

References and Notes

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Effect of Ultrahigh Vacuum on Viability of Microorganisms

Abstract. Three species of resistant microorganisms were exposed for 5 days to an ultrahigh vacuum approaching that of interplanetary space. Since no lethal effect was observed, there is no indication that the vacuum of outer space would prevent transport of viable microorganisms on unsterilized space vehicles.

The effect of ultrahigh vacuum on microorganisms is of current practical interest in connection with the concern that interplanetary vehicles be sterile. Although ordinary laboratory vacuum (10^{-2} to 10^{-4} mm-Hg) is used to preserve microorganisms, Phillips and Hoffman (1) pointed out that the effect of the extreme vacuum of outer space [estimated as low as 10^{-10} mm-Hg (2)] on microorganisms is unknown. Prince (3) reported that microorganisms withstood pressures from 10^{-5} to 5×10^{-4} mm-Hg for 32 days. However, data on the effect of higher vacuums are needed, especially since odd surface effects have been noted (4) on some materials in ultrahigh vacuum (defined as pressures lower than 10^{-8} mm-Hg). The experiment reported here was performed in an 85-liter chamber (5) at the National Research Corporation (6), where pressures as low as 2×10^{-10} mm-Hg have been reached. It was hoped that if the vacuum of outer space is lethal to microorganisms, evidence of it would be obtained at this level of pressure.

Three of the more resistant types of saprophytic microorganisms were ex-

posed to ultrahigh vacuum for 5 days, a period chosen arbitrarily as a little longer than the time required for transit from the earth to the moon. Exposure was at ambient temperature which corresponds to the usual temperature maintained within space vehicles for the proper functioning of instruments. The chamber chosen for these tests operates at room temperatures, not at elevated temperatures as many high-vacuum sources do.

Each of a number of ashless filter paper patches ($\frac{1}{2}$ inch in diameter) was contaminated with one of the three test microorganisms, *Bacillus subtilis* var. *niger* spores, *Aspergillus fumigatus* spores, or *Mycobacterium smegmatis* cells suspended in water, 0.1-percent Tween 20, and Dubos broth base, respectively. After drying over calcium sulfate, three patches of each type were assayed for viable microorganisms to serve as a baseline control.

At the same time that the baseline control patches were assayed, nine test patches, three with each microorganism, were placed in the ultrahigh vacuum chamber. For comparison, similar patches, kept within desiccators in the same room, were maintained under five other environments. These environments were air, atmospheres lacking oxygen or water vapor or both, and low vacuum. At the end of a 5-day exposure period, each patch was removed from its exposure chamber, placed in distilled water, and shaken until the paper patch disintegrated. Samples from serial decimal dilutions were then plated by the spread plate technique, with a trypticase soy agar as the culture medium. The plates were incubated at 37°C , and colonies were counted after the optimum growth period which was 24 hours for *Bacillus subtilis* var. *niger*, 48 hours for *Aspergillus fumigatus*, and 72 hours for *Mycobacterium smegmatis*.

The results shown in Table 1 indicate that, in general, ultrahigh vacuum, ordinary laboratory vacuum, and storage in a nitrogen atmosphere were the environments most conducive for the preservation of microbial viability. Statistically, the recoveries of *Bacillus subtilis* var. *niger* spores or *Aspergillus fumigatus* spores obtained after exposure to these three conditions were not significantly different from each other or from the baseline control. With the vegetative cells of *Mycobacterium*

Table 1. Microorganisms recovered after 5 days of exposure to ultrahigh vacuum and other test conditions. Each entry is an average count for three patch samples. Temperature during test ranged from 23° to 24°C .

Test condition	<i>B. subtilis</i> ($\times 10^4$)	<i>A. fumigatus</i> ($\times 10^4$)	<i>M. smegmatis</i> ($\times 10^2$)
Baseline control	102.2	31.8	469.1
Vacuum, ultrahigh*	118.2	21.9	156.0
Vacuum, lab†	121.9	27.4	106.6
Nitrogen + CaSO_4	96.3	6.4	10.8
Nitrogen only	96.4	23.2	140.9
Air + CaSO_4	37.6	0.1	0.8
Air only	132.1	1.1	3.3

* At 6 hours, pressure was 5.0×10^{-9} mm-Hg; at 24 hours, pressure was 7.2×10^{-10} mm-Hg; and at 5 days, pressure was 3.6×10^{-10} mm-Hg. † Pressure was 4×10^{-2} mm-Hg, produced by a Welch high-vacuum air pump.

smegmatis, the recoveries obtained after exposure to these three conditions were also not significantly different from each other, but were significantly lower than the baseline control assayed 5 days before.

These data furnish no evidence to indicate that the vacuum of outer space would kill microorganisms and thus prevent their conveyance in a viable state on interplanetary vehicles. At the vacuum reached, the effect upon the test organisms was less adverse than exposure to normal atmosphere (7, 8).

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6. The apparatus was operated by the personnel of the National Research Corp.
7. This work was sponsored by the National Aeronautics and Space Administration through an interagency agreement with the U.S. Army Chemical Corps.
8. Since this report was prepared our attention was called to a Hughes Aircraft Company technical memorandum, by E. E. Brueschke, R. H. Suess, and M. Willard (*Planetary and Space Science*, in press), where an opposite conclusion was reached. These authors report that microorganisms survived a pressure of 8×10^{-9} mm-Hg for 10 days but failed to survive 1.2×10^{-8} mm-Hg for 30 days. No control data are given.

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