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STUDIES ON POSSIBLE PROPAGATION OF MICROBIAL CONTAMINATION IN PLANETARY ATMOSPHERES

FIRST QUARTERLY REPORT

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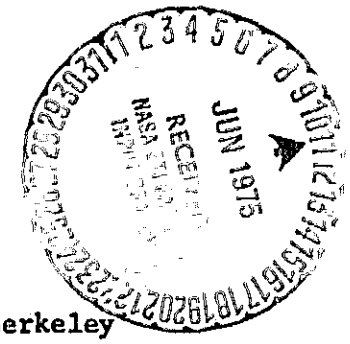

R.L. Dimmick, H. Wolochow and M.A. Chatigny
Naval Biomedical Research Laboratory
School of Public Health, University of California, Berkeley
Naval Supply Center
Oakland, California 94625

Introduction

We are continuing studies on "maintained" aerosols in satisfaction of the overall goal of demonstrating the metabolism and propagation of microbes in clouds as could occur in the course of a "probe" of the atmosphere of Jupiter.

The immediate tasks under investigation at this time, or completed to our satisfaction (see Annual Report, 1974-75), are attempts to show phage production in the aerosol and to initiate studies of airborne anaerobes in anaerobic environments. The former is completed; the latter is under way.

Results and Discussion

1. Phage production

Bacteriophage can be used as a tool to test whether the mechanisms for DNA production remain intact and functional within the airborne bacterial cell. Briefly, when bacteria and phage are mixed in

liquid medium, the following occurs; phage attaches to the bacterial wall within 5 minutes; within 10 minutes the DNA portion of the phage enters the cell (At this point the phage is "dead" in the sense that this particular DNA portion cannot penetrate another bacterial cell ---- it is said to be "incompetent" or in eclipse); the "information" contained in this DNA on "how to manufacture new phage" is impressed onto the bacterial DNA; the bacterial mechanisms then produce "parts" (DNA and protein) for new phage; when enough (a variable quantity) parts are "on hand", new phage particles (5 to 100 per cell) are assembled by a process called maturation, the cell ruptures, and new phage, indistinguishable from the old, is released into the medium. The whole process may occur within 45 minutes to 3 hours after initial mixing.

Studies of airborne phage production have been conducted by the techniques of either mixing bacteria (Escherichia coli, E3000) with T_3 coliphage in the atomizer fluid 3 minutes prior to aerosolization to allow attachment to occur, or by atomizing the two suspensions separately into a common air stream prior to entry into the aerosol chamber. The air environment was 30 C and near saturated humidity.

Attachment and penetration was shown by assaying impinger samples of the air with or without filtering through a 0.4 μm membrane filter. The unfiltered sample measures total intact phage; the filtered sample measures external, unattached intact phage. Initially, we found 2 to 3 times the number of phage in the unfiltered compared to filtered samples,

but as the aerosol aged (180 minute interval), the ratio decreased to 1. A slight increase in the ratio then occurred at the same time that doubling of the phage number occurred (240 minutes), after which the ratio returned to 1.

We found that only about 10% of the intact phage was recovered from the aerosol, if mixing (with time for attachment) was done in the atomizer fluid, compared to the initial phage calculated to have been atomized. We postulate that when fully infected bacteria are atomized, the mechanism for assembling phage particles is blocked in some way within about 90% of the bacteria. The remaining 10% of the bacteria either contain (or lack) a unique physiological attribute permitting phage maturation and not present in the other cells, or else some phage particles are contained within the same particle as a bacterium, but attachment and penetration occur sometime after the particle is airborne and equilibrated to the new environment, thus avoiding the eclipse phase prior to aerosolization, and permitting maturation to occur.

Evidence for the former is stronger because when mixing is allowed to occur in the aerosol, the limited amount of new phage formed appears to have been produced by only about 10% of the airborne cells in which attachment and penetration had occurred, although the phage initially recovered from the aerosol was almost 100% of the theoretical value.

The increase in the number of phage particles was almost equal to the number of viable bacteria as derived from a parallel control run

without phage. Either each cell produced 1 extra phage, which is not typical of phage production *in vitro*, or a few cells produced 4 to 5 phage particles, which can happen *in vitro* when the environment is not optimal. The concept seems reasonable, therefore, that some, but not all, microbes are so damaged by the act of aerosolization that their capacity to participate in phage replication is injured for an indefinite interval. This does not necessarily mean that those same microbes could not replicate their own DNA, for the "block" in the maturation process might be in any of the other steps in the maturation process.

2. Anaerobes

Clostridium butyricum (*C.b.*) and *Bacillus stearothermophilus* (*B.s.*) species have been received from the American Type Culture Collection. Growth of *C.b.*, a strict anaerobe, is slow -- a three-day incubation period is required for assay.

The aerosol chambers have been made anaerobic with N_2, and one run has been accomplished; results were disappointing. The highest initial level achieved was 3×10^4 cells/liter of gas (by electronic particle counter), but none of the cells was viable. We must investigate new methods of sampling and assay for this microbe.

Conclusions

There appears to be little doubt that the biochemical and physiological mechanisms to allow aerobic microbes to propagate in the airborne state do exist. The environmental limits, however, seem to be narrow.

An investigation of the extent of these limits is also limited by the time constraint imposed by gravitational fall-out of particles and the need to contain aerosols in relatively small chambers to achieve quantitative data.

Studies of anaerobes in anaerobic gas will be continued and augmented.