Host-independent Growth of *Bdellovibrio bacteriovorus* in Microbial Extracts

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(Accepted for publication 23 September 1969)

SUMMARY

Three *Bdellovibrio bacteriovorus* wild-type strains tested grew into long forms, up to 80 times their original lengths, when incubated in cell-free microbial extracts. This growth occurred both in extracts of organisms permissive and non-permissive for *Bdellovibrio* parasitic growth, but not in any other medium tested. Under certain osmotic conditions the long forms segmented into chains of cells. Some division of chains occurred. Only approximately 5 % of the *bdellovibrios* grew into long forms. ³H-thymidine was incorporated into the long forms but not into short forms. A component of *Pseudomonas aeruginosa* extracts which supported growth of *Bdellovibrios* was macromolecular, heat stable and resistant to pronase, DNase and RNase.

INTRODUCTION

Bdellovibrio bacteriovorus is a bacterial parasite which multiplies inside susceptible bacterial host organisms. After growing inside the host, it segments into daughter cells which are released from the ghosted host (Scherf, DeVay & Carol, 1966; Starr & Baigent, 1966; Shilo, 1969). The *bdellovibrio*-host system provides an experimentally accessible model for studying certain aspects of intracellular parasitism which are often more difficult to approach with parasites of animals and higher plants (Shilo, 1969). A problem encountered with all such systems is the difficulty of varying the environment in which the parasite grows. In an attempt to minimize this difficulty with *bdellovibrios* we have developed a new system that permits limited extracellular growth and division.

METHODS

Bacterial strains and growth. The *Bdellovibrio bacteriovorus* strain routinely used was a streptomycin-resistant isolate (spontaneous mutation) of strain 109. *Bdellovibrios* were grown in liquid culture using *Escherichia coli* K12 strain P567 as host, according to the conditions of Varon & Shilo (1968). *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* strain 68A were grown on rotary shakers at 37° in nutrient broth; *B. cereus* T, at 30° in nutrient broth; *Micrococcus lysodeikticus*, at 37° on brain heart infusion; and *Saccharomyces cerevisiae*, at 30° in nutrient broth + 2 % (w/v) glucose, adjusted to pH 6.0. *Pseudomonas aeruginosa* was isolated from a patient at Hadassah Hospital, Jerusalem. Other strains were from the collections of the Departments of Microbiological Chemistry and Bacteriology, Hebrew University, Hadassah Medical

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School. Bdellovibrio plaques were assayed on *E. coli* P 567, by the method of Varon & Shilo (1968).

Preparation of cell extracts. Cultures were harvested in late log phase (or for bdellovibrios, soon after host lysis was complete). Organisms were sedimented in a centrifuge, washed once and resuspended in a solution containing 0.3 % (w/v) Difco-Casamino acids (vitamin free), pH 7.5 (hereafter called 'amino acid buffer'). Bacteria were broken by sonic treatment for 5 to 10 min. at 4 to 6 amps in 10 to 30 ml. on a Bronson sonifier model no. 5110. For some experiments *Pseudomonas aeruginosa* organisms were lysed by incubation at 30° (pH 7.8) for 20 min. in a solution containing 30 µg. lysozyme/ml. and 4 mM-EDTA, after which they were incubated for 10 min. at 20° in a solution containing 20 µg. DNase/ml. and 10 mM-MgSO₄. Debris and most remaining intact cells were removed by centrifugation at 20,000 *g* for 15 min. Extracts were then filtered through HA filters (Millipore Corp., Bedford, Mass.) to remove any remaining intact organisms. For some experiments extracts were separated into supernatant and pellet fractions by centrifugation at 120,000 *g* for 2 hr at 4°. In these cases the pellet was washed in amino acid buffer, recentrifuged as above and resuspended in amino acid buffer. Supernatant and pellet fractions were refiltered through HA filters. Extracts or fractions were then dialysed in tubing (No. 18/32, Scientific Instrument Centre, London, England) which had been sterilized by being autoclaved suspended from a capped glass tube into a flask containing the liquid to be dialysed against. Unless stated otherwise, 'bacterial extract' refers to an extract of *P. aeruginosa* prepared by sonication, filtered and dialysed overnight (1:200) against amino acid buffer. These extracts retained 50 to 90 % of their 'activity' (see below) after storage up to 1 month at 4°.

Incubation mixture for induction of long forms. The standard incubation mixture contained 1.5×10^9 bdellovibrios/ml., 5 mg. Difco-Casamino acids (vitamin free), pH 7.5/ml. and bacterial extract or other material being tested for its ability to induce growth. Incubation was at 30°, with shaking, for the time stated. Bdellovibrios were prepared from cultures cooled to 4° when lysis of *Escherichia coli* appeared by phase contrast microscopy to be complete. After 0 to 8 hr, cultures were centrifuged at 750 *g* for 2 min. to remove any precipitate present in the growth medium, and then at 27,000 *g* for 20 min. to pellet the bdellovibrios. These organisms were then resuspended in modified TM buffer (0.01 M-tris (pH 7.5) + metals; Varon & Shilo, 1968), the concentration measured spectrophotometrically and a portion added to the incubation mixture as $\frac{1}{3}$ to $\frac{1}{2}$ the final volume. Bacterial extract was added to a final concentration of 1.0 mg. protein/ml., unless otherwise stated. Protein determinations were by the Biuret method.

Thymidine-incorporation experiments. To measure thymidine incorporation into acid-precipitable material, thymidine-methyl-³H (2 C/MM, Radiochemical Centre, Amersham, England) was added at the designated time to the standard incubation mixture just described, normally to final concentration 3 µC./ml. At the same time, non-labelled thymidine was added to final concentration 0.5×10^{-4} M. At appropriate times, 50 µl. samples were removed and applied to paper discs (Whatman 3 MM). Discs were passed successively through three beakers containing cold 10 % TCA and two beakers containing ethanol, and were dried. Radioactivity was measured in a scintillation counter. For the standard rate-of-incorporation assay, ³H-thymidine was added after the standard incubation mixture had shaken for 12 to 13 hr. A 50 µl. sample was removed 2 hr later and the acid precipitable counts determined as above.

Microscopy. For determining the fraction of long forms in a *Bdellovibrio* population, cultures after 13 hr incubation were suspended in 0.05 M-Na azide plus 0.1 M-Na acetate (pH 6.0) to stop motility and then examined by phase contrast in a Petroff-Hausser counter. Each examination included 450 to 500 organisms. Those organisms which were both noticeably fatter than, and at least $4 \times$ the length of, the normal small bdellovibrios present, were counted as long forms.

Materials. Nutrient broth, casein amino acids, yeast extract, tryptone and brain heart infusion were from Difco Laboratories, Detroit, Mich. RNase, RNase-free DNase and alcohol dehydrogenase were from Worthington Biochemicals, Freehold, N.J. Pronase (grade B) was from Calbiochem, Los Angeles, Cal. Yeast RNA and calf thymus DNA were from Sigma Chemical Co., St. Louis, Mo.

RESULTS

Bdellovibrio growth in host-free cell extracts

When *Bdellovibrio* strain 109 was aerated at 30° in the presence of dilute dialysed cell-free extracts of *Pseudomonas aeruginosa*, approximately 5 % of the organisms grew into long forms. Morphological changes were first observed by phase contrast microscopy after 4 to 5 hr, at which time some of the bdellovibrios were fatter and slightly longer and had lost their motility. Within an hour, some fatter organisms were 3 to 4 times the length of most of the visible bdellovibrios, and were characteristically limp, bending and flopping on their shorter axis in response to random disturbances of the liquid. During the next 5 to 10 hr, progressively longer forms were visible. Over 80 % of these were clearly segmented into units approximately $0.4 \mu \times 0.9 \mu$ (Pl. 1). Chains as long as 100 units appeared.

Partial division of chains less than 15 units long was observed occasionally. In these instances the intact chains appeared to be quivering. Single breaks occurred after some minutes, seemingly at random along the chain. Usually, additional breaks were observed at intervals of several minutes, and single units released swum out of the field. Plate 1, fig. 3-4 shows chains as they appeared during division.

Growth of long forms also occurred in dialysed cell-free extracts of *Escherichia coli*, *Micrococcus lysodeikticus*, *Saccharomyces cerevisiae* and *Bdellovibrio bacteriovorus* strain 109 itself (final protein concentrations of 1 mg./ml.). These forms were indistinguishable by phase contrast microscopy from those of Plate 1. We verified that *M. lysodeikticus* and *S. cerevisiae* are not hosts for *Bdellovibrio* strain 109. Little or no attachment of bdellovibrios to these cells was observed by phase contrast microscopy. No plaques were formed on these strains, even when loops of bdellovibrios at 10^8 /ml. were spotted on cell lawns. Extracellular growth of bdellovibrios did not occur in any of a variety of complex media tested (see below).

In addition to *Bdellovibrio* 109, two strains independently isolated in Jerusalem from sewage were tested for extracellular growth (strains GB and T28, isolated by M. Varon). In *Pseudomonas aeruginosa* extract, each grew into long forms which closely resembled those of Plate 1.

In each of the above instances, only a fraction of the *Bdellovibrios* grew into long forms. Standard incubation mixtures prepared on separate days were estimated to be 6.2, 3.9, 5.3 and 7.0 % long forms (for standard conditions and estimation procedure, see Methods). We were unable to increase this fraction by varying extract con-

centrations, *Bdellovibrio* concentrations, the species source of the extract or the *Bdellovibrio* strain. The length of time after host cell lysis that the bdellovibrios were harvested had little effect. Other instances of phenotypic variation in a genetically homogeneous *Bdellovibrio* population, involving attachment to hosts, have been reported (Varon & Shilo, 1968, 1969a).

Fig. 1

Fig. 2

Fig. 1. Incorporation of ^3H -thymidine during growth of long forms. *Bdellovibrios* were incubated with bacterial extract under standard conditions (see Methods) and 50 μl . samples were removed at various times for determination of acid-precipitable counts. $\bullet$, ^3H -thymidine added at $t = 0$; $\blacktriangle$, ^3H -thymidine added at $t = 12$ hr; open symbols, extract replaced by amino acid buffer in the incubation mixtures.

Fig. 2. Effect of *bdellovibrios* and bacterial extract concentrations on rate of thymidine incorporation. Standard rate of incorporation assays were performed (see Methods). (A) Bacterial extract concentration constant at 1.0 mg. protein/ml. (B) *Bdellovibrio* concentration constant at 1.5×10^9 organisms/ml.

Thymidine incorporation during host-free *Bdellovibrio* growth

Thymidine was incorporated into *bdellovibrios* during incubation in which long forms developed, but not under other host-free conditions. Upon incubation of *Bdellovibrio*, *Pseudomonas* extract and ^3H -thymidine (see Methods) there was a lag period of several hours with little incorporation, followed by a much more rapid incorporation. If ^3H -thymidine was added after the lag period, it was incorporated immediately (Fig. 1). In the latter case, the rate of incorporation was found to vary linearly with both the concentration of cell extract and the *Bdellovibrio* concentration over the ranges shown (Fig. 2). Extracts of *Micrococcus lysodeikticus* stimulated incorporation to approximately the same extent. Separation of organisms according to size, on a sucrose gradient, showed that the label was distributed broadly among large forms, with < 2 % incorporated into *bdellovibrios* of normal size (Fig. 3). Over 90 % of the radioactivity incorporated was in DNase-sensitive material.

On no occasion in this work did we observe long forms without thymidine incorporation or thymidine incorporation without long forms. Neither was ever observed without prior addition of cell extract or an extract fraction. None of the following nutrients induced long forms or thymidine incorporation: nutrient broth, yeast extract, tryptone, brain heart infusion (each at 29 and 35 mg./ml. final concentrations);

bovine serum albumin, haemoglobin, yeast RNA, calf thymus DNA, each at 0.2, 1 and 5 mg./ml. final concentrations, in 4 mg. casein amino acids/ml. pH 7.5; yeast RNA and calf thymus DNA degraded by RNase and DNase at 0.2, 1 and 5 mg./ml., in 4 mg. casein amino acids/ml. pH 7.5.

In view of these results, we considered thymidine incorporation to be a useful supplement of microscopic examination as an indicator of the development of *Bdellovibrio* long forms. In the preliminary characterizations that follow, extract activity is quantitated in terms of the rate of ^3H -thymidine incorporated into bdellovibrios under standard conditions (Methods). For each assay, growth at three or more extract concentrations was examined microscopically after 12 to 14 hr. In every instance this semi-quantitative examination was consistent with the results of the incorporation assay.

Fig. 3

Fig. 4

Fig. 3. Sucrose gradient of *Bdellovibrio* organisms. ^3H -thymidine was added to a standard mixture of bdellovibrios and bacterial extract which had incubated for 12 hr. Following 3 hr additional incubation, 0.3 ml. of the mixture was layered on a 5 ml. gradient of 5 to 20% sucrose in amino acid buffer. The gradient revolved at 2000 r.p.m. for 10 min. at 4° in a clinical centrifuge. The tube was then punctured at the bottom and drops were collected. Recovery in the drops was 70% of the acid-precipitate counts applied to the gradient. $\circ$ — $\circ$, acid-precipitable counts; $\triangle$ — $\triangle$ *Bdellovibrio* plaque forming units.

Fig. 4. Sephadex G-150 chromatography of supernatant fraction of bacterial extract. A 3 ml. sample, containing 40 mg. of bacterial protein and added molecular weight markers was applied to a 100 ml. column of Sephadex G-150 (25 cm. $\times$ 4 cm.²) equilibrated with 0.03 M tris, pH 7.5. Fractions of 2.2 ml. were collected and filter sterilized. Stimulation of ^3H -thymidine incorporation into bdellovibrios was measured by the standard rate of incorporation assay (see Methods), using 0.15 ml. fraction aliquots in 0.3 ml. reaction mixtures. Incorporation of 1000 counts/min. is 1% of the ^3H -thymidine added. ADH is yeast alcohol dehydrogenase, MW 151,000. $\blacktriangle$ — $\blacktriangle$, thymidine incorporation; $\circ$ — $\circ$, optical density (418 nm) due to haemoglobin marker.

An active factor in cell extracts is macromolecular and heat stable

A factor from cell extracts which induced long forms was non-dialysible. The activity in extracts of *Pseudomonas aeruginosa*, *Escherichia coli*, *Micrococcus lysodeikticus*, *Saccharomyces cerevisiae* and *Bdellovibrio bacteriovorus* increased 20 to 100% upon dialysis (based on rate of thymidine incorporation). Upon centrifugation of a dialysed sonicated extract of *P. aeruginosa*, at $120,000 \times g$ for 2 hr, 40 to 60% of the activity was recovered from the pellet and an approximately equal amount from the supernatant. (The same distribution between supernatant and pellet was obtained from extracts of *P. aeruginosa* prepared by lysozyme-EDTA).

The supernatant material was fractionated by G-150 Sephadex chromatography. All recovered activity, representing 65 % of the total applied to the column, was in fractions of molecular weight $\geq 50,000$ (Fig. 4). No inhibitory material was detected in the fractions of lower molecular weight or the fractions between the two activity peaks. Long forms were induced by all fractions which stimulated thymidine incorporation (fractions 2 to 19), although the lengths were variable and, in the area between the peaks, considerably shorter than in the long forms induced by the unfractionated material. The other fractions did not induce long forms of any size. The same pattern of activity was obtained when the molecular weight markers were omitted.

The non-dialysible factor from extracts was heat stable. More than 90 % of the activity from diluted dialysed *Pseudomonas aeruginosa*, *Escherichia coli* and *Micrococcus lysodeikticus* extracts, or the supernatant fractions, remained after boiling for 20 min. When extracts were autoclaved or boiled at very high concentration, a heavy flocculent precipitate appeared, and 50 to 100 % of the activity was lost.

The factor was stable to DNase, RNase and pronase. Dialysed *Pseudomonas aeruginosa* extracts were first diluted to 5 mg. protein/ml. in 0.02 M-tris (pH 7.5) and heated for 5 min at 98°. To test DNase and RNase, MgSO_4 was added to 10 mM, the enzymes added each to 30 $\mu\text{g./ml.}$, and the solution incubated for 30 min. at 25°. Full activity remained, both before and after dialysis. To test pronase, the enzyme (final concentration 100 $\mu\text{g./ml.}$) was added to the diluted heated extract, and the solution incubated at 45°. After 60 min. the mixture was heated for 5 min. at 98° to inactivate the pronase, and was assayed. Full activity remained. (If an extract was incubated at 45° without prior heating at 98°, 30 to 90 % of the activity was lost; the loss of activity varied between extract preparations, but was essentially the same whether or not RNase, DNase or pronase was present.)

An assay for division

^3H -thymidine was incorporated almost exclusively into long forms (Fig. 3). If division occurs, label should appear later in short forms, with a corresponding loss from long forms. Evidence of this was obtained by the direct filtration of long form cultures on cellulose ester (Millipore) filters. Long forms are retained on 1.2 μ (mean pore diameter) filters, while most short forms pass through. Fig. 5A shows that after 15 to 20 hr of incubation label appeared in material which passed through 1.2 μ filters. The results were basically the same when addition of ^3H -thymidine was delayed (Fig. 5B). (Levels of incorporation measured by 0.22 μ filter retained-material, and by acid-precipitable material, were indistinguishable.)

To examine more closely the size distribution of the divided forms, a *Bdellovibrio* mixture labelled as in Fig. 5B was displayed on a sucrose gradient after 14 and 24 hr incubation (Fig. 6). In the period of sedimentation of these gradients, the undivided long forms pelleted, so that only small forms were visible (compare Fig. 3). Figure 6 shows that after 14 hr little or none of the acid-precipitable radioactivity was in the region corresponding to small organisms, while at a later time 50 % of the label was in this region. The radioactivity peak was slightly ahead of the bulk of the *bdellovibrio* organisms probably because the newly divided *bdellovibrios* were more compact than the longer and thinner older organisms and therefore sedimented faster (see below).

Fig. 5. Appearance of label in short forms. ^3H -thymidine was added to a standard incubation mixture at $t = 0$ (A) or $t = 12$ hr. (B). At various times 0.2 ml. samples were removed into 10 ml. of cold amino acid buffer and mixed for 1 min. Equal portions were filtered on 0.22 μ and 1.2 μ filters. Each filter was washed with 50 ml. nutrient broth. $\bullet$ — $\bullet$, Material retained on 0.22 μ filters; $\circ$ — $\circ$, material retained on 1.2 μ filters; $\triangle$ — $\triangle$, material passing through 1.2 μ but retained on 0.22 μ filters (by subtraction).

Fig. 6

Fig. 7

Fig. 6. Sucrose gradients of *Bdellovibrio* organisms. ^3H -thymidine was added to a standard incubation mixture at $t = 12$ hr. At the specified times, 0.3 ml. of the mixture was layered on a sucrose gradient as in Fig. 3 and revolved at 2000 r.p.m. for 30 min. The tubes were then punctured and drops were collected. $\circ$ — $\circ$, Acid-precipitable counts of sample removed at $t = 14$ hr; $\bullet$ — $\bullet$, acid-precipitable counts of sample removed at $t = 24$ hours; $\triangle$ — $\triangle$, *Bdellovibrio* concentration determined by Petroff-Hausser counter.

Fig. 7. Effect of osmolarity on morphology and thymidine incorporation. Standard rate of incorporation assays were performed, except that the usual amino acid concentration of 5 mg./ml. was replaced by various amino acid, sucrose, or NaCl concentrations. After 13 hr incubation, cultures were examined by phase contrast microscopy. $\circ$ — $\bullet$, Amino acid added; $\triangle$ — $\triangle$, sucrose added; $\square$ — $\square$, NaCl added. Open symbols, coiled long forms; closed symbols, segmented, uncoiled forms; half closed symbols, intermediate or both forms.

Two morphologically distinct long forms depending on osmotic conditions

In addition to the segmented long form already described, a tightly coiled non-segmented long form was induced when amino acids were omitted from the standard incubation mixture (Pl. 2, fig. 8-11). The effect was not due to nutrition, since the NaCl or sucrose concentrations also controlled which form appeared (Fig. 7). At intermediate concentrations, forms intermediate between tight-coil and segmented straight forms occurred. The addition of NaCl, sucrose or amino acids after 13 hr growth of long forms in their absence did not induce a conversion of coiled to segmented forms, nor did it stimulate increased thymidine incorporation.

Rich medium induces inclusion bodies in Bdellovibrio short forms

In testing various rich media for growth of long forms, we frequently observed that prominent inclusion bodies, previously unreported in *Bdellovibrio*, were induced in the short forms. A reproducible procedure which we adopted was to incubate bdellovibrios at 30° in cell-free dilute nutrient broth (DNB of Shilo & Bruff, 1965) in which *Pseudomonas aeruginosa* had been heated for 30 min at 65°. Under these conditions, over 70 % of the bdellovibrios developed one or more inclusion bodies within 4 hr (Pl. 2, fig. 12). Despite the small size of the bdellovibrios, these bodies were also visible by phase contrast microscopy.

DISCUSSION

Evidence had accumulated, from the enzymic content of *Bdellovibrio* (Simpson & Robinson, 1968) and its ability to grow equally well on live, heat-killed and u.v.-killed hosts (Varon & Shilo, 1969*b*), that the role of the host bacteria in *Bdellovibrio* infection was limited to satisfying nutritional and/or physical requirements. The growth of bdellovibrios in bacterial extracts reported here further confirms this view. Since extracts of both hosts and non-hosts support growth, it is apparent that factors other than nutrition prevent bdellovibrios from parasitizing certain organisms. A physical barrier against attachment and/or penetration is the likely limitation in many cases. Chemical defences may be relevant as well, however. We noted in this connexion that dialysed and undialysed extracts of *Bacillus cereus* and *B. subtilis* induced few or no long forms, and inhibited the development of long forms when added to other extracts.

Osmotic as well as nutritional conditions affected extracellular growth in our system. At low osmolarity only non-segmenting forms occurred. On the other hand, low osmotic strength was excellent for motility (Varon & Shilo, 1968) and respiration (S. C. Rittenburg, unpublished data) of normal extracellular bdellovibrios. This may reflect the natural dichotomy encountered by bdellovibrios in their normal life cycle. Intracellular growth causes only limited leakage of host material to the extracellular environment (Drucker, 1969), and so presumably occurs at high osmolarity. Motility and maintenance, on the other hand, occur in dilute extracellular environments.

Extracellular bdellovibrios demonstrate considerable metabolic activity (Simpson & Robinson, 1968; Varon & Shilo, 1969*b*). Differences were expected, however, between activities involving motility and maintenance, and those which involve growth. We looked for an activity specific enough to the growing forms to overshadow the corresponding activity in the majority population of the short forms. Experiments with radioactive uracil and leucine showed significant incorporation into both forms. Thymidine, however, was incorporated only into the long forms. Since growth of

long forms in bacterial extracts occurred whether or not thymidine was added for an incorporation measurement, other sources of thymidine were available in the incubation mixtures. Nevertheless, we found that ^3H -thymidine incorporation consistently was correlated with the amount of long forms.

We do not know to what extent the limited extracellular growth in our system is relevant to the intracellular parasitic growth of bdellovibrios. It could instead represent a facultative ability for extracellular growth, with characteristics and controls different from those of the intracellular cycle. There exist mutant *Bdellovibrio* strains which can multiply extracellularly in complex media with no requirement for a factor from bacterial extracts (Shilo & Bruff, 1965; Seidler & Starr, 1968; Dietrich, Denny, Hashimoto & Conti, 1969). The growing forms observed with such strains are significantly shorter than those in our system (M. Starr, personal communication). It is tempting to speculate that chains of up to 100 cells accumulate in our system because the bdellovibrios require a 'divide signal' which would normally occur intracellularly when nutrients were depleted, or when the pressure of the confining host cell wall was sensed. The osmotic requirement in our system also may suggest that growth is proceeding as if intracellularly. Additional studies are needed to clarify the relationship between the three demonstrable forms of *Bdellovibrio* growth (i.e., intracellular, extracellular in microbial extracts and extracellular by selected strains in complex media).

Intracellular parasites need access to the host materials. Many such parasites are permeable to molecules normally excluded from most organisms (Moulder, 1962; Hall & Claus, 1963). A particularly striking case is that of *Clamydiae psittaci*, which changes to a more permeable form during the intracellular stage of its life cycle (Tamura & Manire, 1968; Moulder, 1968). *Bdellovibrios* may well undergo a similar change to a more permeable intracellular form. Since the extracellular long forms reported here may more closely resemble intracellular bdellovibrios, it would be of interest to measure the relative permeability toward various molecules of the long and short forms. Such a permeability change early in intracellular development could exert direct control over many metabolic processes. Thus, DNA synthesis might begin when precursors which are normally excluded from the organism are free to enter. It is possible that thymidine uptake and subsequent incorporation into DNA in our system is an immediate consequence of a permeability change.

Elongation of bdellovibrios after lysis of the host culture has been observed previously (Varon & Shilo, 1968). Typically, bdellovibrios, short and plump upon their release from the host, become thinner, rod-like and somewhat longer, sometimes doubling or tripling their original length. Burger, Drews & Ladwig (1968) briefly reported a different observation, elongation of bdellovibrios into spiral forms in host cell extracts. In none of these cases do the lengths approach those observed in our system, and in no case was segmentation reported.

One of us (A. M. R.) received a grant from the B. de Rothschild Fund for the Advancement of Science and Technology, and an Advanced Studies Fellowship from the Hebrew University. These are gratefully acknowledged.

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EXPLANATION OF PLATES

Figures 5-7 and 11-12 are electron micrographs taken by Dr Martin Kessel of this department. These specimens were prepared by negative staining by either 1 % potassium phosphotungstate (pH 7.0 to 7.2) or 1 % ammonium molybdate. Specimens were viewed in an AEI EM6B electron microscope operating at 60 kv.

PLATE 1

Bdellovibrio long forms grown on *Pseudomonas aeruginosa* extract. Photographs made after 16 hr incubation at standard conditions. Small bdellovibrios are visible in background.

Fig. 1-4. Phase contrast microscopy ($\times 1400$).

Fig. 5. Electron microscopy ($\times 6000$).

Fig. 6. Electron microscopy ($\times 7500$).

Fig. 7. Electron microscopy ($\times 4500$).

PLATE 2

Fig. 8-11. *Bdellovibrio* long forms grown at low osmotic strength (see text). Photographs made after 16 hr incubation.

Fig. 8-9. Typical highly coiled forms. Phase contrast microscopy ($\times 1400$).

Fig. 10. Less typical, less coiled form, showing coils more clearly. Phase contrast microscopy ($\times 1400$).

Fig. 11. Electron micrograph ($\times 17,500$). Highly coiled forms appear partly unravelled in electron microscope preparations.

Fig. 12. *Bdellovibrio* with inclusion bodies ($\times 20,000$).

A. M. REINER AND M. SHILO

(Facing p. 410)

A. M. REINER AND M. SHILO

B