The Survival of Microorganisms in Space
Further Rocket and Balloon Borne Exposure Experiments

John Hotchin, Peter Lorenz, Aletha Markusen, and Curtis Hemenway

Dudley Observatory,
Division of Laboratories and Research of the
New York State Department of Health, and the
State University of New York at Albany

This report describes the results of survival studies of terrestrial microorganisms exposed directly to the space environment on two balloons and two rocket flights. The work is a part of a program to develop technics for the collection of microorganisms in the size range of micrometeorite particles in space or non-terrestrial atmospheres, and their return to earth in a viable state for further study.

Previous survival studies were reported (Hotchin, J., Lorenz, P., Hemenway, C., Nature 206, No. 4983, 442, 1965) in which a few relatively large area samples of microorganisms were exposed on millipore filter cemented to aluminium plates. In the present series of experiments, newly developed technics have resulted in a 25 fold miniaturization resulting in a corresponding increase in the number of experiments performed. This has enabled a statistical evaluation of the results to be made. A total of 756 separate exposure units (each approximately 5 x 5 mm. in size) were flown in the four experiments, and organisms used were coliphage T_1, penicillium roqueforti (THOM) mold spores, poliovirus type I (Pfizer attenuated Sabin vaccine strain), and bacillus subtilis spores. The organisms were deposited either by spraying directly upon the vinyl coated metal units, or by droplet seeding into shallow depressions in the millipore filter membrane coated units. Groups of units were prepared comprising fully exposed, inverted (screened by 2 mm. of Al), and filter protected organisms. All of these were included in the flight set, the back up set, and a laboratory control set. The altitude of the exposures varied from 35 km. in the balloon experiments to 150 km. in the rocket experiments. Times of exposure at altitude was approximately 6 hours for the balloon flights and about 3 minutes for the rocket experiments.

RESULTS

The results of the exposure experiments are too voluminous to be included here in toto, therefore only the main results will be given and the effects of the various factors studied will be related to them. It was found that severe loss of viability occurred during the drying of the viruses for the preparation of the samples. In the coliphage T_1 drying loss could be cut down to 1 or 2 $\log_{10}$ units (titer drops from approximately 10^8 to 10^6 PFU*/area seeded) by careful control of

*PFU = Plaque Forming Units

humidity during and after the drying period. The overall losses of the two control groups (inverted flight set and laboratory control set) are shown in Table 1. It was evident that the droplet seeded laboratory control coliphage showed a smaller fraction recovered (0.00004) than the inverted flight set (0.004). This did not occur with the spray seeded phage and was apparently prevented by evacuation and desiccation of the sprayed virus laboratory control.

Since the survival was better in the inverted flight sets than in the laboratory controls, the results are expressed as surviving fractions relative to the inverted flight set. Table 2 shows the basic results obtained with coliphage T_1. Survival of this bacterial virus which was fully exposed to space up to the altitude of 150 km. fell by a factor of 0.00007 in comparison to a comparable inverted control at the identical location; assuming a straight line inactivation rate the half life of T_1 phage under these conditions is only 20 seconds. The surviving fraction of the other organisms used, under the same exposure conditions, is shown in Table 3. It was evident that the stability of the coliphage was intermediate, and both types of spores were very stable, and also the poliovirus showed some survival in all flight experiments after direct exposure.

The effects of several other conditions of exposure were studied, and the most significant results are expressed in Table 4 in terms of the survival factor of the different conditions used. Seeding the virus in two half volume separate aliquots with complete drying in between gave a 24-fold increase in survival; the reason for this is not clear. In the Dudley rocket flown on November 18, 1965, two days after the Luster rocket, shadowing of the specimens from direct solar radiation was very much higher owing to the angle of flight relative to the sun of an average of approximately 85° from normal compared to approximately 60° in the Luster experiment. This resulted in a 1400 fold higher survival of the coliphage and indicates that the lethal effects of space, noted so far in brief exposures, are probably due to solar sources of radiation. The lower altitude (35 km.) of the balloons was less lethal than the conditions of the Luster rocket. The broth-grown concentrated coliphage apparently contained a protective factor which gave virtually complete protection against solar radiation. The data obtained from the filters indicate that approximately 880 A of gold or 1400 Å aluminum also gives complete protection to the coliphage. It is hoped that study of the spectral transmission of these filters will throw further light on the wave lengths responsible for the lethal effects.

DISCUSSION

These results clearly indicate that the environment of space, so hostile to more complex forms of life, is by no means immediately lethal to various microorganisms. This might be predicted from the known resistance of spores and viruses to vacuum and freeze drying, yet experience with this branch of exobiology supplies definite answers unobtainable by theory alone.

The choice of organisms used has not centered upon the viruses to be most likely stable in space, but has been determined by many factors, not least of which is the health hazard of using some of the more stable agents such as scrapie virus which are severely pathogenic. It is not inconceivable that small viruses such as poliovirus, which have been shown to survive on the fringe of the atmosphere could be carried to great heights during certain weather conditions and thereby obtain some of their unpredictable epidemiological behavior.

The fact that solar radiation appears to be the primary cause of inactivation of microorganisms in space, and that thin layers of appropriate filtering material can offer enormous protection, suggests that this cause of death is not a very great barrier to microbiological interplanetary travel.

SUMMARY

1. A human pathogen poliovirus type 1 can survive at the limits of the atmosphere (balloon altitude c. 35 km.) for long periods, and at rocket altitudes ranging up to 150 km for brief periods.

2. Little or no loss of viability could be detected in bacterial or mold spores (B. subtilis and penicillium) after exposure in space at altitudes up to 150 km. for about 3 minutes.

3. Inactivation of highly dispersed, exposed bacterial virus (coliphage T_1) was rapid at 150 km., but highly aggregated T_1 phage showed a surviving fraction of 10^{-4} after about 3 minutes.

4. The inactivation appears to be due to solar radiation.

5. The inactivating rays were completely screened out by 880 Å of Au or 1400 Å of Al-filter on thin formvar membranes.

6. If fully protected from solar radiation, dried organisms survived better in space than in standard laboratory conditions.

7. Broth-grown coliphage T_1 suspensions contained a protective factor.

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Table 1

The survival of control preparations of T_1 coli phage during drying and storage through the experimental period*

Seeding technic	Set	PFU** seeded	PFU eluted	Fraction recovered	Remarks
Droplet	Laboratory control	8.9	4.5 ± 0.4	0.00004	Held in air at rt° and p***
	Inverted flight set	8.9	6.5 ± 0.3	0.004	Evacuated
Spray	Laboratory control	5.2	2.8 ± 1.0	0.004	Vacuum desiccated
	Inverted flight set	5.2	2.3 ± 0.3	0.001	Evacuated

* Titers expressed throughout in $\log_{10}$.
** PFU = plaque forming units.
***rt° p = room temperature and pressure.

Table 2

The effect of rocket borne exposure to space at 150 km. for about 3 minutes (Luster experiment)

No. of replicate units	PFU/seeded	PFU/recovered		Surviving fraction
		Exposed(a)	Inverted (b)	(a/b)
4	8.9	2.2 ± 0.1	6.5 ± 0.3	0.00007

Table 3

The surviving fraction* of different microorganisms during complete exposure at 150 km. for about 3 minutes

Microorganism	Seeding method	Approximate surviving fraction
T_1 coli phage	Droplet in 1 volume	0.00007
T_1 coli phage	Spray	0
Penicillium spores	Droplet	1
Penicillium spores	Spray	—
Poliovirus type I	Droplet	+ **
Poliovirus type I	Spray	0
B. subtilis spores strain M-1	Droplet	0.35
B. subtilis spores strain M-4	Droplet	1

* Expressed as $\frac{\text{average number of viable organisms on exposed flight units}}{\text{average number of viable organisms on inverted flight units}}$

** The survival of the poliovirus in most flight experiments was not significantly different from the controls, and in all experiments only few plaques were found with this virus.

Table 4

The effect of different exposure conditions on the survival of coli phage T_1 in space (150 km.)

Experiment	Exposure condition	Survival factor**
Luster rocket	Seeding 1/2 vol. 2 x	24 x
Dudley rocket	Shadowing from solar radiation*	1400 x
Balloon I	35 km. altitude	3 x
Balloon II	35 km. altitude	230 x
Luster rocket	3 x concentrated phage in broth medium	6300 x
Luster rocket	Filters ***	14000 x (no loss)

*Due to relative angle of rocket and sunlight.

** Expressed as $\frac{\text{surviving fraction}}{\text{surviving fraction of fully exposed units in Luster rocket}}$

*** 877 Å thick Au or 1400 Å thick Al on formvar membranes. The difference between the survival under the Au filter compared with the Al filter is not significant. Generally, complete survival of the droplet seeded materials was found in all flight experiments under these metal filters.