriolysis (7). A variety of lytic enzymes are present and may be involved in the process of lysis; the proteases, which hydrolyze azocoll and casein, are probably used for hydrolyzing host protein (10).

Another contribution concerns the utilization of host DNA by H-D Bdellovibrio 109D (16). By the use of ^{3}H labeled DNA and cesium

chloride equilibrium density gradients, it was shown that the host DNA is hydrolyzed in an efficient manner that allowed Bdellovibrio to incorporate 73% of the labeled thymidine during growth in a buffer solution. The possibility that the host DNA was hydrolyzed by Bdellovibrio deoxyribonucleases was raised.

In conclusion, bdellovibrios require host cells for: 1) a factor(s) to initiate growth and division (possibly by initiating DNA synthesis), and 2) a supply of specific metabolites as carbon and energy sources (e. g., amino acids, nucleic acid precursors, and possibly vitamins).

Taxonomy

It must be noted that there are three recognized species that comprise the genus Bdellovibrio. The high guanine plus cytosine (50-51% G+C) group is represented by the B. bacteriovorus species. The strains designated 100, 109D, 109J, 6-5-S, and others with 50-51% G+C are in this species. The DNA-DNA homology among isolates in this group is more than 90% but is undetectable with the low G+C strains (41. 8 and 43. 5% G+C). The two low G+C isolates have only 16% DNA homology with each other. Strain A3. 12 with 43. 5% G+C is designated B. starrii and is the only member of the species. Strain Uki2 with 41. 8% G+C is designated B. stolpii and is the only member of this species. The genome size is unique to each species and based on renaturation rates ranges from 60-70% as large as Escherichia coli.

Bdellovibrio Metabolism of Host Macromolecules

In the present study, the utilization of host macromolecules by all three species of *Bdellovibrio* was examined. Bdellovibrios were found to have all the enzymatic machinery necessary for the breakdown of host macromolecules. Nucleases are present, as well as proteases; both will act upon native host macromolecules. The products of these enzymes can be subsequently used by bdellovibrios. A study of the kinetics of host RNA destruction and the following incorporation of RNA precursors into *Bdellovibrio* RNA was made. A clearer understanding of the utilization of the host by *Bdellovibrio* is gained.

MATERIALS AND METHODS

Organisms and Media

H-I Bdellovibrio bacteriovorus 100, 109D, H-D B. bacteriovorus 109D, H-I B. stolpii Uki2, and H-I B. starrii A3. 12 were employed in the present studies. Media and growth conditions have been described (20, 21). Escherichia coli JC411, CSH54, and CSH 42, multiple auxotrophic mutants, were obtained from Dr. L. R. Brown in this department. E. coli JC411 (Met-, Leu-, His-, and Arg-) was used for the production of labeled host cell protein. E. coli CSH 54 (Lac-, Pro-, Trp-, Ura-, His-, and B1-) was used in the preparation of radioactive RNA. Aeromononas hydrophilia ATCC 15467 was used in the preparation in labeled DNA for hydrolysis by Bdellovibrio enzymes and feeding studies. E. coli CSH 42 (Thr-, Leu-, Lac-, Thy-, Mal-, Ilv-, B1, DNA_{ts}, and F+) was used to prepare (^{3}H methyl) thymidine labeled DNA for DNA-RNA hybridization experiments. E. coli CSH 42 was also used to prepare ^{32}P labeled host cells for DNA-RNA hybridization studies. Sprillum serpens was used as a source of autoclaved host cells and cell-free host extracts.

E. coli strains and A. hydrophilia were propagated in modified CM broth (17) appropriately supplemented with growth factors as indicated below. Sprillum serpens was grown in 10% peptone, 0. 3% yeast extract, and 0. 01M Tris buffer, pH 7. 5 (PYE).

Preparation of Labeled Host Protein Hydrolysate

A 5% inoculum of an early stationary phase culture of E. coli JC411 was added to one liter of CM medium supplemented with 0.4% Bacto vitamin free casamino acids and containing 1 mCi of ^{3}H labeled amino acids (algal profile, Schwarz/Mann). The culture was grown to the stationary phase at 25 C, harvested and washed in 25 ml of 0.01 M Tris (hydroxymethyl) aminomethanehydrochloride buffer pH 7.5 (Tris). The cell paste was frozen in an Eaton press and disrupted at 15,000 psi. The disrupted cells were placed in 25 ml of 0.01 M Tris buffer pH 7.5 and incubated with 100 μg/ml RNase and 10 μg/ml DNase (Calbiochem) for 3 hours at 37 C. The preparation was centrifuged at 20,000 x g for 15 minutes at 5 C. Separation of the proteins from hydrolyzed nucleic acids and metabolites was achieved by passing the supernatant through a Sephadex G-100 column. Fractions of 3 ml were collected following the dye front (Blue Dextran 2000). The first six fractions containing the highest 280 nm absorbing material and the highest ^{3}H counts were pooled. These high molecular weight host cell proteins and enzymes were digested with concentrated and partially purified proteases from H-I B. bacteriovorus 100 (7). In the present studies the reaction was run in TCM buffer (0.01 M Tris, pH 7, 5, 2 mM Ca^{++} and 2 mM Mg^{++}) at 40 C for 2 hours. More than 80% of the counts became trichloroacetic acid (TCA) soluble. The hydrolysis was

terminated with 50% TCA (5% final concentration). The material was filtered through a glass fiber filter to remove remaining precipitable counts, neutralized with NaOH, and filter sterilized.

Preparation of Labeled Host RNA Hydrolysate

A 5% inoculum of an early stationary phase culture of E. coli CSH 54 was added to one liter of CM medium supplemented with 0.7% Bacto vitamin free casamino acids, 50 μg/ml tryptophan, 5 μg/ml of thiamine, 20 μg/ml unlabeled uracil, and 250 μCi of (5-^{3}H)uracil. The culture was grown to stationary phase, centrifuged, and resuspended in 25 ml of saline EDTA. The RNA was extracted and purified following the procedure of Moore and McCarthy (17). Some of the purified labeled RNA was hydrolyzed with cell-free spent medium from a stationary phase H-I B. starrii A3.12 culture. This reaction was run at 40 C for 2 hours in the presence of 2 mM Ca^{++} and 2 mM Mg^{++}. The reaction was terminated with TCA, filtered, neutralized with NaOH, and filter sterilized as the protein hydrolysate.

Preparation of Labeled Host DNA Hydrolysate

Aeromonas hydrophilia ATCC 15467 was grown in modified CM broth supplemented with 1% glucose and 2% Bacto vitamin free casamino acids and containing 0.2 μCi/ml of (^{3}H methyl) thymidine. The DNA

was extracted and purified by a modified Marmur technique (24). The DNA was hydrolyzed and treated in the same manner as the RNA.

Assays for Nuclease Activity

Qualitative plate assays were initially used to determine nuclease activity. RNase activity was determined using an acridine orange RNA medium described by Lanyi and Lederberg (15). This RNase test medium is prepared by adding 1 ml of stock solution containing 15 mg/ml of RNA and 1 mg/ml of acridine orange to 100 ml of PYE agar. The agar was autoclaved and then cooled at 50 C for 2 hours to allow the acridine orange to intercalcate between the bases. RNase activity is ascertained by the loss of fluorescence around the growth when observed with an ultraviolet light. When using this procedure for testing for DNase activity, 15 mg/ml of sonicated DNA was used in place of the RNA. In addition, standard DNase test agars were employed. The standard commercial DNase test agars contain 2 mg/ml of bull sperm DNA. Hydrolysis is assayed for by flooding the plates with 2.0 N HCl.

Semi-quantiative assays for RNase and DNase employed the solubilization of ^{3}H labeled RNA or DNA (Figures 2 and 3). The RNase reaction was carried out in a total volume of 2.235 ml containing 2 mM Ca^{++}, 2 mM Mg^{++}, and 25 μg ^{3}H labeled E. coli RNA (specific activity 1850 cpm/μg) with 50 μg cold carrier yeast RNA and

2 ml spent cell-free Bdellovibrio culture medium. The DNase assay was similar but 10 μg of ^{3}H labeled Aeromonas hydrophilia ATCC 15467 DNA (specific activity 4200 cpm/μg) and 100 μg unlabeled calf thymus DNA were substituted for the RNA. The reaction was initiated by the addition of 2 ml of spent culture medium to 0.235 ml of cations and nucleic acids. A control was run with sterile medium replacing the spent culture medium. At specific time intervals, 0.2 ml samples were removed and precipitated with cold 5% TCA.

Experimental Design for Hydrolysate Studies

H-I bdellovibrios were grown on a New Brunswick incubator shaker set at 30 C and 200 rpm. Growth was in PYE (21) supplemented with 0.01 M Tris, pH 7.5, and the necessary isotopes. The experiments were terminated in the early stationary phase (24-36 hours).

Immediately after inoculation (1 to 2 x 10^{7} cells/ml initial density) and after 24-36 hours (1 to 2 x 10^{9} cells/ml), the total counts in the culture were determined by adding 0.1 ml samples of broth to 10 ml of a toluene-Triton X-100 (Beckman Inst. Inc.) fluor. Appropriate samples were also taken to determine the number of cold TCA precipitable counts. Some samples, after initial cold TCA precipitation, were subjected to hot TCA extraction (80-85 C) for 40 minutes. Control experiments with added radioactive DNA and RNA showed that

during this incubation, 90% or more of the nucleic acid is hydrolyzed. Samples were filtered through glass fiber filters (Type AP20, Millipore Corp.) washed with cold 5% TCA and finally washed with cold 70% ethanol. Filters were then dried and counted (13).

A control culture was treated as the radioactive experimental culture to determine background counts. A second, radioactive control was run with some experiments in which the radioactive material was added, but the flask remained uninoculated. No changes in hot extractable or cold precipitable counts were observed in these controls.

Preparation of E. coli CSH 42 DNA for Hybridization Studies

Escherichia coli CSH 42 was grown in modified CM broth supplemented with 1% vitamin free casamino acids, 1% glucose, 5 μg/ml thymidine, and 0.5 μg/ml thiamine and containing 1.0 μCi/ml of (^{3}H methyl) thymidine. The DNA was extracted and purified by a modified Marmur technique (24). This DNA was to be embedded on membrane filters (6). Unlabeled DNA was prepared in a similar manner though no label was added to the growth medium.

Preparation of H-I Bdellovibrio DNA for Hybridization Studies

H-I Bdellovibrio bacteriovorus 109D was grown in PYE broth

containing 0.5 μCi/ml of (^{3}H methyl)thymidine. The DNA was extracted and purified as the E. coli DNA. Unlabeled DNA was prepared in a similar manner though no label was added to the growth medium.

One Step Synchronous Growth Conditions

One step growth conditions for H-D Bdellovibrio bacteriovorus 109D were produced by modifications of the procedures of Seidler and Starr (20) and Varon and Shilo (33). A 12-hour Bdellovibrio lysate containing about 5.0×10^{9} PFU/ml in PYE/10 broth was centrifuged for 10 minutes at 2000 x g at 5 C. The supernatant was then sequentially filtered through 5 μm and 1.2 μm nitrocellulose membrane filters and centrifuged for 20 minutes at 25,500 x g at 5 C. The Bdellovibrio pellet was resuspended to an $O.D._{600}$ of 0.5 or a cell density of 1 to 2×10^{10} PFU/ml. A 12-hour E. coli CSH 42 culture grown in PYE was centrifuged for 10 minutes at 10,000 x g at 5 C. The pellet was resuspended in PYE/10 broth with 2 mM calcium and 2 mM magnesium as added cations to an $O.D._{600}$ of 5.0 or a cell density of about 1.0×10^{10} CFU/ml. Volumes of resuspended Bdellovibrio and E. coli were mixed in a ratio of 2 to 1 in an appropriate amount of PYE/10 plus cations. The multiplicity of infection (MOI) equaled approximately 2. The culture was then incubated on a shaker at 210 rpm at 28-30 C for 6 hours; at this time the burst is completed.

Experimental Design of DNA-RNA Hybridization Studies

The RNA of the host E. coli CSH 42 was labeled by growth for 12 hours in 250 ml CM broth containing 40 μCi/ml of ^{32}P as inorganic phosphate. CM broth contained glucose, 5 mg/ml; NaCl, 2 mg/ml; $MgSO_4$, 1 mg/ml; NH_4Cl, 1 mg/ml; KH_2PO_4, 20 μg/ml; casamino acids, 10 mg/ml; thymidine, 10 μg/ml; thiamine, 5 μg/ml; and 0.02 M Tris buffer, pH 7.5. After 12 hours of growth, an excess of unlabeled phosphate (8 mg/ml) was added (6); growth was allowed to continue for one hour. A one step growth experiment was then started and 40 ml samples were taken hourly for 6 hours from the time of mixing the host and predator until the burst period was completed. These samples for RNA extraction were centrifuged, washed with 0.15 M saline and 0.1 M EDTA, and frozen. The RNA was extracted as described above for the hydrolysate experiments. The E. coli ^{32}P labeled RNA was tested for purity by several means before the hybridization experiments. The bulk ^{32}P RNA was 73% cold TCA precipitable. The RNA was totally hydrolyzed (less than 3% cold TCA precipitable) after 40 minutes in 5% TCA at 85 C, after one hour in 20 μg/ml heat treated pancreatic RNase in 2 X SSC at 37 C, or after 18 hours in 0.1 N NaOH at 37 C. Thus the purified RNA seems to contain no detectable labeled DNA. DNA-RNA direct hybridization studies were performed by the filter technique of Gillespie and Spiegelman (6). Controls were

run using blank filters and Aeromonas hydrophilia DNA containing filters.

Preparation of DNA Containing Filters for DNA-RNA Hybridizations

The labeled DNA of E. coli or H-I B. bacteriovorus 109D was loaded onto the membrane filters (15 cm diameter, B-6; Schleicher and Schuell Co.) by the procedure of Gillespie and Spiegelman (6). Six milligrams of labeled DNA in single-strength standard saline citrate (1 X SSC; 0.15 M NaCl + 0.015 M trisodium citrate, pH 7.0 $\pm$ 0.2) was diluted with 0.1 X SSC to a DNA concentration of 60 μg/ml. The DNA was denatured by adding 10 ml of 1.0 N NaOH to the 100 ml of diluted DNA and maintaining it for 10 minutes at 5 C. The solution was then diluted with 500 ml of ice cold 6 X SSC and neutralized by the addition of about 20 ml of 1 M NaH_2PO_4. The denatured DNA was gravity loaded onto prewashed membrane filters overnight in a cold room. Any remaining solution was filtered by slight vacuum (5 psi) and then the filter was washed with 100 ml of cold 6 X SSC. The filters were dried overnight at room temperature and then for 16 hours at 60-80 C in a vacuum oven. Filters (6 mm diameter) were punched out and stored under vacuum until used. The amount of DNA fixed to the filters was determined by the counts retained on the filter and the known specific activity of the DNA. About 10 μg of DNA were fixed

per filter. The specific activity of the E. coli CSH 42 DNA was 473 cpm/μg. The specific activity of the H-I B. bacteriovorus 109D was 254 cpm/μg.

DNA-RNA Hybridization Procedures

The distribution of the label between host and Bdellovibrio RNA was estimated by direct DNA-RNA hybridization experiments. RNA was extracted from the various time samples obtained during a one step growth experiment. The specific activity of the RNA was determined and 50 μg amounts were added to the hybridization buffer and DNA containing filter. The hybridization buffer was 2 X SSC and 40% DMSO (dimethylsulfoxide). This buffer reduced the Tm by about 32 C. Hybridization was carried out at Tm-25 C, which corresponded to a temperature of 38 C. The total volume in the hybridization vial was 0.5 ml. The DNA filters contained about 10 μg of DNA labeled with (^{3}H methyl)thymidine.

The samples were incubated in a slowly shaking constant temperature water bath for 16-18 hours at 38 C. The filters, after incubation, were passed through three successive washes at 38 C in the hybridization buffer and rinsed three times in 2 X SSC at 38 C to remove any DMSO. The filters were then exposed to 20 μg/ml of heat treated pancreatic RNase for one hour at 38 C. The filters were given a final wash in 2 X SSC, dried overnight at 55 C and counted (13).

The amount of RNA binding was determined from the specific activity of the RNA and the counts retained on the filter. The amount of RNA was then normalized to the amount retained per 10 μg of DNA on the filter.

Measuring Radioactivity

Radioactivity of all TCA precipitates and DNA-RNA hybridization filters was measured by liquid scintillation counting using a fluor of toluene, one liter; PPO (2, 5-diphenyloxazole), 5 g; and POPOP [1, 4-bis-2-(4-methyl-5 phenoxazolyl) benzene], 0. 3 g (Beckman Inst. Inc.). Total radioactivity in the liquid culture was measured by adding 0. 1 ml of sample directly to fluor of the above composition mixed in a ratio of toluene fluor:Triton X-100, 2:1. Counting was done in a Nuclear Chicago Mark I Scintillation Counter. Samples were counted for 10 minutes or until 40, 000 total counts had accumulated. All samples were counted in 10 ml of fluor. Counting efficiency and quenching were determined by the channels ratio method.

Chemicals

All chemicals were from J. T. Baker Co. unless otherwise noted. All media were purchased from Difco. L-aspartic acid (2, 3-^{3}H), specific activity 18 Ci/mmole; L-amino acid mixture ^{3}H labeled, reconstituted, protein hydrolysated (algal profile); glycine uniformly

^{14}C labeled, specific activity 5.2 Ci/mmole; uracil (5-^{3}H), specific activity 18 Ci/mmole; thymidine (methyl-^{3}H), specific activity 8.0 Ci/mmole; adenosine (8-^{3}H), specific activity 19 Ci/mmole; guanosine (8-^{3}H), specific activity 7.4 Ci/mmole were all purchased from Schwartz/Mann.

RESULTS

Preliminary Growth Stimulation Studies with RNA, DNA, and Protein

Preliminary experiments involved measuring growth stimulation of H-I bdellovibrios in growth limiting broth containing 1 mg/ml of Bacto peptone and 0.3 mg/ml of Bacto yeast extract. This medium (PYE/10) resulted in a final cell yield of about 1×10^7 Bdellovibrio/ml from an initial concentration of about 5×10^6 cells/ml. Autoclaved host cells, host cell extracts, and purified cell components were tested for growth stimulation of Bdellovibrio in PYE/10 (Tables 1, 2 and 3). PYE/10 with the addition of 1.5 mg/ml dry weight of autoclaved host cells, yielded greater than 10^9 Bdellovibrio/ml. Experiments with cell-free host extracts showed that 0.375 mg/ml of the extract would stimulate Bdellovibrio growth by some 100 times that of the control (Table 2). The supplementation of PYE/10 with 0.5 mg/ml of protein (bovine albumin) only doubled the cell yield. However, when purified RNA or DNA was added in concentrations of up to 0.5 mg/ml in PYE/10, no additional growth occurred (Table 3).

Enzyme Assays

In the next sequence of studies, the mechanisms used by Bdellovibrio for the breakdown of host macromolecules were examined.

Table 1. Growth responses of H-I Bdellovibrio 100 to autoclaved host cells.

Growth medium tested[a]	Initial titer	Final titer
PYE/10	4×10^7	6×10^7
	2×10^7	1.25×10^7
PYE/10 + 0.375 mg/ml host cells	4×10^7	1.2×10^9
	2×10^7	2.0×10^8
PYE/10 + 0.5 mg/ml host cells	4×10^7	1.7×10^9
	2×10^7	3.0×10^8
PYE/10 + 1.5 mg/ml host cells	4×10^7	3.0×10^9
	2×10^7	1.25×10^9
PYE	4×10^7	2.4×10^9
	2×10^7	1.0×10^9

[a] PYE is composed of 10 mg/ml of peptone and 3 mg/ml of yeast extract. PYE/10 is a 10-fold dilution PYE with 2 mM Ca^{++} and 2 mM Mg^{++} added. All media contain 0.01 M Tris buffer, pH 7.5. The 0.375 mg/ml (dry weight) of host cells corresponds to 7.5×10^8 Spirillum serpens cells.

Table 2. Growth responses of bdellovibrios to cell free host extract.

Growth medium tested[a]	H-I Uki2	H-I A3. 12	H-I 100
PYE/10	6.2×10^7	1.7×10^6	4.0×10^6
PYE/10 + 0.375 mg/ml protein	2.45×10^9	9.5×10^8	7.0×10^7
PYE/10 + 0.075 mg/ml protein	7.7×10^8	5.0×10^8	4.2×10^7
PYE	3.0×10^9	5.0×10^9	1.5×10^8
Initial titer	1.3×10^7	5.5×10^7	1.0×10^6

[a] PYE/10 and PYE are described in Table 1. The cell free host extract was prepared from Spirillum serpens. The cells were broken in an Eaton press, centrifuged, treated with RNase and DNase, and the high molecular weight fractions from a Sephadex G-100 column were pooled. Colony counts were performed after 24 hours when the cultures in PYE had reached early stationary phase.

Table 3. Growth response of Bdellovibrio to RNA and DNA.

Growth medium[a]	H-I Uki2	H-I A3. 12	H-I 100
PYE/10	1.9×10^7	1.3×10^8	1.4×10^7
PYE/10 + 0.1 mg/ml RNA	9.9×10^6	1.7×10^8	1.5×10^7
PYE/10 + 0.5 mg/ml RNA	1.2×10^7	1.2×10^8	2.0×10^7
PYE	2.0×10^9	3.0×10^9	1.4×10^9
Initial titer	1.0×10^7	3.4×10^7	2.6×10^7
PYE/10	1.4×10^7	5.7×10^7	2.0×10^7
PYE/10 + 0.1 mg/ml DNA	1.1×10^7	7.1×10^7	2.3×10^7
PYE/10 + 0.5 mg/ml DNA	2.2×10^7	7.7×10^7	9.2×10^6
PYE	1.7×10^9	3.2×10^9	2.3×10^9
Initial titer	5.0×10^6	2.2×10^7	1.4×10^7

[a] PYE and PYE/10 are described in Table 1. The RNA was purified from crude yeast RNA by the conventional methods (17). DNA was purified from calf thymus DNA (17). Colony counts were performed after 24 hours when the cultures in PYE had reached early stationary phase.

Plate assays were performed to test for extracellular proteolytic, and nucleolytic activities. Protease activity was determined by the hydrolysis of casein in PYE agar. The results in Tables 4, 5 and 6 show H-I B. starrii A3.12 to possess the greatest enzyme activity for all three enzymes tested. On the other hand, H-I B. bacteriovorus 100 is the least active for all three tested. H-I B. stolpii was intermediate in all enzyme activities. Three strains, OX9-2, Se-2, and Se-3, had no detectable DNase activity. A water soluble brownish pigment was produced by three strains, 100, W, and 109D, grown on commercial DNase test agars. This pigment was not seen on any other media.

It has been shown by several groups that bdellovibrios synthesize extracellular proteases, but the function of these enzymes has not been determined (5, 7, 10). The proteases hydrolyze casein, azocoll, and host proteins. In Figure 1, it is seen that ^{3}H labeled Escherichia coli protein is degraded to cold TCA soluble products. The degradation of the protein is almost complete within 20 min.

Nuclease activity had not been previously known; the existence of a Bdellovibrio DNase has been postulated by Rittenberg as a possible mechanism in degrading host DNA (16). Since plate assays gave positive results for all three species (Tables 5 and 6), culture supernatants were tested for the hydrolysis of labeled substrate. The degradation of ^{3}H labeled E. coli RNA by cell-free culture

Table 4. Detection of protease by the hydrolysis of casein.

Culture tested[a]	Amount of hydrolysis[b]
Bdellovibrio starrii A3. 12	+++
B. stolpii Uki2	++
B. bacteriovorus 100	+
" " 109D	+
" " 110	+
" " 114	+
" " 118	+
" " Xty	+
E. coli JC411	-
Pronase 100 μg	++++

[a] These H-I bdellovibrios were plated on PYE agar containing 1% casein (Matrix Mother Culture Media, Galloway West Co.). Incubation was for 24 hours at 28-30 C. Hydrolysis could be observed by clearing zones around the growth.

[b] ++++ = 3-4 cm of clearing; +++ = 2-3 cm of clearing; ++ = 2 cm of clearing; + = 1 cm of clearing; - = no observable clearing

Table 5. DNase plate assays of H-I bdellovibrios.

Culture tested[a]	Hydrolysis on commercial test agar[b]	Hydrolysis on fluorescent test agar
Bdellovibrio starrii A3.12	+++	+++
B. stolpii Uki2	++	++
B. bacteriovorus 100	+	+
" " 109D	+/-	+
" " 110	+/-	+
" " 114	+/-	+/-
" " OX9-2	-	-
" " Xty	+	+
" " Se-2	-	+/-
" " Se-3	-	+/-
W	++	++
3294	++	++
E. coli JC411	-	-
DNase 10 µg	++++	++++

[a] Results are the average of three separate platings on both Difco and BBL commercial test agars. The H-I bdellovibrios were plated on the test agar and incubated from 24-36 hours at 28-30 C. The commercial test agar contained 2 mg/ml bull sperm DNA, 2 µg/ml tryptose and 5 mg/ml NaCl. Hydrolysis was detected by flooding the plates with 2 N HCl. The fluorescent DNase test agar was a modification of the RNase test agar described by Lanyi and Lederberg (15). Fifteen mg/ml of DNA was substituted for the 15 mg/ml of RNA in the stock solution. (See Materials and Methods section and Table 6 for details.) Hydrolysis was observed by loss of fluorescence when the plates were observed under ultraviolet light.

[b] ++++ = 3-4 mm; +++ = 1-2 mm; ++ = 1 mm; + = 1/2 mm; +/- = 1/2 mm or less; - = no observable clearing

Table 6. RNase plate assays of H-I bdellovibrios.

Culture tested[a]	Amount of hydrolysis on fluorescent test agar[b]
Bdellovibrio starrii A3. 12	+++
B. stolpii Uki2	++
B. bacteriovorus 100	+
" " 109D	+/-
" " 110	+/-
" " 114	+/-
Aeromonas hydrophila	+++
E. coli JC411	-
RNase (pancreatic) 10 μg	++++

[a] Results are the average of three separate platings on the test agar. The H-I bdellovibrios were plated on the test agar and incubated from 24-36 hours at 28-30 C. The test agar consisted of PYE agar with the addition of 1% of a stock RNA and acridine orange solution described in the Materials and Methods section. Hydrolysis was observed by the loss of fluorescence around growth when the plates were observed in a darkened room and under ultraviolet light.

[b] ++++ = 3-4 mm of no fluorescence; +++ = 2-3 mm; ++ = 2 mm; + = 1 mm; +/- = 1/2 mm or less; - = no observable clearing

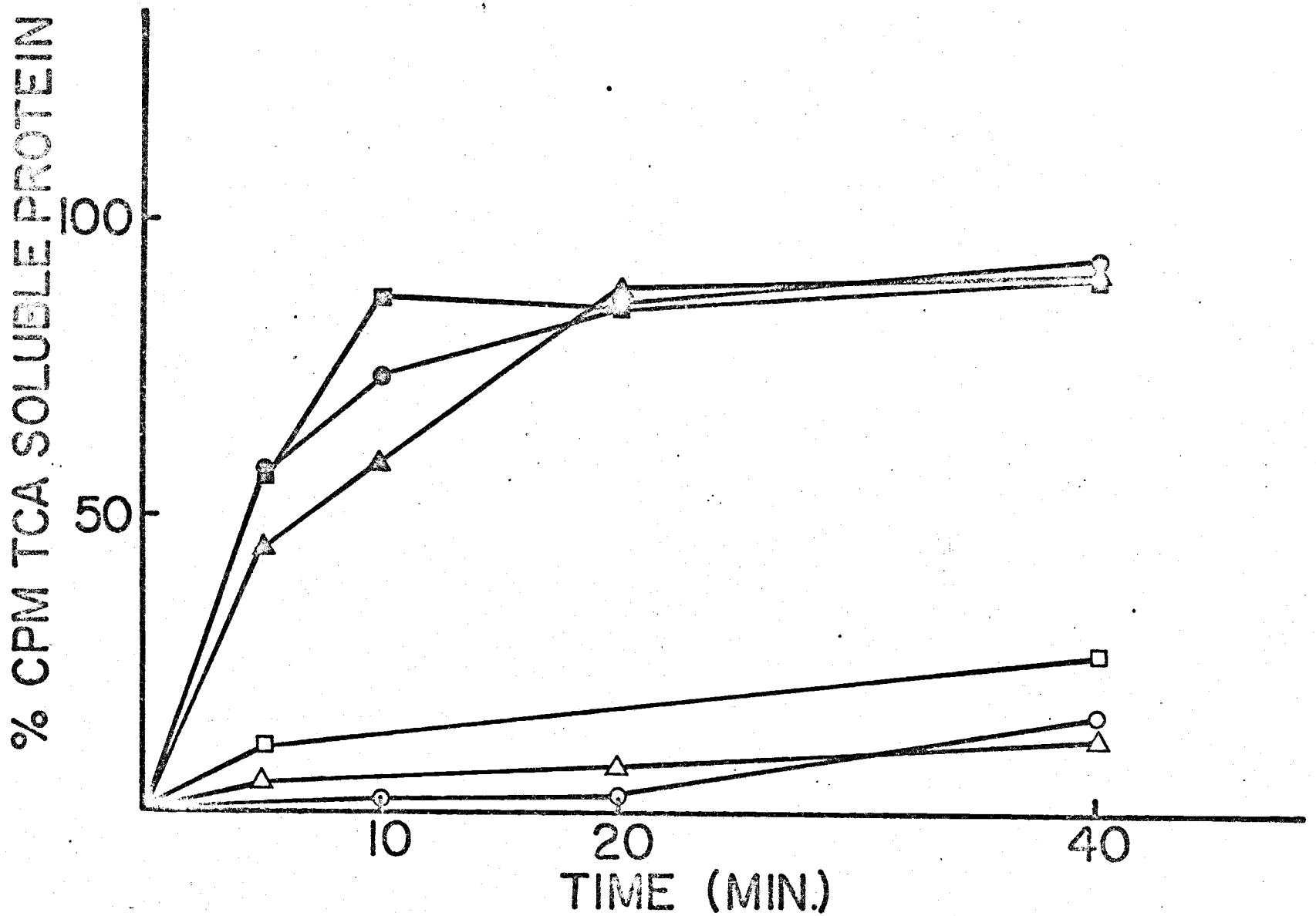
Figure 1. Solubilization of ^{3}H labeled Escherichia coli protein by cell-free spent H-I Bdellovibrio medium. The points represent the average of two experiments. The reaction mixtures contained the following materials: for H-I B. starrii A3. 12 and H-I B. stolpii Uki2: 7 ml TCM buffer, 1 ml ^{3}H protein substrate and 2 ml of spent PYE culture medium; for H-I B. bacteriovorus 100: 4 ml TCM buffer, 1 ml ^{3}H labeled protein substrate and 5 ml of spent PYE culture medium. The reaction was incubated at 40 C.

■ H-I B. starrii A3. 12

● H-I B. stolpii Uki2

▲ H-I B. bacteriovorus 100

Open symbols indicate sterile PYE controls.


% CPM TCA SOLUBLE PROTEIN
100
50
10
20
40
TIME (MIN.)

supernatants is shown in Figure 2. The same species pattern of nuclease activity exists as for the proteases, i. e., with H-I B. starrii A3.12 showing the greatest activity and H-I B. bacteriovorus 100 presenting the lowest activity. Data from the hydrolysis of ^{3}H labeled DNA are very similar, as can be seen in Figure 3.

Utilization of Amino Acids

The utilization of amino acids was to be a starting point for studying the assimilation of protein. H-I bdellovibrios grown in the presence of 15 ^{3}H labeled amino acids (algal profile) incorporate counts into cold TCA precipitable material, but not into hot TCA soluble material (Table 7). Thus there appears to be no detectable levels of label incorporated into nucleic acids.

To test whether Bdellovibrio are capable of synthesizing purines and pyrimidines from the usual amino acid precursors, feeding experiments were conducted with 2, 3 ^{3}H labeled aspartate and uniformily ^{14}C labeled glycine. Table 8 shows that these nucleotide precursors are not incorporated into hot TCA soluble material, although the percent incorporation into other cell components is comparable to that of the algal profile amino acids.

Figure 2. Solubilization of ^{3}H labeled Escherichia coli RNA by cell-free spent H-I Bdellovibrio culture medium. The points represent the average of two or more experiments. Incubation of the reaction was at 40 C. At zero time there were approximately 3,000 cpm per 0.2 ml of sample. See "Assays for Nuclease Activity" section of Materials and Methods for details of the assay.

● H-I B. starrii A3. 12

■ H-I B. stolpii Uki2

▲ H-I B. bacteriovorus 100

Controls showed no loss of counts.

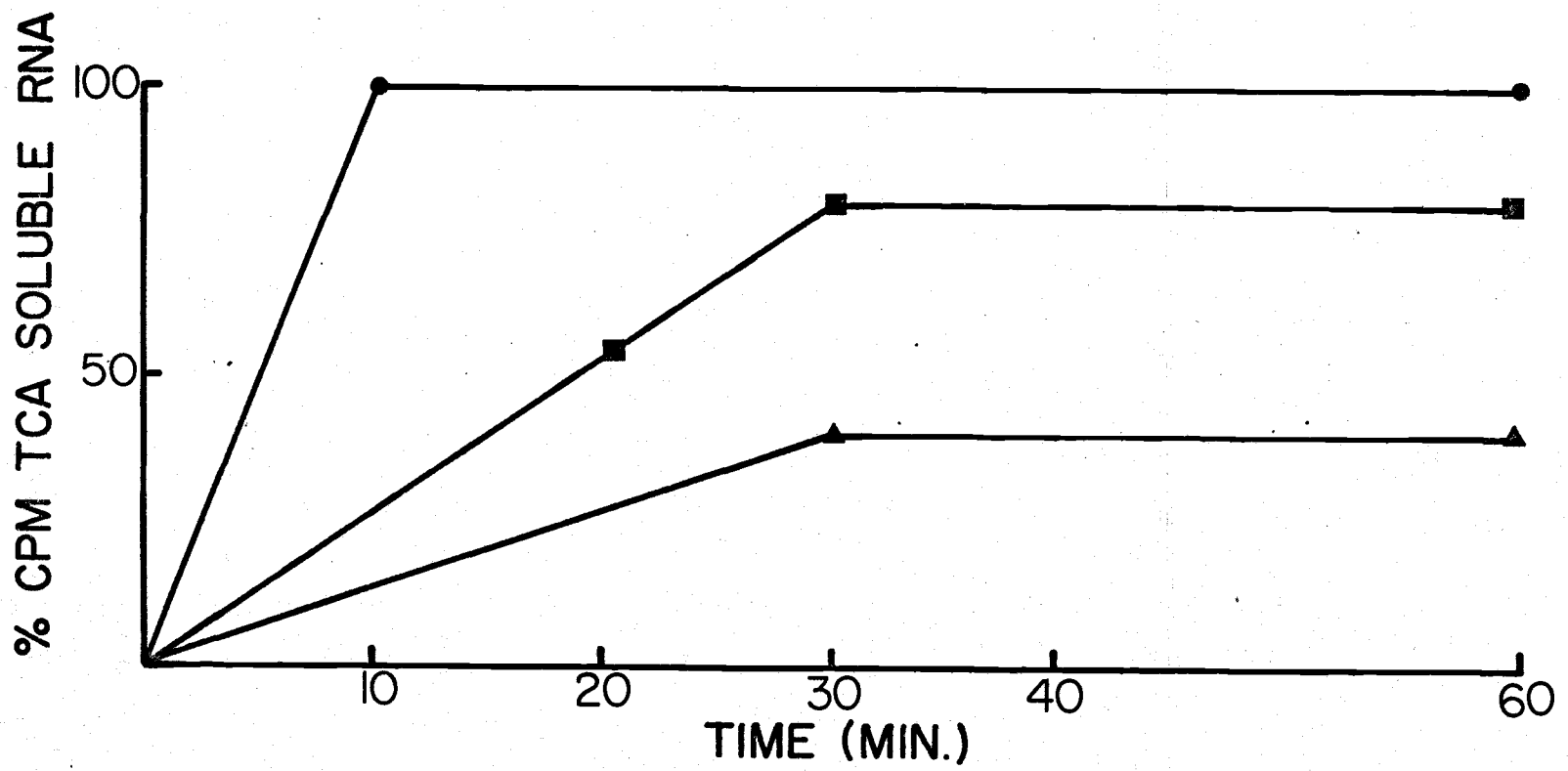

% CPM TCA SOLUBLE RNA
100
50
10
20
30
40
60
TIME (MIN.)

Figure 3. Solubilization of ^{3}H Aeromonas hydrophila DNA by cell-free spent H-I Bdellovibrio culture medium. See the legend to Figure 2 and "Assays for Nuclease Activity" section of Materials and Methods for details of the assay.

■ H-I B. starrii A3. 12

● H-I B. stolpii Uki2

▲ H-I B. bacteriovorus 100

Controls showed no loss of counts.

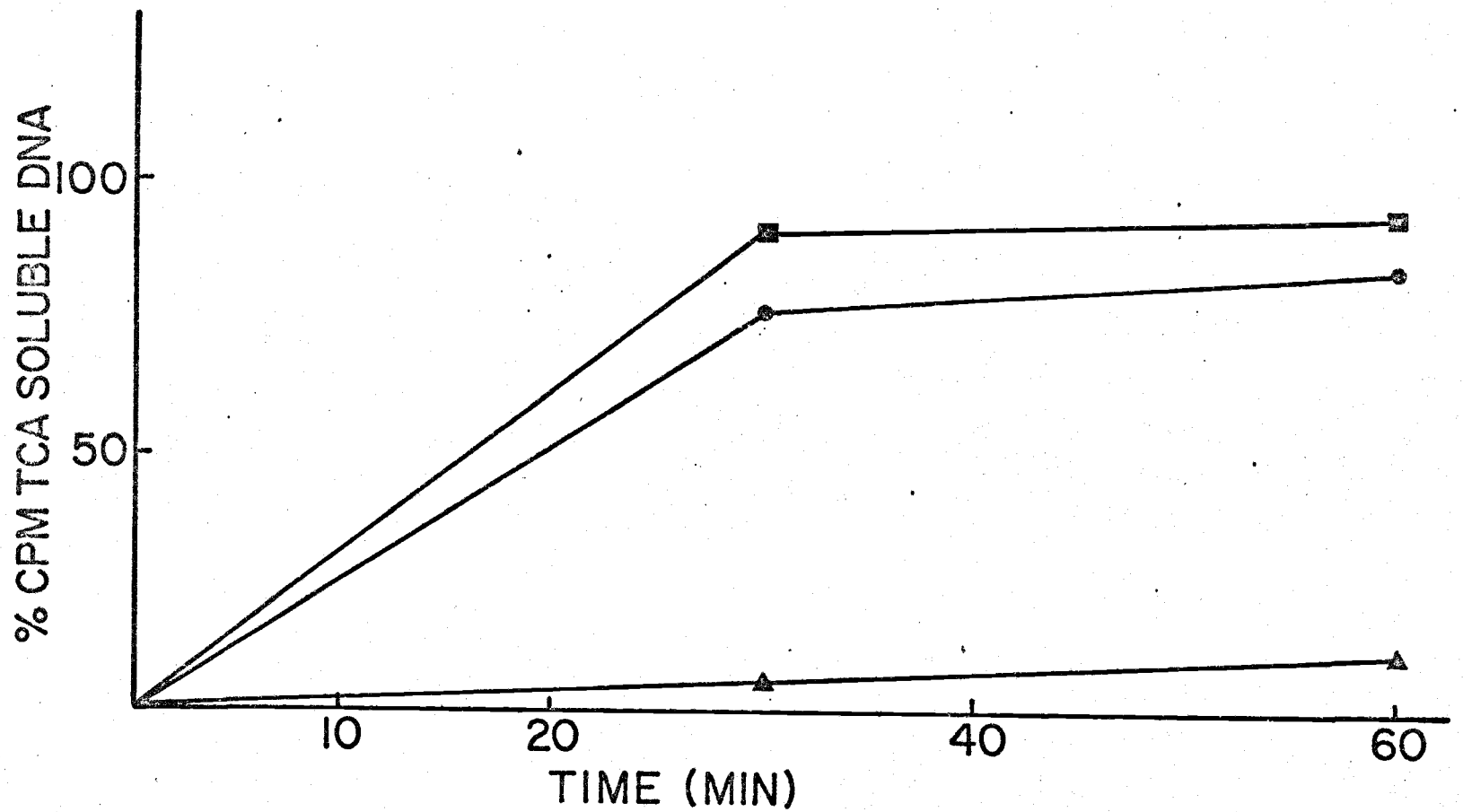

% CPM TCA SOLUBLE DNA
100
50
10
20
40
60
TIME (MIN)

Table 7. Incorporation of ^{3}H labeled amino acid mixture.

	Trial I	Trial II	Trial III
H-I Bdellovibrio starrii A3. 12			
Cpm/0. 1 ml culture	78, 000	47, 000	90, 600
Cpm cold TCA (24 hr)	4, 615	1, 015	5, 670
Cpm remaining from hot TCA extraction	4, 830	1, 233	N. D.
% Cpm cold TCA ppt per 1 O. D. $_{600}$	4%	2. 6%	4. 2%
H-I B. stolpii Uki2			
Cpm/0. 1 ml culture	108, 108	82, 000	81, 088
Cpm cold TCA (24 hr)	3, 012	1, 180	1, 626
Cpm remaining from hot TCA extraction	3, 405	1, 403	1, 840
% Cpm cold TCA ppt per 1 O. D. $_{600}$	1. 6%	1. 1%	1. 2%
H-I B. bacteriovorus 109D			
Cpm/0. 1 ml culture	92, 000	89, 400	
Cpm cold TCA (36 hr)	2, 559	3, 418	
Cpm remaining from hot TCA extraction	2, 668	3, 630	
% Cpm cold TCA ppt per 1 O. D. $_{600}$	2. 8%	2. 5%	

All counts are for 0. 1 ml of sample. There were no detectable cold TCA precipitable counts present at the start of the experiment. The number of cpm of cold and hot TCA precipitable material was determined after 24 or 36 hr of incubation. N. D. indicates not determined.

Table 8. Incorporation of ^{3}H labeled L-aspartic acid + ^{14}C labeled glycine.

	L-aspartic acid		Glycine
	Trial I	Trial II	
H-I Bdellovibrio starrii A3. 12			
Cpm/0. 1 ml culture	108, 108	117, 741	12, 338
Cpm cold TCA (24 hr)	8, 435	10, 476	1, 296
Cpm remaining from hot TCA extraction	9, 562	10, 612	1, 609
% Cpm cold TCA ppt per 1 O. D. $_{600}$	5%	5. 5%	10%
H-I B. stolpii Uki2			
Cpm/0. 1 ml culture	108, 108	103, 272	4, 639
Cpm cold TCA (24 hr)	3, 013	2, 524	322
Cpm remaining from hot TCA extraction	3, 406	2, 654	393
% Cpm cold TCA ppt per 1 O. D. $_{600}$	1. 6%	2. 1%	5. 2%
H-I B. bacteriovorus 100			
Cpm/0. 1 ml culture	95, 291	110, 146	4, 779
Cpm cold TCA (36 hr)	1, 907	1, 163	156
Cpm remaining from hot TCA extraction	2, 317	N. D.	198
% Cpm cold TCA ppt per 1 O. D. $_{600}$	1. 8%	1%	2. 2%

All counts are for 0. 1 ml sample. There were no detectable cold TCA precipitable counts present at the start of the experiment. The number of cpm of cold and hot TCA precipitable material was determined after 24 or 36 hr of incubation.

Utilization of Nucleic Acid Precursors

Before studying the incorporation of the products from Bdellovibrio hydrolyzed nucleic acids, the uptake of several nucleosides was examined. Bdellovibrio takes up labeled uracil, adenosine, guanosine, and thymidine but does not take up uridine or thymine (Table 9; personal communication, R. J. Seidler). The macromolecules labeled by growth in the presence of these substrates are completely solubilized by hot 5% TCA. It should be noted that unlike the amino acid uptake, guanosine and adenosine are usually taken up to greater than 10% of the added label.

Utilization of Bdellovibrio Hydrolyzed Host Macromolecules

To answer the question on the relevance of extracellular enzymes to its metabolism, host hydrolysates were fed to Bdellovibrio. When H-I bdellovibrios are grown in the presence of an E. coli protein hydrolysate, the hydrolysis products become cold TCA precipitable (Table 10). An average of 5.3% of the added label is incorporated per 1.0 O. D.$_{600}$ of Bdellovibrio. Again there is no solubilization of labeled compounds by hot TCA treatments.

In Table 11, it is seen that H-I bdellovibrios grown in the presence of hydrolyzed E. coli RNA incorporate the labeled uracil into cold TCA precipitable material. Ninety-five percent of the

Table 9. Incorporation of ^{3}H labeled adenosine and guanosine.

	Guanosine	Adenosine	
H-I Bdellovibrio starrii A3. 12			
Cpm/0. 1 ml culture	94, 081	75, 099	
Final cpm cold TCA precipitable (24 hr)	19, 422	44, 913	
Cpm remaining from hot TCA extraction	13	25	
% Cpm cold TCA ppt per 1 O. D. $_{600}$	13. 8%	42. 7%	
H-I B. stolpii Uki2		Trial I	Trial II
Cpm/0. 1 ml culture	93, 965	86, 018	66, 417
Final cpm cold TCA precipitable (24 hr)	12, 055	19, 819	16, 057
Cpm remaining from hot TCA extraction	14	41	22
% Cpm cold TCA ppt per 1 O. D. $_{600}$	8. 5%	20. 9%	20. 0%
H-I B. bacteriovorus 100			
Cpm/0. 1 ml culture	98, 037	83, 135	
Final cpm cold TCA precipitable (36 hr)	16, 667	38, 500	
Cpm remaining from hot TCA extraction	13	27	
% Cpm cold TCA ppt per 1 O. D. $_{600}$	12. 3%	34. 3%	

All counts are for 0. 1 ml of sample. There were no detectable cold TCA precipitable counts at the start of the experiment. Final cpm cold TCA precipitable refers to precipitable counts after 24 or 36 hr of incubation. Cpm remaining from hot TCA extraction refers to the number of counts that remain TCA precipitable after 40 mins at 85 C in 5% TCA.

Table 10. Incorporation of ^{3}H labeled Escherichia coli protein hydrolyzed by H-I Bdellovibrio bacteriovorus 100 protease.

	Trial I	Trial II
H-I Bdellovibrio starrii A3. 12		
Cpm/1. 0 ml culture	30, 900	28, 220
Initial cpm cold TCA ppt	60	100
Final cpm cold TCA ppt (24 hr)	1, 840	980
Cpm remaining from hot TCA extraction	1, 750	980
% Cpm cold TCA ppt per 1 O. D. $_{600}$	6%	6. 2%
H-I B. stolpii Uki 2		
Cpm/1. 0 ml culture	32, 960	38, 000
Initial cpm cold TCA ppt	100	0
Final cpm cold TCA ppt (24 hr)	900	1, 040
Cpm remaining from hot TCA extraction	870	1, 020
% Cpm cold TCA ppt per 1 O. D. $_{600}$	4. 8%	4. 7%
H-I B. bacteriovorus 100		
Cpm/1. 0 ml culture	32, 230	33, 370
Initial cpm cold TCA ppt	20	60
Final cpm cold TCA ppt (36 hr)	550	1, 500
Cpm remaining from hot TCA extraction	520	1, 300
% Cpm cold TCA ppt per 1 O. D. $_{600}$	6%	4%

All counts are for 1. 0 ml sample. Initial cpm cold TCA ppt refers to samples taken immediately after inoculation of the medium; final cpm TCA ppt refers to samples taken after 24 or 36 hr. Hot TCA extractions were only carried out after 24 or 36 hr.

Table 11. Incorporation of ^{3}H labeled *E. coli* RNA hydrolyzed by H-I *Bdellovibrio starrii* A3. 12 enzymes.

	Trial I	Trial II
H-I *Bdellovibrio starrii* A3. 12		
Cpm/1. 0 ml culture	6,000	8,470
Initial cpm cold TCA ppt	0	0
Final cpm cold TCA ppt	1,380	1,280
Cpm remaining from hot TCA extraction	60	40
% Cpm cold TCA ppt per 1 O. D. $_{600}$	15. 8%	11. 6%
H-I *B. stolpii* Uki2		
Cpm/1. 0 ml culture	8,450	7,240
Initial cpm cold TCA ppt	0	20
Final cpm cold TCA ppt	870	600
Cpm remaining from hot TCA extraction	50	30
% Cpm cold TCA ppt per 1 O. D. $_{600}$	9. 4%	8. 4%
H-I *B. bacteriovorus* 100		
Cpm/1. 0 ml culture	4,720	8,440
Initial cpm cold TCA ppt	20	0
Final cpm cold TCA ppt	880	2,080
Cpm remaining from hot TCA extraction	40	0
% Cpm cold TCA ppt per 1 O. D. $_{600}$	17. 9%	16%

All counts are for 1. 0 ml of sample. Initial cpm cold TCA ppt refers to samples taken immediately after inoculation of the medium; final cpm cold TCA ppt refers to samples taken after 24 or 36 hr. Hot TCA extractions were only carried out after 24 or 36 hr.

labeled material becomes soluble in hot 5% TCA. From 8.4% to 17.9% of the added labeled material becomes cold TCA precipitable per 1.0 O.D.$_{600}$ of Bdellovibrio culture (Table 11). Similarly, the labeled thymidine from hydrolyzed Aeromonas hydrophila DNA is incorporated by bdellovibrios into cold TCA precipitable material (Table 12). Between 10% and 4.4% of the added labeled material is cold TCA precipitable per 1.0 O.D.$_{600}$ of Bdellovibrio culture. Again as with the RNA, almost all of the radioactive material is soluble in hot 5% TCA.

Kinetics of Host RNA Destruction and Subsequent Utilization of Bdellovibrio

In order to estimate the amount of RNA required to saturate the filters, a portion of the uninfected E. coli culture was used as a source of ^{32}P labeled RNA. The specific activity of the E. coli RNA was determined and amounts of RNA ranging from 1 to 100 μg were allowed to hybridize to filters containing ^{3}H labeled E. coli DNA. As can be seen in Table 13, between 30 and 50 μg of RNA saturated the filters. From this trial experiment, 50 μg amounts of E. coli RNA were hybridized to E. coli and Aeromonas DNA as well as to blank filters (Table 13). It can be seen that the DNA bound only to about 5% of the homologous RNA level. This level corresponds to the expected amount of homology observed between these two genera (personal

Table 12. Incorporation of ^{3}H labeled Aeromonas hydrophila DNA hydrolyzed by H-I Bdellovibrio starrii A3. 12 enzymes.

	Trial I	Trial II
H-I Bdellovibrio starrii A3. 12		
Cpm/1. 0 ml culture	11, 000	12, 660
Initial cpm cold TCA ppt	0	10
Final cpm cold TCA ppt	1, 450	1, 480
Cpm remaining from hot TCA extraction	0	20
% Cpm cold TCA ppt per 1 O. D. $_{600}$	13. 8%	10%
H-I B. stolpii Uki2		
Cpm/1. 0 ml culture	11, 000	12, 390
Initial cpm cold TCA ppt	10	30
Final cpm cold TCA ppt	1, 950	1, 820
Cpm remaining from hot TCA extraction	80	70
% Cpm cold TCA ppt per 1 O. D. $_{600}$	12%	11. 2%
H-I B. bacteriovorus 100		
Cpm/1. 0 ml culture	10, 980	13, 180
Initial cpm cold TCA ppt	10	40
Final cpm cold TCA ppt	2, 110	1, 470
Cpm remaining from hot TCA extraction	20	0
% Cpm cold TCA ppt per 1 O. D. $_{600}$	14. 4%	13. 1%

All counts are for 1. 0 ml of sample. Initial cpm cold TCA ppt refers to samples taken immediately after inoculation of the medium; final cpm cold TCA ppt refers to samples taken after 24 or 36 hr. Hot TCA extractions were only carried out after 24 or 36 hr.

Table 13. *E. coli* RNA saturation levels of DNA containing filters.

Amount *E. coli* RNA in hybridization mixture (μg)	*E. coli* RNA bound to: (μg)
	10 μg *E. coli* DNA
1	0.020
5	0.096
10	0.320
20	0.600
30	0.860
50	0.936
100	0.880
	10 μg Aeromonas DNA
50	0.050
	Blank filters
50	0.001

These values are the average of three separate hybridizations. The mixture refers to the 0.5 ml of the hybridization buffer, 2X SSC and 40% DMSO.

communication, P. Beach). This level of RNase resistant hybrid indicates a good degree of specificity in the reaction. There was virtually no detectable hybrid formed on the blank filters (Table 13). Also, the zero time level of E. coli RNA hybridized to Bdellovibrio DNA was less than 1% of the homologous reaction (Table 15). In all the following hybridization experiments 50 μg amounts of RNA were used.

The growth of H-D B. bacteriovorus 109D on the ^{32}P labeled E. coli CSH42 was monitored by phase microscopic observations and by plaque counts. A typical synchronous life cycle was observed as can be seen by the PFU curve in Figure 4 and the plaque counts in Table 14. The multiplicity of infection (MOI) of the host was 1.7. The average burst size was 4.1 progeny cells per infected E. coli. The slight decrease at 2 hours is common and must be due to cell clumping of the infected host cells, thus giving an artificially lowered titer. In preliminary experiments, the MOI varied from 1.5 to 2.2 and the average burst size varied from 3.8 to 4.3 under growth conditions identical to those used here. Thus, the $^{32}PO_4$ had no noticeable influence on growth kinetics. In the current experiment, the burst began at about 3 hours after infection and was completed by 5 hours.

The RNA that is labeled with ^{32}P changes in its hybridization specificity with time (Figure 4, Table 15). Labeled RNA extracted at

Table 14. Growth of Bdellovibrio in synchronous conditions.

Sample	Time after infection (hr)	Titer	RNA bound to 10 μg Bdellovibrio DNA (μg)
E. coli before mixing	0	1.09×10^{10} CFU/ml	--
Bdellovibrio before mixing	0	8.4×10^{9} PFU/ml	--
Bdellovibrio after mixing	0	1.66×10^{9}	0.00375
Bdellovibrio	1	1.33×10^{9}	0.0215
Bdellovibrio	2	0.81×10^{9}	0.0193
Bdellovibrio	3	1.85×10^{9}	0.0467
Bdellovibrio	4	4.5×10^{9}	0.375
Bdellovibrio	5	6.7×10^{9}	0.671
Bdellovibrio	6	6.8×10^{9}	0.564

$$\text{MOI} = \frac{100 \text{ ml Bdellovibrio} \times 8.4 \times 10^{9} \text{ PFU/ml}}{45 \text{ ml E. coli} \times 1.09 \times 10^{10} \text{ CFU/ml}} = 1.7$$

$$\text{Burst size} = \frac{6.8 \times 10^{9} \text{ final PFU/ml}}{1.66 \times 10^{9} \text{ initial PFU/ml}} = 4.1 \text{ progeny/parent cell}$$

Growth conditions are described in the Materials and Methods section.

CFU - colony forming units; PFU - plaque forming units

Table 15. Results of DNA-RNA hybridizations.[a]

RNA sample time (hr)	μg RNA bound/10 μg DNA		
	Trial I	Trial II	Average
Bdellovibrio DNA on filter			
0	0.003	0.0045	0.00375
1	0.021	0.022	0.0215
2	0.0215	0.017	0.0193
3	0.036	0.0575	0.0467
4	0.35	0.40	0.375
5	0.659	0.684	0.671
6	0.577	0.551	0.564
E. coli DNA on filter			
0	0.625	0.760	0.697
1	0.301	0.389	0.345
2	0.255	0.3089	0.282
3	0.252	0.177	0.215
4	0.194	0.118	0.156
5	0.154	0.122	0.138
6	0.012	0.012	0.012

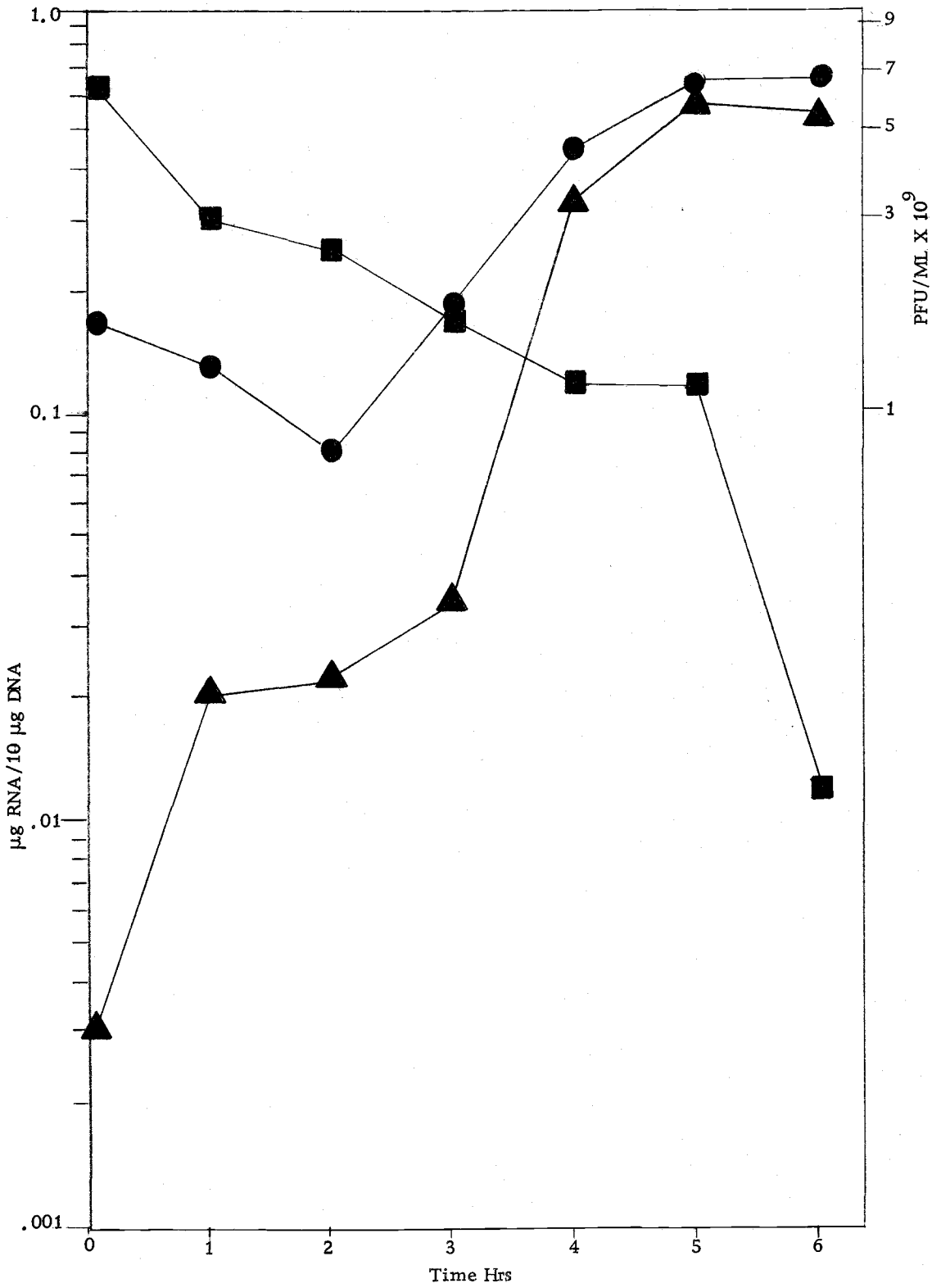
[a]The two groups of hybridizations were run within 24 hours of each other. Three samples were run of each time period with both types of filters: each figure represents an average of three separate filters.

Figure 4. H-D Bdellovibrio bacteriovorus 109D growth curve and specificity of the hybridization reaction. The μg amounts of RNA are the average of six separate hybridization filters. The viable count (PFU/ml) is the average of triplicate counts at two dilutions.

● PFU/ml of H-D B. bacteriovorus 109D

■ μg of labeled RNA hybridizing to 10 μg of Escherichia coli DNA

▲ μg of labeled RNA hybridizing to 10 μg of B. bacteriovorus DNA


1.0
0.1
.01
.001
µg RNA/10 µg DNA
9
7
5
3
1
PFU/ML X 10^9
0
1
2
3
4
5
6
Time Hrs

different times hybridized less effectively with E. coli DNA and more effectively to Bdellovibrio DNA as the infection progressed. At the beginning of the experiment, immediately after mixing the host and Bdellovibrio less than 1% of the homologous labeled RNA hybridizes to the Bdellovibrio DNA containing filters. After 1 hour of incubation there is about a 5-fold increase in the amount of radioactive RNA hybridizing to Bdellovibrio RNA. This value remains the same through the second hour of infection. This increase in Bdellovibrio specific RNA is accompanied by a 50% decrease in the amount of RNA hybridizing to the E. coli DNA.

A major transition in the appearance of radioactive Bdellovibrio specific RNA polyribonucleotides begins between 2 and 3 hours after infection. This is accompanied by a 25% decrease in the amount of RNA that is bound to the E. coli DNA filters.

A very large change in binding specificity occurs during 3 to 5 hours after infection. A 15-fold increase in the RNA binding to Bdellovibrio DNA occurs after some 85% of the host RNA is lost. By 5 hours the labeled RNA hybridized to Bdellovibrio DNA to the same extent as it did to the E. coli DNA at the start of the experiment.

The specific activity of the RNA decreases during the infection cycle (Table 16). The specific activity of the RNA at the beginning of the life cycle is about 35,000 cpm/μg. By 6 hours, when some 99% of the RNA has been turned over, the specific activity of the RNA has fallen to 26,000 cpm/μg.

Table 16. Specific activities of RNA samples.

Time of sample (hr)	Specific activity (cpm/μg)	
	Trial I	Trial II
0	35577	34357
1	39610	39350
2	41704	35942
3	28775	27170
4	28121	24659
5	25872	22983
6	26112	26203

Each value was determined by averaging the counts on three filters. The initial specific activity of purified E. coli RNA was 65, 763 cpm/μg. This specific activity was reduced to about 35, 000 cpm/μg (47% reduction) at zero time upon addition of the Bdellovibrio.

DISCUSSION

Bdellovibrio has the enzymatic mechanisms for the degradation of host protein, RNA, and DNA. The present study has shown the significant functions of these exocellular hydrolytic enzymes. Not only are all three host components hydrolyzed by these enzymes, but the products are subsequently utilized by Bdellovibrio for biosynthetic purposes.

It was demonstrated in a growth limiting medium that autoclaved host cells, cell-free host extracts, and protein could stimulate growth of H-I bdellovibrios (Tables 1, 2, and 3). Purified RNA or DNA in the same medium did not increase Bdellovibrio growth. It appears that the protein can be used as a carbon and energy source. However, nucleic acids are unable solely to provide the requirements for Bdellovibrio growth. Hespell and co-workers (8) have shown that amino acids released from host protein serve as a major energy source for H-D Bdellovibrio 109D during its intracellular growth. They suggested that carbohydrates are not an energy source since neither glucose nor ribose were respired by H-D Bdellovibrio cell suspensions. Therefore, pentoses derived from nucleic acids will not be of use to Bdellovibrio as an energy source. Since acetate was also oxidized, host lipids may be utilized if they can be broken down (8). Therefore, Bdellovibrio is limited in the types of substrates which it can utilize.

In the present study, the extracellular enzymes have only been characterized with respect to activity. The proteases, acting upon a radioactive host protein substrate, hydrolyze it to cold TCA soluble material. H-I B. starrii A3.12 is the most active and H-I B. bacteriovorus 100 the least active (Figure 1). A recent study has indicated that proteolytic activity is due to at least two or three enzymes (7). It has not been determined whether one or all proteases are active specifically in degrading host proteins. It appears that all Bdellovibrio species can totally degrade native host protein. The proteases could provide the amino acids that Bdellovibrio respires. Hespell and colleagues (8) did not suggest any possible mechanism for the degradation of host protein, but noted that they must be hydrolyzed to monomers.

Cell-free culture supernatants contain nucleases which hydrolyze labeled host RNA and DNA. B. starrii A3.12 hydrolyzes the RNA substrate completely in about 10 minutes (Figure 2). B. stolpii Uki2 hydrolyzes some 85% of the host RNA. About 40% of the RNA is hydrolyzed by B. bacteriovorus 100 after 1 hour of incubation (Figure 2). Although the reaction with B. bacteriovorus 100 enzymes and RNA was carried out for 2 hours, no additional hydrolysis occurred. These differences in ability to hydrolyze the purified RNA may indicate different substrate specificities for each nuclease. It is possible that the differences in hydrolysis reflect differences in concentration of nucleases or temperature or pH optima.

When the hydrolysis of DNA is examined (Figure 3), the same general species activity trend exists. Again B. starrii A3.12 hydrolyzes about 95% of the DNA substrate while B. stolpii Uki2 hydrolyzed 85%. Only 24% of the DNA is cold TCA soluble after 1 hour incubation in B. bacteriovorus 100 enzymes. These differences in ability to hydrolyze polynucleotides in vitro may not reflect the exact situation in vivo. It has been demonstrated (16) that after 30 minutes of infection only 30% of the host DNA is bandable in cesium chloride. However, the DNA was still cold TCA precipitable. The RNase and DNase activities are assumed to be distinct, but it is possible that a single nuclease similar to snake venom diesterase is responsible for the hydrolysis of host nucleic acids. Rittenberg (16) suggested several mechanisms that could be involved in the destruction of host DNA. The existence of Bdellovibrio nucleases was one possible mechanism. It is now evident that Bdellovibrio does have the ability to hydrolyze polynucleotides. If nucleases are induced, as the proteases are after 1 hour of infection (personal communication, R. J. Seidler), then the inhibition of host DNA destruction by adding streptomycin at the start of infection (16) may be due to the halt of necessary Bdellovibrio protein synthesis.

Labeled amino acids and host protein hydrolysates are incorporated into TCA insoluble material by growing H-I bdellovibrios (Tables 7, 8, and 10). From 1% to 4% of the added labeled amino

acids is used anabolically for the production of protein. About 4% to 6% of the radioactive label from an added host protein hydrolysate is used in the production of Bdellovibrio protein. It is worth emphasizing that the host protein substrate used in these experiments consisted of native enzymes plus structural proteins. Care was taken to avoid protein degradation. The protein extract was not heated nor subjected to other denaturing agents as was done in previous studies (21, 25). The degradation of the purified proteins would mimic conditions confronting the H-D Bdellovibrio when growing in the intraperiplasmic space of its host. Of the exogenous ^{14}C glutamate added to a culture of unlabeled E. coli and H-D Bdellovibrio, 6% was released as $^{14}CO_2$ and 3% was taken up by Bdellovibrio after 4 hours of infection (8). Similar results were obtained by Hespell et al. (8) using a labeled amino acid mixture in place of ^{14}C glutamate. There is agreement then between the results obtained with H-I and H-D bdellovibrios in terms of amino acid incorporation.

Bdellovibrio macromolecules labeled either by free amino acids or the host protein hydrolysate were not solubilized by hot TCA. This could be caused by biosynthetic deficiencies, which make Bdellovibrio unable to form its own nucleotide bases, or low concentrations of labeled glycine and aspartate in the isotope mixture. Glycine and aspartate are precursors of purines and pyrimidines. The addition of only ^{14}C glycine or ^{3}H aspartate to growing cultures resolved this

point. From 1% to 5% of the aspartic acid and from 2% to 10% of the glycine was incorporated into cold TCA precipitable material. Counts are not reduced by exposure to hot TCA indicating the deficiency for de novo biosynthesis of purines and pyrimidines (Table 8). The consequences of this deficiency are that, during intracellular growth, nucleotide bases for both RNA as well as DNA must be obtained preformed from the host nucleic acids.

H-I bdellovibrios grown in the presence of labeled nucleosides or host RNA or DNA hydrolysates incorporated the label into cold TCA precipitable material. From 8.4% to 17.9% of the ^{3}H-uracil of the added RNA hydrolysate is incorporated into Bdellovibrio nucleic acids (Table 11). H-I bdellovibrios incorporate 10% to 14.4% of the (^{3}H methyl)thymidine from the added host DNA hydrolysate (Table 12). The labeled material from both experiments was totally soluble in hot TCA, indicating that the material is nucleic acids. Studies of H-D forms demonstrated similar uptake of nucleic acid monomers. If H-D bdellovibrios are grown on unlabeled E. coli in the presence of (^{3}H methyl)thymidine, about 6% of the label is incorporated into Bdellovibrio DNA (16). When ^{14}C labeled adenosine is substituted for thymidine, 3.9% of the label is utilized by the parasite. Our experimental conditions, i.e., host DNA or RNA hydrolyzed by Bdellovibrio enzymes and subsequently incorporated by H-I bdellovibrios, approximate the situation encountered by the H-D forms inside the host cell.

Experiments using nucleosides and nucleotides gave some indications as to the chemical form of the breakdown products of nucleic acids that are usable by bdellovibrios. H-I *B. bacteriovorus* 100 and 109D do not incorporate free thymine nor uridine, but readily take up thymidine and uracil (personal communication, R. J. Seidler). Adenosine and guanosine are actively incorporated by H-I bdellovibrios. In contrast to the amount of amino acid incorporation, from 8.5% to 42.7% of these added nucleosides are assimilated into cold TCA precipitable material (Table 9). The labeled material is incorporated into nucleic acids since all the radioactive material is hot TCA soluble. It is inferred that these forms of purines and pyrimidines are made available by enzymatic mechanisms during growth of *Bdellovibrio* within its host.

DNA-RNA hybridization techniques have made it possible to differentiate between host and *Bdellovibrio* RNA species. Using this methodology, it has proven feasible to study the kinetics of destruction of labeled host RNA, and to follow its resynthesis into *Bdellovibrio* specific polynucleotides.

The labeled RNA from uninfected *E. coli* was used for the saturation experiments. The amount of RNA (30-50 μg) required to saturate the *E. coli* DNA containing filters is consistent with other studies using messenger RNA or total RNA (6, 14). It was planned that the 1 hour chase with cold phosphate would remove all the label from the

unstable RNA classes. Although there was some cell growth during this period, it is apparent from the saturation levels that the chase was ineffective. When messenger or total RNA is hybridized to homologous DNA from 3% to 10% of the DNA is saturated (14). Only about 0.3% of the DNA is saturated if ribosomal RNA is hybridized to homologous DNA (6). From about 8.6% to 9.4% of the E. coli DNA was saturated by the uninfected E. coli RNA (Table 13). The labeling of the unstable RNA was fortuitous, since it allowed the destruction of the host messenger and ribosomal RNA to be followed. Since the label from the host was to appear in both messenger and ribosomal RNA of Bdellovibrio, higher concentrations of RNA would be required to saturate the DNA filters than would be required to saturate the DNA with only host ribosomal RNA (14, 24).

During the synchronous life cycle the titer of the Bdellovibrio culture increased from 1.7×10^9 to 6.8×10^9 PFU/ml. As the infection progressed the amount of labeled RNA that hybridized to Bdellovibrio DNA increased while the amount of radioactive RNA binding to E. coli DNA decreased. Bdellovibrio apparently degrades the host RNA to generate the biosynthetic monomers it requires to synthesize its polynucleotides.

Prior to infection less than 0.1% of the E. coli labeled RNA hybridized to Bdellovibrio DNA (Table 15). Almost 7% of the host DNA is saturated by the same RNA sample. This compares with about 9%

hybridization when RNA from uninfected E. coli was used in the preliminary saturation experiments (Table 13). This 22% decrease in the amount of DNA saturated may be due to the dilution from the cold Bdellovibrio RNA. There is a significant change in the specific activity between the uninfected host RNA and the 0 hour sample; the decrease is almost 50% (Table 16). Of the 50 μg of RNA in the hybridization mixture, about 25 μg is labeled, which is slightly less than necessary to saturate the filters (Table 13).

The labeled RNA has undergone major changes during the first hour of infection. Slightly more than one-half of the E. coli RNA has been degraded to a size that either no longer hybridizes to the homologous DNA or that is no longer precipitated by ethanol during the purification procedure. Rittenberg (16) demonstrated that during the first hour of infection all of the host DNA became non-bandable in equilibrium cesium chloride gradients. This DNA, however, was still cold acid precipitable. It appears that the degradation of the RNA and DNA occurs simultaneously although significantly about 50% of the RNA remains host specific at 1 hour.

After hour 1 of infection there is a continuous and incremental drop of about 25% per hour in the amount of labeled RNA that remains hybridizable to host DNA (Figure 4). Between the fifth and sixth hours of infection (Figure 4, Table 15) there is a final 10-fold drop in host specific RNA. This decrease occurs after the burst is almost

complete. It may indicate a loss into the supernatant of the remaining unhydrolyzed RNA from the broken host cells.

This type of degradation may indicate a controlled process in which the nucleic acids are broken down as needed, so that monomers do not escape out of the damaged host by diffusion. In the utilization of host DNA, it was suggested that the process is closely controlled to allow maximum utilization of the host DNA (16). It appears that the DNA is quickly degraded to intermediate size polynucleotides and they are further broken down to mononucleotides as needed. There must be a balance between the rates of degradation and synthesis.

The synthesis of Bdellovibrio specific RNA from radioactive host RNA occurs in a discontinuous fashion during the infection cycle (Figure 4, Table 15). Bdellovibrio specific RNA is synthesized from the degradation products of host nucleic acids during the first hour of infection (Table 15). Immediately after Bdellovibrio enters the host the process of acquiring host nucleic acid monomers must begin. There is no detectable synthesis of Bdellovibrio DNA during the first hour of infection (16). The synthesis of DNA was followed by isolating DNA in cesium chloride gradients. If Bdellovibrio specific DNA synthesis had been determined by DNA-DNA hybridization, synthesis not observed by the former technique may be discovered by this more sensitive approach.

During the second hour there is no further increase in Bdellovibrio specific RNA; thus could be due to a channeling of nucleotides into DNA synthesis. Immediately following the first hour of infection incorporation of exogenous thymidine increases greatly (personal communication, R. J. Seidler). The first period of DNA synthesis observed by Rittenberg (16) occurred from 45 to 105 minutes after infection.

The incorporation of ^{32}P labeled host material into Bdellovibrio RNA continues for the rest of the infection cycle. Major increases in Bdellovibrio specific RNA occurred immediately prior to the burst period (Figure 4). The greatest amount of RNA biosynthesis occurring during the third and fourth hours of infection is parallel to the active Bdellovibrio mass increase which is microscopically observable during this period (20). DNA synthesis resumes between 180 and 225 minutes after initiation of infection (16). Thus both DNA and RNA synthesis appears to occur concomitantly during the later part of the infection cycle. It appears that early in the cycle DNA synthesis may have preference over RNA synthesis.

During the first three hours of infection the specific activity of the RNA remains constant (Table 16). Following hour 3 of infection, the specific activity of the RNA falls to about 74% of the initial value and remains constant for the duration of the infection. This fall in specific activity occurs concomitantly with the period of maximum

Bdellovibrio RNA biosynthesis. When Bdellovibrio DNA synthesis occurs from 180 to 225 minutes, the specific activity falls some 30% from its previous level and by 210 minutes it remains constant at that level (16). It was suggested that non-DNA host precursors were the cause of the drop in specific activity. It seems likely that the RNA falls in specific activity because there is about a 25% non-utilization of host nucleic acid precursors possibly caused by turnover of unlabeled Bdellovibrio components.

The studies of Bdellovibrio metabolism show that RNA, DNA, and protein are all hydrolyzed and assimilated by the parasite. DNA-RNA hybridization experiments confirmed that the results obtained with the H-I bdellovibrios correspond to the situation with the H-D forms. The utilization of the host RNA resembles that of the host DNA; both appear to be under close control that allows for maximum utilization of the host macromolecules in a controlled continuous fashion throughout the life cycle. There is very little of the host that escapes destruction and eventual metabolism by bdellovibrios.

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