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MICROBIOLOGICAL EXPLORATION OF THE STRATOSPHERE

Development and Flights of the Mark II and III Sampling Systems

by Paul D. Pederson, Jr.

Prepared by
LITTON SYSTEMS, INC.
Minneapolis, Minn.
for Goddard Space Flight Center

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ABSTRACT

Research to determine the existence and identity of viable microorganisms in the stratosphere (begun under Contract NASr-81 and continued under Contract NASw-648) was extended under Contract NAS5-3888 with a scheduled period of performance between 1 June 1964 and 1 October 1967. This report covers all effort expended under the above contract and includes the description of two sampling systems (Mark II and Mark III) which employ low-pressure drop media for the collection of viable microorganisms at low air densities. A total of seven operationally successful flights were conducted with samples obtained from altitude profiles ranging from 10,000 to 90,000 feet.

Emphasis was on contamination control techniques to minimize or eliminate microbial background during all procedures associated with sample acquisition and analysis.

Organisms recovered existed in low numbers generally varying inversely with altitude in the range of 10^{-2} to 10^{-4} organisms per ambient ft^3 air. A variety of bacterial and fungal species were isolated. Consideration was given to the problem of statistical analysis of these data and results are presented using nonparametric techniques.

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I. INTRODUCTION

Since January 1962, a continuous research effort has been conducted by the Applied Science Division of Litton Systems, Inc. (formerly General Mills' Electronics Division) to determine the existence and, if possible, the identity of viable microorganisms in the stratosphere. The original contract to support this research effort was NASr-81 (January 1962 to December 1962) under which a modification of an existing stratospheric sampling device was employed for two flights.[1] A second contract NASw-648 (March 1963 to May 1964) funded four additional flights of the original sampling system. The description of the sampling equipment, as well as experimental data and operational procedures from these six flights have been presented in two final technical reports to NASA,[2, 3] and in two publications.[4, 5]

In June 1964, a new contract (NAS5-3888) was awarded by NASA Goddard Space Flight Center, to continue work initiated under the previous contracts and to extend the precautions necessary to insure the biological integrity of the equipment, the operational program, and the analytical procedures. During the tenure of NAS5-3888, a modification of the original sampler was designed, fabricated, evaluated and flown seven times. The improved sampler (subsequently referred to as Mark II) employed the same basic principles as the original equipment (Mark I) but incorporated a number of modifications which minimized extraneous non-stratospheric contamination (background).

The first part of the report presented herein (Section IV) deals with the description of the Mark II sampler, its pre-flight evaluation, the improvements made in contamination control techniques, and with the data collected from stratospheric flights. The operations utilizing this sampling system were completed in November 1965.

Subsequent effort under Contract NAS5-3888 was devoted to a complete redesign of the sampling system including the development of a more sophisticated simplified device which incorporates the same physical collection principles as utilized in the Mark I and II systems. This system will be referred to as the Mark III and is presented in detail in Section V of this report.

II. LITERATURE REVIEW

A review of the pertinent literature dealing with atmospheric microbiology was presented in the final report on Contract NAS4-648.[2] In that review, a chronological listing of investigations of high-altitude microbiology showed that the single serious effort to sample microorganisms in the stratosphere prior to 1962 was that of Rogers and Meier.[6] Since this review was compiled, the National Aeronautics and Space Administration sponsored a conference on Atmospheric Biology[7] and the published proceedings of that conference contain some more recent contributions to the field. Nonetheless, the only papers at the symposium which dealt with actual experimental flights above the tropopause were those of Soffen[8] and the paper presented in conjunction with Contract NASr-81.[5] Soffen's conclusion seemed to substantiate those previously drawn concerning maximum limits of microorganisms ($<1 \times 10^{-2}/ft^2$). These data were not sufficiently quantitative or qualitative enough to amplify any further conclusions. The work of others as well as that presented here were subject to confounding error from non-stratospheric contamination. Other papers presented dealt with investigations at lower altitudes ($<20,000$ ft) or with theoretical or non-biological parameters.

A recent series of reports by workers at the U. S. Air Force School of Aerospace Medicine[9-13] might be pertinent to the subject. These papers deal with aeroplane samplings of microorganisms from masses between 2000 and 10,000 ft. Although these altitudes are rather low, the conclusions reached about population stability at higher altitudes ($\sim$10 to 30 organisms/m^3 = $\sim$0.3 to 1.0 ft^3) might lend credence to the conclusion that viable microorganisms do exist in the stratosphere, albeit at considerably lower concentration. Of even greater interest was their characterization data which indicated that

Cladosporium sp. and Alternaria sp. were the predominant genera of fungi at all altitudes, and that at high altitudes over the sea, fungi comprised 76 to 82% of the total biological population.

A modified cosmic dust collector was used to collect viable microorganisms from altitudes between 60 and 180 kilometers during the flight of an Aerobee rocket.[14] The collection mechanism was impaction on a thin film which was subsequently analyzed by classical methods. The results indicated that population levels were not significantly greater than background and that larger effective volumes of air must be sampled.

III. APPROACH TO THE PROBLEM

The difficulties encountered by previous investigators and the various approaches taken toward solution of these difficulties have been described in considerable detail in the cited final reports.[2, 3] Essentially, the problems can be grouped into two major areas:

1) To collect micron-sized particles efficiently from very large and known quantities of stratospheric air without impairing the viability of living particles

2) To minimize and monitor all sources of extraneous contamination so that the sample would truly represent the viable entities which exist in the stratosphere.

Experience with the Mark I sampler showed that the first problem area, namely the mechanical collection and culturing of stratospheric samples, is more amenable to solution than the contamination control area. This is not to say that contamination control cannot be effected. It does emphasize, however, that when dealing with such low microbial concentration as 1×10^{-2} to 1×10^{-3} organisms per cubic foot, the "noise" contributions from every potential source becomes extremely significant and that distinguishing between "signal" and "noise" borders on the limits of bacteriological state-of-the-art.

Consequently, emphasis during this phase of the program was on contamination control and monitoring. The decision was made to employ the same techniques of particle collection, the same general concept of sampling during controlled descent, and the same methods for elution and culturing of viable particles from the filters, as were successfully employed previously.

Both the Mark II and Mark III (modification and redesign) sampling systems presented here were developed to provide more positive sealing mechanisms, increased sterilizibility, more reliable prelaunch, flight and impact protection, faster and simpler disassembly

and analysis procedures. The analytical techniques and background contamination monitoring methods were improved minimizing noise from these sources, with a more exact definition of the extent of extraneous contamination inherent in the overall operational program.

IV. DEVELOPMENT AND FLIGHTS OF THE MARK II SAMPLING SYSTEM

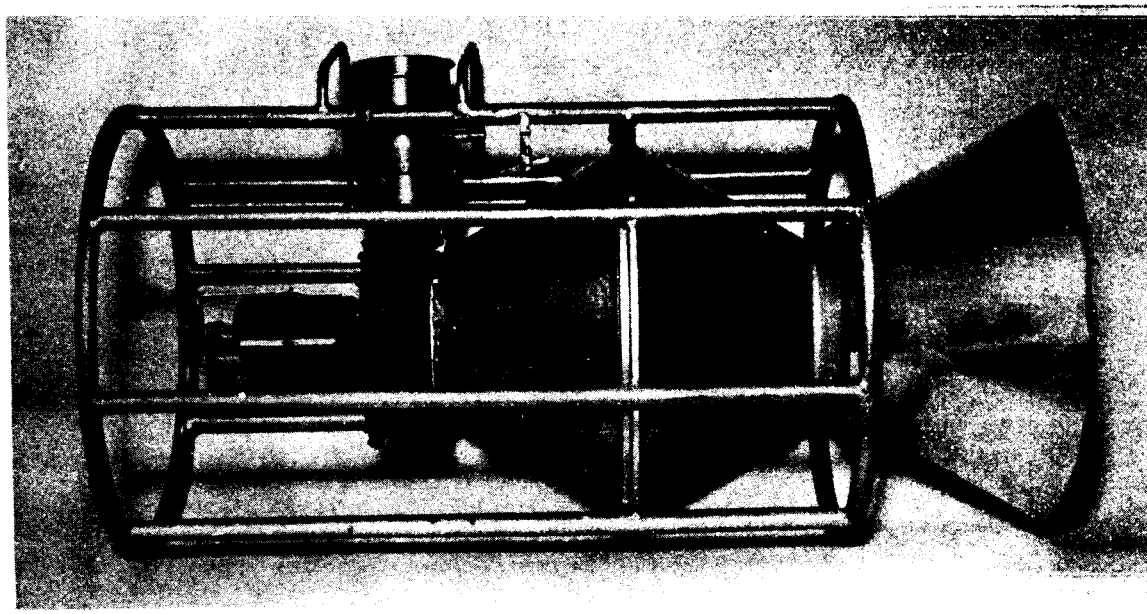
A. Design Considerations

The basic principles of the stratospheric air sampling equipment used in the past and present studies have been previously discussed.[2, 3] A photographic and diagramatic sketch of the Mark I sampler is reproduced in Figure 1.

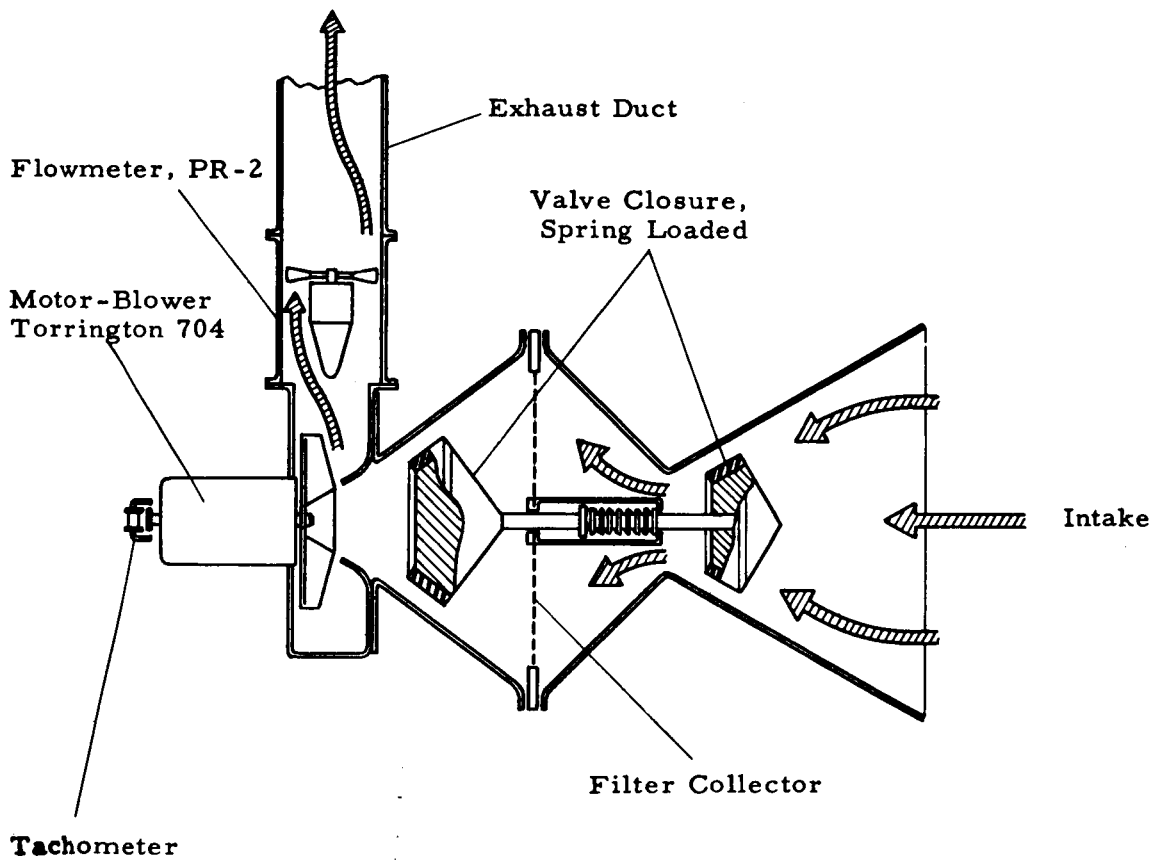
Figure 2 is a drawing of the Mark II sampler developed under this contract. Although the external appearance is similar to the Mark I sampler, the following important modifications have been incorporated:

1) The Mark I sampler employed sealing gates mounted at both ends of a spring-loaded, self-locking shaft, which was cocked open during assembly, and which remained open during sterilizing, storage, launch, and ascent. During all of these steps the integrity of the interior was maintained by dust covers on the sampler's intake and exhaust. The Mark II sampler employed similar dust covers and sealing gates but, in addition, used a linear actuator system to open and close the sealing gates. Thus, the units were launched while sealed, the gates opened just prior to sampling in the stratosphere, and re-sealed themselves just after the sample was acquired. The linear actuator was driven by 1/50-hp Globe motors (5A1414) operated at 27 volts dc, drawing less than 1 ampere. Each actuator was fitted with a Teflon® thrust bearing and lubricated with molybdenum disulfide. Each was capable of raising 20 lb dead weight through a 5-inch opening or closing cycle in 30 to 40 seconds at -100°C. At the terminus of an opening or closing cycle, open microswitches turned off the actuator and simultaneously flashed a light in the flight data recorder to note satisfactory operation of the gates.

® Teflon is a registered trademark of duPont for its fluorocarbon resins.



a) Unit Ready for Attachment to Gondola



b) Airflow Pattern through Unit in "Cocked-Open" Position

Figure 1. Diagram of Mark I Sampler

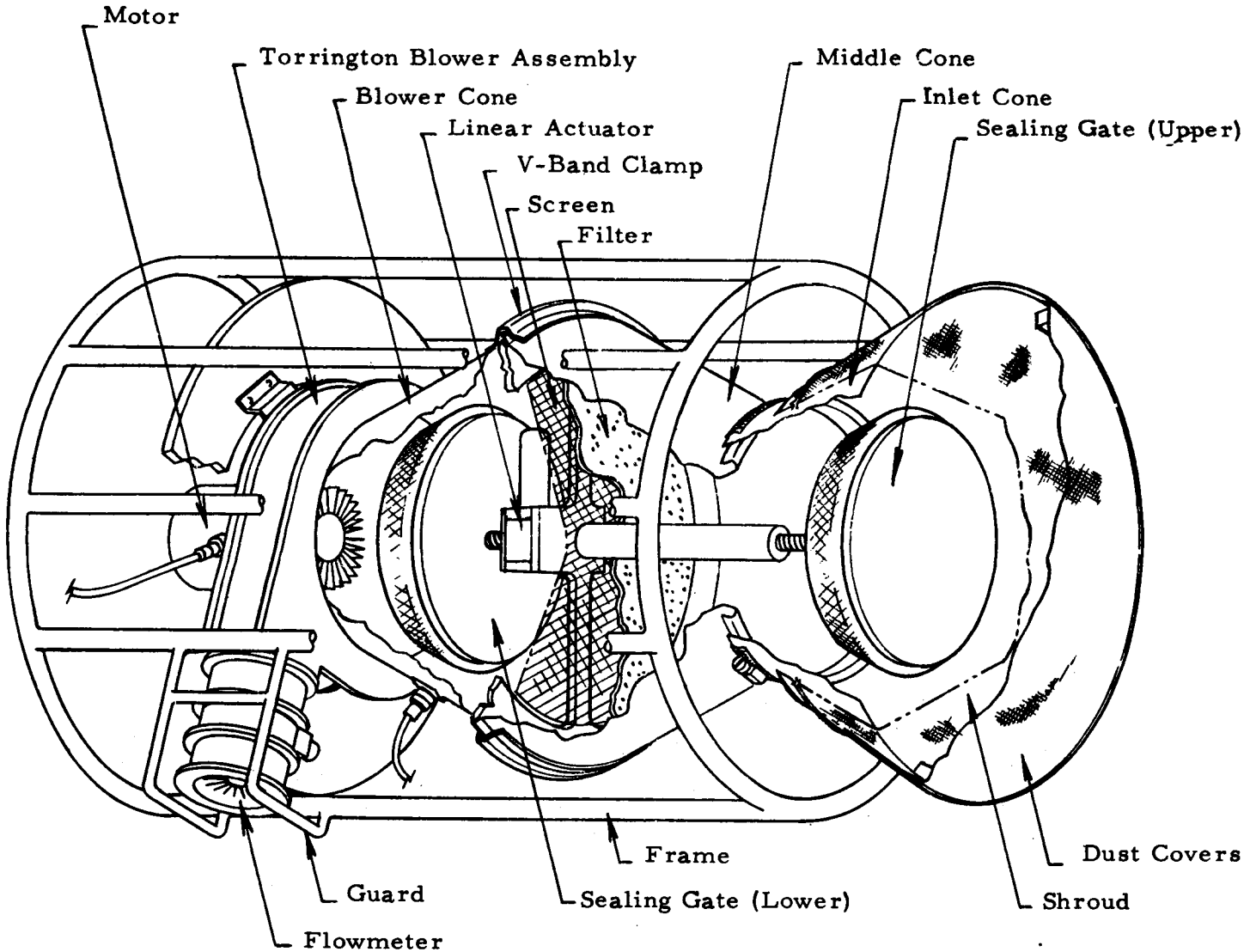


Figure 2. Diagram of Mark II Sampler

2) In the Mark I units, the spun aluminum skins of the samplers were bolted to a stainless steel flange which was part of the tubular frame. The Mark II units contained an independent stainless steel filter holder (oriented perpendicularly to the linear actuator center shaft) to which the aluminum cones were clamped with a "V" band clamp (Marmon). This clamp facilitated assembly and, furthermore, eliminated a large number of manipulations during aseptic disassembly, thus minimizing contamination that could be introduced in the analysis area. Microbiological integrity at the spinning-filter holder surfaces was maintained by Mortite seals and an autoclave tape gasket under the "V" band clamp.

3) In the Mark I unit, the whole sampler was one module. The Mark II unit consisted of three separate modules: the tubular aluminum frame, the blower-motor-flowmeter assembly, and the sampling system itself (filter holder-center shaft-linear actuator, the filters, the sealing gates, the aluminum skin, and the inlet cone-dust cover assembly). The blower-motor-flowmeter module was independently sterilizable in ethylene oxide; the sampling module was independently autoclavable; and the frame was not sterilized. The three modules could be prepared for flight separately and aseptically mated immediately before launch. This modular construction facilitated sterile storage during flight delays (i.e., the integrity of the sterile components was not comprised until the launch was imminent) and, furthermore, aided in the rapid recovery of the sampler module if impact occurred in awkward terrain. In addition, the separability of the sampling module from the frame permitted ready access to the filter surface in the clean room where the bacteriological analyses were conducted.

4) The design of the jettisonable dust covers for the Mark II unit was improved. A flanged aluminum disc gasketed with polyurethane foam was fitted tightly to the inlet cone spinning and was held in place with a muslin shroud cinched by cord around the inlet cone. The dust covers and shrouds were released in the same fashion as previously (firing squibs at altitude to part the cinch cord). However, during the present

program, this operation took place while the interior sealing gates (operated by the linear actuator) were still guarding the sterile integrity of the interior. The flowmeter exhaust port was protected by an aluminum cap attached by cord to the inlet cone dust cover. When the bottom dust covers were jettisoned in the stratosphere, the aluminum caps were simultaneously pulled off and jettisoned.

5) The complete sampler module was designed to be placed in a muslin bag for autoclaving and storage. Thus, there were three separate and redundant physical barriers to contamination:

   a) Interior sealing gates

   b) Dust-cover-muslin shroud and aluminum flowmeter cap

   c) Muslin autoclave bag.

   Using these three barriers, it was possible to maintain interior sterility during every phase of the operation before flight.

6) The sealing gates were conical wooden plugs loosely covered with multiple layers of nonabsorbent cotton that was held in place by loose nylon hair nets (see Figure 3). These gates could tolerate the extreme temperatures to which the samplers were exposed (120°C in autoclave to -100°C in test chamber) without impairment of function.

   During some flights, sealing gates using silicone rubber "O" ring seals were fitted to several units to check their efficiency.

B. The Gondola Instrumentation

Five of the Mark II units were attached to the same gondola built for the previous contract (Figure 4). The gondola contained the batteries, Baracoder and 5-watt transmitter, camera box, and the sampler and balloon control units. Each operation (e.g., release helium, drop ballast, open or close gates, start motors, etc.) was

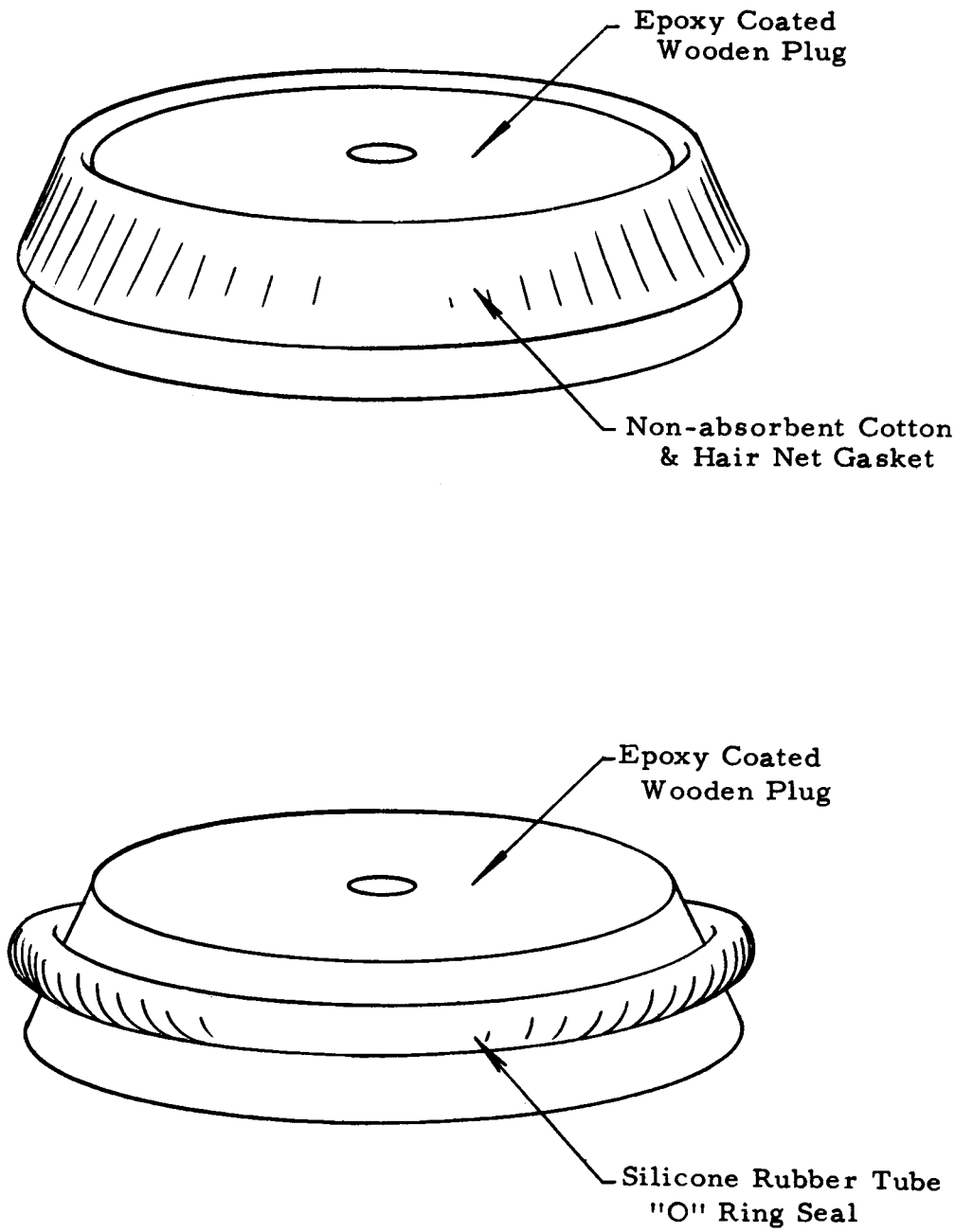


Figure 3. Sealing Gates

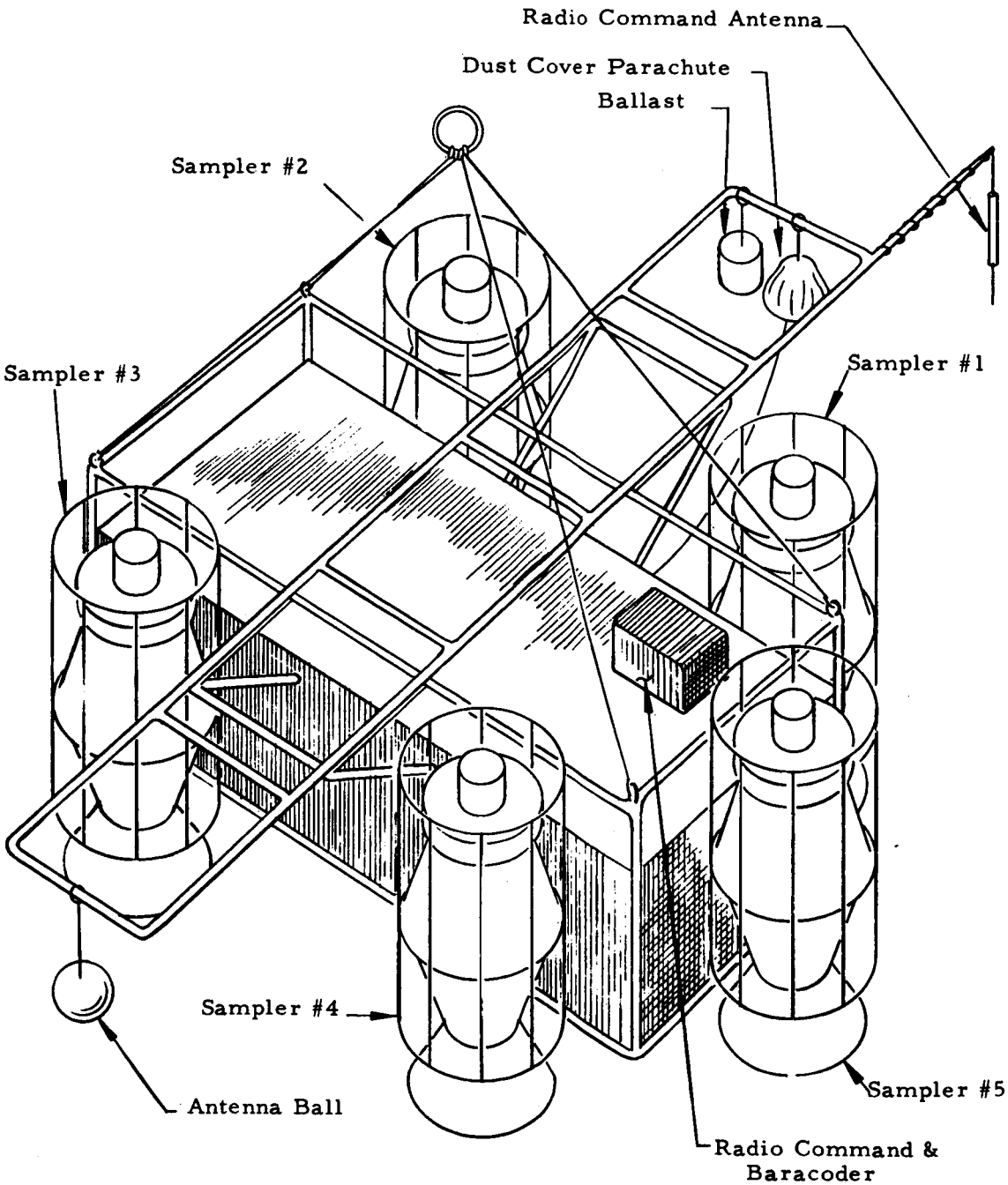


Figure 4. Payload Diagram

controlled by a triple redundant switching system (altitude-pressure; time; radio command). A typical program for the gondola circuitry is given in Table 1.

This program is similar to the ones employed during the previous flights under Contracts NASr-81 and NASw-648. One important modification was the incorporation of an emergency overriding circuit designed to close all of the sealing gates during descent at 10,000 feet.

Although a given program was fixed for any given flight, the gondola circuitry was designed to provide for any desired program changes between flights. Thus, it was possible to program sampling through any discrete altitude profile by one or more samplers operating concurrently, depending on the segment of stratosphere of greatest interest.

A diagram of the balloon and complete flight train is shown in Figure 5.

C. Preflight Evaluation

During the design and fabrication phases of the Mark II sampler program, a number of mechanical and micorbiological evaluations were performed to check out our concepts, the samplers parts and prototypes and ultimately the complete sampler units themselves. This section of the report summarizes the results of these evaluations.

1. Mechanical Equipment

The testing programs carried out since 1962 and the empirical experience gained during previous stratospheric flights showed that the basic sampling equipment of the Mark I unit could tolerate the extreme environmental exposures imposed during preparation and operation. It was known that the filters (polyurethane foam), the

Table 1. In-Flight Programming

Event	Altitude x 1000 ft	Event and Time (min)	Function	Sensor
0.1	5	---	Drop 1750 Kc antenna	Pressure switch
0.2	5	---	Thermistor system on	Pressure switch
0.3	5	---	Activate camera box functions	Pressure switch
0.3+	10	---	Arm thermal delay 1 & 2	Pressure switch
0.4	75	---	Drop ballast if needed	Radio command
0.5	50	---	Radio command from sampler 4 to sampler 2	Pressure switch
0.6	75	---	Radio command switched to balloon valve open	Pressure switch
0.7	75	---	Radio command switched from sampler 3 to sampler 1	Pressure switch
0.8	80	---	Radio command switched from balloon valves close to dust covers	Pressure switch
1.1	80	---	Start timer delay	Pressure switch
1.2	80	---	Drop all dust covers	Pressure switch
1.3	80	---	Drop dust cover & timer backup	Radio command
1.4	90	---	Open balloon valves at float	Radio command
1.5	90	1.1 + 20 M	Open gates, start sampler 1	Delay timer
1.6	90	1.1 + 20 M	Open balloon valves, backup	Delay timer
1.7	90	1.1 + 20 M	Open sampler 5	Delay timer

Table 1 (Continued)

Event	Altitude x 1000 ft	Event and Time (min)	Function	Sensor
1.8	90	1.1+20 M	Start sampler time 1	Delay timer
1.9	90	1.1+22 M	Backup, sampler 1, 5 & balloon valves	Radio command
1.10	88	1.8+50 M	Stop sampler 1, close gates	Sampler timer
1.11	84	---	Stop sampler 1, close gates (backup)	Pressure switch
2.1	1.8-6 K	---	Open gates, start sampler 2	Pressure switch
2.2	1.8-6 K	---	Start sampler timer 2	Pressure switch
2.3	83	---	Open gates, start sampler 2 (backup)	Radio command
2.4	70	---	Radio command switched from dust cover to close balloon valve	Pressure switch
2.5	65	---	Close balloon valve upon reaching 550 fpm descent	Radio command
2.6	65	---	Radio command from sampler 1 to sampler 3	Pressure switch
2.7	65	---	Radio command switched from balloon valves to ballast	Pressure switch
2.8	62	2.2+50 M	Stop sampler 2, close gates	Sampler time
2.9	60	---	Stop sampler 2, close gates (backup)	Pressure switch
3.1	60	---	Open gates, start sampler 3	Pressure switch

Table 1 (Continued)

Event	Altitude x 1000 ft	Event and Time (min)	Function	Sensor
3.2	60	---	Start sampler timer 3	Pressure switch
3.3	58	---	Open gates, start sampler & timer (backup)	Radio command
3.4	40	---	Radio command switched from sampler 2 to sampler 4	Pressure switch
3.5	35	3.4+50 M	Stop sampler 3, close gates	Sampler timer
3.6	30	---	Stop sampler 3, close gates (backup)	Pressure switch
4.1	30	---	Drop ballast to slow descent	Radio command
4.2	30	---	Open gates, start sampler 4	Pressure switch
4.3	30	---	Start sampler timer 4	Pressure switch
4.4	28	---	Open gates, start sampler 4 & timer (backup)	Radio command
4.5	12	4.4+50 M	Stop samplers 4 & 5, close gates	Sampler timer
5.1	10	---	Close all samplers (backup)	Pressure switch
5.2	10	---	Arm impact switches	Pressure switch
5.3	10	---	Energize thermal delay	Pressure switch
5.4	10	5.1+3 M	Close all doors (backup)	Thermal delay
5.5	10	5.4+1 M	Remove power from doors	Thermal delay
5.6	0	---	Destroy balloon by rip panel	Impact limit switches

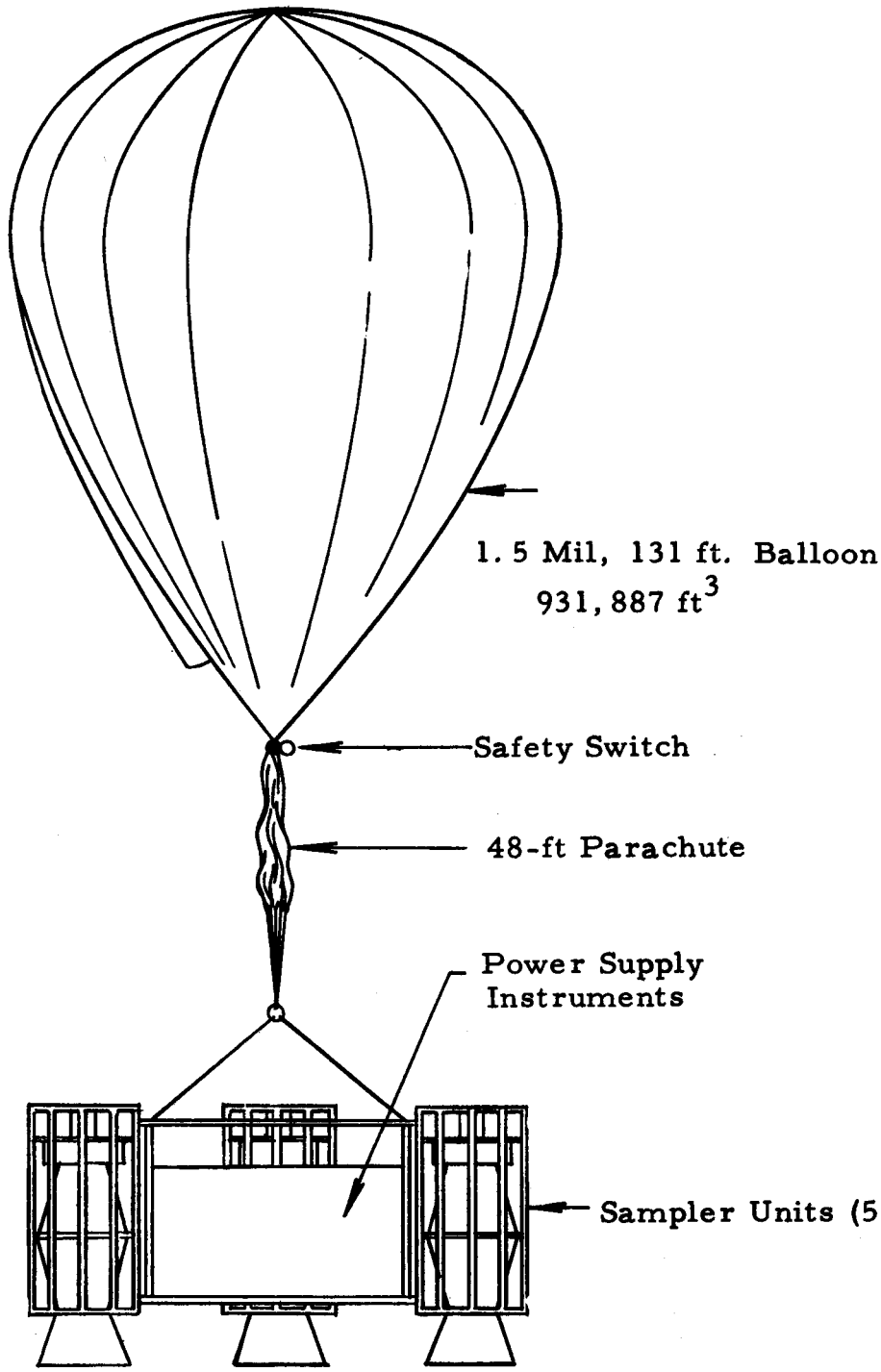


Figure 5. Diagram of Flight Train

spinnings, shrouds, cords, gasketing and sealing tapes employed would withstand moist-heat sterilization and would subsequently perform their function in the stratosphere. However, the Mark II sampler incorporated certain modifications which had to be tested before flight. The item of greatest concern was the linear actuator assembly. Consequently, each motor purchased, and each linear actuator fabricated, was exposed to a number of autoclaving-cold exposure cycles and tested for mechanical reliability before incorporation into a sampler unit.

The tests involved autoclaving at 121°C for one hour, followed by freezing in an environmental chamber at $< -55°C$ and 10 mb. While in the chamber, power was applied and the performance of the motors and actuators was observed. The parts were certified as reliable when they could perform satisfactorily after each of three hot-cold cycles. The criterion for satisfactory performance in the case of the motors was the ability to generate 10,000 rpm with a delay of <20 seconds. The criterion for the linear actuators was the ability to complete an opening or closing cycle against a 20-lb dead load in <1 minute.

The blower-motor-flowmeter modules were placed in polyethylene bags and exposed to ethylene oxide for 48 hours. After this time they were compared to an untreated assembly. To be certified as reliable, their performance after gas sterilization had to be equal to the untreated standard.

After the sampler units had been assembled, sealing plugs (described above) were "custom made" and fitted for each individual sampler. The plugs were then attached to the linear actuator shaft, and the complete unit subjected to a hot-cold cycle and mechanical test to verify the proper fit and closure of each unit.

Only after completion of all of these trials were the sampling units judged ready for actual flight preparation, i.e., final assembly and sterilization.

2. Sampling Efficiency and Recovery

In the previous reports, data were presented relative to the efficiency of polyurethane foam as a filter for micron-sized particles at various simulated altitudes. These data showed that at altitudes above 45,000 feet, one-inch thick, 80-pore foam had efficiencies of >99%. Further and more detailed trials of this nature were conducted during this program. Using a Collison aerosol generator and suspension of Bacillus subtilis spores sprayed into a wind tunnel, polyurethane foam was tested for both spore retention and viable particle recoverability. Sheets of 1/4-inch foam were mounted in frames and were backed up by membrane filters to measure actual slippage. Duplicate frames held only the membrane filters to measure the absolute number of organisms. Sampling was conducted at the approximate flow rates programmed for the samplers (550 fpm face velocity).

The filter efficiency trials, summarized in Table 2, showed that at altitudes above 40,000 feet, two layers of 1/4-inch, 100-pore foam would retain ≥90% of a one-micron challenge aerosol, and that below 20,000 feet three layers of this filter would provide >75% retention.

Viable recovery tests showed that at least 64% of the theoretical number of organisms present were eluted and cultured from the filter. When the filters were treated with a glycerol-water mixture (50 to 50 V/V), the subsequent recovery was 85% of theoretical. This suggested that the filters be glycerol treated prior to final assembly and autoclaving.

Table 2. Polyurethane Sampling Efficiency

Filter	Treatment	Efficiency (%)
at 86,000 feet; 550 fpm face velocity		
1/4" x 100 pore	None	83.14
1/4" x 100 pore	50:50 V/V glycerine	97.36
1/2" x 80 pore	None	97.8
1/2" x 80 pore	50:50 V/V glycerine	98.7
at 15,700 feet; 550 fpm face velocity		
2 x 1/4" x 100 pore	None	47.8
2 x 1/4" x 100 pore	50:50 V/V glycerine	42.2

3. Mating Techniques

It was previously mentioned that one of the innovations employed in the Mark II sampling system was the modular design concept in which the sampling section and the motor-blower-flowmeter section could be assembled and sterilized separately, and then be aseptically mated to each other and the frame immediately prior to flight.

Many trials were conducted to measure the extent of contamination introduced during mating. The modules were sterilized, each mating surface being protected with multiple muslin wrappers held in place with rubber bands. The blower-motor section was removed from its plastic bag and bolted to the frame; the sampler section was removed from its muslin autoclaving bag and held in proximal apposition to the blower section. Within ten seconds, the muslin wrappers from the mating surfaces were removed, the two modules were joined, the joint was taped secure with autoclave tape, and the sampler module was bolted to the frame. During this operation the sampler interior was still protected by the closed sealing gate and the

dust cover-shroud over the inlet cone; the flowmeter exhaust was still protected by its aluminum cap. Exhaustive swabbing of the interior mating surfaces after this operation revealed no extraneous contamination.

4. Controlling and Monitoring Microbial Noise

In previous reports, elaborate discussions were presented about the sources of extraneous contamination and about the approaches we employed to define and minimize contributions from these sources. Work on the Mark II system did not add any significant new contamination control approaches but did amplify and extend the earlier ones. Most of these measures were made possible by the design modifications listed in Section IV. A.

1) The samplers were sterilized in muslin bags, rather than in paper wrappers, and were kept in the unopened autoclave bag until immediately prior to launch, at which time they were aseptically mated to the other modules (see above).

2) The sealing gates and the linear actuator system provided an extra physical barrier against extraneous contamination since the samplers could be launched in a sealed position and not opened until they reached the stratosphere.

3) The independent "backup" circuit which closed all samplers after descent through 10,000 feet added a redundant control measure to override any malfunction of the gondola circuitry, and ensured the protection of the samplers from post-impact contamination even if they were not adequately sealed immediately after a stratospheric sampling cycle.

4) The units were all decontaminated with peracetic acid before disassembly in the clean room. This minimized contamination from organisms that might be aerosolized during disassembly.

5) The modular design and "V" band clamp permitted rapid disassembly and a less cumbersome access to the filter material during dissection. This minimized contamination from personnel who, in the case of the Mark I unit, often had to work at awkward angles and in difficult positions.

6) The filters were dissected and handled with sterile instruments in a clean room. The microorganisms were eluted with sterile buffer in sterile plastic bags; the buffer was filtered through preincubated sterile membrane filters, and the nutrient plates were kept closed until incubation was complete. Many preliminary trials showed that a sterile filter could be thus dissected and extracted with a contamination contribution of <3 organisms per square foot of filter, i.e., <0.2 organisms per filter segment. Similar trials which involved the complete post recovery analysis procedure (disassembly of a sterilized unit, dissection, extraction, plating, etc.) showed that our technique never contributed more than 10 organisms per square foot of filter.

Contamination monitoring was conducted in several ways:

1) Spore strips (Amsco-Spordex) were taped into the interior of the sampler module and blower-flowmeter module before sterilization. After recovery, during disassembly, the spore strips were cultured in order to verify the sterility of the sampler interior. In every case, the spore strips indicated adequate sterilizing exposures.

2) Eight polyurethane "diffusion pads"[3] were taped to strategic locations of the sampler interior upstream and downstream from the filter screen. These pads were designed to distinguish between microorganisms that entered during stratospheric sampling and those which gained entrance at impact through mechanical leaks or malfunctioning seals. If the diffusion pads on a sampler had ten or more contaminants on them and if the contaminants were qualitatively similar to those isolated from the filter, it was assumed that the filter did not truly contain an unconfounded stratospheric sample; the data were discounted.

3) For each flight, two samplers served as contamination controls: the first was flown; its sealing gates were opened at the top of the highest profile and closed at the bottom of the lowest profile, but its blower was inoperative and it did not acquire a sample; this was the "impact control". A second sampler was assembled and sterilized together with all of the others but never left the laboratory. It was taken apart and analyzed to serve as a "prelaunch" and lab technique control.

4) Immediately prior to launch, the balloon and the nonsterile hardware (gondola, framework, parachute, etc.) was dusted with ZnCdS fluorescent particles. After biological analysis, the filter pads were examined for these particles to ascertain the extent of contamination that might be acquired from the vehicle systems.

5) A number of trials were performed to verify the integrity of the sampling units and, simultaneously, to precisely determine the total noise level that would be encountered by impacting in a heavily contaminated environment when all seals functioned properly. These trials involved the dropping of a sterilized sealed sampler from a height of 10 feet onto hard packed earth thoroughly sprinkled with lyophilized B. subtilis spores ($\sim 10^{12}$ organisms/ft^2). The maximum contamination that was thereby contributed (including "impact contamination", post impact contamination, and the contribution from disassembly and analysis) was 16 organisms per square foot of filter. It was evident, therefore, that if the linear actuator performed according to design and program, the noise level would be sufficiently low to place confidence in a sample with 20 organisms per filter pad and a low diffusion pad count.

6) These noise levels were verified (unfortunately) during analysis of an aborted flight (Flight 1, see below) when units which had never opened or sampled but impacted in the sealed condition yielded counts of 2, 1, 9, 13, and 8, respectively, on their filter pads and 1, 0, 2, 0, and 2, respectively, on their diffusion pads. Sixty-nine of the 82 segments were sterile, 19 had one contaminant, and the other four had between two and seven contaminants each.

5. Environmental Testing of Complete System

After all of the preliminary development and testing had been completed, the entire sampling system (sampling units and gondola instrumentation) was assembled for test in an environmental chamber. The chamber was cooled to -55°C and the barometric pressure gradually lowered to simulate the actual rate of rise (1000 to 2000 fpm). The following program was carried out:

5,000 ft (simulated):	Antenna dropped
80,000 ft (simulated):	Dust covers and shrouds dropped
80,000 ft (simulated):	Helium valve circuit energized
80,000 ft (simulated): +20 minutes	Sampler 1: Seals open; blower started Sampler 2: Seals open
80,000 ft (simulated): +70 minutes	Sampler 1: Blower stop; seals closed
74,000 ft (simulated):	Sampler 2: Seals open; blower started
60,000 ft (simulated):	Sampler 2: Blower stop; seals closed Sampler 3: Seals open; blower starts
30,000 ft (simulated):	Sampler 3: Blower stop; seals closed Sampler 4: Seals open; blower starts
10,000 ft (simulated):	Sampler 4: Blower stop; seals close Sampler 5: Seals close
10,000 ft (simulated): +3 minutes	Overriding circuit opens to close seals not previously closed.

The units performed flawlessly; all the seals were tightly closed; all the circuitry seemed ready to fly.

D. Flight Preparation and Post-Flight Analysis Technique

Each experimental flight in a program of this nature must be considered as a separate probe rather than as one of a series of replicates. The data from any single flight will depend on certain controllable variables such as sampler operation and bacteriological

assay techniques, as well as on certain uncontrollable variables such as the season of the year, local meteorological conditions, and microbiological distribution in given air masses at given times. Any scientific experiments which deal with such uncontrolled variables must depend on a large number of observations in order to deduce meaningful generalities from specific probes. Furthermore, it is essential that the controllable variables be scrupulously standardized to avoid confounding the limited data which becomes available.

Standardization of sampling and analysis techniques was effected by employing the same equipment each time, by preparing and launching the equipment in an identical fashion each time, by sampling through the same general altitude profiles, and by performing all of the analyses in the same clean room, with the same technical personnel and using the same techniques for each flight. Previous sections of the report described the sampling equipment and instrumentation. This section will deal with the procedures for preparation, launch and analytical program.

Preparation for a flight required a minimum of one week in the laboratory and a concurrent week for instrument checkout and flight train preparation by the flight operations team. In the laboratory, the filters were thoroughly washed and treated with glycerol-water. The linear actuators were lubricated, new sealing plugs were custom-fitted, and the sampling modules were assembled. The spore strips and diffusion pads were taped into place; the metal-metal contact surfaces between the aluminum skin and the filter holder flange were sealed with Mortite and were taped on the outside with an autoclave tape gasket before fitting the "V" band clamp. The dust covers were fitted to the inlet cone and secured with a cinched muslin shroud. The exhaust-port which would be mated to the blower was covered with muslin. The fit of the sealing plugs was rechecked and the whole unit

was placed in its autoclave bag which was closed tightly with a drawstring. The only part of the sampler still exposed was the electronic connector plug to the linear actuator.

Each sampler was autoclaved with the sealing gates open (to permit free access of saturated steam) for one hour at 120°C. After autoclaving, the units (still bagged) were dried at 3 mb in a vacuum chamber for seven hours. After drying, the sealing gates were closed without taking the samplers out of the bags. (Proper closure was noted by the marked increase in amperage drawn when the plugs were being forced against the sampler throats just prior to the limit switch closure.) The closed, sterile samplers were then stored in their bags until a few hours prior to launch.

The blower-motor-flowmeter modules were concurrently sterilized by exposure to ethylene oxide in sealed plastic bags for at least 48 hours. These modules were bolted to the frames while still enclosed in the plastic, i.e., the bolts were fitted through the polyethylene. These assemblies were then stored until a few hours prior to launch.

The gondola instruments and circuitry were re-checked and the program re-verified. The batteries were recharged and the radio communication net involving the tracking vehicles, the balloon, the spotter aircraft and the control center was checked out. Concurrent with these activities, a weather-watch was initiated to choose the most propitious time for launch.*

*Although not an integral portion of this report, it should be pointed out that the launch operations are among the most dramatic and exciting aspects of the program. To ensure a high probability of successful flights, one must consider such meteorological parameters as low surface winds at launch site (< 8 knots): clear skies (< 40% cloud cover); suitable stratospheric conditions, i.e., no marked "shear layers" or inversions; and the direction and intensity of high-altitude winds which will determine the track and impact site. In the latter case, one must think ahead about the terrain at

(continued)

Once the flight operations officer decided that all conditions for a launch were met, final preparation began--usually 4 or 5 hours before actual lift-off. The sample modules were removed from their autoclave bags, the polyethylene shrouds were cut away from the blower-motor assemblies and the modules were aseptically mated (see Section C. 3). The sampling units were then attached to the gondola and the pressure tap tubes and electrical plugs were connected to their respective receptacles, i.e., motor, flowmeter, dust cover, squibs, linear actuators, etc. The five inlet cone dust covers were attached to each other and to the flowmeter caps, and all were tied to a parachute. Ballast was tied onto the frame, the antenna were fitted, the radio command and baracoder were secured, and the entire payload was wrapped in polyethylene and transported to the flight line by truck.

The complete payload was weighed and suspended from the launch truck, and the appropriate volume of helium was metered into the balloon; during this period final checks of the equipment were carried out and the squibs were armed. The ZnCdS fluorescent particles were dusted over the non-sterile hardware, and environmental samples of air, soil and vegetation were gathered.

After launch, the flight was monitored and tracked by a spotting aircraft, two ground recovery vehicles, and the control center. Since the probable track and approximate impact site had already been

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* (continued).

impact site (i.e., presence of large bodies of water, rugged forest land, etc.) and make suitable preparations for rapid and non-contaminating recovery. Since a complete flight would be cancelled and postponed if any of the abovementioned conditions were unfavorable, it can be seen that the scheduling and performance of balloon-borne exploration involves considerably more uncertainty than routine bacteriological probes.

determined, the vehicles were dispatched to that location. A typical flight terminated 6 to 7 hours after launch, and in most cases, the recovery vehicles were within 10 to 20 miles of the touchdown site at the time of impact.

The detailed flight descriptions for each probe are presented below (Section E). In general, however, the intent was to raise the sampling equipment to maximum altitude (90,000 ft) at ~1000 fpm; to jettison the dust covers, shrouds and flowmeter caps as the payload was rising past 80,000 feet; to float at maximum altitude for 20 minutes before sampling; to start a slow descent (500 to 700 fpm); and to sample different altitude profiles during this descent. The total payload weight was in the order of 870 lb and the vehicle was a 131-foot, 1.5-mil balloon which was filled with about 20,000 ft^3 of helium and which expanded to 932,000 ft^3 at altitude when fully inflated.

The recovery crew tried to reach the impact site as soon after touchdown as possible. The payload was inspected for mechanical damage, in particular, for any breaks in integrity which might have permitted entry of extraneous contaminants. All switches and plugs were disconnected and the sampling modules (protected now only by the sealing gates) were removed from the units by untaping the sampler-blower mating flange and removing the securing bolts which held the sampler attached to the frame. The sampling modules were replaced in their original autoclaving muslin bags and returned immediately to the laboratory. The rest of the payload was then recovered and returned.

In the laboratory, the clean room had been prepared for disassembly and analysis. All surfaces had been physically cleaned and then sprayed with a solution of 2.0% peracetic acid. The tables were draped with sterile covers, and the wrapped sterile instruments and equipment were placed on them. The ventilating equipment (70 changes per hour downflow) was turned on and allow to run for

20 minutes to purge the area. After this time, no one was permitted to enter the room unless garbed in sterile surgical gowns, capped, masked and gloved.

The sampler modules were thoroughly cleaned with water and detergent in the laboratory outside the clean room and then were sprayed liberally with the peracetic acid decontaminant. The "V" band clamp was removed, and the autoclave tape gasketing was also sprayed with decontaminant, followed by slitting with a sterile scapel. (At this stage, the top cone was being held in place by the top sealing gate locked into the closed position; the bottom cone was bolted to the filter ring through the same sockets which had been used to secure the sampler to the frame. Sterile integrity at the spinning-filter ring surface was still maintained by the mortite seals.)

The sampler modules were then placed in the clean room and decontaminated once more with peracetic acid. Suitably attired technicians removed the inlet sealing plug and exposed the filter for the first time since it had been exposed in flight. Each filter was then aseptically dissected into sixteen equal-sized segments (the segment lines were premarked and coded during assembly before autoclaving), and each segment was aseptically transferred to and sealed into a sterile Capran® bag. Similarly, the diffusion pads taped to the interior were removed with sterile forceps and sealed in sterile bags. After all of the samplers had been disassembled and the filters dissected and bagged, the spore strips were taken out and cultured. The bags were refrigerated, and the room prepared for bacteriological analysis.

® Capran is a registered trademark of the Allied Chemical Corporation for nylon films.

The disassembled sampler parts were removed and the room was again washed down and decontaminated. Sterile membrane filter apparatus was brought in along with bottles of sterile phosphate buffer (pH 7.2), the filter segments to be analyzed, and plates of tryptone-glucose-extract agar (DIFCO) on which membrane filters (47-mm dia. and 0.45-micron pore size) had been pre-incubated. The bags were aseptically cut open, the buffer was added (100 ml per segment), and the microorganisms on the filters were eluted by hand kneading. The eluents were then filtered through the sterile membranes which were replaced on their respective petri plates and reincubated at 35°C for 24 hours, then at room temperature (20°C) for 6 days.

Complete analysis after a flight usually took two days (one day for disassembly and dissection; one day for eluting and culturing). After incubation of the bacteriological samples from the stratosphere, fluorescent particle counting of representative segments was carried out with an ultraviolet microscope, and the environmental samples were cultured routinely.

E. Flight Descriptions and Sampling Information

The Mark II sampler was flown seven times. During the first two flights in late 1964, the balloons failed during ascent through the tropopause, aborting the flights before samples could be acquired. These probes, consequently, served only as contamination controls to supplement our experience with biological background factors. The remaining five flights, launched from June through October 1965, were operationally successful. In each case the vehicle raised the payload to the desired altitude, and in each case one or more samples of stratospheric air were acquired. However, even these successful flights were not without some difficulties. During flights 3 and 5, either an electrical or a mechanical malfunction occurred, and no

sample was obtained below 30,000 feet. Similarly, during flight 6, impact damage to two units impaired the biological integrity of the samples which were successfully acquired above 75,000 feet. Furthermore, there were occasions during the program when the sealing apparatus itself malfunctioned, and permitted some ground-borne dust to contaminate the sample filters. The mechanical sampling data are summarized in Table 3. Notwithstanding the sporadic difficulties, it should be pointed out from a historical point of view, that this program achieved, quantitatively and qualitatively, what had never been done before. Sterile samplers were repeatedly exposed at a known series of altitudes between the tropopause and 90,000 feet. Several hundred thousand cubic feet (ambient) of stratosphere were aseptically sampled from defined profiles; the samples were safely recovered and analyzed for viable microorganisms; and sufficient controls were incorporated into the flight and analysis program to provide an insight into the reliability of the data. At the very least, the feasibility of microbiological exploration of the stratosphere was demonstrated.

In any program of this nature, wherein aseptic sampling must be conducted with externally contaminated hardware, questions will always arise about the contribution of noise from these sources. This is particularly true in a situation where the sampling equipment is suspended from a very large balloon with an unknown and variable level of microbial contamination. Sampling during descent precluded this source (i.e., the samplers were "in front" of the vehicle) and by designing the samplers to draw in air at their approximate rate of descent (i.e., to sample isokinetically through a "virgin" profile). The data from Table 3, however, demonstrate that the descent rates were certainly not constant. Consequently, it was necessary to employ a monitoring technique which would measure fallout from the vehicle and hardware. This technique consisted of the ZnCdS

Table 3. Summary of Seven Flights: Sampling Data (Mark II)

Flight/ Sampler	Date	Impact Site	Profile Sampled	Sample Size (ambient ft^3)	Descent Rate While Open (fpm)	Remarks
Flight 1	10/14/64	Bloomington, Minnesota	---	---	---	Balloon rupture at 40,000 ft
Flight 2	11/14/64	Somerset, Wisconsin	---	---	---	Balloon rupture at 35,000 ft
Flight 3	6/7/65	Scandia, Minnesota				
Sampler 1			89K-86K	45,200	50	Seals okay
Sampler 2			86K-60K	43,400	200	Seals okay
Sampler 3			60K-40K	35,000	600	Seals okay
Sampler 4			---	---	---	Fuse blew on actuator; no sample
Sampler 5			Impact control	---	50-600	Seals okay
Flight 4	7/22/65	Shieldsville, Minnesota				
Sampler 1			90K-88K	45,000	32	Seals okay*
Sampler 2			84K-77K	37,200	161	Questionable seal
Sampler 3			59K-54K	43,400	99	Seals okay
Sampler 4			39K-10K	18,600	707	Seals okay

Table 3 (Continued)

Flight/ Sampler	Date	Impact Site	Profile Sampled	Sample Size (ambient ft^3)	Descent Rate While Open (fpm)	Remarks
Flight 4 (continued)						
Sampler 5			Impact control	---	32-707	Questionable seal
Flight 5	8/4/65	Hayfield, Minnesota				
Sampler 1			89K-86K	45,200	52	Seals okay*
Sampler 2			82K-70K	41,800	250	Questionable seals
Sampler 3			60K-30K	18,350	562	Seals okay
Sampler 4			---	---	---	Seals okay-blower malfunction
Sampler 5			Impact control	---	52-562	Seals okay
Flight 6	8/19/65	Bear Valley, Wisconsin				
Sampler 1			89K-87K	46,800	37	Impact damage Questionable seals*
Sampler 2			82K-76K	42,100	100	Impact damage Questionable seals*

Table 3 (Continued)

Flight/ Sampler	Date	Impact Site	Profile Sampled	Sample Size (ambient ft^3)	Descent Rate While Open (fpm)	Remarks
Flight 6 (continued)						
Sampler 3			60K-46K	17,000	270	Seals okay
Sampler 4			29K-9K	13,200	730	Seals okay
Sampler 5			Impact control	---	37-730	Questionable seals*
Flight 7	10/1/65	Babcock, Wisconsin				
Sampler 1			80K-67K	49,000	280	Seals okay
Sampler 2			80K-67K	55,200	280	Seals okay
Sampler 3			40K-10K	16,350	830	Seals okay
Sampler 4			40K-10K	21,000	830	Seals okay
Sampler 5			Impact control	---	280-830	Seals okay

*Silicone rubber "O" ring seals on these units; all other units fitted with cotton seals.

fluorescent particle dusting mentioned above (Sections C. 4 and D). Table 4 summarizes the FP analyses from three flights. It can be seen that in no case was there a significant difference between those samplers that were pulling air and those that were inoperative; indeed, in no case was there found that polyurethane foam exposed in the stratosphere (filters or diffusion pads) had a higher count of FP's than those obtained from the manufacturer. It appears that the lot-to-lot variation in fluorescence was the most significant variable, and that no important fallout from the balloon or gondola had entered the samplers.

Table 4. In-Flight Contamination: Summary of FP Analyses (fluorescent particles/in.^{2})

Flight		Operative Samplers	Inoperative Samplers
3	Filter Surface	5	4
	Diffusion Pads	14	15
4	Filter Surface	4	3
	Diffusion Pads	11	9
5	Filter Surface	1	1
	Diffusion Pads	3	11

F. Bacteriological Analyses in Data Evaluation

The bacteriological data from all flights are summarized in Table 5. This table presents a sampler-by-sampler tabulation of the total numbers of viable organisms cultured, the predominant types isolated, and the condition of the spore strips. Furthermore, to

Table 5. Summary of Bacteriological Analyses

Flight/ Sampler	Designation	Spore Strips	Viable Microorganisms Recovered	
			Filters	Diffusion Pads
1/1	No sample taken	Sterile	2-Diphtheroid; gram (-) rod	1-Gram (-) rod
2	No sample taken	Sterile	1-Unidentified mold	0
3	No sample taken	Sterile	9-Diphtheroid; gram (-) rod	2-Gram (-) rods
4	No sample taken	Sterile	13-Diphtheroid; micrococci	0
5	No sample taken	Sterile	8-Cladosporium sp; Diphtheroid	2-Gram (-) rods
2/1	No sample taken	Sterile	Not analyzed	Not analyzed
2	No sample taken	Sterile	Not analyzed	Not analyzed
3	No sample taken	Sterile	Not analyzed	Not analyzed
4	No sample taken	Sterile	Not analyzed	Not analyzed
5	No sample taken	Sterile	Not analyzed	Not analyzed
3/1	89-86K	Sterile	7-Bacillus sp.; Alternaria sp; gram (-) rods	1-Actinomycete
2	86-60K	Sterile	7-Bacillus sp.; Alternaria sp; gram (-) rods	1-Actinomycete
3	60-40K	Sterile	0	0
4	No sample taken	Sterile	3-Bacillus sp.; Actinomycete; micrococcus	1-Micrococcus

Table 5 (Continued)

Flight/ Sampler	Designation	Strips	Viable Microorganisms Recovered	
			Filters	Diffusion Pads
3/5	Impact control	Sterile	2-Gram (-) rods; actinomycete	0
6	Lab control	Sterile	4-Gram (-) pleomorphic rods; micrococcus	0
4/1	90-88K	Sterile	13-Gram (-) rods; diphtheroids	3-Unidentified molds; gram (±) rods
2	84-77K (questionable seals)	Sterile	321-Cladosporium sp; unidentified molds; actinomycetes; diphtheroids	108-Not identified
3	59-54K	Sterile	8-Diphtheroids; actinomycetes	14-gram (±) rods
4	39-10K	Sterile	462-Cladosporium sp; Alteraria sp; yeasts	13-Cladosporium sp; yeasts
5	Impact control (questionable seals)	Sterile	48-Cladosporium sp; diphtheroids; yeasts	45-Cladosporium sp; Alternaria sp; yeasts
6	Lab control	Sterile	0	0
5/1	89-86K	Sterile	19-Yeasts; diphtheroids; Alternaria sp; actinomycetes; gram (-) rods	4-Gram (-) rods; diphtheroids; Bacillus sp.

Table 5 (Continued)

Flight/ Sampler	Designation	Spore Strips	Viable Microorganisms Recovered	
			Filters	Diffusion Pads
5/2	82-70K (questionable seals)	Sterile	500-Bacillus sp.	500-Bacillus sp.
3	60-30K	Sterile	117-Micrococci; gram (-) rods; diphtheroids; Cladosporium sp	7-Micrococci; actinomycete
4	No sample taken	Sterile	14-Diptheroids; unidentified molds; Bacillus sp; micrococci; gram (-) rods	0
5	Impact control	Sterile	5-Diphtheroids; micrococci; unidentified molds	2-Bacillus sp; gram (±) rod
6	Lab control	Sterile	0	0
6/1	89-87K (impact damage)	Sterile	>3000-Diphtheroids; gram (-) rods; Bacillus sp.	700-Not identified
2	(Impact damage)	Sterile	>3000-Diphtheroids; gram (-) rods; Bacillus sp.	700-Not identified
3	60-40K	Sterile	37-Gram (-) rods; diphtheroids; Bacillus sp; Cladosporium sp	3-Gram (-) rods; unidentified molds
4	29-9K	Sterile	60-Micrococci; gram (-) rods; Bacillus sp.; Alternaria sp	6-Cladosporium sp; unidentified molds

Table 5 (Continued)

Flight/ Sampler	Designation	Spore Strips	Viable Microorganisms Recovered	
			Filters	Diffusion Pads
6/5	Impact control (questionable seals)	Sterile	90-Cladosporium sp; yeast; unidentified mold	25-Cladosporium sp; unidentified molds
6	Lab control	Sterile	0	0
7/1	80-67K	Sterile	70-Gram (-) rods; diphtheroids; Bacillus sp; Cladosporium sp	30-Gram (-) rods; diphtheroids; Bacillus sp
2	80-67K	Sterile	26-Diphtheroids; yeasts; micrococci	5-Micrococci; unidentified molds; actinomycetes; gram (-) rods
3	40-10K	Sterile	65-Gram (-) rods; diphtheroids; micrococci	4-Micrococci; gram (-) rods
4	40-10K	Sterile	147-Micrococci; gram (-) rods	5-Unidentified molds; yeasts; micrococci
5	Impact control	Sterile	15-Diphtheroids; micrococci; gram (-) rods	3-Actinomycetes; micrococcus; gram (-) rods
6	Lab control	Sterile	0	0

facilitate identification of a given sample and to aid in the evaluation thereof, some pertinent information previously presented in Table 3 is repeated here.

It is not difficult to obtain bacteriological data from a program of this type. Indeed, it is relatively easy merely to fly a sampler, recover the filter, culture it, and present the results. On the other hand, it is extremely difficult to interpret such data, knowing the many possible sources of extraneous contamination which are involved. Even simple statements about whether or not a sample is contaminated cannot be made without equivocation. Thus, it is not difficult to interpret results from sample 3, flight 3: no viable organisms recovered from either filter pads or diffusion pads can be understood and can be used to establish a maximum population limit for that profile. Similarly, it is not difficult to interpret results from sampler 1, Flight 6: in this case obvious impact damage had occurred and both filter pads and diffusion pads were heavily contaminated with non-stratospheric dust, probably occurring during impact. It is the borderline area between perfect samples and grossly contaminated samples that contributes to confusion. In order to glean meaning from these samples, it is necessary to consider the following parameters:

1) Physical integrity of sampler and seals as observed visually by the recovery crew

2) Fluorescent particle analysis

3) Ratio of viable recovery from sampling filter and diffusion pads of same sampler

4) Similarity or difference between predominating species on sampling filter and diffusion pads of same sampler

5) Comparison of numbers and types of organisms in an operative sampler and one which did not obtain a sample during the same filter (if that one is itself valid)

6) Comparison of samplers which flew and the laboratory control which did not (if that one itself is valid)

7) Comparison of bacteriological data from the flight program with the preliminary "dry runs" conducted to establish noise levels.

After all the abovementioned criteria have been considered, it is still necessary to make a subjective decision to select those pieces of information which are probably the most reliable. This type of evaluation is summarized in Table 6 which pinpoints the significant reasons for validating or invalidating the bacteriological results from a given sample.

Ultimately, a sample was accepted as valid if the counts on the diffusion pads from that unit fell within or below the normal experienced noise level; if the count on the filter pad itself was significantly higher than the experienced noise level (without a concomitant increase in diffusion pad count); or if there was a marked difference in the types of organisms isolated from the filter and the diffusion pad. In every case, samplers with questionable physical integrity were invalidated, even if all of the other validating criteria were met.

From Table 6, it can be seen that this program yielded thirteen samples which the investigators regarded as most probably valid. The physical and biological characteristics of these samples are summarized in Table 7. There is no doubt that some of the biological information (numbers and types) presented in this table are contamination from a non-stratospheric source; nevertheless, on the basis of our best judgment and experiment, these data represent as true a picture of stratospheric microbiology as has been possible to acquire with the Mark II sampling system.

When the results of the Mark II program, as summarized above, are compared with the data previously obtained with the less reliable and cruder Mark I sampler, some striking qualitative and quantitative

Table 6. Selection of Valid Samples

Flight/Sample	Validating Reasons	Invalidating Reasons	Decision
1/All	---	Aborted flight	Invalid
1/All	---	Aborted flight	Invalid
3/1	FP* Physical integrity Low DP count** Qualitative difference between filter and DP	Filter count within experienced noise level Qualitative similarity between this and inoperative samplers #4 and #5	Valid
3/2	Same as #1	Same as #1	Valid
3/3	Perfect sample	---	Valid
3/4	---	No sample taken	Invalid
4/1	FP Physical integrity Low DP count Qualitative difference between filter and DP	Filter count within experienced noise level	Valid
4/2	FP	Questionable seals High count on DP	Invalid
4/3	FP Physical integrity Qualitative difference between filter and DP	DP count higher than filter Filter count within experienced noise level	Valid

*FP = Fluorescent Particle Data

**DP = Diffusion Pad Data

Table 6 (Continued)

Flight/Sample	Validating Reasons	Invalidating Reasons	Decision
4/4	FP Physical integrity Filter count significantly higher than experienced noise level Filter count significantly higher than DP	DP count on borderline of acceptance Qualitative similarity between filter and DP	Valid
5/1	FP Physical integrity Low DP count Filter count higher than experienced noise level	Qualitative similarity between filter and DP Quantitative similarity between this and inoperative #4	Valid
5/2	FP	Questionable seals High count on DP	Invalid
5/3	FP Physical integrity Low DP count Filter count significantly higher than experienced noise level Count significantly higher than inoperative #4 and #5	Qualitative similarity between filter and DP Qualitative similarity between this and inoperative #4	Valid
5/4	---	No sample taken	Invalid
6/1	---	Impact damage High DP count	Invalid

Table 6 (Continued)

Flight/Sample	Validating Reasons	Invalidating Reasons	Decision
6/2	---	Impact damage High DP count	Invalid
6/3	FP Physical integrity Low DP count Filter count higher than experienced noise	Qualitative similarity between filter and DP	Valid
6/4	Same as #3 Qualitative difference between filter and DP	---	Valid
7/1	FP Physical integrity Filter count higher than experienced noise	High DP count Qualitative similarity between filter and DP	Invalid
7/2	FP Physical integrity Filter count higher than experienced noise Low DP count Qualitative difference between filter and DP	Qualitative similarity between this and inoperative #5	Valid
7/3	FP Physical integrity Low DP count Filter count higher than experienced noise	Qualitative similarity between filter and DP Qualitative similarity between this and inoperative #5	Valid
7/4	Same as #3	Same as #3	Valid

Table 7. Summary of Data: Valid Samples

Flight/ Sample	Date 1965	Profile (ft x 10^3)	Volume (ft^3 ambient)	Viable Microorganisms Recovered	
				Numbers	Predominant Types
3/1	6/7	89-86	45,200	7	Alternaria sp; Bacillus sp; gram (-) rods
3/2	6/7	86-60	43,400	7	Alternaria sp; Bacillus sp; gram (-) rods
3/3	6/7	60-40	35,000	<1	
4/1	7/22	90-88	45,000	13	Gram (-) rods; diphtheroids
4/3	7/22	59-54	43,400	8	Diphtheroids; actinomycetes
4/4	7/22	39-10	18,600	462	Cladosporium sp; Alternaria sp; yeasts
5/1	8/4	89-86	45,200	19	Yeasts, diphtheroids; Alternaria sp; actinomycetes; gram (-) rods
5/2	8/4	60-30	18,350	117	Micrococci; gram (-) rods; diphtheroids; Cladosporium sp
6/3	8/19	60-46	17,000	37	Gram (-) rods; diphtheroids; Bacillus sp; Cladosporium sp
6/4	8/10	29-9	13,200	60	Micrococci; gram (-) rods; Bacillus sp; Alternaria sp
7/2	10/1	80-67	55,200	26	Diphtheroids; yeasts; micrococci
7/3	10/1	40-10	16,350	65	Gram (-) rods; diphtheroids; micrococci
7/4	10/1	40-10	21,000	147	Micrococci; gram (-) rods

similarities become evident. One becomes impressed with the repeated isolation from the upper altitudes of a limited variety of microflora, among which diphtheroids, gram negative rods, micrococci, Cladosporium sp. and Alternaria sp. predominate. It may, of course, be argued that these are common terrestrial contaminants and that we are repeatedly observing the same type of post-impact noise. However, the consistency of encounter with the above-mentioned varieties and the relatively infrequent isolation of the common soil bacilli, actinomycetes, and other fungi lends credence to the assumption that we are actually dealing with more than simple terrestrial noise.

Similarly, the quantitative estimates of stratospheric microflora, previously published, become better defined. Table 8 summarizes the maximum, minimum and mean counts for three general profiles as determined with the Mark II samplers and then presents grand mean counts based on the four years of balloon sampling experiments with both Mark I and Mark II.

Table 8. Viable Organisms in the Stratosphere

Sampler	Altitude (microbial counts/ambient ft^3)		
	10,000 to 30,000 ft	30,000 to 60,000 ft	60,000 to 90,000 ft
Mark II			
Maximum	462/18,000 = 2.6×10^{-2}	117/18,000 = 6.5×10^{-3}	26/55,000 = 4.7×10^{-4}
Minimum	60/13,000 = 4.6×10^{-3}	0/35,000 = 3×10^{-5}	7/45,000 = 1.5×10^{-4}
Mean	734/68,000 = 1.1×10^{-2}	162/113,000 = 1.4×10^{-3}	75/233,000 = 3.3×10^{-4}
Mark I and Mark II			
Mean	846/74,000 = 1.1×10^{-2}	678/283,400 = 2.4×10^{-3}	227/474,000 = 4.8×10^{-4}

V. DEVELOPMENT AND FLIGHTS OF THE MARK III SAMPLING SYSTEM

A. Introduction

During the several years of operational experience acquired with the Mark I and II sampling systems, several important considerations and conclusions were developed regarding the equipment and analytical procedures. They are as follows:

1) Background or incidental contamination. The amount of background noise encountered with the various types of controls employed indicated either that the controls were not functioning as intended or the amount of background contamination was a level at times equal to or greater than the acquired sample.

2) The Mark I and II sampling systems used four or five separate sampling modules employing one or more as a control along with the diffusion pad internal controls. The question arises when analyzing results based on this system is whether it is valid to compare four or five different data sets when the contamination controlling function built into each device function at an unknown efficiency level. The reliability of each sampler module is therefore questionable, however, if all functions were completely operational in all samplers and no contamination was was encountered on any control surfaces of any sampler modules, then a certain amount of significance could be ascribed to the results. The Mark I and II systems did not lend themselves to "zero" background operation for reasons previously discussed.

3) The Mark I and II systems were not designed in a way that data acquired could be analyzed using statistical methods for dealing with low sample numbers. Interpretation of control results with the previous sampler was subjective in nature and exact confidence limits or probabilities of occurrence could not be placed on any data gathered. For these reasons, previous data were analyzed on a qualitative basis and subjective interpretations of location of sampled organisms were set forth.

4) Many components of the samplers and gondola instrumentation had been flown many times, resulting in sustained damage from ground impact conditions. The reliability of the equipment due to impact damage was therefore becoming an increasingly important factor in the overall program.

5) The total instrumentation burden and need for redundant systems required by many interflight design and programming changes rendered the operation of the total system during flight conditions very unwildy and complex.

For the above reasons, a complete redesign of the sampling system was undertaken, the design of which was primarily to eliminate or reliably control background contamination and to produce and fly a more reliable total sampling system.

B. Experimental Design Parameters

The Mark III sampling system was designed to allow any data gathered to be analyzable by at least one of several statistical techniques in addition to the incorporation of more reliable and simplified operation. Previous operation of the Mark I and II systems revealed that if any organisms existed at stratospheric altitudes, the numbers were very low; therefore, when dealing with small numbers, it was essential to have the ability to make a confidence statement regarding the sample as compared to its controls. To gain this end, along with simplified operation, required a complete redesign where the same basic collection parameters were employed but all sampling functions were performed by a single sampling unit. The use of a single sampler module to acquire air from all attitude profiles eliminates the need for comparing multiple samplers with all the vagarities of assessing multiple equipment and control functions.

The proven collection efficiencies and analytical procedures were retained (i.e., polyurethane foam filters were used as a collection media, and extraction and refiltration were employed as per the previous procedures). Perhaps the most important procedural change was the ability now to conduct analytical procedures within sterile isolators, thus precluding the need for a large clean room and the use of a "surgical" type technique for handling the samplers during analysis. In summary, the Mark III system enabled the following general experimental design parameters to be effected:

1) The data obtained would be amenable to statistical analysis

2) All samples were acquired with a single sampler module

3) Analysis would be performed in a "sterile" atmosphere in an isolator where operator-introduced contamination would be further minimized

4) Other sources of background contamination would be minimized by engineering and design techniques discussed in a following section.

C. Engineering Design

1. Sampler Module

The sampler module as designed was aimed at elimination of extraneous background interference but incorporating the proven physical collection and analytical characteristics of the previous biological sampling system. The Mark III system uses the same filter material and cross-sectional area, however, the packaging concept is radically different allowing for greatly simplified preparation and assay procedures. Figure 6 presents a cutaway view of the sampler showing the doors open and a spool of polyurethane filter

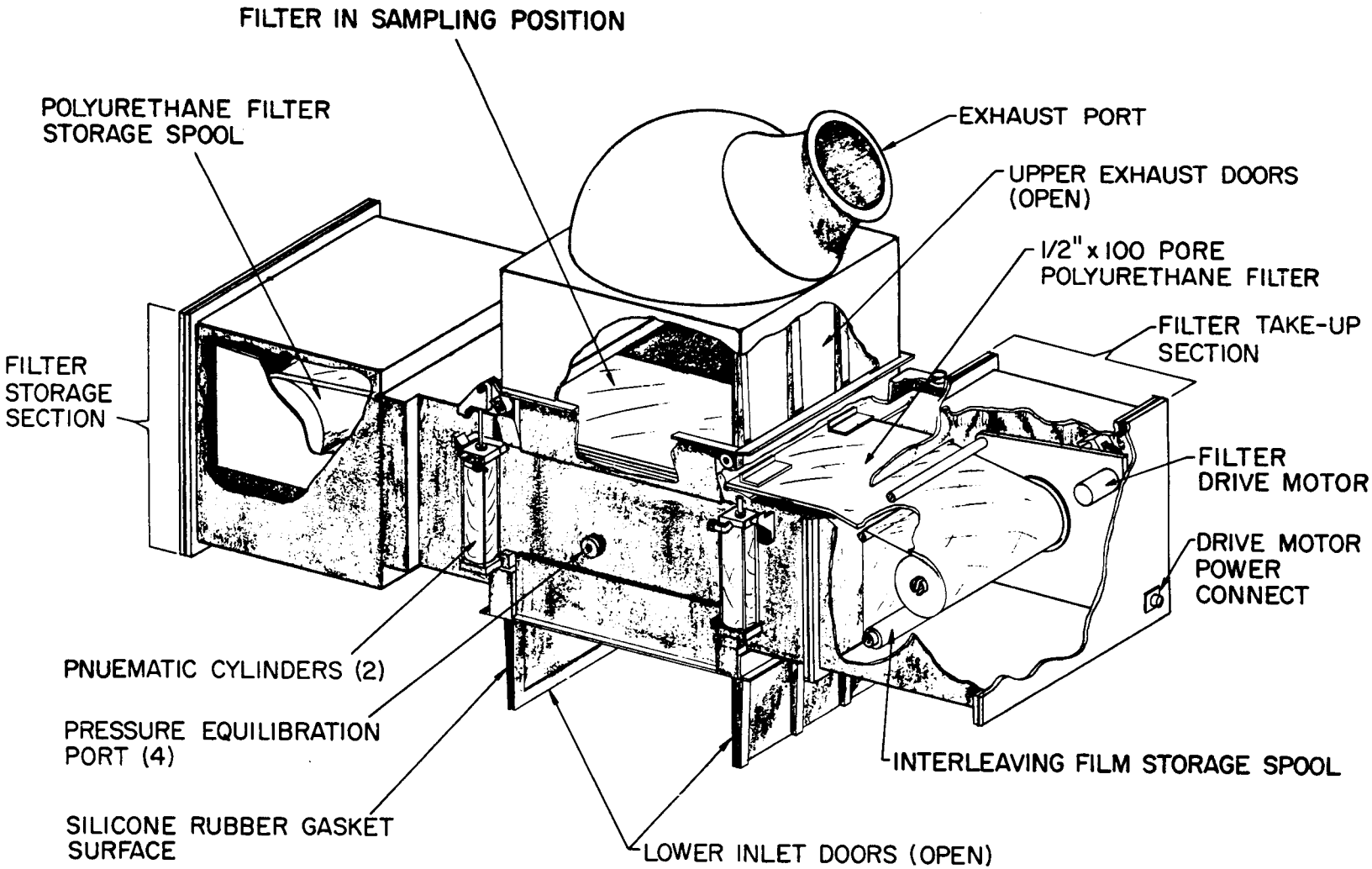


Figure 6. Sampler Module (doors open)

media in place. Figure 7 shows a similar view with the main door closed and no filter material in the sampler. The sampler module is composed of three basic components: filter storage section, main sampling plenum, and filter takeup section.

The basic operation of the sampler is similar to that found in many still cameras, where sequential frames of the film correspond to the various altitude profiles sampled, a section of filter material remains stationary throughout each sample profile and is advanced prior to initiating the following sequence at the next (lower) profile. As the sampler is operated, filter media is advanced from the storage section through the sampling plenum to the takeup section. In the takeup section a 0.001-inch thickness of impervious polyamide film of the same width as the filter is interleaved with the filter, effectively separating the layers on the spool. This measure prevents cross contamination of the filter.

A system of upper and lower doors actuated by two pneumatic cylinders is shown in Figure 6. The two sets of double doors are hinged and spring-loaded such that when no pressure is applied to the pneumatic cylinder the doors are held in a closed position. Silicone rubber gasketing on the door contact points and a system of knife edged mating surfaces serve to seal this module from sources of exterior contamination. A platen which raises and clamps the filter into the sampling position is also activated as the pneumatic cylinders open the upper and lower doors.

After the sampling sequence, the filter material is advanced through a narrow slot, the width of the filter, into the takeup section. This takeup module is equipped with a spring-loaded door, the configuration of which can be seen in Figure 8. When all sampling sequences have been completed and upon reaching a certain altitude on descent, the filter is wound on the takeup section, and the gate is

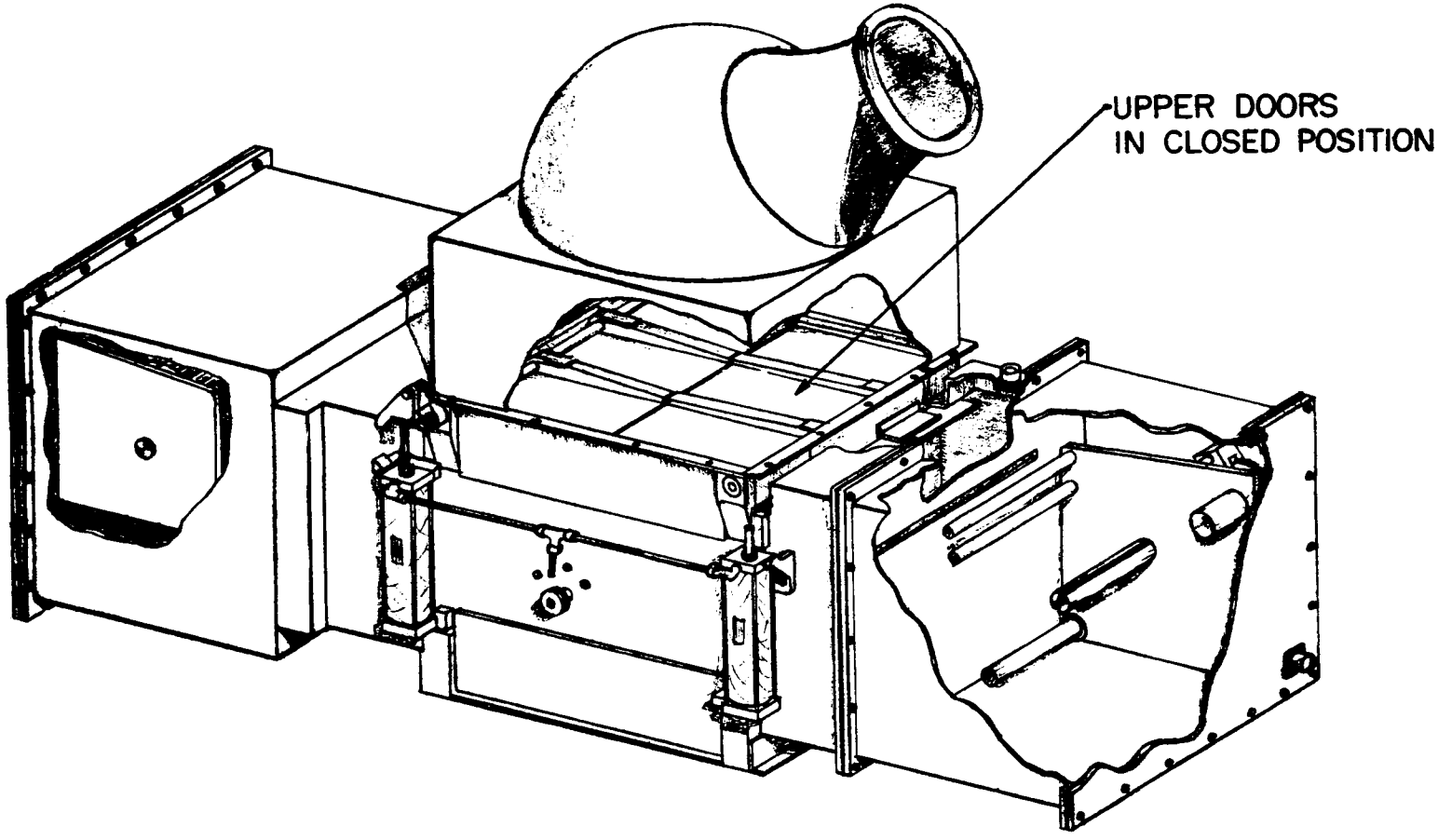


Figure 7. Sampler Module (no filter or inter-leaving film - doors closed)

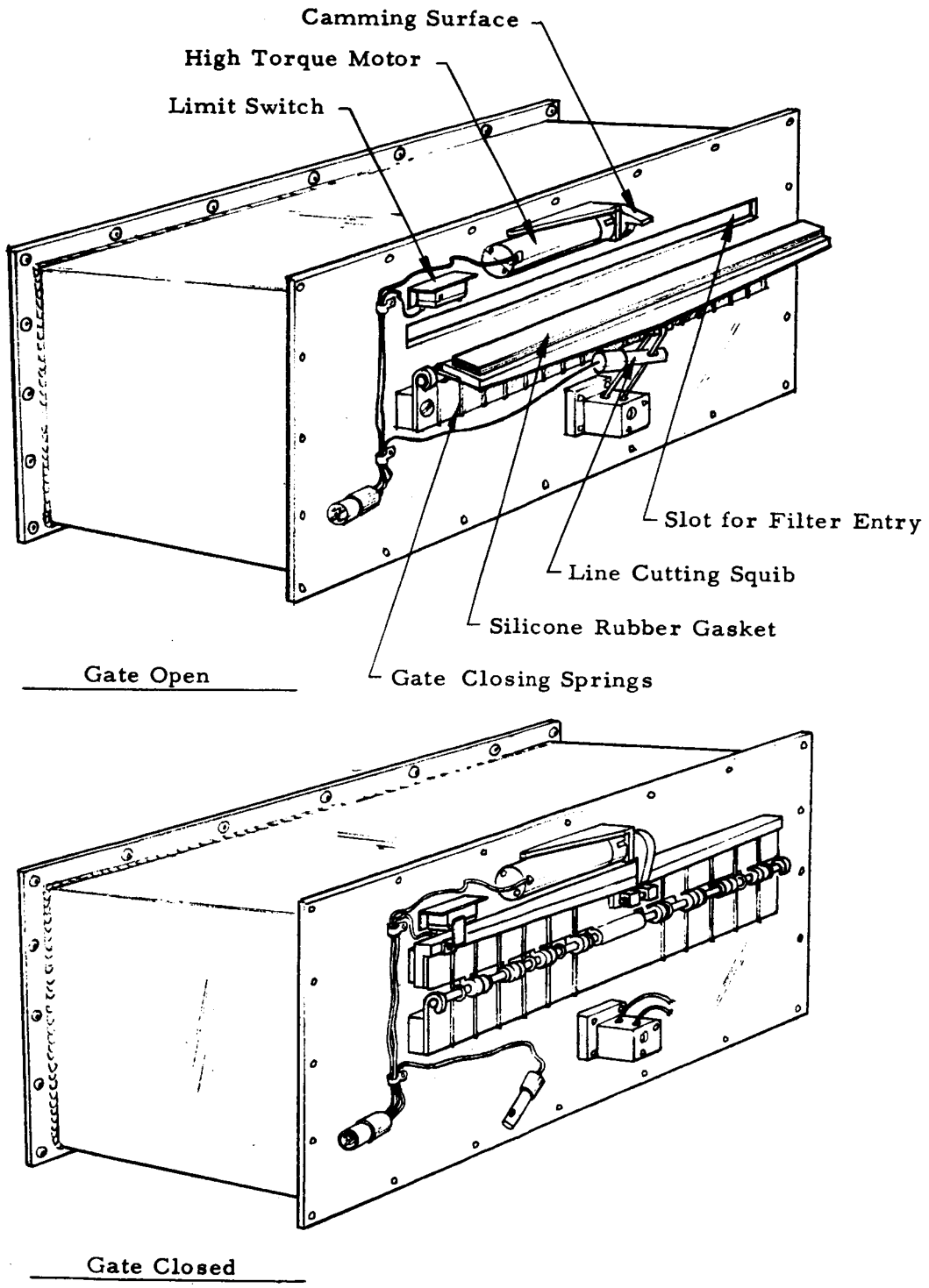


Figure 8. Filter Takeup Section

closed by a line cutting squib. The gate is gasketed by silicone rubber against metal. As the line cutter is fired, a low-speed, high-torque electric motor compresses the gasket to a predetermined point where it is stopped by a limit switch. When this operation has been completed, the samples are sealed in a small module which can be conveniently transported and manipulated during analysis. The dimensions of the takeup section are 10-3/4" x 8-1/4" x 14".

The control of filter movement through the sampler was by a series of alternating magnets imbedded along edges of the filter material. As the filter advances toward the takeup section, it passes under a set of two magnetic reed switches located inside the takeup section (see Figures 15 and 16, programmer circuitry). The use of this system allows the filter to be pre-marked permitting accurate determination of areas where stratospheric air had been sampled. This also simplified analysis steps since all samples and controls were pre-coded.

Inlet and exhaust duct fittings complete the sampler module components. The outlet (exhaust) fitting leads to the air moving source (air ejector). Inlet fittings consisting of a crushable diverging section, dust cover and shroud arrangement are similar in design and purpose to that used with the Mark I and II sampling systems. The design of the ducting allowed isokinetic flow through the sampler.

2. Ejector Air Moving System

Prior to the use of the Mark III design, battery-powered high rpm blowers were used to pull air through the filters. Electromechanical systems such as this have always been a source of considerable unreliability. Carbon dust, oil and battery vapors introduce the hazard of spurious contamination and motors overheat easily in the

thin atmosphere and burn out. At the highest profiles of interest, blowers operate inefficiently, thus compounding the problem with increasing attitude.

The air ejector pump offers an attractive approach to this problem. This uncomplicated device uses no moving parts. Power is derived from a tank of compressed gas and electrical power is needed only to actuate valves, allowing the use of dry cells. There is nothing to wear or burn out.

Under contract with the Division of Biology and Medicine, U. S. AEC, this laboratory has utilized theoretical and experimental studies to design a balloon-borne ejector powered filter sampler.[15] Although the ejector sampler was initially developed to operate at altitudes of 100,000 feet and greater, calculations indicated that it is a competitive system at lower altitudes, especially if comparisons include reliability factors.

Calculations have been made based on previous design data to determine the ejector performance. The work involving the theoretical development of continuity and momentum equations has been done by A. McFarland and supported in part by the AEC.[15, 16, 17]

Basically, the air ejector pump is a simple device, as illustrated in Figure 9. A jet of high-velocity primary gas is injected into a mixing tube, with the resulting expansion of the gas the surrounding secondary air is entrained. The turbulent exchange of momentum between the primary and secondary gases produces a region of reduced pressure and a net flow through the system.

Two types of ejectors have been described in the literature. In one type the primary and secondary gases mix in a tube of a constant area; in the other type mixing takes place under constant pressure conditions in a diffuser. The constant area ejector was chosen because low air density tends to reduce the performance of a diffuser.

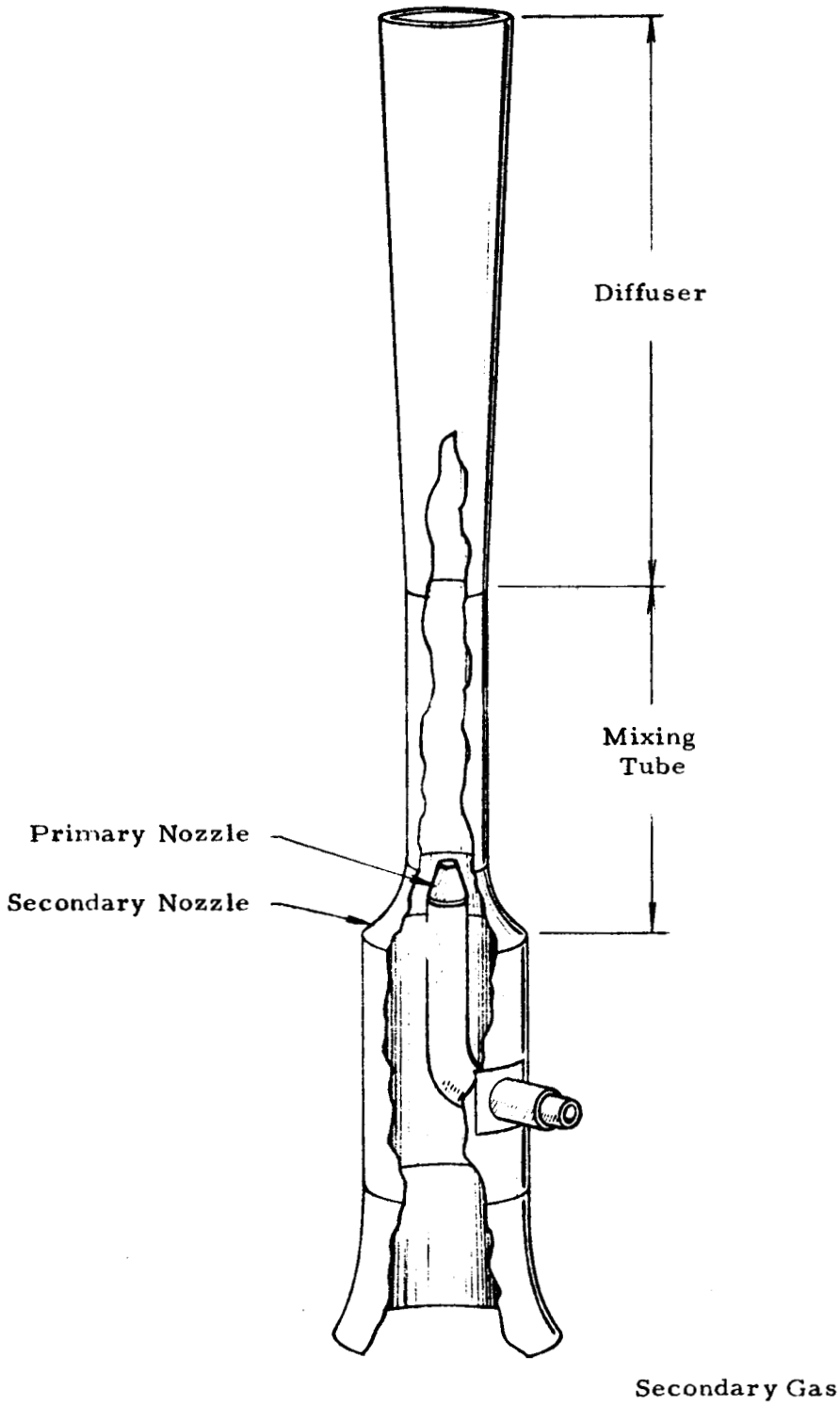


Figure 9. Ejector Pump

In McFarland's analysis, which is patterned after Chisholm's,[18] the airflow is considered to be unidimensional, steady, frictionless and adiabatic, when the two gases, primary and secondary, are both air and at the same total temperature and with sonic flow at the primary nozzle. The following equations have been generated.

List of Symbols

a = sound speed

A_1 = area, primary nozzle

A_2 = area, secondary nozzle

A_9 = area, diffuser inlet and tube

m_1 = mass flow, primary gas

m_2 = mass flow, secondary gas

M_9 = Mach no. diffuser inlet

M_2 = Mach no. secondary nozzle

P_a = pressure at altitude

P_b = static downstream pressure

P_{o1} = total pressure, primary gas

P_{o2} = total pressure, secondary gas

Q_a = volume flow rate, air ingested ambient

Q_2 = volume flow rate, secondary nozzle

T_o = stagnation temperature

V_2 = air velocity, secondary nozzle

ϕ = mass ratio, mass secondary air/mass primary air

r = isentropic exponent (air = 1.4)

η_d = diffuser efficiency

P_a = density, air ambient

P_2 = density, air at secondary nozzle.

Continuity

$$\frac{P_{o1}}{P_a}\frac{A_1}{A_9}\left(\frac{2}{r+1}\right)^{\frac{r+1}{2(r-1)}} + \frac{P_{o2}}{P_a}\frac{A_2}{A_9}M_2\left(1+\frac{r-1}{2}M_2^2\right)^{\frac{-(r+1)}{2(r-1)}} = M_9\left(\frac{1+\frac{r-1}{2}M_9^2}{1+\frac{r}{2}\eta_d M_9^2}\right)^{1/2}$$

Momentum

$$\frac{P_{o1}}{P_a}\frac{A_1}{A_9}\frac{2^{\frac{r}{r-1}}}{(1+r)^{\frac{1}{r-1}}} + \frac{P_{o2}}{P_a}\frac{A_2}{A_9}\frac{(1+r\,M_2^2)}{\left(1+\frac{r-1}{2}M_2^2\right)^{\frac{r}{r-1}}} = \frac{1+r\,M_9^2}{1+\frac{r}{2}\eta_d M_9^2}$$

The measure of the ejector performance is the ratio ϕ of the mass flow rate m_2 or air drawn into the air ejector per unit mass ratio of m, of primary gas expanded. The mass ratio can be then expressed as:

$$\phi = \frac{m_2}{m_1} = \frac{P_{o2}}{P_{o1}}\frac{A_2}{A_1}M_2\left[\frac{1+r}{2\left(1+\frac{r-1}{2}M_2^2\right)}\right]^{\frac{r+1}{2(r-1)}}$$

These three equations have been solved numerically on a Control Data Model G15D computer. A family of curves generated from these equations define ejector configuration in addition to defining optimum primary and secondary ratios. These relationships are presented in Figures 10, 11, 12 and 13. The performance of the ejector pump is substantially independent of size, provided similarity of geometry is observed.

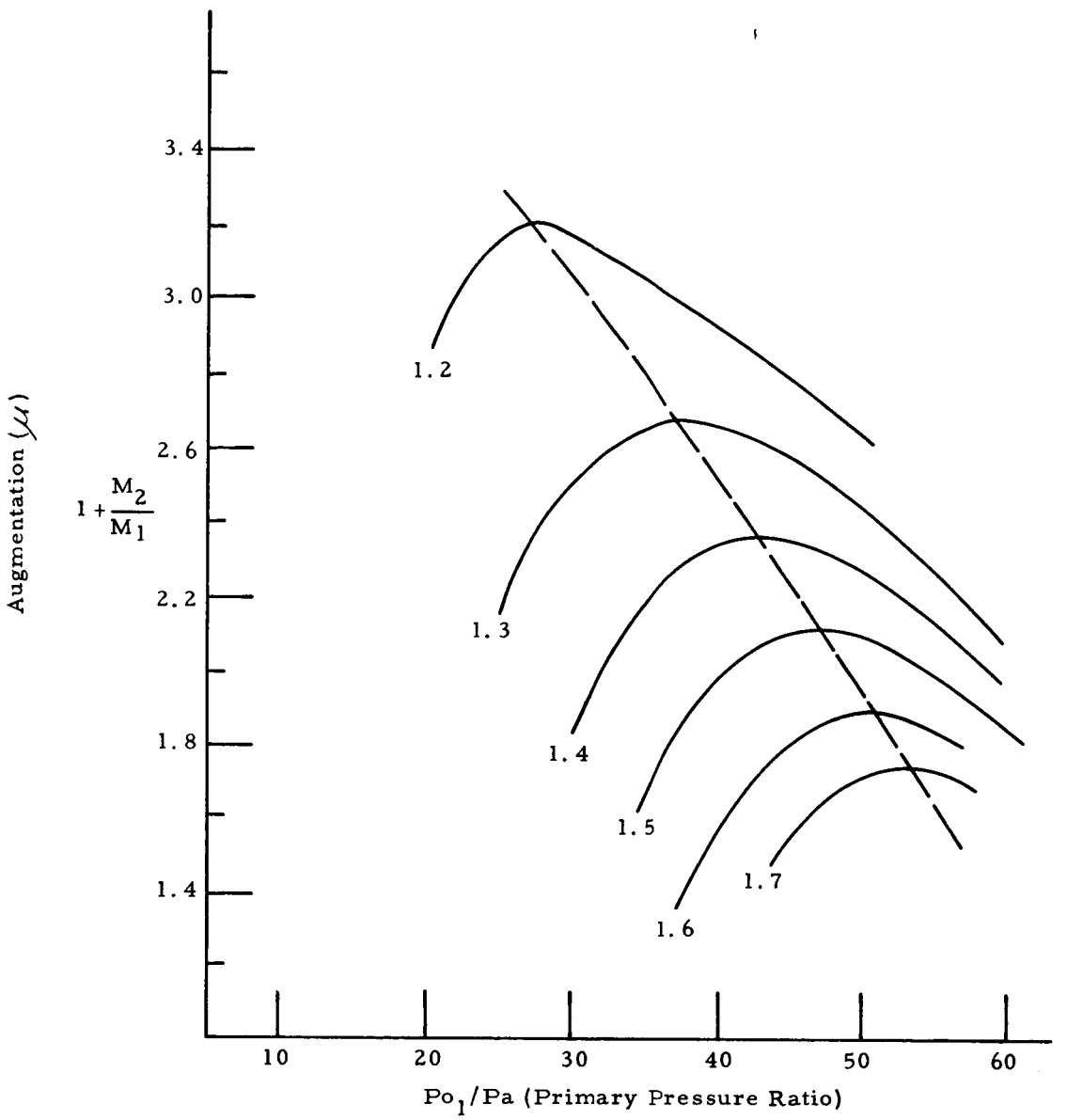


Figure 10. Mass Augmentation

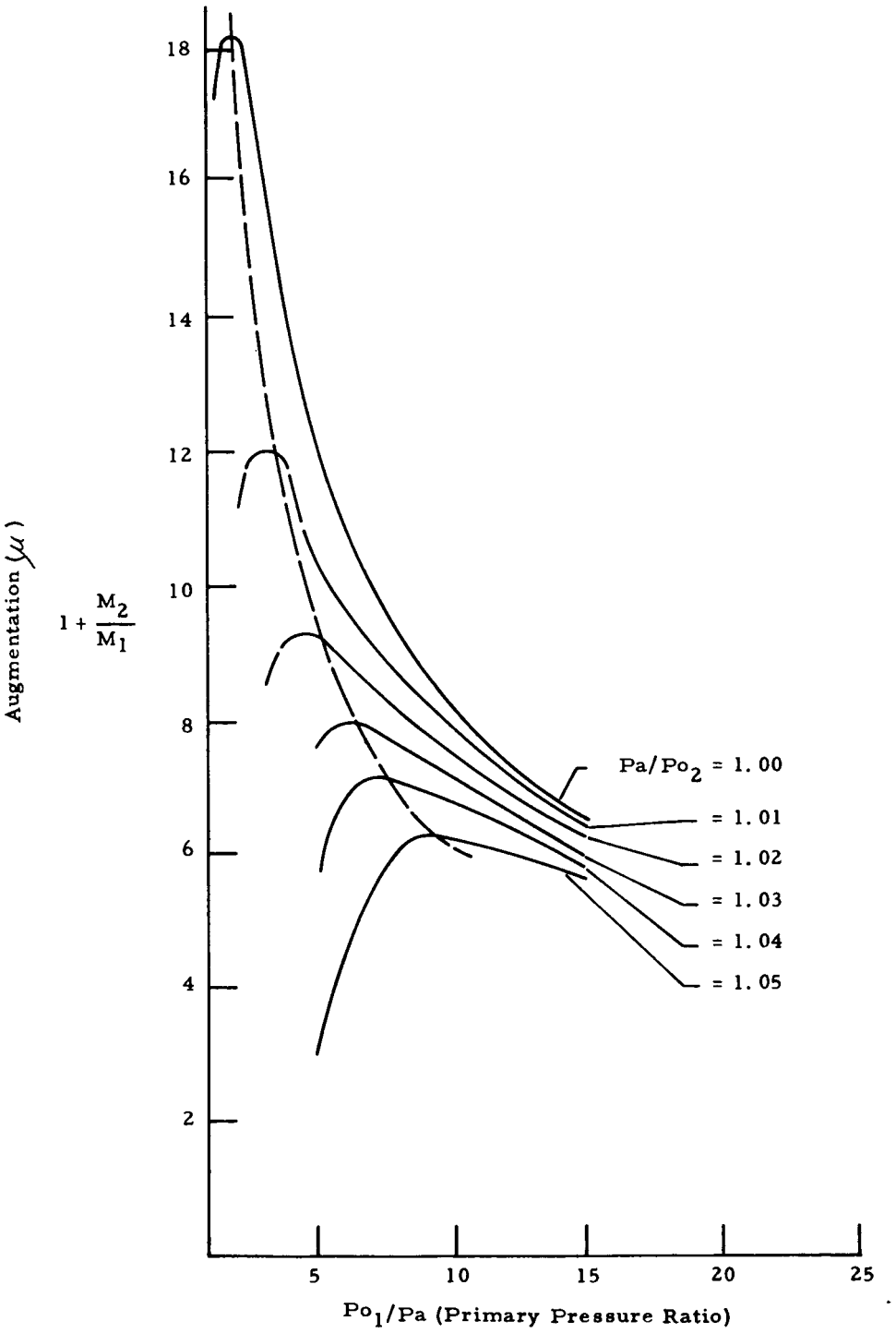


Figure 11. Mass Augmentation

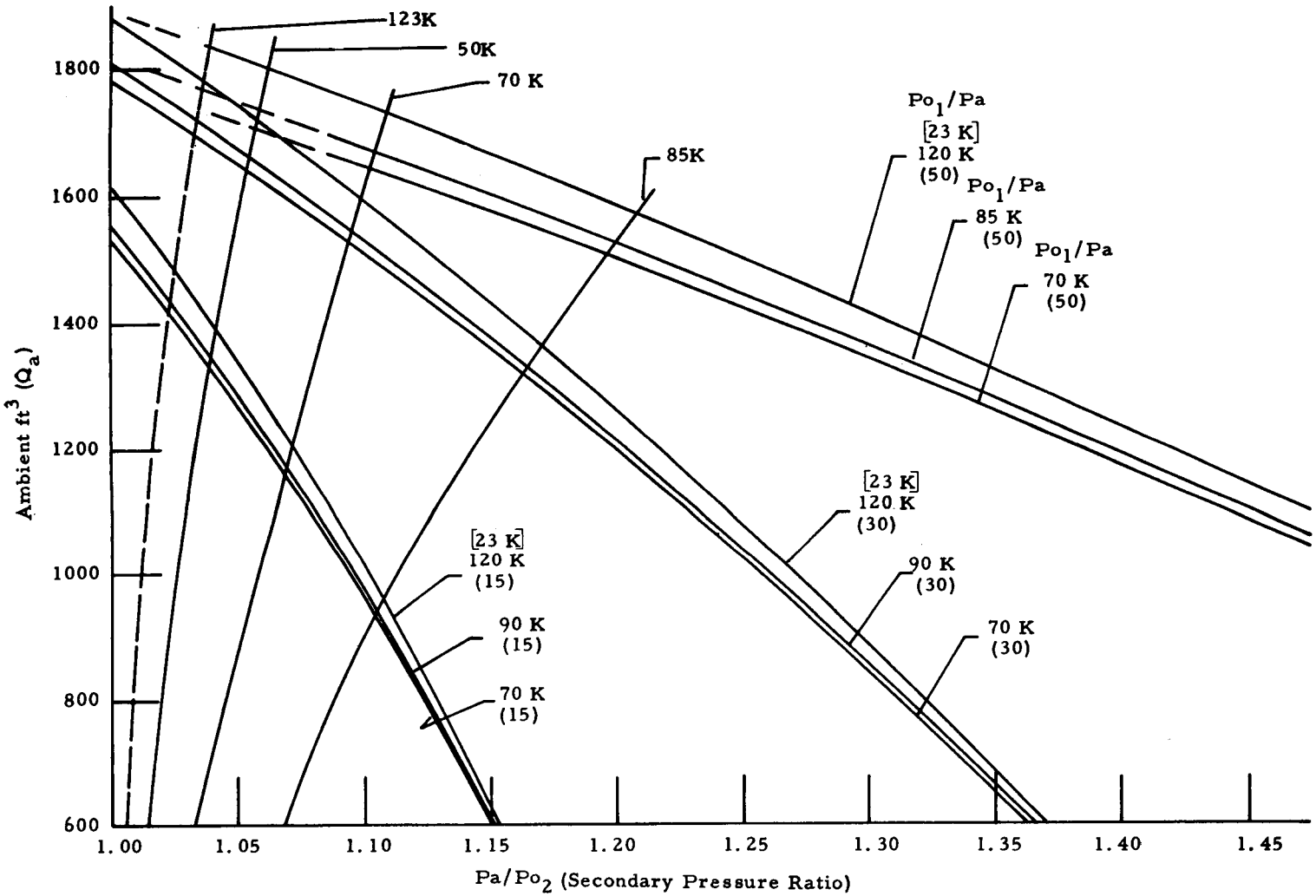


Figure 12. Ejector Pump Performance - NASA Mark III Sampler

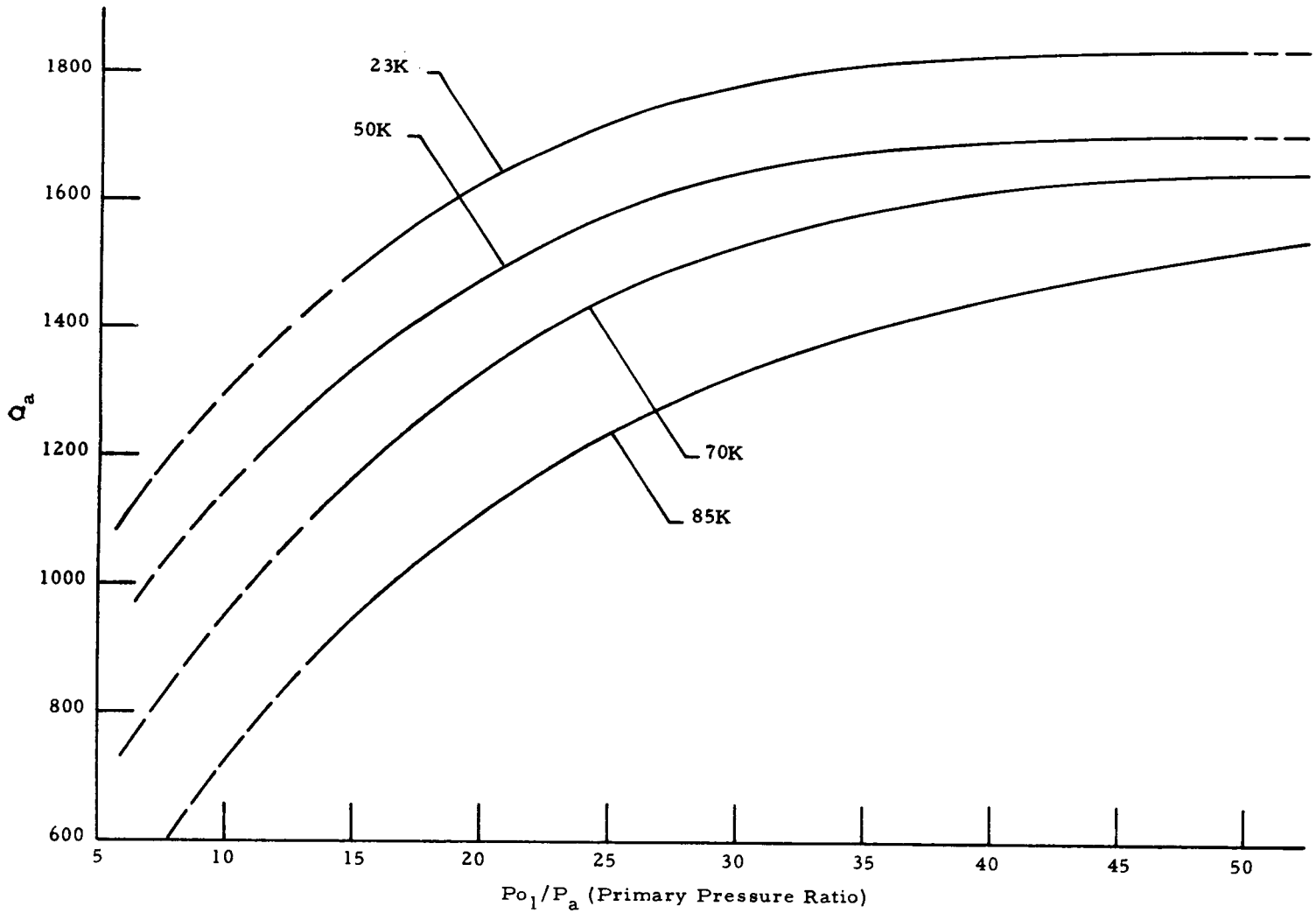


Figure 13. Performance Curve - NASA Biosampler

Data extrapolated from these curves are expressed below and represent the flow performance expected from the ejector designed for the Mark III flights.

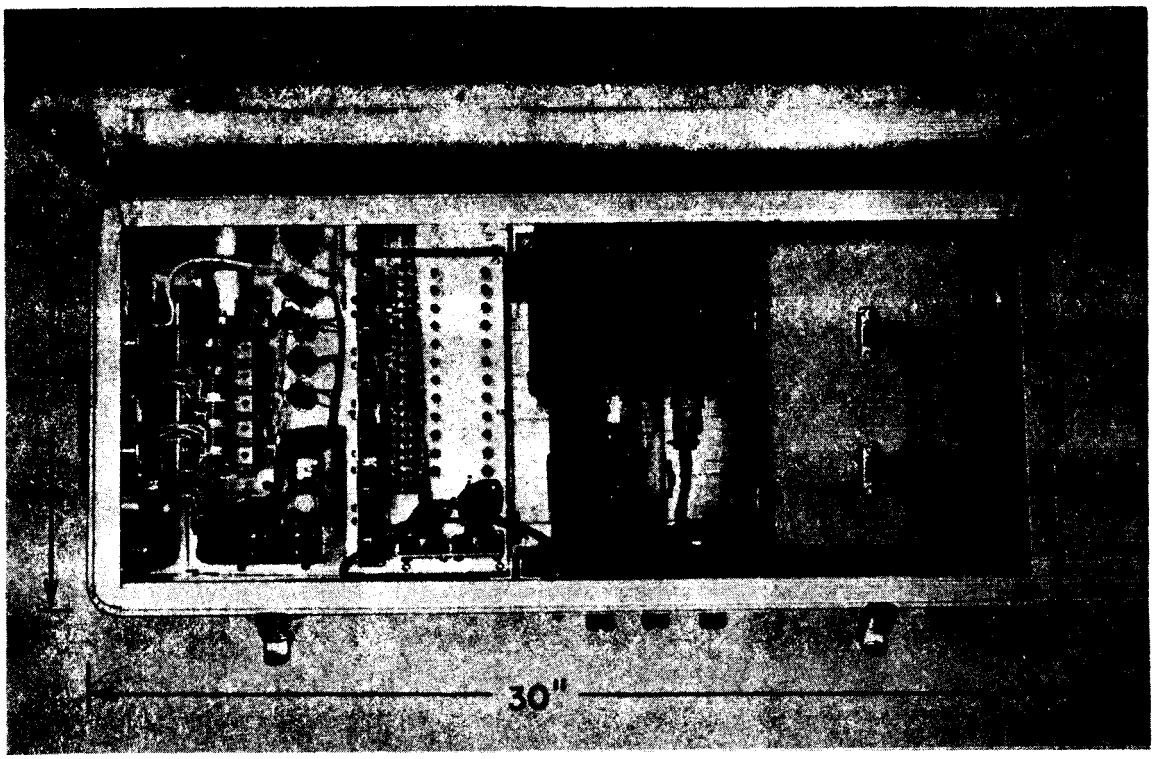
	Profile (ft K)			
	90 to 80	80 to 60	60 to 40	40 to 10
Ambient volume sampled (ft^3)	61,000	47,500	36,000	31,200
Sampling rate (ft^3/min)	1,050	1,160	900	780
Sampling time (min)	58	41	40	40
$\overline{X}$ altitude (ft K)	85	70	50	20
Air processed (lb)	130	210	420	1,268

Gas for each sampling sequence was supplied by a high-pressure spherical titanium tank. Each was capable of holding ~60 pounds of nitrogen and four such tanks were carried on each flight. The flow of gas from the tanks is controlled by a manifold with a series of squib-actuated, spring-loaded toggle valves.

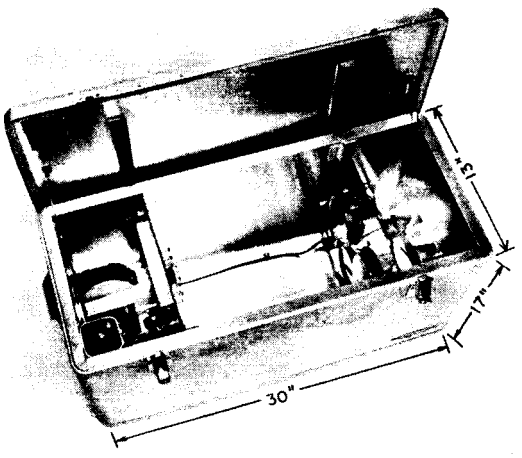
3. Flight Instrumentation

On-board instruments are carried in two modules: the flight programmer and flight data recorder. Both modules are packaged in insulated aluminum containers 30" x 13".

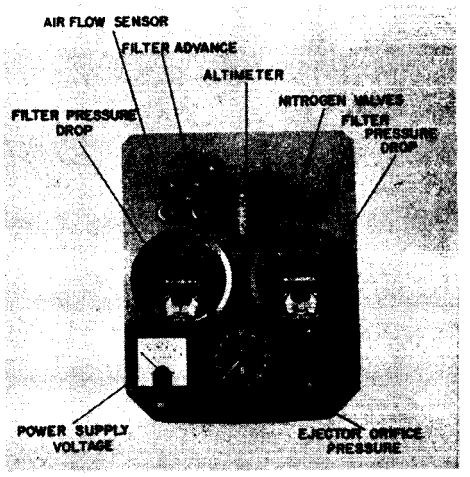
a. Programmer. - Two photographs of this assembly are presented in Figures 14(a) and (b). Detailed electrical circuitry is given in Figures 15, 16 and 17. The basic purpose of this module is to control sampler functions and to some extent serve as a source of telemetered information. Table 9 gives a sequence of operations performed by the programmer module during flight.



(a) Programmer Module Detail



(b) Data Recorder Module



(c) Data Recorder Photo Panel

Figure 14. Flight Instrumentation

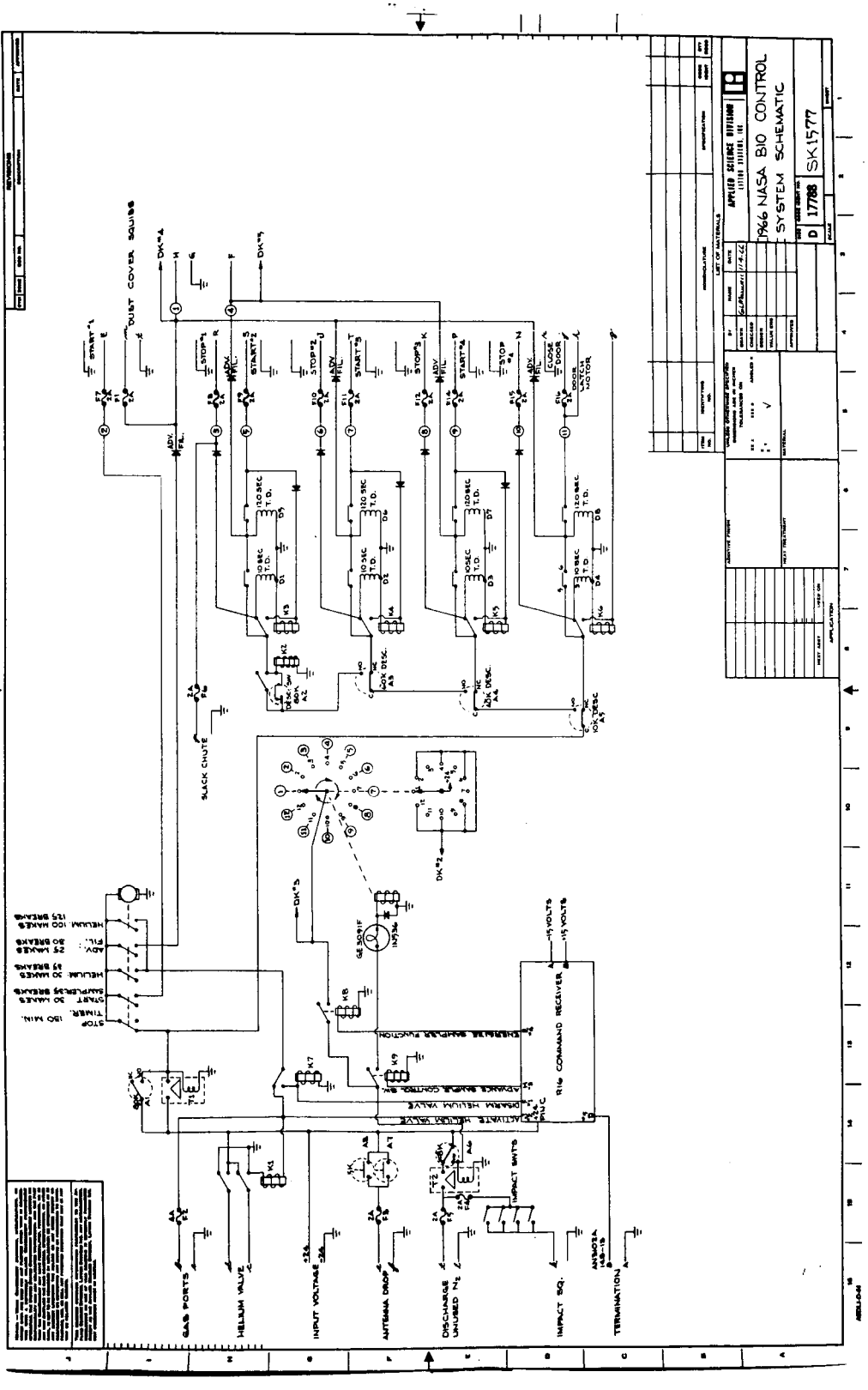


Figure 15. Control System Schematic

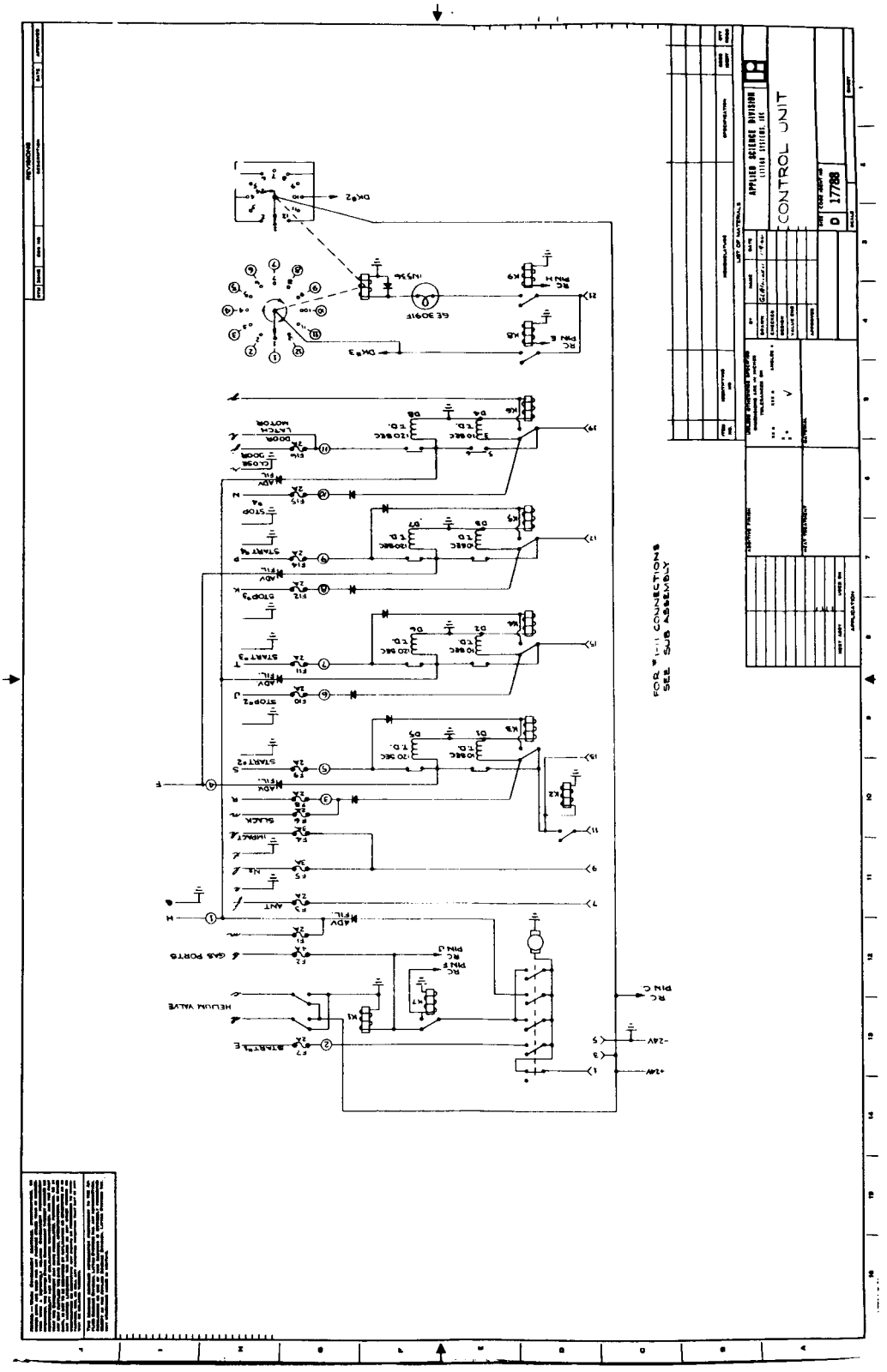


Figure 16. Control Unit

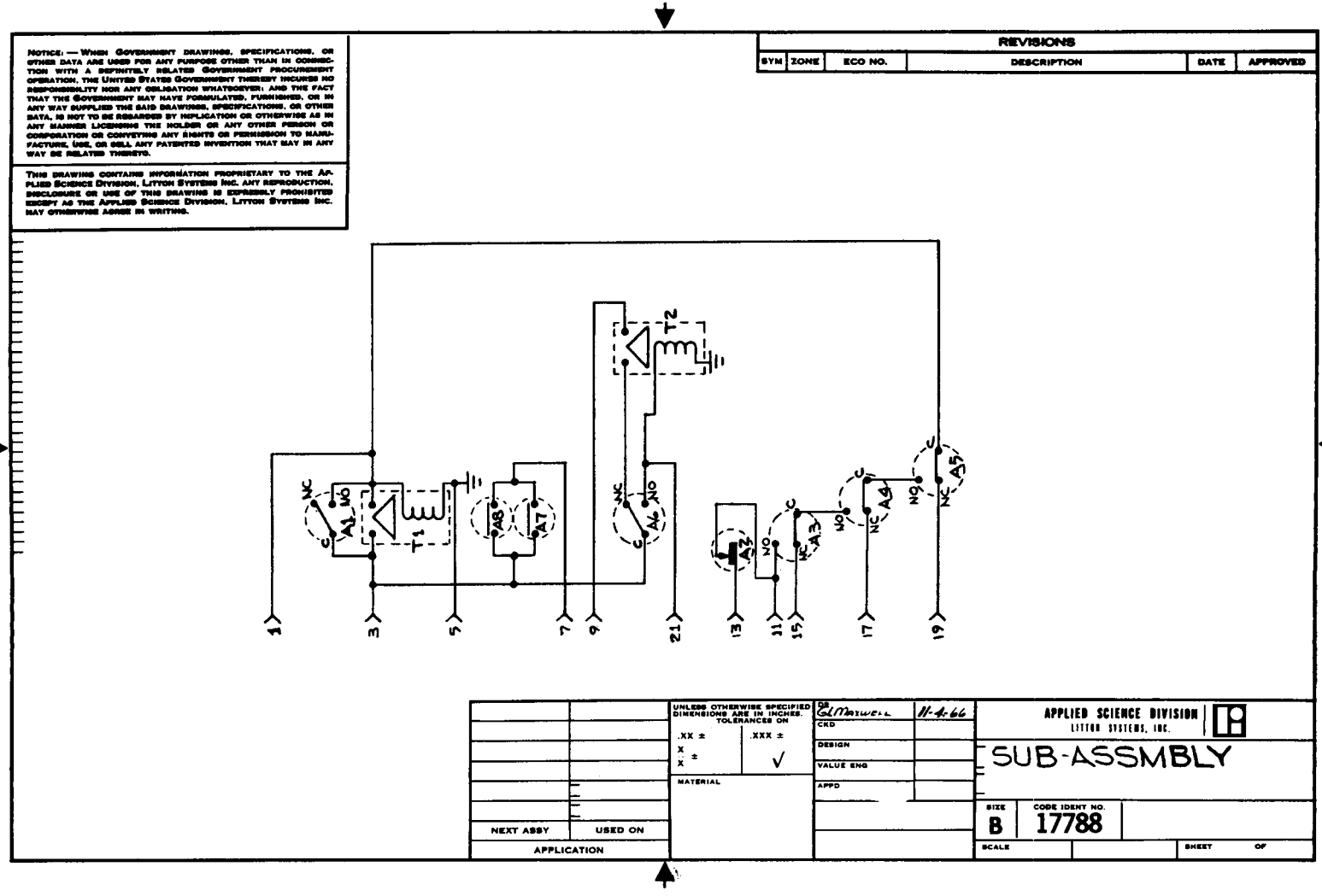


Figure 17. Connection Subassembly

Table 9. Programmer Operation Sequence

5K on ascent	Antenna drop squibs.
8K on ascent	Arm command functions #3 and #4.
71K on ascent	Start camera motor.
80K on ascent	Program delay time starts.
80K plus 25 minutes	Advance filter paper. Dust cover drops. Double keying #4 telemetered (code NXN).
80K plus 30 minutes	#1 sampler starts. Double keying #1 telemetered (code NKS). Electric helium valve opens; telemetered frequency shift (F.S.K.)* Helium ports open.
80K plus 35 minutes	Electric helium valve closes (cease F.S.K.).
80K plus 100 minutes	Electric helium valve opens; start F.S.K.
80K plus 125 minutes	Electric helium valve closes; cease F.S.K.
80K plus 130 minutes	Program timer stops.
80K on descent	#1 sampler stops. DK #1 stops. Slack chute squibs fire.
80K on descent plus 10 seconds	Advance filter. Double keying #5 (code NDT).
80K on descent plus 180 seconds	#2 sampler starts. Double keying #1 starts; NKS.
60K on descent	Stop #2 sampler. Double keying #1 stops.

Table 9 (Continued)

60K on descent plus 10 seconds	Advance filter. Double keying #4 starts; NXN.
60K on descent plus 180 seconds	#3 sampler starts. Double keying #1 starts; NKS.
40K on descent	#3 sampler stops. Double keying #1 stops.
40K on descent plus 10 seconds	Advance filter. Double keying #5 starts; NDT.
40K on descent plus 180 seconds	#4 sampler starts. Double keying #1 starts; NKS.
10K on descent	#4 sampler stops. Double keying #1 stops.
10K on descent plus 10 seconds	Advance filter. Double keying #4 starts; NXN.
10K on descent plus 180 seconds	Close door on takeup spool section. Takeup section door sealing motor operates. Double keying stops.
8K on descent	Discharge unused N_2. Arm impact switches. Disarm command functions #3 and #4.

* FSK = Frequency Shift Keying.

A separate radio command assembly provides an overriding control on the samplers in flight operations, adding a redundant safety factor to the acquisition of samples from each profile. In addition to controlling sampler function, the radio command may operate helium valves on the balloon or jettison the entire payload should the situation warrant. A list of these functions is presented in Table 10. Although designed expressly for the Mark III system, the functions of the program and telemetry are similar to and for the same purpose as those employed on the previous sampling systems.

b. Data Recorder. - The function of this module is to indicate certain operational occurrences and record them by means of a motion picture camera. Figure 18 presents a schematic diagram of the functions recorded by the camera and is self-explanatory. Two photographs of the recorder module are shown in Figures 14(b) and (c).

The two instrument packages, plus the use of a PR-3 flowmeter and recording system, allow all pertinent parameters to be recorded and/or telemetered plus provide for backup control systems in the event of failure of a particular operational function.

4. Gondola

The gondola frame holding all the flight instruments and equipment was initially fabricated from tubular magnesium. The damage from impact largely destroyed the gondola during the first two flights; therefore, aluminum was substituted during the third and final operation. Figure 19 presents a schematic of the gondola with the modular components attached. Salient features of the design include:

1) Central mounting of the sampler with the inlet ducting through the central axis of the frame

Table 10. Radio Command Functions

Channel No.	Code	
1	1792	Disarm control unit electric helium valve circuit.
2	17941*	Open electric helium valve.
3	1796	Advance sampler control switch. Double keying #2 telemetered (code NGS) on alternate positions #2, 4, 6, 8, 10 and 12.
4	17981*	Energize sampler function. Double keying #3 telemetered (code NNA). Controller Position and Function 1. Advance filter (1st, 3rd and 5th positions). 2. Open valve #1. 3. Close valve #1; slack chute. 4. Advance filter (2nd and 4th positions). 5. Open valve #2. 6. Close valve #2. 7. Open valve #3. 8. Close valve #3. 9. Open valve #4. 10. Close valve #4. 11. Close and latch door. 12. Blank position.
5	17981*	Termination (release balloon).

NOTE: * Denotes hold last digit (#1) on dial till double keying changes when required, or to hold helium valve open.

Telemetered Double Keying Code

D.K. #1	Pressure differential indicator
D.K. #2	Sampler control switch position
D.K. #3	Sampler control switch activation
D.K. #4	Advance filter (1st, 3rd, and 5th positions)
D.K. #5	Advance filter (2nd and 4th positions)

NOTE: Highest D.K. number overrides all other double keying codes.

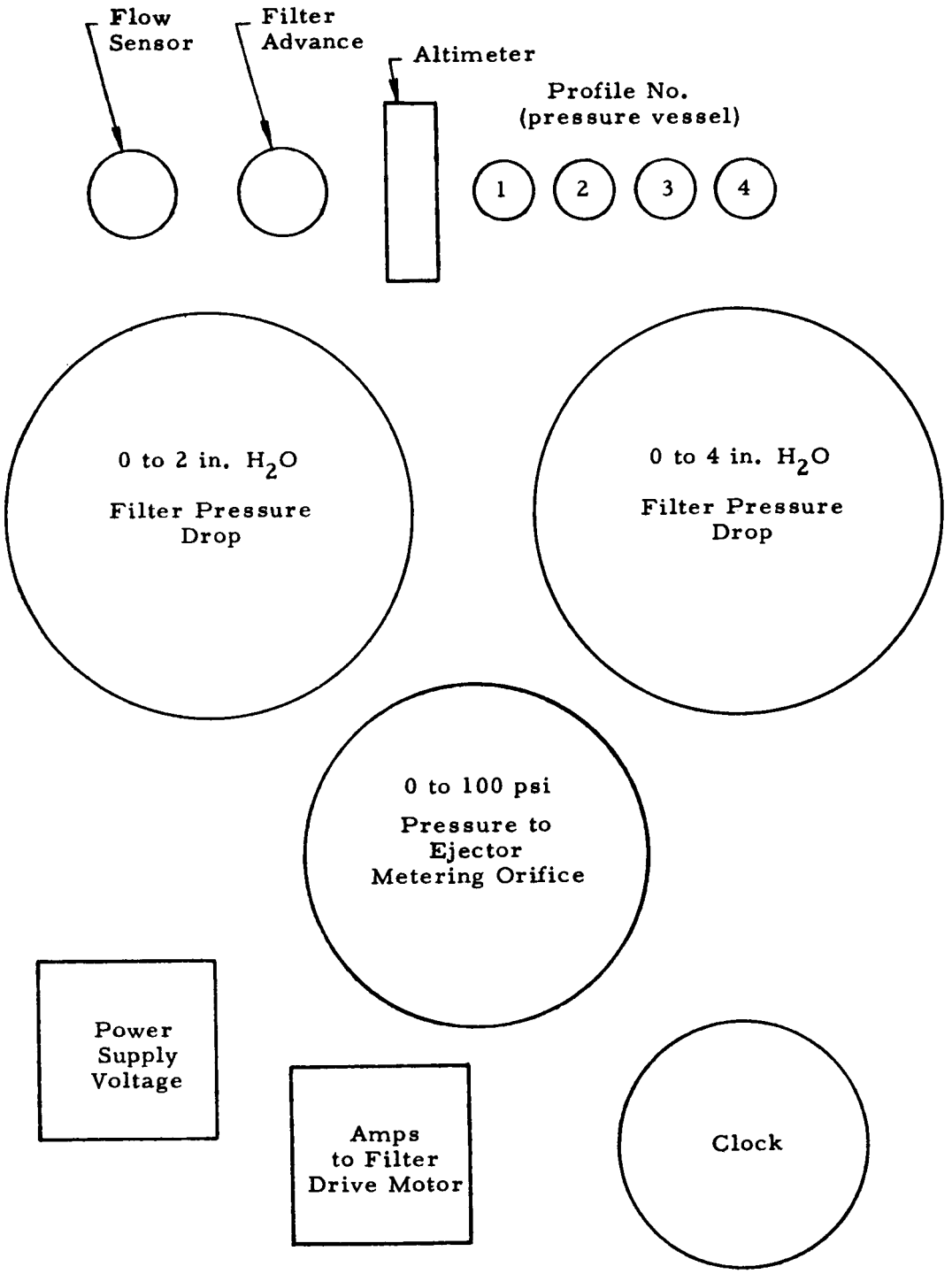


Figure 18. Photo Panel Function

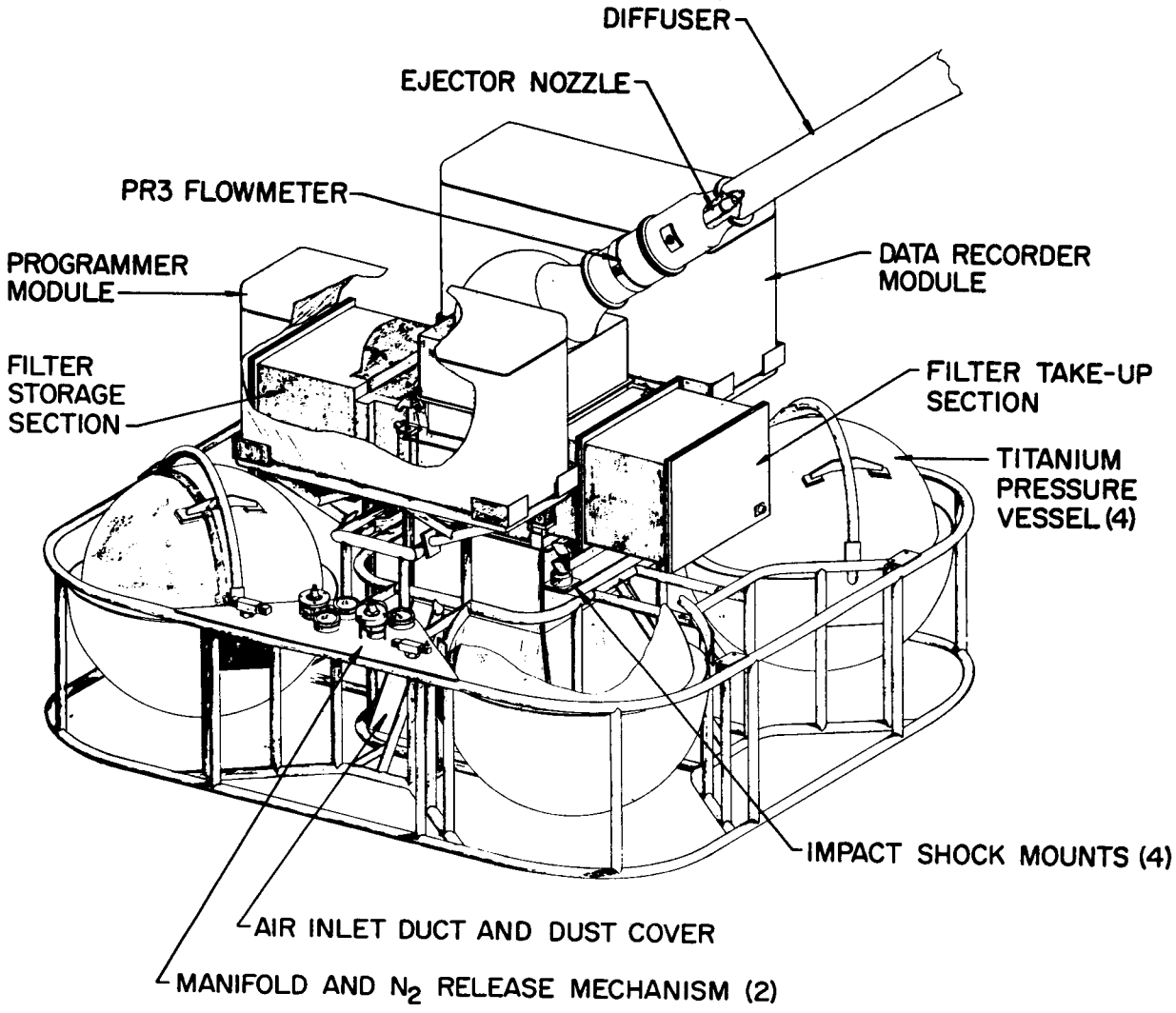


Figure 19. Mark III Sampler Payload

2) Four point shock absorbers between the sampler and frame

3) Foamed polyurethane and fiberglass hemispheres containing the four titanium pressure vessels.

5. Balloon

Specifications are as follows:

Manufacturer:	Winzen Research, Minneapolis
Material:	Strato film (polyethylene), Winzen manufacturer
Film gauge:	1.5 mil
Volume:	1,001,800 ft^3
Type:	Natural shape, tailored and taped
Inflated height:	121.9 ft
Diameter:	134.6 ft
Ducts:	One high at 113 ft; one low at 80 ft
Valving:	One EV13 electric operated; two gas ports
Destruct device:	Rip panel.

A schematic diagram of the balloon, including other components of the flight train, is given in Figure 20.

D. Analytical Procedures

1. Biological Analysis

The basic method of dissecting pre-coded sections of filter, placing in sterile bags, adding water, eluting the microorganisms, followed by membrane filtration has not changed. The procedural mechanics have been modified with the object of reducing extraneous contamination.

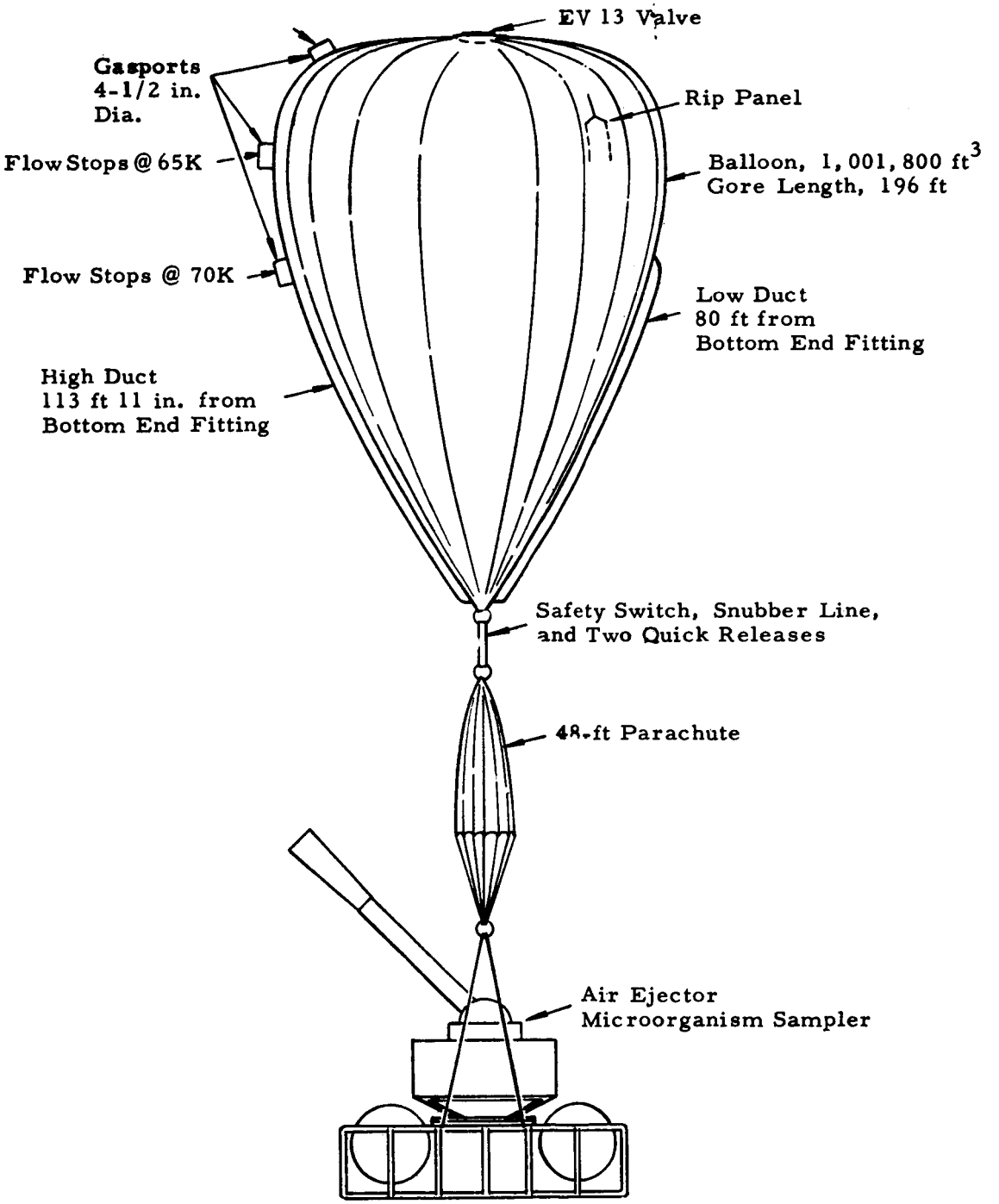


Figure 20. Mark III Flight Train

a. Isolators (Figure 21). - Of greatest significance is the use of a series of four isolators for conducting all biological analytical operations. The configuration consists of a single decontamination chamber (isolator) with a passthrough airlock leading to a series of three chambers serving as the analysis area. All chambers are held at a pressure greater than ambient where the highest pressure is found in the main chamber area, with lower pressures in the lock and decontaminating chamber. Sterile air supply to these areas is provided by pressure filtration through two Army Chemical Corps absolute particulate and charcoal cannisters. These filters are sterilized by autoclaving and installed just prior to chamber decontamination.

Also provided in the chamber are apparatus for membrane filtration and a supply of pnosphate buffer extraction fluid. The membrane filter apparatus is operated by an external vacuum supply and connected by means of quick disconnect fittings. Extraction fluid is provided from a large, brass, epoxy lined cannister of 15-liter capacity which is exterior to the chambers. The fluid is admitted to the chambers through an interface broken by a quick disconnect coupling. Air required for pressurizing this device is filtered through membranes eliminating contamination from this source. All of the above components and supplies are steam sterilized. At a point immediately prior to use, the phosphate buffer diluent is filtered through a membrane. This step serves two functions: it is a "fail safe" sterilization method and it also gives diluent containing low particulate count.

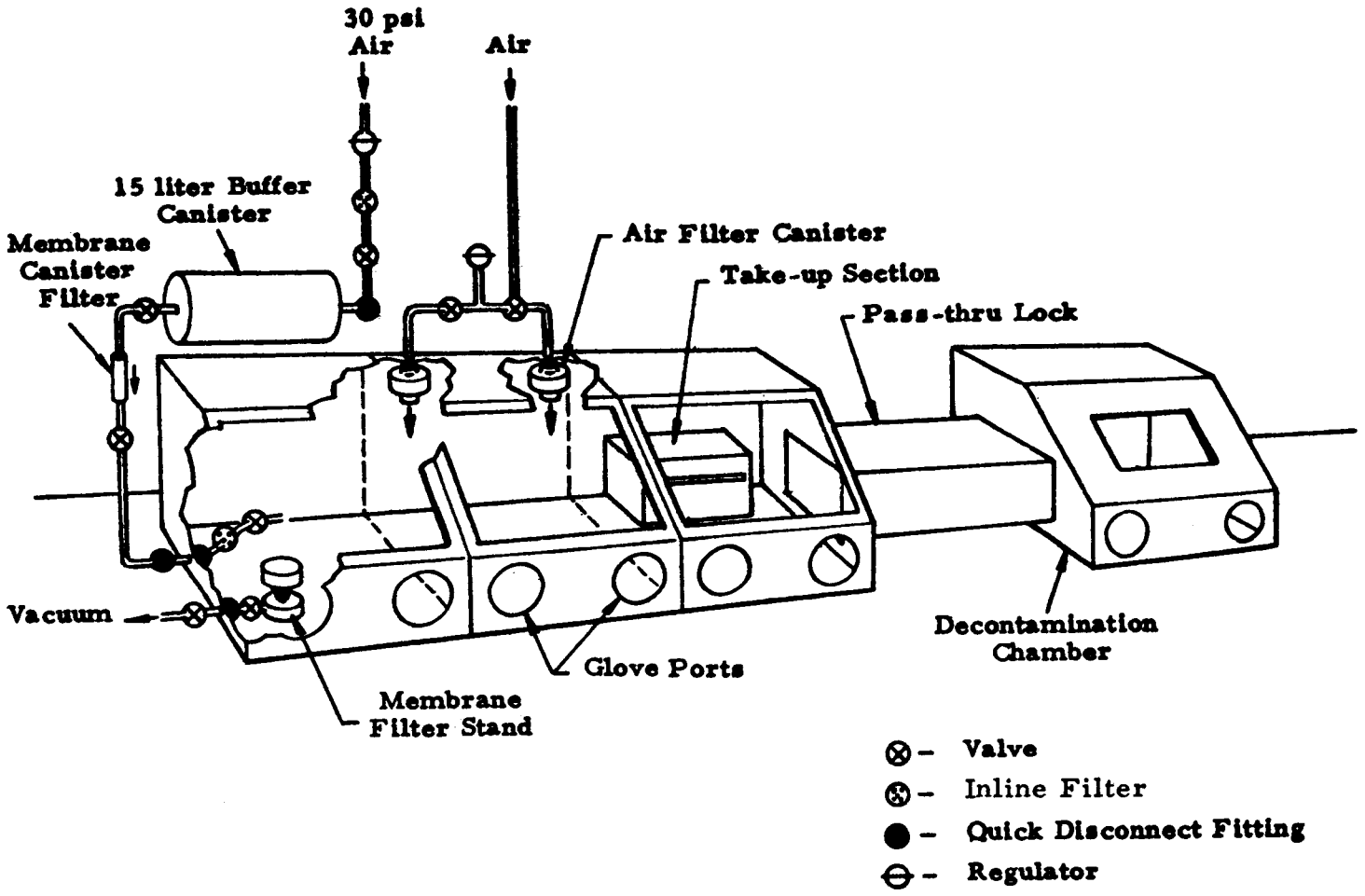


Figure 21. Sample Analysis and Decontamination Chambers

b. Decontamination and Sterilization Procedures

Isolators. - Since the isolator is the location where the filter samples are exposed and manipulated, careful consideration was given to the problem of assuring that contamination would not be incurred in this area. As mentioned previously, provision is made for positive pressure sterile air precluding migration of particulate contamination through small leaks in the isolator structure. Two basic problems exist: destroying the viable microorganisms resident on the chamber interior working surfaces and maintaining a sterile environment during the introduction of material into the chambers and during bio-analysis.

The chambers were effectively sterilized by spraying with a 2% per-acetic acid (PAA) solution in distilled water. This concentration and compound was selected on the basis of its known properties of a broad spectrum "cold sterilant". Per-acetic acid is also little effected by organic material or other common neutralizers and also leaves no residual after evaporation from surfaces. Application of PAA was accomplished by the use of a self-contained single-phase spraying device pressurized with air to 125 psi. This device contains 500 ml which in one filling was adequate for application to the entire isolator. During the spraying process, care was taken to apply to all interior surfaces including complete coverage of all gloves. After spraying the chamber pressure was increased to ~1.0 in. H_2O and allowed to ventilate for at least 24 hours prior to use. No PAA was present in the chamber during any analysis procedure. When operating, the pressure was reduced to ~0.5 in. H_2O.

The effectiveness of this process for destroying resident flora of the isolator was determined by measuring the density of organisms on various surfaces before and after treatment, and by measuring the

reduction of flora on surfaces artificially contaminated with bacterial spores. In the first instance, surface contact (Rodac) samples were taken with a density of 1.87 organisms/cm^2 established, yielding an extrapolated microbial load of 9.9×10^4 organisms for the total interior isolator surfaces. The large majority of the organisms isolated from these samples were Bacillus subtilis var globigii (Bg), a common spore contaminant found in these laboratories. Twenty-four hours after spraying with 500 ml PAA and ventilating, additional samples were acquired. These samples after treatment yielded a total of two organisms, both which were Bg. Calculations based on these two indicated a level of 5.9×10^{-3} organisms/cm^2 or 313 present on all isolator surfaces. These extrapolations may be misleading since they are based on the isolation of only two organisms from many samples acquired and may be due to spurious contamination (non-isolator).

Realizing that the contact sample method for evaluating the number of organisms surviving PAA spraying has some difficulties, an experimental exposure system was devised. Stainless steel strips were uniformly innoculated (aerosol deposition) with dry, lyophilized Bg spores. The strip dimensions were 4 x 7 cm and contained 4.8×10^6 viable Bg spores. Strips were exposed to the PAA spray in the chambers during several trial decontaminating runs (placement was random throughout the chamber). The strips were quantitated by placing the strip in a sterile polyethylene bag, adding 100 ml phosphate buffer, then vigorously rubbing the surfaces of the polished strip to remove any bacteria. Aliquots of this extract were either plated using standard dilutions and pour plates or directly plated after membrane filtration. The results indicated that the efficiency of PAA spraying is to some extent dependent upon the locus of the surface within the chamber. Test strips exposed with a surface hidden (underside) were not completely decontaminated; strips with little or

no surface hidden were effectively sterilized. Comparative decade reductions were 1.57×10^6 for strips with one partially hidden surface and 4.8×10^6 (sterile) for totally exposed strips. The strips that were not sterilized had a mean recovery of 7.7 Bg out of a possible 4.8×10^6 present prior to treatment. Based on these data, gathered with a hardy organisms used as simulant, it is probable that no organisms remain viable at any readily accessible chamber surface. Considering that prior to treatment with PAA and after cursory cleaning procedures, a load of $\sim 10^5$ exists; therefore, a decade decrease of at least 10^6 leaves a theoretical 0.1 organism per chamber. Hence, for all practical purposes, a sterile condition exists.

Other Sterilization and Handling Procedures. - All items passing into the analysis chamber were pre-sterilized either by ethylene oxide or steam (autoclave) and sprayed with PAA in the decontamination chamber. With the exception of the sampler takeup section module, all supplies passed into the chamber were double wrapped in Kraft paper. These materials are spayed with 2% PAA and allowed to stand in the decontamination chamber for 15 minutes. After this time interval, the first covering is removed and the item is passed through the lock into the main analysis chamber. Here, the last wrap is removed and passed back through the lock.

Decontamination of the module containing the sample differs from the above procedure in the following ways: A pre-wash of PAA is applied under a standard hood and the module is scrubbed to remove as much soil as possible, about 1/2-hour contact time is used; wrappings are not used with this item. During this operation, tape seals along gasketed surfaces are removed and the area under the tape sprayed with the PAA solution. After final PAA spraying in the decontamination chamber, the module is wiped dry with sterile toweling prior to insertion through the airlock.

Tables 11 and 12 present a list of materials sterilized by heat and ethylene oxide and the respective time, concentration, temperature relationships. All the items tested were monitored for sterility with the use of spore strips placed in locations where penetration of heat or vapor would be minimal. Sterility of these strips indicates complete sterility throughout the material.

Table 11. Autoclaved Items used in Analysis Chamber (121°C - 15 psi saturated steam)

Item	Exposure Time (min)
Sampler Module	60-90
Air Filter Cannister	30
Pressure Container for Buffer	60
Membrane Filtration Apparatus	30
Capran Bags	30
Culture Media	30
Toweling	60
Drapes	60
In-Line Filter Holder and Valve	30
Electrical Connections to Filter Drive Motor and Power Supply	30
Instruments:	
Forceps	30
Screwdrivers	30
Blades	30
Knife Handles	30
Hemostats	30
Pencils	30

Table 12. Items Sterilized with Ethylene Oxide (20°C, >75% RH, >100 mgm/ℓ for >48 hr)

PR-3 Flowmeter, Ejector and Diffuser Assembly
Polyethylene Bags
Antennas
Plastic Petri Dishes*
Membrane Filters*

* These items were sterile as received; they were wrapped and treated again primarily to sterilize the coverings.

c. Analysis of Filtered Sample. - At the impact site, the filter takeup section was removed from the sampler and inspected for damage to malfunctioning seal mechanisms. Spore strips placed in other sections of the sampler module are at this time removed and placed in sterile containers for subsequent analysis. The samples, still retained in the sealed takeup section, are then transported to the laboratory.

Preparation of material for analysis is initiated several days prior to expected flight. The chambers were decontaminated and ventilated according to the procedures outlined previously. The floor of the chamber was draped in sterile muslin. Petri plates containing trypone glucose extract agar were poured directly in the chamber. After the medium solidified, membrane filters (0.45-micron pore size and 47-mm diameter) were implanted on the agar surfaces and incubated until used for filtering the extracted sample. This pre-incubation step serves two important functions: it allows some measure of contamination control to be exercised prior to analysis (plates and filters showing growth are not used), and it eliminates a time-consuming operation of opening sealed packets of filters during analysis.

Prior to insertion of the takeup section module, all supplies needed to complete the analysis are decontaminated and passed into the chamber. At this point, the decontaminated and dried sampler module is inserted into the chamber and analysis is begun. The spool and backing plate containing the filter and inter-leaving film is unbolted from the outer case and this case removed from the chamber. An electrical connection is made to the filter drive motor allowing the motor to unwind the filter from the spool. This function is controlled from outside of the chambers. The chambers are arranged such that three persons can perform the analysis, allowing for completion in less than four hours. One operator sections the pre-coded filter and inter-leaving film, places it in a plastic bag held by the second operator who codes a petri dish corresponding to a specific sample. A third operator adds diluent to the sample, extracts, filters through the membrane (from the petri dish) and replaces the membrane with the filtered sample on the agar surface. After analysis has been completed, all materials with the exception of the filtered samples are removed from the chamber. The sterile chamber also serves as an incubator, thereby eliminating contamination incurred by normal incubation procedures. Incubation is for one week at 20°C. Spore strips from various locations are placed in tubes of fluid thioglycollale and also incubated for one week.

After incubation the plates are removed and samples enumerated and characterized by the following methods:

1) The filters containing the developed colonies (if any) are examined with the aid of a stereo microscope and the discrete colonies counted.

2) Bacteria-like colonies were streaked on TGE plates and a gram stain made of the original culture.

3) Fungal colonies were transferred to Sabouraud dextrose agar plates and mounts made using transparent tape and permanent lacto phenol cotton blue mounts.

4) Colonial and microscopic characterizations were performed on both the original and subcultured colonies.

Tubes containing the spore strip controls were examined for evidence of growth (turbidity) after one week incubation.

2. Fluorescent Particle Tracer Analysis

Fluorescent zinc cadmium sulfide (ZnCdS) was used as an indicator of extraneous contamination during the Mark III operation. These particles can be obtained in the size range of bacteria (one micron) and of various characteristic fluorescent colors. These particles were applied to various flight equipment surfaces (exterior) in hopes of determining migration onto the sample surface during the operational and analytical procedures. Since these particles are insoluble in the extraction fluid, they are retained on the membrane. After biological analysis is completed, the filters are examined with a fluorescence microscope and the particles counted visually.

3. Flight Preparation Procedures

Prior to actual flight operation, all mechanical and electrical functions of the sampling system were carefully checked for satisfactory performance. Essentially all subsystems or modules were dismantled and their conditions evaluated. At this time, any necessary changes (modifications or additions to the equipment or program) were effected.

When a system subcomponents had been qualified for use, the complete payload was assembled in the test laboratory. This included the controls to the balloon scheduled for the particular flight. The titanium tanks were filled with liquid N_2 and allowed to reach operating pressures. At this time a complete flight program was conducted by using a series of exterior connections to the flight

programmer, stimulating the barometric functions.* After satisfactory system performance was obtained, preparation for flight was initiated. Concurrently, the sampler was removed from the gondola frame and the following series of operations performed.

1) The sampler unit was disassembled into its three modular forms.

2) The filter drive motor, takeup section motor and pneumatic cylinder were checked for proper lubrication and function.

3) A strip of polyurethane filter and inter-leaving film was pre-coded. Care was taken during this step to minimize particulate contamination on these surfaces. Rubber-impregnated magnets were then countersunk into the polyurethane filter at the appropriate locations.

4) The filter was assembled into the storage module, threaded through the sampling plenum, and attached to the takeup spool. At this point, the filter was advanced through its normal sampling sequence to check for magnetic reed switch function and ease of filter and inter-leaving film travel.

5) The filter was removed from the sampler, wrapped and autoclaved for one hour. This step was carried out to reduce the biological burden to a minimum during the final sterilization of the complete sampler module.

6) All gasket surfaces and seals were checked and fresh membrane filters and cotton backing placed in the pressure equilibration ports.

7) The filter and inter-leaving film were reassembled into the sampler and spore strips placed at strategic locations in the filter roll and other interior sampler surfaces.

* The first flight used a direct switching technique simulating the altitude. Ground checks for the last two flights were conducted by placing all pressure-sensitive components in a large bell jar, allowing them to function normally according to their pressure switches.

8) The sampler modules were bolted to the main sampler body and the exhaust and inlet fittings attached. A dust cover was attached to the inlet cone and the nylon shroud cinched in place.

9) All exposed gasketed interfaces were further sealed with a layer of pressure-sensitive autoclave tape.

10) A final circuitry check was made on the filter drive and takeup section door closing motors.

11) The total sampling system was then wrapped in a layer of muslin cloth prior to autoclaving.

12) Sterilization was accomplished by autoclaving at 121°C for 90 minutes followed by a fast exhaust and an extended vacuum drying.

Concurrently, the ejector flowmeter diffuser subassembly was sealed in a doubler layer polyethylene bag and sterilized with ethylene oxide.

After autoclaving, the sampler was stored in its muslin wrapping until final assembly prior to flight. The final sampler preparations prior to launch were as follows:

Since the normal time for balloon launching is at dawn, final preparation was started at about midnight the night before. Prior to attaching the sampler to the gondola, all other modules were attached and given a preliminary checkout. The sampler was mounted to the gondola shock mounts by bolting through the muslin shroud, thereby affording the greatest amount of protection during further assembly and transportation operations. Also attached through the muslin were pressure and electrical connections to the sampler. The assembled payload, less the air ejector assembly, was then ready for truck transport to the launching site. At the launch site, the ejector assembly was attached to the sampler exhaust port by cutting away the muslin cover on the sampler and polyethylene cover around the

flowmeter portion of the ejector. The ejector assembly was held close to the sampler at which time fabric caps were removed from the two mating surfaces. Each surface was quickly sprayed with PAA, joined, clamped together and sealed.

E. Sampler Evaluation

This section of the report describes the environmental and biological testing performed prior to use of the sampling system. The purpose was to determine whether the system would perform reliably and with validity in the stratosphere.

1. Environmental Testing

Components, mechanical and electrical, used in modules other than the sampler had previous histories of satisfactory performance; therefore, only confirmatory tests were conducted. The sampler module required complete environmental assessment because the operational configuration and components had not been previously used for high-altitude applications. This module also must survive the autoclaving process. In effect the sampler must function normally after autoclaving for at least one hour followed by undetermined storage time, then temperatures to -60°C at ambient air densities to 100 K ft.

a. Autoclave-Freeze Tests on Sampler Components. - The following components were individually tested under autoclave (121°C/1 hr) and freeze (-30°F/6 hr) and approved for use in the sampler module. Prior to testing the electric motors and pneumatic cylinders were degreased and relubricated with molydenum disulfide.

Filter drive motor
Takeup section gate sealing motor
Sealed magnetic reed switches

Rubber-impregnated magnets
Silicone adhesive
Sized steel shot ballast
Epoxy nozzles
Reefing line cutting squibs
Limit switches.

Several types of paint were tested for effect after autoclaving and none were found to be satisfactory. No finish was applied to the iridite aluminum skin surfaces of the sampler.

b. Cold Tests - Sampler Module. - Several tests were conducted at various external and internal temperatures to determine the operative characteristics of the filter advance mechanism. A summary of these results are presented in Table 13. Although near the tropopause temperature may be lower than -60°F, radiant heating from the sun and insulation generally keep the internal components well above ambient temperature.

c. Autoclave and Cold Test - Sampler Module. - Several trials were conducted where all functions pertinent to the operation of the sampler module were evaluated. Environmental conditions were autoclaving for one hour, 16 hours at room conditions followed by a seven-hour cold soak at -25°F.

The operations evaluated were: filter advance (reed switches and magnets), filter advance motor and gear train, takeup section spring doors and squib release, door closing motor and limit switches, and pneumatic main doors. After exposure to -25°F for seven hours, the sampler interior temperature had fallen to -12°F. All functions performed as intended, with the exception that power requirements for releasing the filter from the storage spool were excessive. It was

Table 13. Sampler Module Cold Tests

Filter Position	Time (sec)	Amperes (mA at 24 volts)
-40°F Exterior, -15°F Interior		
Start to No. 1	49	400
No. 1-2	28	375
No. 2-3	24	375
No. 3-4	21	500
No. 4 to Takeup	20	500
-35°F Exterior, -10°F Interior		
Start to No. 1	51	375
No. 1-2	29	375
No. 2-3	24	375
No. 3-4	20	375
No. 4 to Takeup	20	375
-38°F Exterior, 0° Interior		
Start to No 1	50	375
No. 1-2	29.5	375
No. 2-3	23.5	375
No. 3-4	20.6	375
No. 4 to Takeup	20	375

found that tape holding filter to the spool performed unsatisfactory. The situation was remedied by simply milling a slot in the spool, precluding the need for any fastening. Satisfactory performance throughout these tests gave good indication that the system would perform as programmed during flight.

Concurrently with these operations, the programmer, flight data recorder and manifold system were cold tested in their modular form.

A final autoclave-cold test was performed which included test of all minor modifications such as the filter release mechanism.

After preparation, the sampler was sterilized by autoclaving for one hour using the fast exhaust and dry cycle. The sampler was allowed to equilibrate to room temperature overnight and then placed in a cold chamber at -40°F for seven hours. All sampler functions were then tested in the cold at the end of seven hours' exposure. Results were as follows:

Profile	T (°F)	mA	t (sec)
1	-40	0.5	67
2	-40	0.4-0.5	35
3	-40	0.4-0.5	27.5
4	-40	0.4-0.5	22
To Takeup		max 0.7	30, all filter in storage

After the filter was advanced to the takeup section, the sealing gate was released with a squib and the door sealed with the low rpm high-torque motor. The motor was shut off via a limit switch after 11 seconds of operation. After all the above functions were executed, the upstream and downstream doors were activated with 50 psi air; all doors worked smoothly at this pressure.

2. Biological Evaluation

a. Retention of Sampled Organisms. - During the normal sampler operation, the filter is moved and slightly abraded across metal edge surfaces when advancing into the takeup section. A series of experiments were designed to determine whether organisms present (collected) on the filter matrix were wiped from the filter and transferred to the knife edge. Transfers from the knife edge to subsequent sections of filter were also determined. Removal and transfer of collected material would serve to confound any data gathered during a normal flight operation.

Samples of filter were prepared by filtering aerosols of lyophilized Bacillus globigii (Bg) and Serratia marcescens (Sm) using 1/2-inch x 100-pore polyurethane foam as the filter matrix. Sections of filter containing the sampled organisms were abraded across a sterile foil-covered knife edge for a distance of one foot. The sterile foil overlay facilitated subsequent analysis of any particles (organisms) removed from the filter to the foil. Numbers of organisms removed from the filter were determined by analyzing the foil (extraction in polyethylene bag) and extraction of the filter. Re-entrainment from knife edge surfaces was evaluated by abrading a second sterile section of filter across the knife edge. Enumeration of these samples yielded an accurate estimation of the proportion of sample removed from the primary filter area.

Results indicated that from 1.13×10^{-2} to 4.4×10^{-4} percent of the sample was removed by a one-foot pass over a knife edge. Virtually all of the removed sample (85%) was re-entrained by a subsequent pass of a sterile one-foot filter section. The type of organism (Sm or Bg) was not a factor. The variation in percent

transferred was to some extent related to the number originally present on the filter surface. Based on these data, it is evident that transfer of organisms will not be a significant factor.

b. Filtration Efficiency. - Although the collection efficiency of open-pore polyurethane foam has been determined previously for the Mark II sampler, a slightly different configuration of filter type and a new testing procedure warranted a repeated evaluation for the Mark III unit. The Mark II sampler utilized multiple layers of 1/4-inch x 100-pore polyurethane, while the Mark III unit operates with a single thickness of 1/2 inch x 100 pores.

The tests were conducted in a low-pressure (high-altitude) tunnel utilizing mono-dispersed uranine dye particles. The particulate was generated via a spinning disk to a uniform diameter of 1.5 microns. All electrically charged particles were removed by precipitation. Filtration efficiency was measured by comparing the amount of aerosol collected in a section of 1/2-inch x 100-pore polyurethane test filter to an absolute filter located downstream of the test filter. A schematic drawing of the test fixture is presented in Figure 22.

Previous efficiency data were collected using Bg spores generated in a Collison unit for a test aerosol. Although most of the electron dense spores were removed by a charged particle precipitor, the high-voltage bipolar ion source was not used because of the unknown effect on viability. Hence, these data presented a slight increased efficiency based on electrostatic effects in addition to the normal inertial impaction expected with uncharged particles. It was noted that a medium performance felt filter with 1-micron particles, 20% efficiency was obtained with neutral particles and 96% efficiency with particles carrying only $\sim 300\ \bar{e}$ charges per particle. Because the electron charge of the stratospheric particulate is unknown, current evaluations were made with the neutral particles.

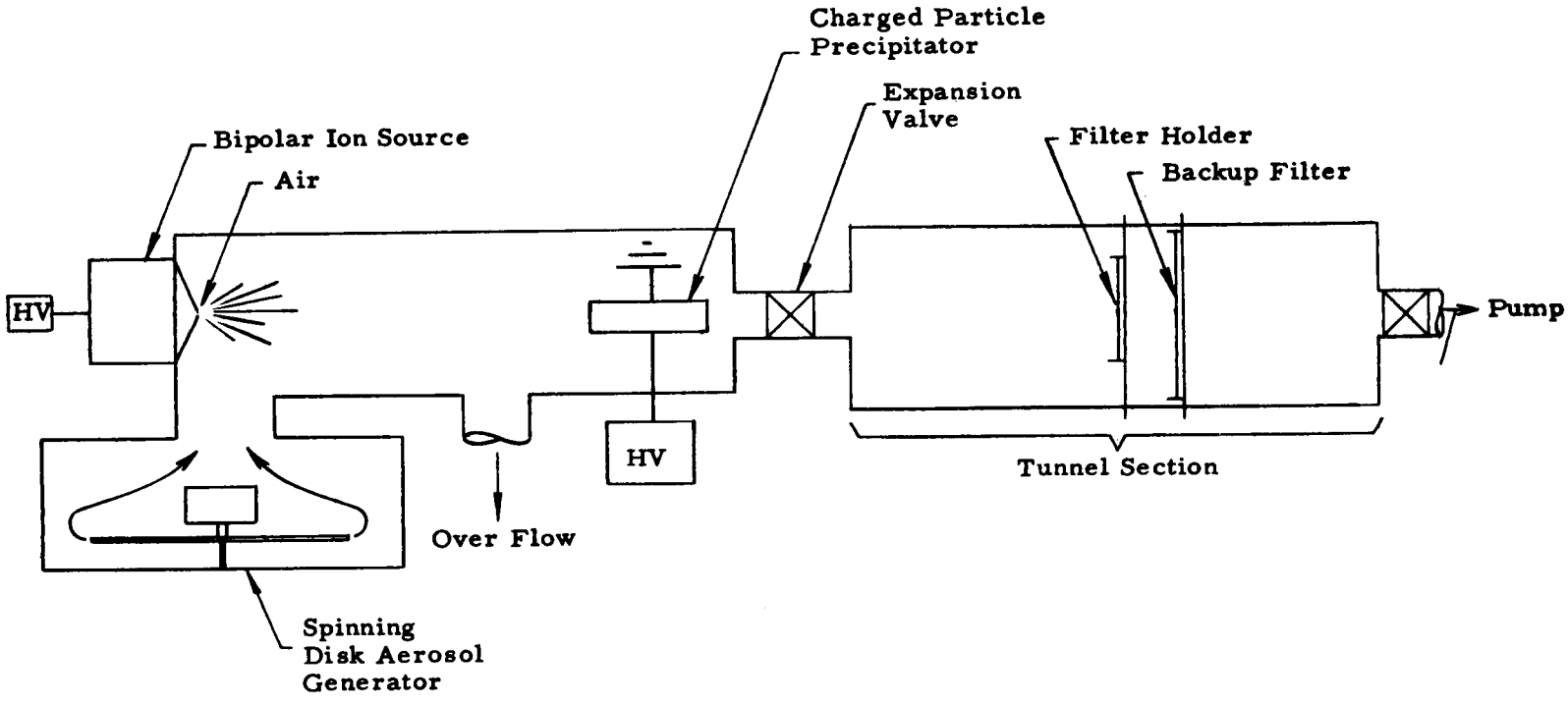


Figure 22. Low-Pressure Filter Test Apparatus

Table 14. Efficiency of 100-Pore, 1/2-Inch Polyurethane at Low Pressures (face velocity = 550 fpm)

Altitude (ft K)	Pressure (mm Hg)	Efficiency (percent)
20	360	67
40	150	72
60	57.5	85.5
80	24.4	91.3

With inertial impaction as the predominating collection mechanism, the data will plot as a straight line on log-probability scale (efficiency against the slip correction factor). The slip correction factor is a function of the mean free path of the air molecules and, therefore, of the altitude. These data conform quite well to the theoretical and the slope of the line.

Although the efficiency for neutral charged aerosols is somewhat lower than experienced previously, the tests as conducted express the least efficient condition. An increase in particle diameter, face velocity or electron charge would enhance the collection efficiency.

c. Location and Migration of Sampled Aerosols within the Sampler Unit. - This test configuration was designed to determine the location and ultimate distribution of organisms after collection and advance into the filter takeup section. The experimental procedures were conducted as follows:

1) The sampler was fitted with filter as per a normal flight operation, and autoclaved for one hour.

2) After sterilization, the sampler unit was mounted on a frame in a 300 ft^3 aerosol chamber. A Gelman Hurricane portable blower was mounted to the exhaust flanges of the sampler to provide near normal airflow.

3) An aerosol of dry, lyophilized Sm was generated in the chamber utilizing a continuous screw feed disseminator.

4) The filter was advanced from the flight control location to profile number 1. The pneumatic doors were opened and the blower started. The Sm aerosol was sampled at location number 1 for 5 minutes, after which the blower was stopped and the doors closed.

5) After sampling, the remainder of the filter was advanced into the takeup section, the door fired closed and sealed with the motor cam drive.

6) Filters were analyzed for Sm using the extraction procedure. The non-permeable inter-leaving film was also analyzed. A schematic diagram of the test filter and the percentage of the aerosol recovered at various locations is given in Table 15.

It is evident by the data presented that only a very small amount of the sampled aerosol (0.24%) migrates to any other filter or inter-leaving film location. The data suggest that a very large sample would be required (i.e., >100 organisms) to bias the adjacent control areas or compromise the filters for subsequent or past sampling profiles. Because the aerosol does remain deposited where sampled, the statistical analysis will have more validity.

d. Residual Effect of Decontaminating Procedures. - The purpose of these experiments was to determine the effect of the peracetic acid spray decontamination on collected organisms. Peracetic acid (2%) is used initially to sterilize the analysis chambers in addition to decontaminating all materials that pass into the chambers.

Table 15. Location of Sampled Aerosol

Filter Schematic and Percent Recovery

E	4	D	3	C	2	B	1*	A	FC

Direction of Filter Movement ——→

	Percent of Sampled Aerosol	
Filter Area	Polyurethane Filter	Adjacent Inter-Leaving Film
FC	2.38×10^{-3}	0
A	1.88×10^{-2}	0
1*	99.76	2.45×10^{-2}
B	0.191	1.90×10^{-3}
2	2.85×10^{-3}	7.14×10^{-4}
C	2.14×10^{-3}	0
3	7.14×10^{-4}	0
D	$< 2.38 \times 10^{-4}$	0
4	7.14×10^{-4}	0
E	$< 2.38 \times 10^{-4}$	0

* Aerosol sampled at this location.

Realizing that PAA is effective in relatively small concentrations in the vapor state, it has been considered that concommitant destruction of microorganisms on the filter sample during decontamination and analysis by residual cidal effect could be a confounding factor.

To determine effect of the various PAA treatments, a filter sample was prepared with a uniform amount of labile trace organism (Sm). One-half of the filter sample was placed in the sampler takeup section and processed employing the standard PAA sterilization and decontamination techniques. The remaining half of the filter was used as a control, i.e., no PAA treatment was used in conjunction with its analysis. Any quantitative difference can be attributed to the effect of the PAA treatment. The mean recoveries of Sm from the sampler were as follows: 1.38×10^5/segment for the series analyzed after PAA treatment and 2.38×10^5/segment for the control series. A "t" test for means indicated that there was no significant difference at the 0.5 level.

e. Background Contamination and Sterility Evaluation. - Two full-scale sampler analyses were conducted using procedures developed for use in analyzing flight data. These tests were intended, as much as possible, to evaluate the total background contamination level from all sources. All preparation and analysis procedures are described in previous sections of this report.

A strip of filter 9-1/2 feet long containing 8 linear feet of sample surface was pre-marked into four flight profile areas of 1 ft^2 each and one flight control (FC) area of 1 ft^2. The flight control is that section of filter which is located in the sampling portion until just prior to the first sampling sequence at 90,000 feet. Each of these areas (the profiles and FC) are separated by 1/2 ft^2 control areas (A to F). All sections of the filter are divided into subsections of equal area, thus the FC and samples 1 to 4 have 16 subsections,

whereas the A to F inter-sample controls contain 8 each. A schematic diagram showing this arrangement is presented in Figure 23.

The preparations peculiar to these tests were as follows:

After autoclaving and equilibration to room temperature, the filter was advanced into the takeup section and the unit sealed with the spring-loaded door and high-torque motor. The sealed sampler was then exposed to an aerosol of 10^7 Bg /ft^3 in a configuration similar to that of flight and impact. Shock loading was applied intermittently during the 15-minute aerosol exposure. This biological challenge and shock exposure gave an indication of structural integrity of the hardware and effectiveness of peracetic acid for destruction of resistant spores.

Samples were incubated for seven days at room temperature using the analysis chamber as the incubator. In addition to analyzing the sampler filters and inter-leaving film, sterile sections of filter and large quantities (>1000 ml) of extraction fluid were also analyzed for further control purposes. Spore strips placed through the sampler were used to establish adequate sterilization.

Test 1 Results. - One bacterial colony was observed from inter-sampler control B. The other 127 filter sections were sterile. All filter, extraction fluid and sport strip controls were sterile.

Test 2 Results. - Two organisms were isolated from the sampler filter, a filamentatious fungi from segment 3, profile 1, and an orange-pigmented bacteria from inter-sample control C, segment 6. The remaining 126 samples were sterile as were all spore strips, filter and buffer controls. The average background "noise" encountered with the Mark II sampler was on the order of ten viable

A,B,C,D,E & F = INTER-SAMPLE CONTROLS

1 ≈ 90 – 80K
2 ≈ 80 – 60K
3 ≈ 60 – 40K
4 ≈ 40 – 10K

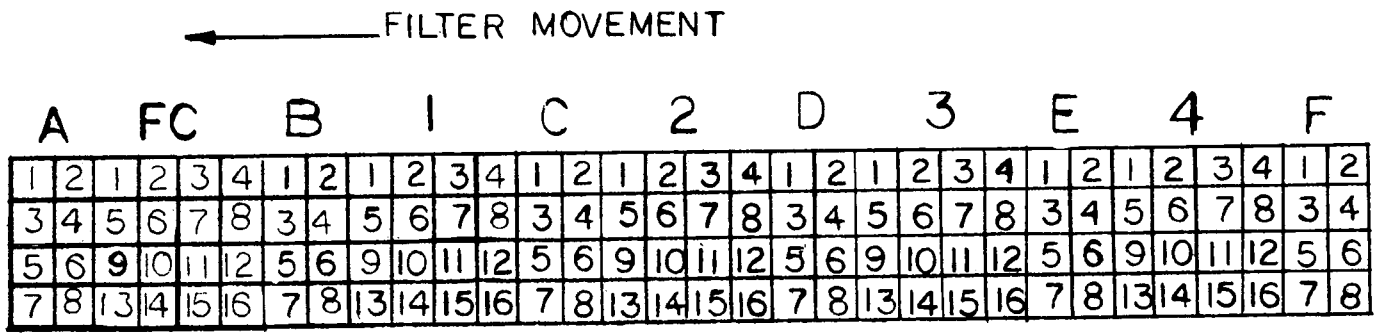


Figure 23. Filter Schematic

organisms per ft^2 of the filter surface. From the above data, it is calculated that background level is in the order of 10^{-2} viable organisms per ft^2, a three-decade sensitivity increase.

F. Mark III Flight Descriptions and Results

1. Mark III Flight No. 1 - Launched 24 October 1966

All modules of the sampling system were assembled to the gondola and subjected to a complete pre-flight checkout during the week of 17 October 1966. Final flight preparation and a weather watch was initiated on 18 October 1966. The sample module was prepared for sterilization on 23 October and autoclaved. Weather forecasting indicated that meteorological conditions on the morning of 24 October would be suitable. The sampler was mounted to the gondola through the muslin shroud to minimize contamination during transport to the launch area. Final system checkout was performed on the flight line. The payload was launched utilizing the same dynamic downwind truck launching procedures as used for the Mark II balloon and payload. Conditions at launch were: 4- to 8-knot surface winds, clear skies, tropopause at 39K with a temperature of -57°F, and a shear of 80 knots. A projected track of 270 degrees, with impact 10 miles south of Madison, Wisconsin, was plotted before launch. All prelaunch and launch activites proceeded uneventfully. Lift off was at 8:32 CDT. The rate of rise was normal; telemetry checked out following the antenna drop at 5K ft. At 10:23 a.m. at an ascending altitude of 80K, telemetry rpm decreased and finally stopped. From this time, all contact, except visual, was lost. At 1:45 p.m. no obvious descent was observed; the radio command operational sequence was initiated at this time. Descent was not visually noted until the emergency timer released the payload at 4:30 p.m. Descent by parachute and impact occurred at 5:07 p.m. on a heavily wooded hillside near

Merrilan, Wisconsin. No damage was noted to the sampler, gas bottles or either instrument module; however, about one-half of the magnesium frame was damaged quite extensively. The main sampler doors were opened at the impact site to determine if the filter had advanced into the takeup section. At this time it was observed that the filter had advanced to the number three position, probably occurring at 80K on descent. The takeup section door was not closed, but was closed at the impact site by cutting the squibbing line and allowing the door to seal against the partially wound filter. All gas bottles had landed with full pressure with the exception of No. 3 which had lost a slight amount of nitrogen. The various subassemblies were removed from the gondola, loaded, and shipped by truck to the laboratory. Balloon end fittings and valves were recovered from the balloon which impacted in a field about five miles from payload impact.

a. Results - Flight No. 1. - The complete programmer failure at 80K ascent was due to a faulty power switching relay. Subsequently, the main power lead burned open and all instrumentation ceased to function. During the time power was applied, the dust cover and shroud were dropped, and the filter was advanced.

It was decided to perform a biological analysis on the portion of the filter that had advanced into the takeup section. The results indicated that a relatively large amount of contamination occurred probably during the opening of the main sampler door prior to manual sealing of the takeup section. There was a direct relationship between the distance of the filter from the door seal and the number of microorganisms recovered from the filter. Inter-sample control (A) had a total of $70/ft^2$, while the filter for profile 2 contained $700/ft^2$. These data indicate obvious contamination entering through the open takeup section door. Bacteria and fungi recovered seemed to

be representative of common saphrophytic species. Seventy-four plate and filter controls were zero, and a total of 1200 ml of extraction buffer were also zero. Spore strips used to control sterilization procedures were negative with the exception of one strip found in the ethylene oxide sterilized diffuser-flowmeter ejector assembly.

2. Mark III Flight No. 2 - Launched 19 November 1966

Damage and malfunction incurred during the previous flight required the following repairs and modifications to the flight hardware. The gondola framing was reconstructed. Two modifications were made to the sampler module: Additional pressure equilibration ports were added to the filter storage section and inlet ducting to eliminate the possibility of dead airspaces during sterilization and also to allow rapid pressure equilibration during flight. It was noted that the filter matrix would take a rather permanent set after autoclaving and storage for long periods of time. The storage compartment required that the filter be compressed. Some areas of the material were then permanently disfigured as to thickness and width. Because the pressure drop and efficiency characteristics are unknown and variable, it was decided that a new compartment of increased size was necessary. One of an appropriate size was designed and fabricated allowing the filter to be stored in a non-stressed state.

A redesign of the toggle valving system for release of nitrogen made it possible to eliminate all but one of the relays which malfunctioned during the previous flight. This in turn led to a simplified programmer circuit. During programmer modification listed above an additional telemetry function was incorporated: a signal was sent and recorded when pressure drop was noted, thus indicating starting and stopping of the sampling process.

Pre-flight preparation and evaluation were begun during the week of 7 November 1966. Modifications had been completed by this date. The payload was assembled and subjected to a complete system check with operation by the flight programmer. All pressure-dependent functions were located on a separate programmer subassembly. This subassembly was placed in a bell jar to simulate altitude during the evaluation. All functions checked out perfectly. Final reassembly for flight was begun on 15 November 1966.

A weather watch was initiated on 14 November. The weather service indicated that conditions on Saturday, 19 November 1966, would be suitable for flight. Final preparations for launch were started at 2400 hours, 18 November 1966. The sampler module was mounted to the gondola through the muslin shroud, and as many pressure and electrical connections as possible were made in the laboratory. Transport to the flight center was by stake truck at 0400 hours. The conditions at launch were: ground temperature 0°C, -55 to -70°C from 40K to maximum altitude, surface wind 7 to 8 knots, with cloud cover 50% at launch time.

The payload was launched uneventfully at 0816 CST, 19 November 1966. A rate of rise to float altitude was established at 755 fpm. A maximum altitude of 93K was achieved at 1010 CST with a noticeable descent at 1030 CST, all helium valving seemd to function as programmed. The controllable EV-13 valve was closed at 1030 CST. No telemetry indication was received for the No. 1 sampling sequence (float to 80K), and repeated radio command signals failed. At 1100 CST, a telemetry signal was received indicating activation of the No. 2 sequence (1100 CST at 80K). The remainder of the flight data telemetry indicated normal sampler performance. An average descent rate of 643 fpm was established between 80 and 10K. Impact occurred five miles East of Boscobel, Wisconsin, at 1255 CST, 19 November 1966. Damage at impact was unusually heavy; the flight

train caught in a large tree, thus not allowing the payload to touch down. Suspension in the tree precluded release of the balloon by microswitches located at the bottom corners of the gondola. Most damage occurred during the time the payload was caught in the tree, however, the ejector system and programmer module were found some hundred yards from the impact site, having been torn off during previous passage through a large tree.

Impact damage incurred included:

Total destruction of basic gondola frame

Diffuser nozzle and air ejector

Insulated boxes for both programmer and data recorder

Internal damage to programmer included destruction of a multi-staged synchronous timing unit

Upper and lower inlet plenums for sampler module.

No damage was observed to the sampler or filter takeup section.

a. Results - Flight No. 2. - The sampler section containing the filters was detached from the gondola at the impact site and transported to the laboratory by car. Visual examination indicated no breaks in sampler takeup section integrity. Analysis was initiated on the following morning (20 November) following previously described procedure.

The location of microorganisms recovered from the filters and impermeable inter-leaving film is shown in Figure 24, with the summary of quantitative data presented in Table 16. The qualitative characterization of isolated organisms is presented in Table 17. All filter, extraction buffer and spore strip controls from all locations were sterile. Examination and comparison of the type of organism

A, B, C, D, E & F = INTER-SAMPLE CONTROLS

1 ≈ 90 – 80K

2 ≈ 80 – 60K

3 ≈ 60 – 40K

4 ≈ 40 – 10K

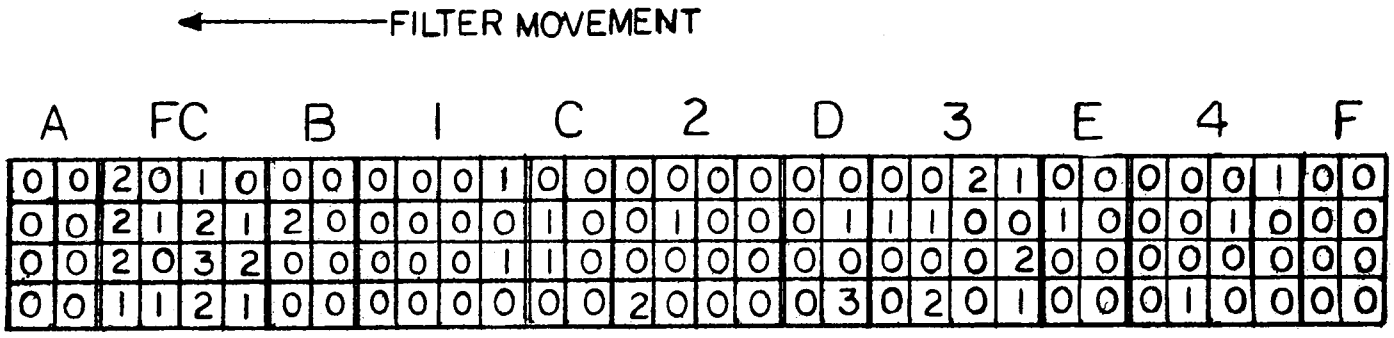


Figure 24. Location of Microorganisms - Mark III, Flight No. 2

Table 16. Quantitative Data - Mark III, Flight No. 2

Location on Filter Matrix	Total Recovered Organisms	$\overline{X}/ft^2$ Filter	Total Recovered from Inter-Leaving Film	Ambient ft^3 Sampled
A	0	0	0	---
Flight Control	21	21	0	---
B	2	4	0	---
No. 1	2	2	0	0
C	2	4	0	---
No. 2	3	3	0	26,590
D	4	8	2	---
No. 3	10	10	Not Done	6,120
E	1	2	1	---
No. 4	3	3	1	855
F	0	0	0	---

Table 17. Qualitative Data - Mark III, Flight No. 2

Location	Segment Number	Microscopic Morphology
Control A	---	None recovered
Flight Control	1	Yeast, thin (+) rod
	3	Yeast
	5	Two streptomycetes
	6	An uncharacterized bacteria
	7	Two yeast
	8	An uncharacterized bacteria
	9	Diphtheroid, long (-) rod
	11	Filamentous fungi, yeast, pleomorphic (±) rod
	12	Filamentous fungi, spore-forming rod
	13	Yeast
	14	Filamentous fungi
	15	Yeast, small (-) rod
	16	Yeast
Control B	3	Short bipolar (-) rod, yeast
Profile 1	4	Small (+) cocci tetrads (packets)
	12	Medium (+) rods
Control C	3	Small (+) cocci packets
	5	Medium (-) rod
Profile 2	6	Short (+) rod with spores
	13	Two yeast
Control D	4	Small (+) cocci tetrads
	8	Three small (+) cocci tetrads

Table 17 (Continued)

Location	Segment Number	Microscopic Morphology
Profile 3	3	Short (+) rod with spores, medium (+) rod
	4	Medium (+) rod questionable spores
	5	Medium (+) diphtheroid
	6	Medium (+) rod with spores
	12	Spore-forming rod, medium (+) cocci packets
	14	Medium pleomorphic spore-forming rod
	16	Small (±) cocci packets
Control E	3	Large (+) spore-forming rod
Profile 4	4	Medium (+) rod
	7	Medium (+) rod with spores
	14	Medium (+) rod
Control F	---	Non recovered

Inter-Leaving Film:

Control D	Two medium (+) rods
Control E	Thin (+) rods
Profile 4	Long (+) rods

(All other inter-leaving film sterile)

All filter, extraction buffer, and spore strips were sterile.

recovered on a sample-to-sample or sample-to-control basis indicates no striking qualitative relationship, therefore, analysis was based on quantitative differences.

The most salient feature of the quantitative data are the comparatively large number of organisms recovered from the flight control (FC). No other section of filter had a higher sample-to-control ratio. These flight control data are disconcerting when considering that air was not drawn through this filter section, i.e., it advanced just prior to the first sequence of sampling. The magnitude of the flight control is in itself more than difficult to explain: the apparent difference between it and its adjacent controls defines logical interpretation. The filter was sterile after autoclaving since all spore strips within the filter and sampler showed no growth after one week. It is doubtful that the analytical technique was contributory; all samples were analyzed randomly plus the "A" control was sterile. All plate, filter and diluent controls were sterile. If one proposed that some of the organisms are due to impact contamination, it is not logical that one of the innermost layers of filter contained most of the organisms (flight control) and the section of filter closest to the source of contamination (Control F) was sterile. There are four sources of contamination which seem most likely to have caused the discrepancy on the flight control and the higher-than-expected noise level throughout the filter strip.

1) Initial backflow from the air ejector could have deposited organisms on the FC although the filter should have moved out of the air path before the ejector was started. A fluorescent particle tracer study is designed to determine whether there is a problem.

2) The distribution of organisms is somewhat indicative of contamination after autoclaving and before the filter was advanced to the No. 1 sampling position. Experiments undertaken in the developmental phase of this program indicated that the integrity of the sampler unit including the takeup section was nearly perfect.

3) The occurrence of uncontrolled "noise" as a result of the analysis techniques is still a possibility and the peculiar distribution could be, in fact, caused by introduction of extraneous contamination at some point.

4) It is conceivable that the FC portion of the filter did not advance until a sample at the No. 2 profile had actually been collected. Both telemetry and flight recordings indicate that power was applied to the filter drive motor for the correct time duration. The occurrence of this phenomenon is very improbable.

Preliminary statistical analysis indicated that these data are not normally distributed (which was expected) thus ruling out many of the more powerful statistical tools. Analysis of data from flights 2 and 3 gave unequal variances between sample and control, which further complicated the data. Initially, it was planned to use an analysis of co-variance technique, however, the data suggested the use of a less powerful, but more applicable, distribution-free analysis. This treatment is deferred to a following section.

Mechanical malfunction occurred in two areas during this flight. Three of five hermetically sealed reefing line cutters failed to operate. These cutters were supplied having the wrong temperature limitations and failed to fire at flight temperature. Therefore, nitrogen bottles for profiles 1, 3 and 4 did not release any gas, all sampling was effected by the No. 2 bottle at its respective profile and the overrun to the two lower profiles. Some trouble was also experienced with the toggle valves jamming open, however, this was easily corrected.

All other functions were as planned, programmer and data recorder operation was near perfect. The payload descent rate was higher than anticipated although this may have been due to the added weight of the full titanium pressure vessels.

b. Fluorescent Particle Analysis. - Selected samples from each filter location were observed for ZnCdS particles of the type to be used as tracer compounds. As no tracer was employed on this flight, these data served as background levels for subsequent flights. Three colors were tabulated: yellow, green and red. Analysis indicated that a high background of yellow and green ZnCdS particles were present, precluding their use as a tracer. Only one red particle was found on all samples analyzed, therefore, this color was selected for the future flights.

3. Mark III Flight No. 3 - Launched 12 May 1967

During the unfavorable winter month flight conditions, several modifications were made on the existing flight hardware.

A new mounting arrangement for the N_2 manifold was fabricated for use with the same type of squib released toggle valve system as used during the last flight. Prior to acceptance for flight, the arrangement was subjected to repeated low-temperature testing.

The failure of three hermetically sealed reefing line cutter squibs during the course of the last flight prompted a study effort toward the solution of this problem. Some reefing line cutters from the same shipment used for the flight were found to misfire at -65°F.

A test was conducted using 50 standard 2-grain, powder squibs. Treatment of the squibs included autoclaving for 1-1/2 hours at 121°C followed by exposure at -35°F for 24 hours with explosion of all 50 charges at -100°C using a standard 24-volt power supply. All squibs

tested, fired under these conditions. Considering these favorable results, future flights were flown using standard squibs where a cutting operation was required.

Considering the high stress impacts encountered at the termination of the previous flights, it was decided to have the pressure vessels tested by an independent environmental testing company. Each tank was tested at 3800 psi water and certified leak free. Normal operation is with 3200 psi nitrogen.

Modification of the programmer included replacement of the insulating and shielding containers and rebuilding of some damaged components.

Two procedural changes were made for the third flight. The polyethylene (gas sterilized) bags used for extracting the sample from the filters were replaced by an autoclavable heat sealable 1.0-mil polyamide film. The number of inter-sample subsections per profile was decreased from 16 to 9 and the adjacent controls from 16 to 6. The total area of filter and control surface was not changed. Reducing the number of discrete inter-profile and control samples was intended to reduce the background noise (background is at least a partial function of sample number) in addition to reducing the time expended for analysis.

The final pre-flight evaluation and checkout was initiated on 8 May 1967, subjecting the payload to a complete system check. All functions performed perfectly. Final assembly for flight was begun on 11 May 1967.

Conditions of the surface winds, winds aloft and cloud cover suitable for launch were forecast for the morning of Friday, 21 May. Final preparations for flight were initiated at 0100 hours of this day. As before, the sampler was mounted on the aluminum gondola by bolting through the muslin shroud, thereby offering the protection of

the shroud until just prior to launch. As many electrical and pressure connections as possible were made through the muslin while the payload was still in the laboratory. The assembled payload was transported to the flight line by a stake truck at 0300 hours. At the flight line, the final checkout was performed, the diffuser-ejector-flowmeter unit was removed from its plastic bag and aseptically mated to the sampler exhaust port. Several minutes prior to launch, the remaining muslin shrouding was cut away and the dust cover squibs armed. Several grams of red zinc cadmium sulfide tracer were dusted on the lower gondola and sampler surfaces using a powder blower.

The payload was launched uneventfully at 0721 CDT, 12 May 1967. The conditions at launch were: ground temperature 2°C, surface wind from the SSW at 2 knots, and sky condition 8000 scattered with a high thin cirrus. The temperature at altitude ranged from -20 to -58°C at 19K to 90K ft with the minimum temperature at 55K ft and a mean temperature of ~ -50°C. Average ascent rate was 661 fpm with maximum altitude of 90K ft reached at 0937. The maximum wind velocity was 60 knots encountered at 40K ft. The helium valve was opened at 0944 with first noticeable descent occurring at 0948. The filter was advanced to the No. 1 profile location at 90K ft, but there were no telemetry indications of airflow; camera box data verified the fact that no sampler had been obtained at this float altitude. At 80.5K ft the filter was advanced to the No. 2 position and sampling was initiated. The No. 2 sequence was continued for 53 minutes until an altitude of 59.2K ft was achieved on descent; at this time the filter was advanced and the No. 3 sequence started. The No. 3 profile was terminated at 38.5Kft after ~54 minutes of operation and the No. 4 filter advanced and sampling was again initiated. At 46 minutes of operation at 1334 hours, the N_2 pressure vessel was essentially

emptied and sampling ceased at an altitude of 14K ft. Telemetry signals indicated that after completion of the last profile, the remaining filter had advanced to the takeup section.

The payload impacted at 1356 hours, 5 miles west of Tomahawk, Wisconsin. At impact, as it was observed by the recovery aircraft, the balloon did not separate from the payload as programmed but rather dragged across the ground dislodging the sampler, instrument modules and air ejector system. The weight decrease imposed by the release of these components resulted in a free lift great enough to cause a rapid ascent of the remaining attached payload.

The sampler and other recoverable payload modules were picked up at the impact area at 1430 hours, 13 May, trucked to Tomahawk, then flown to Minneapolis.

During ascent after impact, the tracking aircraft attempted to radio command a signal to sever the payload from the balloon, however, all attempts to command any function failed. No beacon signal was received by any monitoring stations and the concensus was that all telemetry and command functions had been eliminated by impact shock. At sundown, 12 May, the balloon and payload was fixed 25 miles SSE of Escanaba, Michigan. The Lake Michigan Coast Guard was alerted and a cutter dispatched to effect a recovery should the payload land in the lake during the night. The altitude of the derilect fluctuated, but it remained in the stratosphere. On 13 May, the position was over the lower Michigan penninsula at an altitude of 62K ft. On 14 May a.m., the package had passed north of Toronto over Canadian territory. Later on 14 May, the last report indicated that the derilect had headed out over Boston in an easterly course still at high altitude and evidently well into a fast jet stream.

At the time of this report the balance of the payload had not been recovered. Items lost included the following: gondola frame, balloon valves and fittings, titanium pressure vessels, manifold system, and diffuser-ejector-flowmeter assembly.

Biological analysis was initiated on the morning of 13 May and proceeded according to methods previously outlined with the following two exceptions.

Damage to the external electrical fitting which allows the filter to be unwound from the takeup section was incurred at impact. This damage required that the filter be stripped from the takeup spool manually which increased the amount of handling during analysis.

After the analysis had been completed, the chamber gloves (3 pair) were scrubbed with wet sterile sections of polyurethane foam to remove any contaminating organisms picked up during analysis procedures. These foam sections were then bagged, extracted and plated using standard procedures.

The location of organisms recovered are shown in Figure 25. Table 18 presents a summary of all the quantitative data, including total volume of air sampled and airflow rate. Qualitative data are shown in Table 19. All spore strip, filter and extraction fluid controls were sterile, however, a total of three organisms were isolated from the chamber gloves after analysis.

The quantitative data again presents an equivocol situation. A relatively large concentration of organisms found on control D (16 organisms distributed, 2, 5 and 9) and the single section containing 20 organisms found in the No. 4 profile is unusual considering the balance of the distribution. The high number found on control D comprises any statement made about adjacent sections.

A, B, C, D, E & F = INTER-SAMPLE CONTROLS

1≈ 90–80K
2≈ 80–60K
3≈ 60–40K
4≈ 40–10K

FILTER MOVEMENT

A		FC		B		1		C		2		D		3		E		4		F	
0	0	0	0	0	1	0	0	0	1	0	1	2	3	2	0	0	20	0	1	1	
0	0	1	1	0	0	0	0	0	1	1	0	5	8	0	0	1	1	1	1	0	
0	1	1	0	2	0	0	1	0	0	1	1	9	4	2	0	1	1	1	2	0	

Figure 25. Location of Microorganisms - Mark III, Flight No. 3

Table 18. Quantitative Data - Mark III, Flight No. 3

Filter Location	Total Organisms Recovered	$\overline{X}/ft^2$ Filter	Total Recovered from Inter-Leaving Film	Ambient ft^3 Sampled	Ambient ft^3/min Flow Rate
A	0	0	0		
Flight Control	4	4	0		
B	2	4	0		
No. 1	2	4	1	No Sample	---
C	0	0	0		
No. 2	6	6	1	53,671	990.2
D	16	32	2		
No. 3	19	19	0	39,929	864.2
E	2	4	0		
No. 4	28	28	0	26,210	586.3
F	1	2	1		

Table 19. Qualitative Data - Mark III, Flight No. 3

Location	Segment Number	Morphology
Control A	---	None recovered
Flight Control	5	Fungi, no characteristic conidia, filamentous
	6	Fungi, dense hyphae, no conidia, filamentous
	7	Fungi, sessile micro-conidia, pseudo-mycelia with intercalary chlamydospores
	8	Fungi-abundant rectangular, hyaline arthrospores resemble Coccidiodes immitis
Control B	3	Black colony, scant aeriel hyphae, pseudomycelia, probably a yeast
	3	Yeast
Profile 1	1	Medium gram (+) rod
	9	Medium gram (+) rod
Control C	---	None recovered
Profile 2	1	Questionable, may be artifact, no subculture growth
	3	Small short gram (+) diphtheroid
	4	Irregular gram (+) rod, spore former
	5	Medium gram (+) rod, spore former
	8	Irregular gram (+) rod, spore former
	9	Medium gram (+) rod, chains, spores questionable

Table 19 (Continued)

Location	Segment Number	Morphology
Control D	1	Yeast
	1	(Fungi) Aspergillus sp.
	2	Gram (±) coccobacilli
	2	Small (+) diphtheroid
	2	Pleomorphic thin gram (-) rod
	2	Short gram (-) bipolar rods
	2	Large gram (+) rod
	3	Yeast with pseudomycelia
	3	Yeast with chlamydospores questionable
	3	Yeast
	3	Yeast
	3	Gram (±) coccobacilli
	3	Small gram (+) diphtheroid
	3	Medium gram (+) rod, spores
	3	Small gram (+) coccobaccili
	3	Small gram (+) diphtheroid
Profile 3	1	Medium gram (-) rod
	1	Questionable isolate, no microscopic evidence or subculture growth
	1	Fungi, chlamydospores, plus a few large micro-conidia
	2	Medium gram (+) rod
	2	Medium gram (+) spore-forming rod
	4	Short gram (-) rod
	4	Medium gram (+) spore-forming rod

Table 19 (Continued)

Location	Segment Number	Morphology
Profile 3	4	Medium gram (+) coccobacilli
	4	Medium gram (±) diphtheroid (3)
	4	Small gram (-) rod
	4	Yeast
	7	Medium gram (-) rod
	7	Pleomorphic gram (-) rod
	7	Small gram (+) rod with spores
	7	Yeast
	8	Medium gram (+) diphtheroid
	8	Short gram (-) bipolar rod
Control E	2	Medium gram (+) rod with spores
	3	Short gram (-) rod
Profile 4	1	(4) yeast with chlamydospores (probably Candida sp.)
	1	(4) black colony, yeast with chlamydospores
	1	(2) yeast, salmon colony pigment
	1	Short gram (+) rod with spores
	1	Gram (+) rod, diphtheroid
	1	(3) yeast, white colony
	1	Pleomorphic gram (+) diphtheroid
	1	Actinomycetales
	1	Medium gram (+) diphtheroid
	3	Yeast (salmon colony pigment)
	4	Pinpoint colony, no microscopic evidence, may be artifact
	5	Fungi, chlamydospores, terminal, no characteristic conidia

Table 19 (Continued)

Location	Segment Number	Morphology
Profile 4	6	Fungi, dense mycelia, abundant conidia (yeast)
	7	Yeast
	8	Fungi, no characteristic structures
	9	Fungi, no characteristic structures
Control F	1	Fungi, numerous chlamydospores and arthrospores
Inter-Leaving Film	1	Large gram (+) rod, chains
	2	Yeast
	D	Medium gram (+) rod
	E	(2) medium gram (+) rods
	F	Medium (+) rod diphtheroid
Station 1 Gloves	1	Mixedspore-forming gram (+) rods
Station 2 Gloves	2	Mixed spore-forming (±) rods
Station 3 Gloves	3	Medium gram (+) rods, spores

The qualitative data are suggestive of impact contamination since many organisms (fungi 41%) were types commonly associated with soil. Laboratory contamination would be confined largely to spore-forming rods (especially Bg in these labs). Although some contaminants were isolated from all three pair of gloves, their numbers were not large. If faulty gloves were responsible for transferring contaminants into the chamber one would most certainly find micrococci; it is

interesting to note that no micrococci were recovered at any location or from any control. Faulty sterilizing operation is unlikely since spore strips placed at locations where sterilization would be most difficult were all sterile. The type of organisms recovered, notably the fungi, are quite heat labile, thus their surviving heat treatment would be improbable.

a. Fluorescent Particle Tracer Analysis. - As mentioned previously, red ZnCdS fluorescent particles were applied to the lower portions of the payload. These particles were to act as an inanimate tracer simulating organisms present at these locations throughout the flight. Any microorganisms on the exterior surfaces of the equipment could conceivably pose a problem if they were able to be dislodged during a sampling sequence and enter the sampler inlet area. Theoretically, if the system is functioning as designed, no possibility of contamination through this mechanism is possible since the sampling is isokinetic. Red ZnCdS was selected because prior analysis showed that a very low background existed for this color.

After the extracted and filtered samples were incubated, enumerated and subcultured, the membrane filter pads containing all the particulate sampled were examined by fluorescence microscopy for red ZnCdS particles. Two passes across the 47-mm diameter filter were made using the 100X objective; by using this technique, slightly more than 10% of the total filter area was scanned with the microscope. The data were normalized for total filter area and a summary of results is presented in Table 20. It is immediately obvious that the control surfaces contain fewer fluorescent particles. The rather high general level of these particles was at first disturbing but it is adequately explainable. Large numbers of particles were applied to the gondola and sampler surfaces, in the order of 10^{10} to 10^{11} individual particles.

Table 20. Fluorescent Particle Analysis - Mark III, Flight No. 3

Filter Location	Red ZnCdS/ft^2 Filter
A	0.00
Flight Control	33.57
B	34.77
No. 1	0.00
C	8.61
No. 2	58.86
D	0.00
No. 3	169.47
E	26.16
No. 4	156.96
F	26.16

Red ZnCdS/ft^2 Filter

	Control Surfaces	Sample Surfaces
$\bar{X}$	16.16	128.43
Number Analyzed	28	17

The decontamination steps prior to analysis are designed to render the takeup section surfaces biologically clean (sterile) but no attempt was made to remove particulate matter, except which is incidentally removed by the standard PAA washing and spraying procedures. Therefore, in all probability there were large numbers of FP introduced into the chamber. This, coupled with the extra handling required because of the damaged electrical circuit, effectively explains the presence of FP on the filter; it does not, however, satisfactorily explain the discrepancy in means between the control and sample filters. There is nearly a factor of eight difference between the number of particles recovered on filter surfaces as compared to control areas.

G. Statistical Considerations

A detailed treatment of both the biological and fluorescept particle data suggested that:

1) The variances between controls and sample filters are significantly different.

2) The distribution is not normal but is probably distributed according to a modified Poisson statistic.

3) Since analysis by normal theory statistical methods assumes equal variances and a well-defined or reasonably assumed distribution, these methods are not applicable. Because the data distribution is unknown, these data would be most amenable to nonparametric statistical techniques.[19]

1. Data Evaluation

Examination of the data collected from the second and third flights reveals several factors which make the use of common normal theory statistical procedures incorrect. These factors at the same time suggest the use of nonparametric methods. Since the distribution

parameter for viable organisms in the stratosphere is unknown, any assumption based on this distribution is not valid. Any levels of significances or confidence limits calculated based on an erroneous assumed distribution would not be appropriate. Population distributions will not affect the level of significance when nonparametric methods are used. Data transformations were not successful in forcing data into a classical distribution.

a. Variation in Volume of Air Sampled. - The volumes of ambient air sampled varies when comparing profiles within a flight or when comparing the same profile on different flights. Therefore, the numbers of viable organisms recovered for various profiles and flights are not directly comparable without some correction factor. For example, about seven times more air was acquired during profile 3 as during profile 4 in Flight No. 2. If the concentration of organisms from these two altitude ranges is identical and not zero, one would expect the sevenfold more organisms on profile 3. In Flight No. 3, approximately 1.5 as much air was sampled for profile 3 as for profile 4. Yet these four abovementioned samples, plus others from yet another altitude range, must be compared in some way. Transformation of data by a simple multiplication is incorrect since background is relatively large.

The nonparametric tests used will make use of ranks rather than rational data. Because the volumes of air sampled decrease with decreasing altitude (and expected increase in concentration of viable organisms), the ranking method should give a conservative test of a hypothesis of identical concentrations at all altitudes. That is, detection of any increasing concentration of organisms or altitude decreases will occur with less probability than if equal air volumes were sampled, but if a significant increase is found, the α error will be lower than that calculated.

b. Population Distribution and Variances at Sampled Profiles. - Initial examination of the data indicated a Poisson distribution, hence data for which variances can be controlled by a square root transformation. The data collected, however, do not conform to the usual Poisson distribution as shown by a x^2 goodness of fit test. These data seem to conform to a distribution form often associated with spore enumeration. Here a Poisson distribution is modified by a conditional probability of various size multi-organism clumps associated with the Poisson event. Unfortunately the data from two operationally successful flights are not sufficient to determine the exact distribution.

The nonparametric tests used make only the very general assumptions about population distribution functions and therefore are not affected by the lack of information of the exact distribution functions as listed previously.

c. Variation in Number of Samples Analyzed. - If none of the organisms on the sample filters resulted from contamination, variation in samples analyzed would be of little importance. However, the pattern of organisms present on the control surfaces indicate that this is not the case. Spearman's rank correlation coefficient was used to test for correlation between control and adjacent treated surfaces and was found to be non-significant. While lack of correlation should not be interpreted as proof that no relationship exists (the coefficient is significant at somewhere below the 20% level), it is, however, an indication that at least some of the contamination occurs during analysis. If this is true, then there is a greater chance of analytical contamination during Flight No. 2 where more samples were analyzed.

The number of samples analyzed is not important when using non-parametric methods since in-flight comparisons are made only after the results of both flights are combined.

2. Analysis

a. Test a. - To approach the problem of the presence or absence of viable microorganisms in the stratosphere, a Wilcoxon Signed Rank Test was used on pairs of the sample and control surfaces. No attempt was made at this time to differentiate between various altitudes sampled or between the individual control surfaces used. The sub-samples from the sample surfaces of Flight No. 2 were combined to form four units, each one representing a "row" of sub-samples from the filter strip. The number of organisms found on each of these subsections is then paired with the number of organisms found on the subsection representing the corresponding row of the control surface. The total area and number of sub-samples represented by a unit of treated filter surface and control surface should be equal; the combination of control areas designed as profile 1, controls C, D, E and F fit this requirement and are considered to be areas most similar to the filter through which air was sampled. There were four pairs of control and sample areas from Flight No. 2. Similar pairing effected for Flight No. 3 resulted in three pairs since the number of sub-samples was decreased. This pairing is necessary since results from Flight No. 2 are not directly compared with results from Flight No. 3. This particular choice of pairing compares the sampled surfaces from which any organism moves laterally, with the control surface which moves in the same fashion, i.e., both surfaces reside at the same location with the sampler at all times.

The results are given below:

Flight	Row	Number of Organisms Recovered: Sample	Control	Difference
2	I	4	1	+3
2	II	4	3	+1
2	III	2	2	0
2	IV	6	3	+3
3	I	28	4	+24
3	II	13	6	+7
3	III	12	11	+1

The procedure was to rank the difference disregarding sign and then find the sum of negative ranks. There were no negative differences, thus the sum of negative is zero in this instance. The results show that the sample always has more (or equal number) of organisms than its paired control. This result is significant at the 0.025 level.

The significant result that the distribution of organisms to which the control and treated areas are exposed do not have the same median and therefore viable organisms exist in at least some of the altitude layers sampled.

b. Test b. - Two additional nonparametric tests were conducted to expand results of the Wilcoxon Test. The first of these is a Friedman two-way ANOVA. This test is the nonparametric equivalent of a parametric technique previously considered for use. The No. 1 profile and controls are included in the analyses for both Flights No. 2 and 3. This test uses total number of organism per sample profile filter within each flight. The organisms found on the inter-leaving film are added to their corresponding adjacent areas of sample or control filter.

The Friedman test is conducted as follows:

	Number of Organisms			
Flight	No. 1	No. 2	No. 3	No. 4
2	2	3	10	4
3	3	7	19	28

and ranking the totals within each flight:

	Rank for			
Flight	No. 1	No. 2	No. 3	No. 4
2	1	2	4	3
3	1	2	3	4

The null hypothesis (H_o) is that the four sample profiles within each flight represent identical distributions. If this H_o is true, the above rankings occur with a probability of 0.167 which is the significance level (α level) of this test. It should be considered that if profile 4 from Flight No. 2 showed more organisms than profile 3, the α level would be increased to the 0.05 level. An increased level of significance could be expected for reasons which were discussed in Section 1.a.

The total number of organisms recovered from the control areas of Flight No. 2 shows good fit to a Poisson distribution with mean equal to 1.38. Thus, there is at least a 50% chance that least three of the organisms found on the filter and inter-leaving film for profile 4 of Flight No. 2 are the result of contamination. No valid correction which would result in reversal of rankings of profiles 3 and 4 of Flight No. 2 is possible.

Consideration must be given to the importance of the 0.167 level of significance. Generally, α levels of 0.05 and 0.01 are used to connote cause for rejection of the H_o, however, these levels were set as a matter of custom and must not be considered as a strict rule.

c. Test c. - The third test conducted is the Wilcoxon Signed Rank Test conducted separately for each profile sampled versus the adjacent control areas.

Results of these tests are:

		Totals for			Totals for			Totals for		
Flight	Row	#2	Control	Diff	#3	Control	Diff	#4	Control	Diff
2	I	0	0	0	0	+3		0	+1	
	II	1	2	-1	2	0		1	0	
	III	0	1	-1	0	+2		0	0	
	IV	2	3	-1	3	0		0	+1	
3	I	2	2	0	2	+3		1	+20	
	II	2	5	-3	6	+2		1	+2	
	III	2	9	-3	10	-4		1	+3	

These three tests show extreme differences in results at the three altitudes sampled. At the higher altitude (#2), there is no evidence of presence of viable organisms. In fact, there are more organisms present on the adjacent control surfaces in all cases (except for ties). At altitude #3, there is more evidence of organisms present, but this evidence is not strong enough to be significant. At altitude #4, there is sufficient evidence to make a statement of presence of viable organisms with an α level of 0.05. These results strengthen the results found previously: that for at least one altitude, organisms are present. It should be noted that these three tests are not independent since each control serves as a control for samples from two profiles.

3. Discussion

Because of the factors discussed previously, the small amount of data and the presence of a relatively large amount of organisms on the control surfaces, analysis of these data presented many interesting problems. Interpretation of results of the three tests overlap. However, the three must be viewed in combination to reveal the whole picture.

None of the tests are made without some reservations, but they represent the best available for these data. Control areas used for various tests are chosen: 1) as those of the same size and having the same number of sub-samples as for the treated units used, and 2) as those most closely related to all treated surfaces with which they are compared. Thus, the size and composition of the control group varies with the test.

The Wilcoxon Test (test a) indicates the presence of significantly more organisms on the combined treated filter area than on comparable control surfaces. This plus the Friedman Test (test b) point to the presence of viable organisms in at least one of the altitude layers sampled. Test No. 2 includes a greater array of control area and in that respect is a better test of overall treated area versus control area. Test b does not require the more-or-less artificial pairing used in test a to eliminate the direct comparison of samples from the two flights. All sample totals used in test b can be considered as independent samples. In these respects, it is a better test. One would expect the true α level of test a to be greater than that calculated (because of non-independence of some of the samples) and that of test b to be less than that calculated. Test c (individual Wilcoxon for the three altitudes) gives added information about which of the altitudes contribute to the differences found in the first two tests.

Considering these results, a confident, positive statement can be made that there are viable organisms at the lower profile sampled, i.e., from 40 to 10K ft. No equally positive statement can be issued for higher altitudes. Certainly there is lack of evidence for presence of any appreciable concentration of organisms at the highest altitude (60 to 80K ft) but the level of confidence in this statement cannot be ascertained from these data.

VI. SUMMARY AND CONCLUSIONS

Essentially, this program extended the previous work conducted under NASA Contracts NASr-81 and NASw-648. Two generations of sampling devices were designed, fabricated, tested and flown. Prime consideration was given to preclude some of the contamination problems inherent with previous devices. They were successful, but only partially so, since background contamination was not completely eliminated. During the program, contamination control measures were improved with the development of more sophisticated monitoring systems. These measures were effective but led to greater difficulty in data validation.

The data obtained are encouraging because of the substantiate work done in the past and provide more precise definitions of the stratospheric microflora than were heretofore available. Loss of equipment during Flight No. 3 necessitated cancellation of two remaining probes, eliminating the chance to obtain confirming data. If, for example, the data from these flights generally conformed to that already attained, significant statements could have been made concerning the higher profiles and more exact limits placed on low-altitude populations.

The techniques developed for analysis of very small numbers of microorganisms in the presence of great amounts of natural interfering flora must be considered as an advance of "state-of-the-art" technology. The need for appropriate statistical tools for analysis of small samples with unknown population distributions cannot be minimized, along with the absolute requirement for an experimental design amenable to statistical treatment.

Parenthetically, it may appear ironic that while scientific efforts are evermore geared to detecting life on other planets, the research effort presented here with all its drawbacks, reports the only recent and controlled effort to discover life in our own stratosphere.

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