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AN EXPERIMENT TO DETECT MICROORGANISMS IN THE UPPER ATMOSPHERE FLOWN ON AEROBEE NASA 4.150

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GODDARD SPACE FLIGHT CENTER

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ABSTRACT

A cosmic dust collector was modified and flown on the Aerobee flight NASA 4.150, September 28, 1965, to collect microorganisms from the atmosphere. Detection was performed on samples from the recovered collector by classical growth methods and by the firefly luciferase assay for adenosine triphosphate. These methods showed no statistically significant results to indicate the presence of microorganisms.

Microbiological tests were run to determine best methods of organism removal, recovery, and assay. Sterility effectiveness was determined on the collector, the sampling film, and on procedures for removal and assay. Data are given on flight conditions, preliminary tests, assay, and sample volume.

Future flights with increased sample size will be necessary to improve statistical reliability. Isolation environment and assay sensitivity will be improved as well for coming flights.

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AN EXPERIMENT TO DETECT MICROORGANISMS IN THE UPPER ATMOSPHERE FLOWN ON AEROBEE NASA 4.150

INTRODUCTION

An experiment to detect microorganisms in the upper atmosphere was carried by Aerobee flight NASA 4.150 GA-GI-GB, which was successfully launched from site L-21 at the White Sands Missile Range by Naval Ordnance Test Facility personnel. The launch was on September 28, 1965, at 2:09:58:375 MST with impact occurring at approximately 2:16 a.m. The experiment hardware consisted of a cosmic dust collector that was modified for this application.

The purpose of this investigation was to examine the problems associated with collecting microorganisms in the earth's atmosphere and to check out systems for sterilization, isolation, and assay for microorganisms by growth on agar plate and by firefly luciferase ATP (adenosine triphosphate) determination.

COLLECTOR DESCRIPTION

The collector (Figure 1) consists of a cylinder that holds a spool of film which moves over a section of the solid circumference of the cylinder to a second spool. A third spool supplies film that "sandwiches" with the back of the exposed film and rolls up with it on the second spool. The cylinder, which extends from and retracts into the body of the collector, is sealed by an O-ring when retracted. The collector flange is mounted flush with the side of the rocket extension. Cylinder extension is at right angles to the longitudinal axis of the rocket with the collecting surface of the moving film normal to the flight vector (Figure 2). It was assumed that the velocity of the rocket, the high static charge on the film, and the sandwiching would maintain microorganisms at the altitude position on the film where impact occurred and that no migration would occur.

The collector was originally designed for collecting cosmic dust and with minor modification was considered suitable for collecting microorganisms. The modifications were necessary to insure that the device could withstand sterilization by dry heat at 135° C for 24 hours and consisted of:

- Using a polyimide film similar to Mylar* (i.e., Kapton* or "H" film - 1 mil thickness)

*Registered Trademark, E. I. du Pont de Nemours and Co., Inc., Wilmington, Delaware

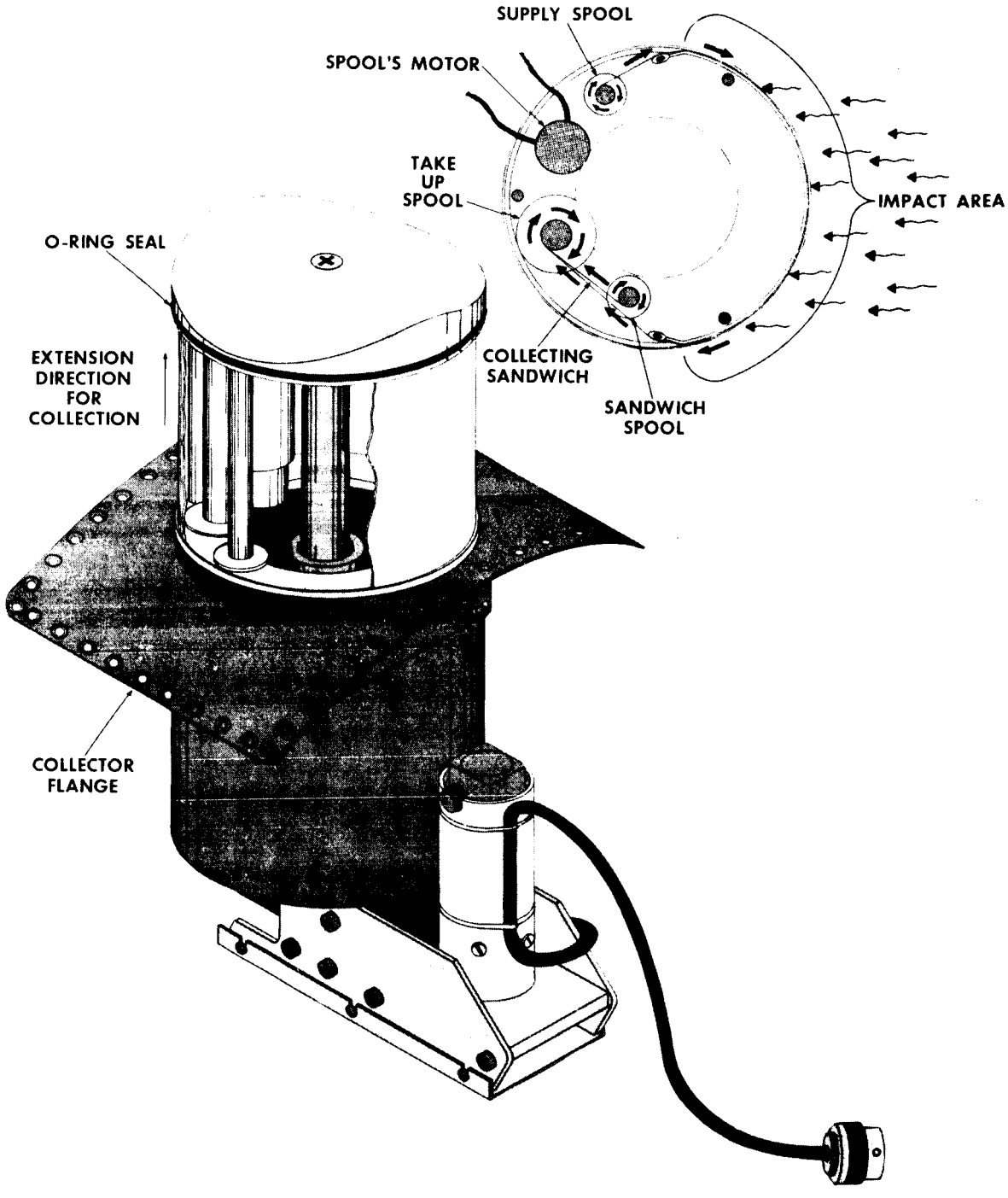

Figure 1. Collector Configuration

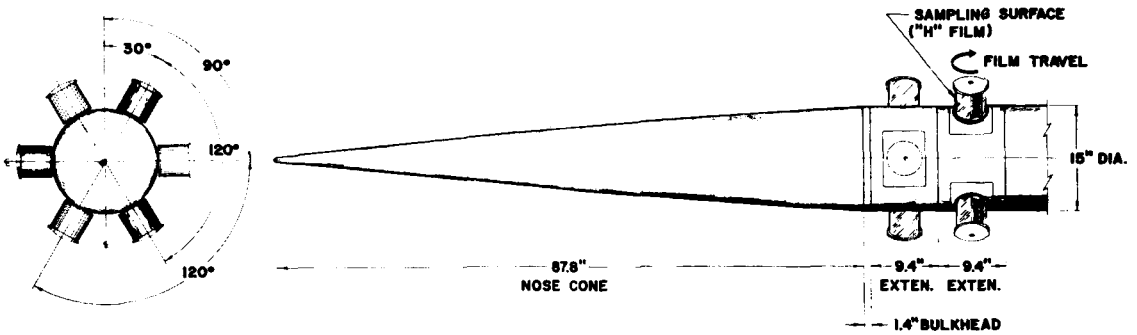

Figure 2. Flight Configuration

- Replacing the nylon film guide (backing) with Teflon*
- Replacing a bleeder squib for pressure equalization with a stainless steel cartridge fitted with cotton and membrane filters

RECOVERY METHOD

Film Selection

Film materials of Mylar, Teflon, stainless steel, filter paper, polypropylene, and Kapton were tested to determine the effects of dry heat sterilization (135° C for 24 hours). Teflon, Kapton, and stainless steel were unaffected by sterilization conditions; however, stainless steel was eliminated because it was not sufficiently flexible and Teflon because of availability problems.

Further tests were performed on the Kapton film to determine the efficiency of recovery of viable organisms. Kapton showed good recovery efficiency: 100 percent at time of drying on film and after 24 hours 55 percent for vegetative cells and 100 percent for spores. Kapton was therefore chosen for further testing to select a recovery method and to determine the degree of recovery after microorganism impaction.

Selection of Recovery Method

Experimentation was performed to determine the best method of recovering bacteria from the film: pour plate method, cotton swab, contact with agar surface, or spread plate method. Results indicated the pour plate method is best when the concentration of organisms is expected to be low.

*du Pont Registered Trademark

Recovery after Impaction

Various methods of impacting dried organisms onto the film were used in tests to determine the percent recovery of viable cells from the film.

An aerosol of Bacillus subtilis var. niger spores was generated and samples collected on the film held in an Anderson sampler.* Comparison was made with collection on an agar surface in the Anderson. In the two sets of tests large number of colonies were observed. Both times more colonies developed on the Kapton film.

A gelatin capsule containing very fine sand with its natural flora of microorganisms was impacted onto the film by a compressed air pistol from about 4 inches away. In two attempts 100 percent recovery was achieved compared to a sample that was not impacted.

Known numbers of lyophilized spores and vegetative cells of Bacillus subtilis var. niger and cells of Serratia marcescens were air pistol impacted on the Kapton film. Because of the very large number of cells impacted and since a large number of cells were recovered, it is difficult to say if any loss was due to impaction.

Vegetative cells of B. subtilis var. niger were impacted by an air pistol from a distance of about 8 inches onto moving, sterilized Kapton mounted in a sterilized collector. Segments of the film were numbered and enumeration of the viable organisms determined after 24 hours. Large numbers of organisms were recovered on the appropriate segments; no attempt was made at quantitative recovery. In this test the segments were removed inside a sterile plastic isolator. Growth of colonies was negative on controls of nonexposed film.

STERILITY AND MONITORING

Tests were made to determine the effectiveness of sterilizing the collector. Tests were also performed to determine the possibility of system contamination from various sources such as from leaks in the collector mechanism, from the procedures used in removing the film from the collector, from the outside of the rocket, or by transferring organisms from film strips that were exposed to those that were not exposed.

*Model 0800, Andersen Consulting Service, Provo, Utah

Collector Sterility

A sterilization procedure employing dry heat at 135° C for 24 hours was selected. The collector, loaded with Kapton film with and without the added test microorganism (Bacillus stearothermophilus), was exposed to this time-temperature treatment. Results showed that the procedure was adequate for sterilization of the collector and the film.

Collector Leak

To determine whether gross leaks were present that had allowed the entrance of potential contaminants, the collector was helium leak tested. The postflight test was performed using Lubriplate* to lubricate the drive shaft and other moving parts and Dow vacuum grease for the O-ring seal; the collector was then sterilized. After sterilization the test was accomplished by injecting helium to a pressure of 5 psi and checking all interfaces for leaks with a helium leak detector.**

The filter cartridge showed an instantaneous leak of greater than 1.5×10^{-5} cc/sec. All other leaks combined (five areas) totaled approximately 6.5×10^{-6} cc/sec. The major leak was through the drive shaft assembly. Assuming that all leaks were through one hole, the diameter of the hole would be 15 microns. (See Appendix A for calculations.)

Additional tests were performed and it was noted that:

- The Lubriplate at elevated temperatures (135° C) was extremely fluid and drained from the drive shaft assembly.

- Apiezon*** was much more effective for lubricating and sealing this collector. This high-temperature lubricant maintained a seal such that no leak was detectable.

The use of Apiezon is anticipated on future flights, but it will be necessary to test the operation of the collector in a simulated space environment since volatilization, etc., might occur with this new lubricant.

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*Walsco Electronics Company, Rockford, Illinois

**Consolidated Electrodynamics Corporation, Pasadena, California

***J. G. Biddle, Plymouth Meeting, Pennsylvania

Film Removal

The removal of the film from the collector for assay was performed aseptically by employing a plastic isolator. In this test the isolator was sterilized with a 2 percent peracetic acid spray, as was the isolator entry tunnel and surface of the collector as it was brought into the isolator. The interior of the isolator was sprayed with an aerosol of B. subtilis var. niger spores. Cotton swab samples of the interior of the isolator were taken after sterilization. No spores were recovered with any of the swab samples after sterilization.

Toxicity controls were run to determine if the sealed microbiological culture media inside the isolator during sterilization would retain any peracetic acid and be toxic to organisms later cultured in them. Staphylococcus aureus, Escherichia coli, and B. subtilis var. niger cultures were capable of growing in the media.

Rocket Nose Cone Fallout

Because range regulations made it impossible to keep the rocket covered during the last 2 hours before launch, contamination from the outside of the rocket was considered a serious problem. Fallout studies of microorganisms from the atmosphere were performed at the Goddard Space Flight Center by determining the level of microbial contamination accumulating on the surface of the rocket nose cone. Tests were made both inside the laboratory and on the third-story roof of the laboratory building.

Tests were performed at various times following decontamination of the nose cone. A mixture of 20 percent formalin and 80 percent isopropyl alcohol was used to wipe down the nose cone. Rodac* plate (agar contact) counts 15 minutes after decontamination indicated that the particles per square foot of rocket surface in the laboratory ranged from 3 to 72 viable particles, and on the roof top from 20 to 253. These results indicate that the build-up after decontamination is so rapid and so great that wipedown of the rocket surface would not be an effective decontamination technique; however, a wipedown was done for the purpose of decontaminating for other experiments on the flight.

Cross Contamination

The possibility of transferring organisms from film strips that were exposed to those that were not exposed was examined. Contaminated and sterile

*Tradename, Baltimore Biological Laboratories, Baltimore, Maryland

strips of film were alternately cut using the same scissors and forceps. All the strips were subsequently cultured and results showed that a highly contaminated strip may have allowed transfer of a very small number of organisms to sterile strips by means of the scissors or forceps used. Ninety colonies were detected on the contaminated strips and only one each on four out of five sterile strips. Controls showed one each on three out of sixteen strips tested. It was concluded that with a very small number of organisms present on the exposed sections of film from the flight, it was possible but highly improbable that scissors or forceps would transfer these organisms during subsequent assay procedure.

FLIGHT OPERATIONS

Collector Preparation

The collectors were prepared for flight as follows: the Kapton film was marked at 1 3/4-inch intervals to establish reference marks, wiped with cheese cloth, and rolled and mounted into the collector. The collector cartridges were filled with filter material and mounted in place. The O-ring was lubricated with silicone vacuum grease and the drive shafts with Lubriplate. Two collectors, the flight model and the back-up, were assembled.

The collectors were wrapped in Kraft paper and sterilized in dry heat at 135°C for 24 hours. Electrically operated extension and short film rolling was done within a laminar-flow clean bench to make sure the units still operated; they were then packaged in a fiberglass carrying case and transported to the launch site. The flight model was mounted in the rocket 8 hours before the flight. Decontamination of the nose cone and payload sections of the rocket was performed by wiping with ethanol and distilled water.

Flight

The launch occurred at 2:09:58:375 a.m. MST on September 28, 1965, with impact taking place at approximately 2:16 a.m. The film started to roll at launch and was jammed at 640 seconds. Burnout velocity was 5588 ft per second, 51.8 seconds into the flight, at a mean sea level altitude of 124,872 ft. A peak altitude of 117.7 miles MSL was attained at 233.0 seconds; the total flight time was 437.0 seconds. The ground temperature at launch was 21°C, and the wind velocity was 7 knots from 186 degrees. The collector opened at 64 seconds, was fully opened at 73.5 seconds, started to close at 373.5 seconds, and was fully closed at 383.0 seconds. The time-altitude relationships are:

Time (sec)	Altitude (meters)
64.025	55919.21
73.525	70231.41
373.525	87510.29
383.025	74354.54

White Sands Missile Range Final Data Report Number 19501 gives the smoothed position, velocity, acceleration, and trajectory angle data as obtained from the reduction of FPS-16 tracking data. The velocity curves (ascent and descent) are given in Figure 3, which shows the total corrected velocity vs. altitude with the corresponding strip number that was exposed at that altitude. Since the strips were moving during exposure, they "saw" different ranges of altitude (as estimated in Figure 4) and were exposed to different volumes of the space environment as related to the position of the strip numbers on the velocity curve (Figure 3). The total sampling volume was calculated from the trajectory data and is estimated between 63,852 and 65,550 cubic feet. The volume per strip section was also calculated (Appendix B).

Collector Recovery

The rocket payload impacted about 3 miles off range and was recovered at 7:30 a.m. on September 28, 1965; sunrise was about 6:00 a.m. The rocket

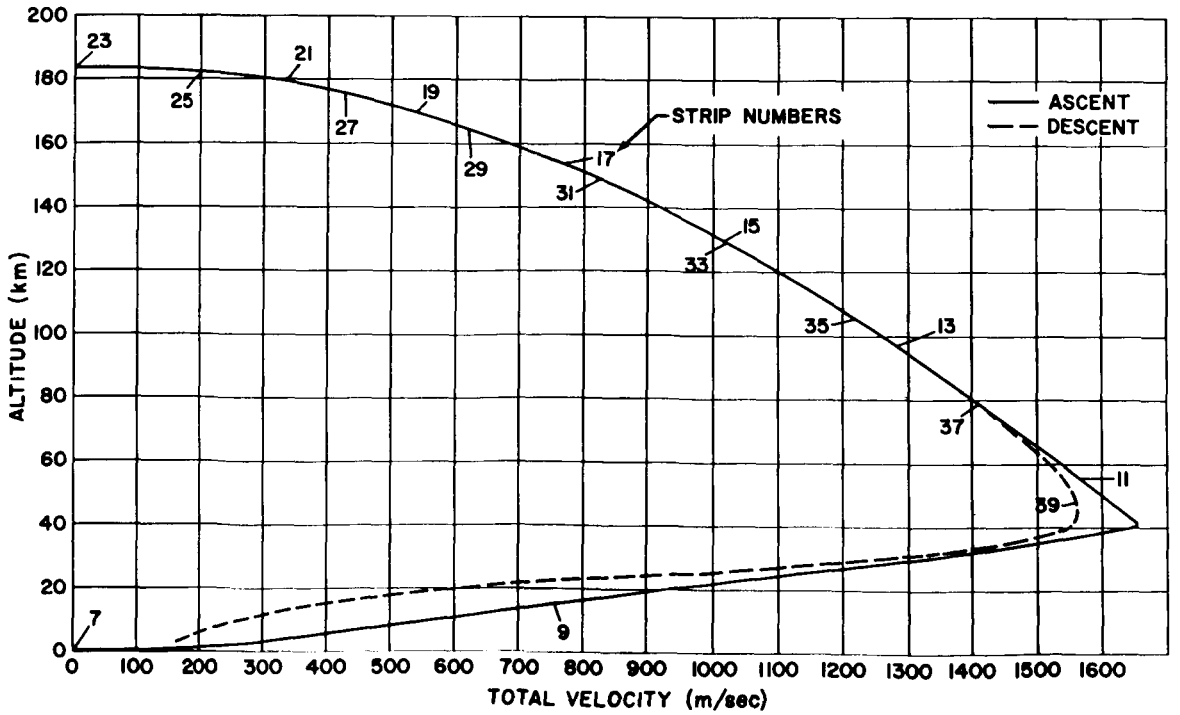

Figure 3. Altitude vs Total Velocity

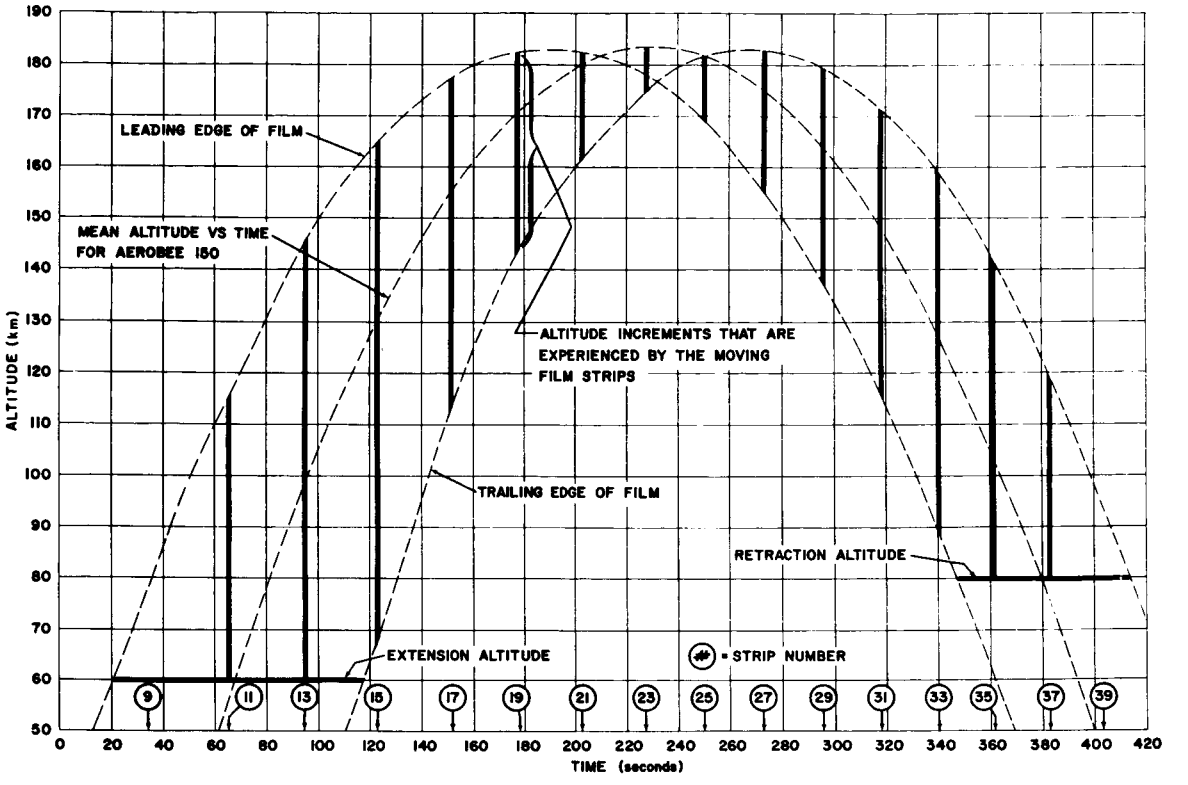

Figure 4. Altitude Collection Profile

payload was intact and the collector was lying upwards on the sunbaked mud of the desert; many tufts of grass spotted the terrain. The rocket skin temperature was 23°C and the mud temperature one-half inch below the surface was 20°C. Samples of the surface of the collector and the sandy mud were collected and stored in sterile containers. The collector was removed from the rocket payload and returned to the Goddard Space Flight Center, arriving at 8:00 p.m. EST, September 28, 1965. During transport the collector was protected against overheating (>23°C).

POSTRECOVERY OPERATIONS

Upon return of the collector to the laboratory the film was aseptically removed, and growth and ATP assays were performed.

Film Removal and Processing

After removal of the motor the collector was placed in the isolator tunnel and peracetic acid spray administered; 30 minutes later the collector was moved into the sterile main part of the isolator. The film was removed and

unrolled, and strips were cut for analysis using three pairs of sterile forceps and scissors: intervals of film from lines 35 to 40 were cut with one pair, from lines 34 to 9 with another pair, and lines 1 to 7 and the SC (unexposed) strips with the third pair. The film was cut into 3 1/2-inch sections corresponding to calculated altitude levels determined by their sequential location, rate of film traverse, and rocket velocity during exposure. It was determined that the sections exposed on extension were 9 through 15 and on retraction were 35 through 40.

Each section of sandwiched film was cut down the center and one-half of each section was put into a sterile 15 cm petri dish; the other half was put into a 250 ml Erlenmeyer flask. The two layers of sandwiched film in the petri plates were separated with forceps and washed by shaking for 15 minutes with 15 ml of sterile distilled water. The strips in the Erlenmeyer were covered with a 1:1 mixture of N-butanol-TRIS buffer and shaken for 5 minutes; the mixture was then allowed to separate into a water phase and a butanol phase. Aliquots of the aqueous phase were removed with a needle and syringe for ATP assay. All of these processes were accomplished within the isolator.

Growth Assay

Film—The petri plates were removed from the isolator and mechanically shaken for 15 minutes. Fifty milliliters of Difco Tryptic Soy Agar (suitable for cultivation of both bacteria and fungi) was added to each plate in a laminar cross-flow clean bench; the plates were incubated at 35°C for 108 hours and then at 30 ±2°C for 108 hours. Colonies that grew were transferred to agar slant cultures to verify viability and to be maintained for species identification. Standard methods for identification were used on all viable colonies (Appendix C).

Table 1 gives the results showing interval of film, number of colonies per section, and species detected. The four film intervals designated SC in Table 1 were single layers of Kapton which were not unrolled in flight and which were removed from the collector within the isolator. The nine samples designated SAC in Table 1 were sterile agar poured into empty and sterile petri plates in the clean bench.

Results showed as many colonies on the unexposed film per unit as on the sample or exposed film (exposed 0.40 organisms per unit assayed; unexposed, 0.55 organisms per unit assayed). Under the assumption of a mean value of 0.45 organisms per unit of film, the Poisson distribution predicts that the expected occupancy should be zero in 64 percent of the film intervals, one particle in 29 percent and two or more in 7 percent. This prediction is consistent with the results in which there were 58 percent with zero count, 38 percent with one, and 4 percent with two; therefore, the assumption that organisms detected

Table 1
Results of Growth Assay on Flown Film

Section of Film Cultured	No. of Colonies Per Plate	Microbial Species Isolated
SC 1	0	—
SC 2	1	B. subtilis
SC 3	0	—
SC 4	2	B. subtilis var. niger, Penicillium sp.
1-0	1	Aspergillis niger
3-1 Unexposed	0	—
5-3 film	0	—
7-5	0	—
9-7	1	Bacillus megaterium
	5 colonies/9 strips	
11-9	1	Sarcina urea
13-11	0	—
15-13	0	—
17-15	0	—
19-17	1	Diphteroide sp.
21-19	(2*)	Bacillus subtilis var. niger Sarcina urea
23-21	1	Cryptococcus sp.
25-23	1	B. cereus
27-25	0	—
29-27	0	—
31-29	0	—
33-31	1	Flavobacterium rhenanum
35-33	0	—
36-35	0	—
38-36	1	Penicillium sp.
40-38	0	—
	6 colonies/15 strips	

*Laboratory contaminants

NOTE: SC, single layers of film not unrolled in flight and removed from collector within the isolator. SAC, sterile agar in sterile petri plates

Table 1 (Continued)
Results of Growth Assay on Flown Film

Section of Film Cultured	No. of Colonies Per Plate	Microbial Species Isolated
SAC 1	0	—
SAC 2	0	—
SAC 3	0	—
SAC 4	0	—
SAC 5	0	—
SAC 6	0	—
SAC 7	0	—
SAC 8	0	—
SAC 9	0	—

were collected purely at random independent of altitude and opening of sampler was not rejected.

Desert Sand—Assay of desert sand taken at the impact site was made by plate count and streak plates.

In the plate count assay 1 gm sample of the sand was suspended in 100 ml of 0.85 percent saline and shaken mechanically for 30 minutes. One ml aliquots were removed and serial dilutions were made to 10^{-3}. Plate counts in quadruplicate were made of each dilution by the pour plate method.

In the streak plate assay, microorganisms in the sand were isolated by streaking one loopful of the sand suspension on Tryptic Soy Agar plates. This medium was used since it was also used for the isolation of microorganisms from the collector.

Plates were incubated at 35°C, 55°C, and at room temperature (28-30°C). Plate counts demonstrated that the sand contained 1.47×10^6 mesophilic, aerobic microorganisms per gram. No thermophiles were isolated from the sand after incubation at 55°C. Microorganisms isolated from the sand include:

Bacillus subtilis var. niger

Bacillus megaterium

Sarcina lutea

Micrococcus ruber

Streptomyces sp. (2)

Bacillus sp.

Cladosporium sp.

The only organisms which were similar to those isolated from the Kapton film were B. subtilis and B. megaterium; however, these organisms are widely distributed in nature and have been isolated in this laboratory in other experiments.

ATP Assay

The firefly bioluminescent enzyme (luciferase) can be used as a quantitative assay method for ATP determination. ATP is an integral part of metabolic processes and is considered a compound characteristic of living things. The amount of light emitted from the reaction of luciferase and luciferin is proportional to the ATP added to the reaction. The ATP must be extracted from the microorganisms by a chemical solvent such as butanol before reaction with luciferase-luciferin can take place.* The reaction is initiated by the injection of 0.01 ml of ATP solution into a mixture containing 0.1 ml of purified luciferase, 0.1 ml of Mg SO_4 (0.01 molar), and 0.1 ml of luciferin (0.5 mg/ml). Light emission is read before injection to obtain the "inherent light" reading and then the "reaction light" is read following injection; both readings are taken for 60 seconds.

Table 2 shows the inherent light and the response from samples from three extraction syringes obtained from the isolator. The first four samples (designated Blank) shown in Table 2 were taken from a syringe containing a butanol-TRIS mixture containing no film or ATP. As shown, the Blank light average is -1559 counts per 60 seconds. The next seven samples (designated Control) were taken from the syringe containing the solution derived using unexposed portions of Kapton film. (These samples correspond to the microbiological assay samples designed SC.) The Control samples gave an average of 42 counts per 60 seconds. (The Control response readings were corrected by subtracting the inherent light and the Blank average of -1559.) The last eight samples (designated Sample) were taken from the syringe containing the solution derived using all exposed portions of the Kapton film. The Sample readings were also corrected for inherent light and the Blank average of -1559 which resulted in an average of 125 counts per 60 seconds.

*NASA CR-411: "The Design and Fabrication of an Instrument for the Detection of Adenosine Triphosphate (ATP)." March 1966

Table 2
Results of ATP Assay on Flown Film

Sample No.	Inherent Light Average	Response	Net Response	Corrected Net Response
Blank				
1	2377	802	-1575	
2	2225	728	-1497	
3	2333	736	-1597	
4	2227	661	-1566	
			Average: -1559	
Control				
1	2678	985	-1693	-134
2	2455	839	-1616	- 57
3	2553	1248	-1305	254
4	2463	1396	-1067	492
5	3213	1745	-1468	91
6	3172	1132	-2040	-481
7	2297	867	-1430	129
				Average: 42
Sample				
1	2300	870	-1430	129
2	2061	929	-1132	427
3	1983	762	-1221	338
4	2428	1105	-1323	236
5	2792	969	-1823	-264
6	2551	1021	-1530	29
7	2426	911	-1515	44
8	2530	1031	-1499	60
				Average: 125

A Wilcoxon Ranking Test* was applied showing a $T_{sample} = 52.5$, while the tabled value is $T_{0.05} = 38$. Therefore, no significant difference was detected between the corrected Sample reading and the corrected Control readings at the 5 percent level of statistical significance.

CONCLUSIONS

Growth Assay

It appears that the major problem associated with low level microbial determination lies in the difficulty in obtaining a negligible or zero background or control level. Sterility precautions and challenges showed zero level of background in the preflight testing and in the isolator procedures with the flight hardware; however, the unexposed film from the flight showed as much contamination as the exposed film. The reason for this has not been determined, but it seems reasonable to say that the only probable source of contamination on the unexposed film resulted from the 15 micron leak in the collector drive shaft assembly. This potential contamination precludes stating that colonies observed actually resulted from organisms collected at altitude from the atmosphere sampled.

The bleeding rate through the squib and through the drive shaft is at least in the ratio of 100 to 10; since the leak rate of the squib saturated the leak detector, only a lower limit of the ratio is known. The percentage of air leaking through the drive shaft then is 10 percent or less and leakage inward would occur when there was a large pressure differential on descent after closing. This would correspond to the strip of unexposed film cut as samples and designated SC. It is possible that contamination of the unexposed film came from the drive shaft. Another possible source of contamination on exposed or unexposed film is the narrow edge of the rolled spool of film which is open to organisms settling on it at any time they are present within collector. Position of collected microorganisms on the film was not determined since the detection method was washing instead of direct plating. Film interval 21-19 is thought to be a contaminant from the laboratory because a chip of glass from the cover of the petri plate fell into the plate before the agar was added.

*Snedecor, George W.: Statistical Methods, Fifth ed., Iowa State University Press, Ames, 1956

The species determination indicated that the organisms collected could have all been contaminants since they are species found widely distributed in nature or are common laboratory contaminants.

ATP Assay

If one assumes that the growth assay will detect only 80 percent of the organisms present, there would be 7-8 organisms in the collected sample. This number is below the sensitivity level of the ATP assay system.

It is concluded that no definite statement can be made that microorganisms were collected in the upper atmosphere during this flight. Future flights will include more collectors to improve the statistical validity of the data as well as improvements in the sensitivity of the ATP assay and use of an additional assay system for the presence of FMN (flavomononucleotide). The probability of contamination will be reduced by all processing being done inside an isolator and the collectors being leak resistant.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the valuable statistical consultation provided by Dr. Edward Batschelet of Catholic University. The cosmic dust collector was provided by the Astrophysics Branch. The sample volume calculations were done by Richard H. Ott, Jr., Sounding Rocket Branch. Preliminary microbial evaluation was performed by Mrs. Jean Mardres and the ATP assay by Emmett Chappelle, Space Biology Branch.

APPENDIX A
CALCULATION OF LEAK RATE

Calculation of the collector gas leak is based on the following.* Leak is considered to be singular and all inclusive. The leak path is considered to be long with respect to the molecular pathlength and the flow is limited by the collision of helium molecules with the walls of the leak path.

Basic equation

$$F = \frac{2}{3} - \frac{a^3}{l} v_a \quad \text{or}$$

$$F = 30.48 \frac{a^3}{l} \left(\frac{T}{M}\right)^{1/2} \text{liters-sec}^{-1}$$

where F = leak rate in liters/sec. = measured at

6.9×10^{-6} ml/sec. = 6.9×10^{-9} liters/sec.

a = radius in cm of the leak

l = leak path length = 11 cm

v_a = can be substituted by $\sqrt{\frac{T}{M}}$

where T = room temperature = 293.16°K

M = molecular weight of gas (grams/mole)

since the test was made with Helium,

M = 4.00026

$$6.9 \times 10^{-9} = 30.48 \frac{a^3}{11} \left(\frac{293.16}{4.0026}\right)^{1/2}$$

so: $a^3 = 27 \times 10^{-11}$ cm

$2a = 6 \times 10^{0.6}$ μ = 15μ = diameter of leak under worst case conditions.

*Dushman, Saul, and Lafferty, J. M., editors: Scientific Foundations of Vacuum Technique, Second ed. John Wiley and Sons, Inc., New York, N.Y., 1962

APPENDIX B
CALCULATION OF SAMPLE VOLUME

The calculations of the volume of space sampled by the collector were made for each film section and total volume determined. The determinations of maximum and minimum volumes were established based on lateral and longitudinal magnetometer data along with vehicle radar position data to estimate the rocket lateral and longitudinal velocities.

The following procedure was computerized:

1. Determination of the rocket lateral and longitudinal velocity

   a. A time history of the rocket velocity vector in an earth-fixed coordinate system (east, north, vertical) is obtained from the rocket radar position data from White Sands Missile Range (XV, YV, ZV).

   b. A time history of the magnetic vector in earth-fixed coordinates is obtained by using the corresponding latitude, longitude, and altitude as inputs to the Jensen and Cain Spherical Harmonic Analysis of the Geomagnetic Field (XB, YB, ZB).

   c. The longitudinal component of the magnetic field is determined using the output of the longitudinal magnetometer (B_{LONG}). The angle between the rocket longitudinal axis and the magnetic field vector is then determined from the following equation:

$$O_B = \cos^{-1}\left(B_{LONG} \Big/ \sqrt{(XB)^2 + (YB)^2 + (XB)^2}\right)$$

      Assumption: The longitudinal magnetometer is not affected by magnetic noise in the payload. Very little payload effect on the lateral magnetometer is in evidence upon investigation of the peak to peak motion of the telemetry trace.

   d. The time history of the upper and lower limits of the longitudinal velocity is obtained from the dot product of the velocity vector and the rocket longitudinal vector (both in earth-fixed coordinates).

      Assumption: At burnout, the rocket vector is equivalent to the velocity vector.

      This is normally accurate to within 1 degree.

Assumption: The maximum excursion of the rocket longitudinal axis in the plane containing itself and the magnetic vector is the maximum allowable in a plane perpendicular to this excursion. This leads to two different locations of the rocket vector each representing an extreme condition.

e. The output of the magnetometer is sinusoidal for a rolling vehicle with the maximum and minimum corresponding to the alignment of the magnetometer along or opposite the projection of the magnetic field vector on the rocket lateral plane. A time history of the lateral magnetic axis in rocket coordinates for each rocket position extreme is calculated at the maximum and minimum points of the lateral magnetometer trace.

f. The lateral experiment axis is located in rocket coordinates at every point in time since its location relative to the lateral magnetometer axis in the lateral plane is known. The time history of the upper and lower limits of the lateral velocity vector is obtained from the dot product of the velocity vector and the lateral experiment vector.

2. Determination of the volume of space on each film section

a. The projected areas of the film on the collector is calculated along both directions of the rocket longitudinal and lateral axes.

b. The volume limits are calculated by a multiplication of the proper projected area by the limits of the corresponding longitudinal or lateral velocity, and this value by some time increment. For the purposes of accuracy, the film is divided into 0.01 inch strips and a time increment chosen in which the collection film moves 0.01 inch. Thus, a calculation is made of the volume of space on the film every time section moves 0.01 inch.

c. The volume of space represented by each 350 divisions (3.5 inches), representing a film section, is calculated along with the total volume of the collection film.

Assumption: The collection cylinder was considered operational from 68.8 seconds to 378.3 seconds after launch.

3. Results

Film Section	Maximum Volume (ft^3)	Minimum Volume (ft^3)
9-11	307	287
11-13	3849	3822
13-15	9648	9388
15-17	10608	10454
17-19	8273	7859
19-21	5660	5364
21-23	3856	3762
23-25	2181	2165
25-27	1373	1353
27-29	2345	2282
29-31	2083	2030
31-33	3707	8601
33-35	4893	4788
35-37	2575	2555
37-39	2218	2186
39-41	1974	1956
Total:	65,550	63,852

APPENDIX C
IDENTIFICATION OF ISOLATES FROM COLLECTOR

Aspergillus niger (Strip 1-0)

Rapid growing, flat, woolly; white becoming black with spores; no reverse pigment

Hyphae branched and septate

Conidophore consists of stalk, vesicle, and sterigmata

Conidium: dematiaceous; occur in unbranched chains from sterigmata

Bacillus megaterium (Strip 9-7)

Gram positive spore-forming rod

Motile

Growth abundant on agar slant (TSA). Varies from smooth to rough on subculture. Some browning with age.

Agar colony (original isolate): large, rough, and concentrically ridged

Acetylmethylcarbinol not produced

Citrates utilized as sole source of carbon

Nitrites not produced from nitrates

Indole not produced

Litmus milk: peptonization and reduction

Urease production ± (slight)

Methyl red negative

Acid from mannitol and lactose; no gas

Trypticase soy broth clear with flocculent sediment

Sarcina urea (Strip 11-9 and 21-19)

Gram positive cocci, occurring singly, in pairs and packets

Motile

Growth on trypticase soy agar: grayish white, mucoid

Uniform turbidity and slight pellicle in broth

Produces urease

Sarcina urea (Strip 11-9 and 21-19)

Does not produce indole

Nitrites produced from nitrates

Acetylmethylcarbinol produced

No change in litmus milk

Methyl red test negative

No acid from mannitol or lactose

Hydrogen sulfide not produced

Diphteroide sp. (Strip 17-19)

Agar colonies: large, grayish white, smooth

Agar slant: grayish white, smooth, entire; slow growing

Gram positive bacilli; very short, evenly strained, and occur in typical palisades

No spores

No acid in mannitol

Bacillus subtilis var. niger (Strip 21-19)

Gram positive spore-forming rods; abundant spores 48 hour

Motile

Agar colony (original isolate); orange pigment, rough wrinkled

Agar slant: browning with age; growth abundant, orange, spreading, wrinkled

Nitrite produced from nitrate

Indole not produced

Acetylmethylcarbinol produced

Urease not produced

Acid from mannitol; no acid from lactose

Cryptococcus sp. (Strip 23-21)

Growth on TSA potato dextrose agar, slow, smooth, pasty and dirty white; grows at 25°C but not at 35°C

Cryptococcus sp. (Strip 23-21)

Budding cells resembling yeast; no hyphae, only blastospores; produces a capsule

Nonpathogenic (probably, since no growth at 35°C)

Bacillus cereus (Strip 25-23)

Gram positive spore-forming rods; long filaments and tangled chains; numerous spores in 24 to 48 hours

Agar colony (original isolate): large, rough, colorless with mottled dry appearance

Agar slant: abundant growth, rough, whitish, opaque

Nitrites produced from nitrates

Acetylmethylcarbinol produced

Urease not produced

Methyl red negative

No acid from lactose or mannitol

Litmus milk: peptonization and coagulation 5 days

Indole not produced

Citrate not utilized

Flavobacterium rhenanum (Strip 33-31)

Gram negative rods occurring singly and chains; irregularly arranged; short rods

Agar colony (original isolate): yellow, soft, entire

Trypticase soy agar slant; yellowish-orange, soft, entire

Trypticase soy broth: uniform turbidity with yellow orange pellicle on bottom

Motile

Nitrites produced from nitrate

Indole not produced

Acetymethylcarbinol not produced

Urease not produced

Flavobacterium rhenanum (Strip 33-31)

Methyl red negative

Litmus milk: peptonization and soft coagulum in 5 days

No acid from lactose or mannitol; lactose alkaline 5 days

Penicillium sp. (Strip 38-36 and SC 4)

Rapid growing, flat powdery and green; reverse pigment absent

Hyphae branched and septate

Conidiphore includes stalk, metulae, and sterigmata

Sterigmata are brush-like

Conidium occur in unbranched chains and are elliptical