Early Effects of *Bdellovibrio* Infection on the Syntheses of Protein and RNA of Host Bacteria.

Mazal Varon, Ilana Drucker and Moshe Shilo

Department of Microbiological Chemsitry

Hebrew University, Hadassah Medical School

Jerusalem, Israel

Received September 2, 1969

SUMMARY. *Bdellovibrio bacteriovorus* 109 inhibited the induced synthesis of β-galactosidase of host *E. coli* bacteria. RNA synthesis of the host was inhibited 3 min after infection; this was followed by the inhibition of protein synthesis 5-6 min later. The halt in RNA synthesis also occurred in host-parasite mixtures where penetration of the parasite was blocked by streptomycin.

The parasitic life cycle of *Bdellovibrio bacteriovorus* in its host bacterium consists of several steps (1-5): attachment to the host cell, penetration into the host periplasm, and intracellular growth and reproduction. The complete cycle terminates in the lysis of the host several hours after infection. However, the instantaneous cessation of movement observed in motile hosts upon attachment (6) indicated that damage to the host occurs very early in its interaction with the parasite.

S.C. Rittenberg found in our laboratory that 5-10 min after mixing a lactose permeaseless *E. coli* mutant with *Bdellovibrio*, the host became permeable to lactose. This finding indicates that damage to the cell membrane occurs within the first few minutes after the parasite attaches. The experiments described in this communication show that the syntheses of protein and RNA of the host are also disturbed very soon after attachment.

MATERIALS AND METHODS. Methyl β-D-thiogalactoside (TMG) and O-nitrophenyl- β-D-galactopyranoside (ONPG) were purchesed from Sigma. L-^{14}C leucine (10 mC/mM) and 5,6 -^{3}H uracil (1000 mC/mM) were obtained from the Radiochemical

Centre, Amersham, England, and streptomycin sulfate from Rafa, Jerusalem.

Bdellovibrio bacteriovorus 109 was grown at 30° for 22 hr in dilute nutrient broth (7) (DNB) containing 10^{10} host cells/ml from an inoculum of 5×10^7 bdellovibrios/ml. The cells were prepared for experiments and their number estimated as described previously (3).

E. coli K-12 P678 (streptomycin resistant) was grown in enriched nutrient broth of Seidler and Starr (4) with the addition of streptomycin (50 µg/ml). E. coli B was grown in a minimal medium (8) supplemented with 0.2% glycerol (for experiments of β-galactosidase induction) or in Difco nutrient broth (for leucine incorporation). Growth conditions of the hosts and the estimation of their number have been described (3). All experiments were performed with host concentrations of $3-5 \times 10^8$ cells/ml. Bdellovibrios were added at a multiplicity of infection (MOI) as described in the figures.

Enzyme activity was measured in 0.5 ml toluenized cells to which were added 0.5 ml distilled water and 4 ml of 0.03 M phosphate buffer (pH 7) containing 500 µg ONPG/ml. After incubation at room temperature, the reaction was stopped with 0.5 ml Na_2CO_3 1 M; optical density was measured with a Klett colorimeter (filter 42). One unit of enzyme activity is defined as that amount of enzyme which caused a change of 10 Klett units/min.

Radioactive L-leucine or uracil were added to the culture to a final concentration of 1 µC/ml. Samples of 0.2 ml were removed at different times and added to 5 ml 5% trichloroacetic acid. Material insoluble in hot trichloroacetic acid (in the case of leucine) or in cold trichloroacetic acid (in the case of uracil) was collected on Millipore filters (HA, 0.45 µ) and washed with 30 ml 5% trichloroacetic acid and 30 ml water. Radioactivity was measured with a Packard scintillation counter.

RESULTS AND DISCUSSION. The study of the effect of bdellovibrio infection on the synthesis of β-galactosidase of the host and on its incorporation of protein and RNA precursors was possible because of the low activity of β-galactosidase in the bdellovibrios and their inefficient incorpo-

ration of amino acids and uracil, as compared to the host bacteria. Furthermore, kinetic study of the early effects of Bdellovibrio on the host required a system in which the entire host population is infected within a very short time. This is not readily obtained in bdellovibrio infection, and even under the best conditions for attachment a significant percent of host cells remain uninfected for many minutes after mixing host and parasite. This percent, roughly estimated by viability counts of the hosts, was clearly lower when the MOI was increased. For this reason, MOIs as high as 8-17 were used; with such MOIs the viable count of the host dropped by 50% within 2-4 min (Fig. 1). As a criterion for the integrity of infected cells, the release of β-galactosidase into the medium was measured: during the first hour after addition of

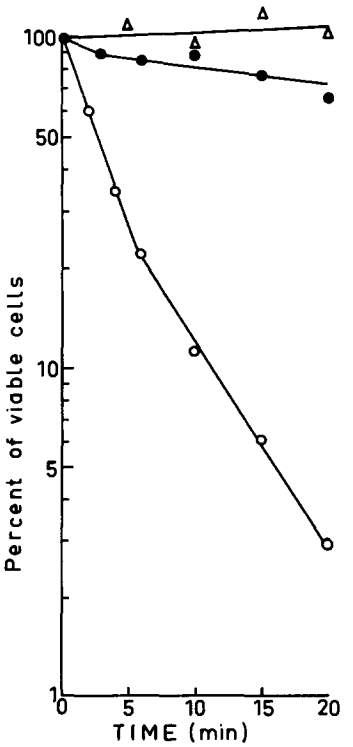

Figure 1. Killing of E. coli K 12 P678 by Bdellovibrio in absence and presence of streptomycin. Host and parasite bacteria were preincubated separately at 30° for 5 min before mixing at MOI of 10. Viability counts were made on nutrient agar plates containing streptomycin (100 µg/ml). Symbols: uninfected culture (Δ); culture infected in the presence of streptomycin 50 µg/ml (●); culture infected in the absence of streptomycin (O).

bdellovibrios no more than 1.2% of the total enzyme content leaked out of the cells.

Experiments on induced synthesis of β-galactosidase in bdellovibrio-infected E. coli are summarized in Figure 2. When Bdellovibrio was added to pre-induced host cells (curve B) the inhibitory effect of the parasite was expressed within 3–5 min. Enzyme synthesis slowed down and then ceased altogether after another 18–20 min. When the host cells were simultaneously exposed to Bdellovibrio and the inducer of β-galactosidase (curve D), enzyme synthesis was nearly completely suppressed. The inhibitory effect of Bdellovibrio on enzyme synthesis may be interpreted as interference with the synthesis of

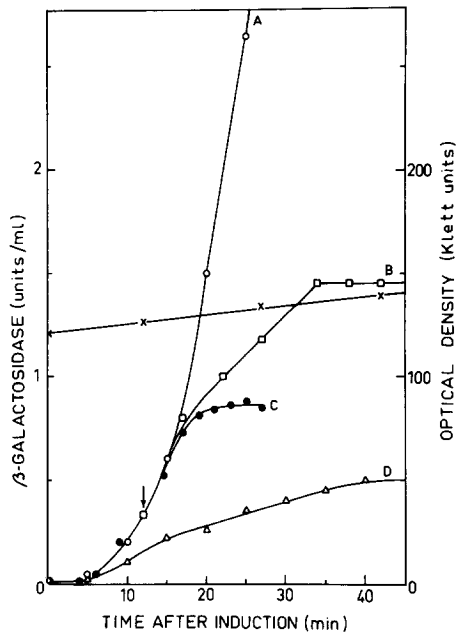

Figure 2. Kinetics of β-galactosidase induction by TMG and the effect of bdellovibrio infection, or inducer removal. E. coli B was pre-incubated in DNB medium for 20 min at 30° , and then TMG was added to a final concentration of 5 x 10^{-4} M. Bdellovibrio at MOI of 8 was added together with the inducer (Δ) or 12 min later (□) (at time indicated by arrow. In a parallel experiment, the inducer was removed from an uninfected culture after 12 min by filtration on Millipore filter (DA, 0.65 μ) and the cells were resuspended in inducer-free medium prewarmed to 30°(●)Kinetics of β-galactosidase induction in an uninfected culture (o) and the increase in turbidity (Klett 42) of the same culture (x) are shown. Two ml samples treated with 0.05 ml toluene for 30 min at 37° and stored in ice served for enzyme assay.

enzyme-specific messenger RNA of the host. In such a case the general kinetics should be similar to the effect of inducer removal. Curve C shows that the rate of β-galactosidase synthesis after removal of the inducer changes at about the same time, but the cessation of synthesis is attained 12-13 min earlier than with Bdellovibrio infection. A possible explanation for the difference between curves B and C could be that while removal of the inducer immediately affects the whole population, a relatively long time is required for the infection of 95% or more of the host cells by Bdellovibrio.

Figure 3 shows that the rate of incorporation of uracil by E.coli K 12 into acid precipitable material changed 3 min after infection, and con-

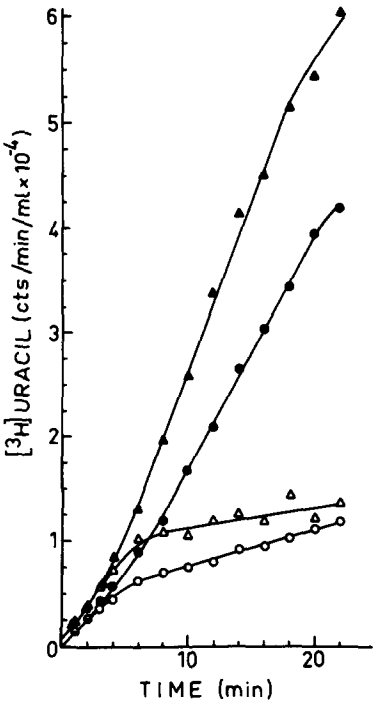

Figure 3. Effect of Bdellovibrio infection on the incorporation of uracil by E. coli K 12 P678 in the presence and absence of streptomycin. Labeled uracil was added at 0-time to host suspension preincubated for 5 min in DNB medium at 30°. The suspension was immediately divided into two parts: one was infected with Bdellovibrio at a MOI of 17 (Δ) and the second was diluted with an equal volume of medium (▲). In a parallel experiment, streptomycin (50 μg/ml) was added to the bdellovibrios (O) or the medium (●) 1 min before mixing them with the host.

tinued thereafter at a reduced rate. The same results were obtained with E. coli B.

It was previously shown (3) that although streptomycin does not affect attachment of Bdellovibrio to its host, it prevents penetration into the host cells. In order to determine whether attachment is sufficient to inhibit RNA synthesis or whether penetration is also necessary for this inhibition, we measured the incorporation of uracil by E. coli K 12 infected with Bdellovibrio in the presence of streptomycin. Figure 3 shows that the rate of uracil incorporation was similarly inhibited by bdellovibrio infection in the presence and absence of streptomycin. In the former case, however, the host bacteria are not killed(Fig. 1); thus, the bdellovibrio-induced inhibition of uracil incorporation has to be reversible.

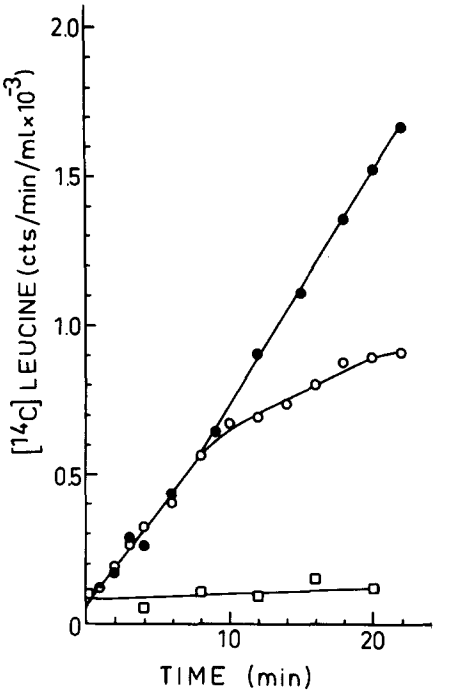

Figure 4. Effect of bdellovibrio infection on the incorporation of leucine by E. coli B. Labeled leucine was added at 0-time to host suspension preincubated for 5 min in DNB medium at 30°. Symbols: uninfected culture (●); culture infected with Bdellovibrio at MOI of 9 (O); Bdellovibrio (7×10^9 cells/ml) in suspension with UV-irradiated (5) host cells (□).

Figure 4 describes the effect of bdellovibrio infection on the incorporation of leucine by E. coli B into acid precipitable material. The incorporation rate changed sharply 8-9 min after infection. Incorporation of a mixture of amino acids in a separate experiment gave essentially the same results.

Infection of E. coli by Bdellovibrio reduces the rates of both RNA and protein syntheses. Although the inhibited rates varied in different experiments, the times at which the rates changed were fairly constant and the inhibition of RNA synthesis preceded that of protein by 5-6 min. This suggests that the primary effect of bdellovibrio infection could be the RNA-synthesis inhibition, which in turn may then lead to inhibition of protein synthesis.

Inhibition of bacterial macromolecular synthesis is known to occur after bacteriophage and colicin infection (9-14). In the case of T-even and λ bacteriophages it has been shown that the inhibition of bacterial protein synthesis is partially due to the inhibition of host RNA synthesis. Kaempfer and Magasanik (9) suggested that changes in surface properties might affect the transcription of bacterial DNA into mRNA. Cohen (15) mentioned that bacteria infected by T-even phages swell rapidly, indicating membrane damage. He suggested that the bacteriophage infection separates the transcription apparatus in the membrane from the genome, preventing further transcription. The findings of Rittenberg (mentioned above) that the cell membrane is damaged very early in the process of bdellovibrio infection together with our findings that inhibition of RNA synthesis is effected while the parasite is still outside the host cell, further indicate the link between damage to the cell envelope and the inhibition of the transcription process.

Acknowledgements

The authors are indebted to Miss N. Lachman for technical assistance, to Dr. Y. Halpern for critical reading of the manuscript, and to Mrs. B. Golek for aid in its preparation.

References

1. Scherff, R.H., J.E. DeVay and T.W. Carroll, Phytopathology 56: 627 (1966).
2. Starr, M.P. and N.L. Baigent, J. Bacteriol. 91: 2006 (1966).
3. Varon, M. and M. Shilo, J. Bacteriol. 95: 744 (1968).
4. Seidler, R.J. and M.P. Starr, J. Bacteriol. 97: 912 (1969).
5. Varon, M. and M. Shilo, J. Bacteriol. 99: 136 (1969).
6. Stolp, H. and M.P. Starr, Antonie van Leeuwenhoek 29: 217 (1963).
7. Shilo, M. and B. Bruff, J. gen. Microbiol. 40: 317 (1965).
8. Davis, B.D. and E.S. Mingioli, J. Bacteriol. 60: 17 (1950).
9. Kaempfer, R.O.R. and B. Magasanik, J. Mol. Biol. 27: 453 (1967).
10. Nomura, M., B.D. Hall and S. Spiegelman, J. Mol. Biol. 2: 306 (1960).
11. Watanabe, H. and M. Watanabe, J. Virol. 2: 1400 (1968).
12. Benzer, S., Biochim. Biophys. Acta 11: 383 (1953).
13. Terzi, M. and C. Levinthal, J. Mol. Biol. 26: 525 (1967).
14. Nomura, M., Ann. Rev. Microbiol. 21: 257 (1967).
15. Cohen, S.S., Virus-Induced Enzymes, Columbia University Press, N.Y. 1968.