CLEAN ASSEMBLY & STERILIZATION LABORATORY

FINAL REPORT

for

NATIONAL AERONAUTICS & SPACE ADMINISTRATION

GODDARD SPACE FLIGHT CENTER

Prepared by

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INDEX

SUMMARY

The GSFC Clean Assembly and Sterilization Laboratory (CASL) was designed and constructed to provide a flexible research and development laboratory for the development of sterilization and clean-room procedures, and yet also to function as a research laboratory for more basic problems related to planetary quarantine technology. The design also allowed for the use of the facility for the processing of spacecraft parts and assemblies for flight experiments developed by Goddard and other experimenters. The thirty months of laboratory operation have demonstrated that the two lines of work, hardware processing and planetary quarantine research, should not be conducted at the same time, although the laboratory design accommodates both. During this period of operation, the operational and research functions have been emphasized. Breadboard and flight experiment hardware were processed as test models of sterile assembly procedures.

The facility has been brought to operational status by the installation, testing, use, and modification of the clean-room and sterilization equipment; by the development of monitoring, cleaning, decontamination, and maintenance procedures; and especially by the constructive interaction of the staff members. Their varied background in this setting has resulted in a strongly motivated, creative research program.

The combination of extensive clean-room, personnel-entry regimen, sterilization and other microbiological facilities permitted the development of unique research procedures by the staff. This combination is not available elsewhere. It provided research opportunities for the study of microbiological shedding from the skin and containment, resulting in the substantial reduction of the biological contamination from clean-room operating personnel.

Confronted with the recurring problem of the recovery of low numbers of microorganisms, several procedures were developed which improved both the sensitivity and the efficiency of routine techniques. These ranged from direct culture of polyurethane collection material to the application of newly developed, high-volume air samplers in biological surveillance of the clean-room.

With a Class 100 clean room available for subculture routines, it was possible to develop data defining the short-time, high temperature parameters for aerobic and anaerobic spores on film surfaces necessary for biological acceptance of sterile insertion techniques in spacecraft repair and assembly procedures.

In the absence of the CASL laboratory complex, developed through operational experience, the shedding studies leading to environmental conditions in which the sterile assembly of the candidate, planetary biology experiment (Wolftrap) was demonstrated successfully, would have been most difficult, if not impossible. With the operating laboratory, sterile assembly of complex devices on clean benches outside the clean room has been accomplished repeatedly. Studies in progress at the time of facility shutdown pointed toward the realization of low cost, containment garments preventing any biological contamination from operators, yet requiring no life support equipment and permitting freedom of motion. Substantial savings in sterilization facilities, equipment and spacecraft processing time are possible if operating and containment techniques such as those developed in CASL can be used by local and other NASA planetary projects.

The unique quality of the laboratory lies in the interaction of the various elements of equipment and physical facilities contained in it. This laboratory must retain its integrity in order to retain its character and usefulness. The value of the laboratory for planetary programs is the R & D capability in planetary quarantine procedures which resides in its operational status, adequately staffed and supplied. In parts, it represents only isolated pieces of equipment obtainable by any other laboratory group. Whole, it is a substantial resource for planetary projects requiring specific techniques for meeting quarantine requirements.

INTRODUCTION

Objectives

To develop a flexible laboratory which can provide facilities with operational procedures and routines for research in planetary quarantine application and also function as a processing laboratory for experimenters with assemblies which must meet planetary quarantine strictures, the following objectives must be met:

1. The acquisition, installation, certification, testing, and maintenance procedures for the essential equipment not already provided in the laboratory and the appropriate procedural development for that already installed.
2. The adequate staffing of the laboratory and the training of technical personnel to an acceptable level of performance.
3. The collection, by a variety of techniques, of the available expertise which exists in the scientific community in general and in the NASA community, specifically.
4. The establishment of appropriate communication channels with the technical monitor, other NASA scientists, of publication procedures, and of methods of handling data.
5. In the operation of a microbiological laboratory, the problem of assay techniques must be constantly dealt with. As new materials are used, an assay procedure yielding reliable data must be developed. It is apparent, then that continuing development of imaginative assay procedures must be an objective in a facility of this kind.

As these objectives began to be fulfilled, it was possible to consider research objectives for the facility. As it developed, some research subjects were an outgrowth of the attempt to attain operational status; i.e., the study of microbiological skin shedding. Other research projects developed as directly assigned tasks. What might be considered the ultimate

research objective was developed as a combination of operational status and developed research techniques. This involved the investigation of techniques for sterile assembly. The concept that this technique can be done is a revolutionary one. The development of techniques associated with approaches to sterile assembly is important to NASA, but also, the spin-off from these procedures would have innumerable applications for medicine, pharmaceutical manufacture and sterile-fill operations.

Operational Stature

It appears that a period of one year would be required as a minimum—— to bring a facility of this type from the existing physical plant to a functional, operating, staffed facility.

Since this facility is unique, so are the problems encountered in the process of start-up and in the acquisition of staff qualified to handle the variety of equipment and procedures required by this laboratory.

A number of requirements were met in order to attain operational status:

1. The clean room itself was particle and leak tested as part of the original construction contract. Subsequently it was cleaned and decontaminated on a regular basis. Some monitoring was carried out to determine the efficiency of the microbiological barrier. Maintenance of the filtering system was required.
2. Techniques for operation of the associated shower and dressing areas were developed in coordination with the study and development of clothing design and procedures and techniques for use in the clean room.
3. A system of maintenance of supplies, parts and operational status was developed for the various types of sterilizers adjoining the clean room.
4. Complete equipping of the microbiological laboratory to allow for the variety of assay techniques required was accomplished. The accumulation and balance of equipment and supplies necessary to meet requirements is essential since the usable data acquired from this facility is essentially

produced as microbiological data.

5. The aesthetic appearance of a clean assembly laboratory must be maintained for practical, functional and psychological reasons. The care, cleaning, design and maintenance of the facility's physical surroundings is of paramount importance. For these reasons time and effort were expended to attain these objectives to the greatest degree possible. Valuable procedures for maintenance of clothing, floors and supplies were developed.

The status of the research projects can be accessed as follows:

1. Three papers were published based on work carried out in this facility:
   1. "Dry heat resistance of spores of Bacillus subtilis var. niger on Kapton and Teflon film at high temperatures."
   2. "Reduction of microbiological shedding in clean rooms."
   3. "Improved method for pouring Rodac plates."

   Three more papers are in preparation
   1. "Sterile assembly and disassembly of the Wolftrap experiment."
   2. "Dry heat resistance of spores of Clostridium sporogenes on Kapton and Teflon film at high temperatures."
   3. "Design and testing of clean room clothing to reduce microbiological shedding."

The research supporting these publications has been performed and will be discussed in detail later. Some corollary research for these projects and other tasks has been carried out and will also be discussed.

FACILITY DESCRIPTION

The CASL facility is built around a Class 100 vertical-flow clean room and can be functionally subdivided, if need-be, into the following areas:

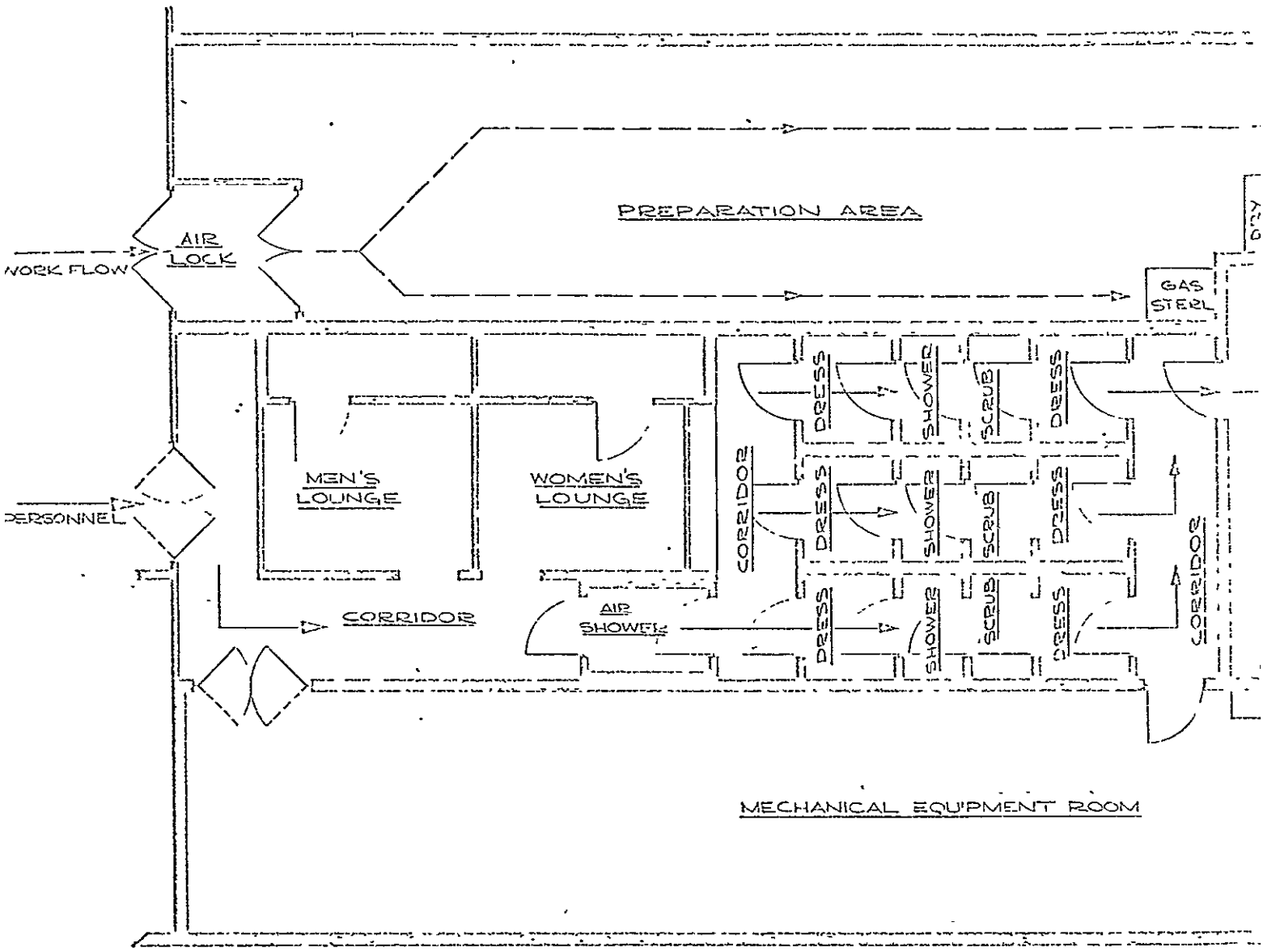
1. Bio-monitoring laboratory
2. Isolation area
3. Mechanical equipment and storage area
4. Down-flow clean room and associated shower and dressing areas
5. Preparation area for materials received into the laboratory for processing and/or assembly
6. Equipment test area

Opening into the clean room are three double-door sterilizers, the largest is a 48 x 54 x 84 vessel which can be used for dry-heat or ethylene-oxide sterilization. Large size, adaptable sterilizers provide the facility with the flexible capability to sterilize large loads, such as small space probes and/or related hardware. A second, smaller, dry-heat sterilizer, 20 x 20 x 40 in. is used for the sterilization of small components, tools, and various materials used in the operation of the clean room. The third, a 20 x 20 x 40 in. ethylene-oxide sterilizer, is used for routine gaseous sterilization of laboratory equipment, clean-room clothing, and other items which cannot withstand the dry-heat sterilization temperatures of steam sterilization.

Also attached to and opening into the clean room by way of a small, three-way, air lock is the Blickman apparatus. This is a stainless steel, glove-box isolator consisting of three separate compartments with a double-door, combination steam and ethylene-oxide sterilizer attached to the end not opening into the clean room. The Blickman apparatus is located in the Bio-monitoring Laboratory which also houses a steam sterilizer, water still, incubators, refrigerators, laminar flow work benches, ultrasonic cleaner, stove, water baths, and laboratory benches, which would constitute most of the typical equipment found in a modern microbiological

laboratory. In the room labeled Isolation Area are office cubicles, refrigerators, an anaerobic incubator, an incubator-shaker, two hot air ovens, a stainless-steel laboratory, counter with drawers. The Mechanical Equipment Room contains the air-handling equipment and air conditioning for the clean room and adjacent dressing and lounge area. Also found in this room are an in-line vacuum cleaner, air compressor and shelving and cabinets for storage. Enclosed in the dressing area adjacent to the clean-room are a men's and women's lounge, each with toilet facilities and a sink, an air shower, and three identical dressing-shower corridors leading to the clean-room corridor. The preparation area is a work area with some shop facilities containing a washing machine and dryer, work bench, two laminar flow clean benches and a large ultrasonic cleaner. The equipment test area which is elevated 18 inches higher than the preparation area, contains the smaller dry-heat and ethylene-oxide sterilizers. From this elevated area, activity and personnel in the clean room can be observed through a large window. A television system is installed throughout the facility allowing the camera or monitor to be placed in any work area including the clean room.

Much of the equipment is movable and the use or function of a given area need not be rigidly enforced. If need-be, the design of the facilities allows the institution of a rigorous routing for flow of materials.


PREPARATION AREA
AIR LOCK
WORK FLOW
PERSONNEL
MEN'S LOUNGE
WOMEN'S LOUNGE
CORRIDOR
AIR SHOWER
CORRIDOR
DRESS
SHOWER
SCRUB
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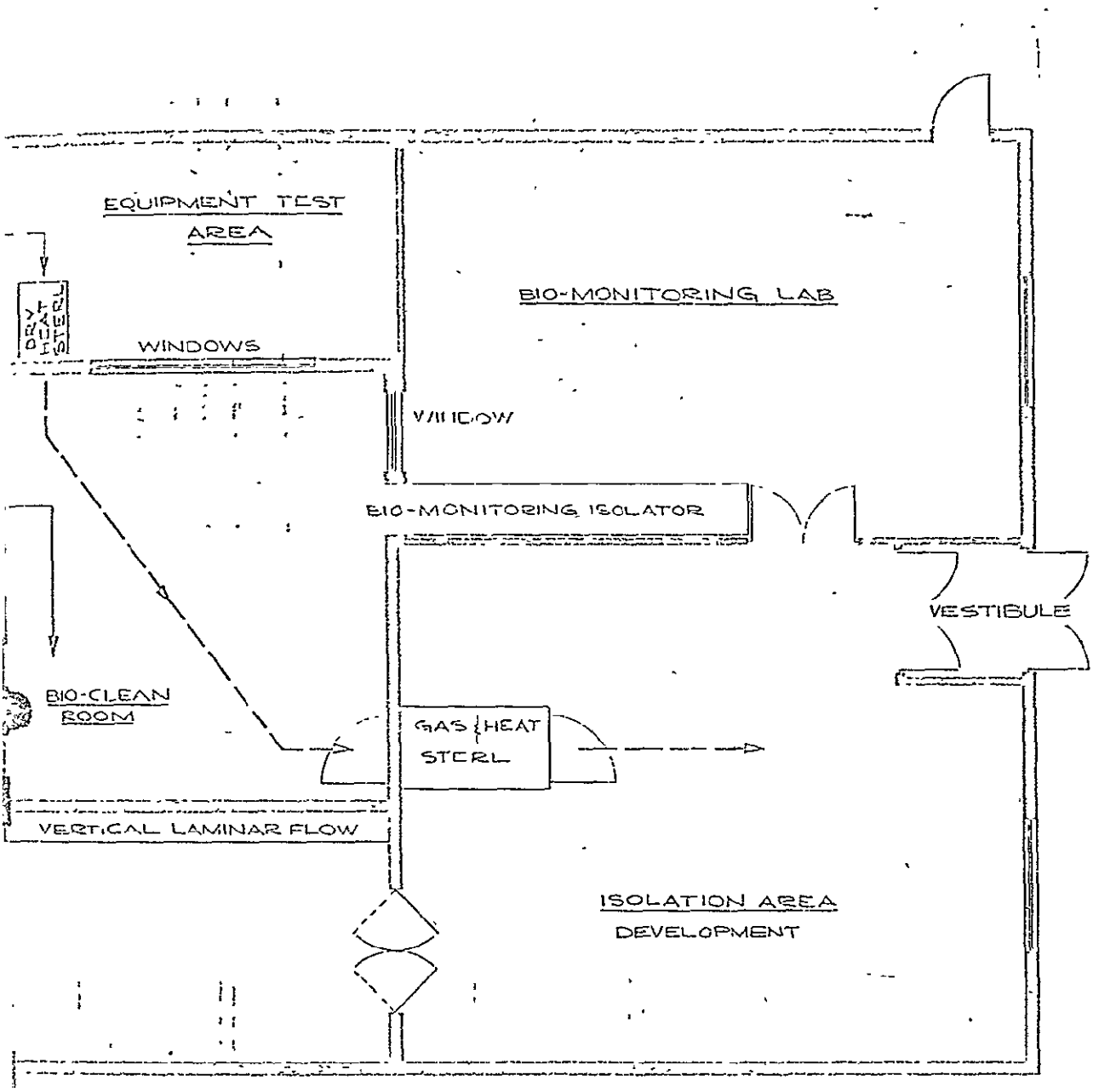
DRESS
CORRIDOR
GAS STERL
MECHANICAL EQUIPMENT ROOM

REVISIONS

ZONE	DESCRIPTION	DATE	APP'D



REQ'D	PART NO	DESCRIPTION	MATL	MATL SPEC	UNIT WT
		LIST OF MATERIAL			

UNLESS OTHERWISE SPECIFIED	NAME	INIT	DATE
DIMENSIONS ARE IN INCHES	APPROVED		
TOLERANCES ON	DRAWN Schultz	GD	7/9/72
FRACTIONS ± DECIMALS ± ANGLES ±	CHECKED		
	APPROVED		
FINISH	APPROVED		
NEXT ASSY	USED ON		

EXPERIMENTAL STERILZATION AND CLEAN ASSEMBLY FACILITY

SCALE 1/2" = 1'-0" | WT | SHEET OF

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
GODDARD SPACE FLIGHT CENTER
GREENBELT, MARYLAND

MD 2191

SIZE | CODE | SHEET OF

OPERATION OF CLEAN ASSEMBLY AND STERILIZATION LABORATORY

The following sections describe tasks and procedures performed to make the facility operable and enable subsequent maintenance of suitable operating conditions required for the clean room.

Entrance Procedure

All persons entering the clean room leave all of their street clothes in a locker room and put on a sterile hospital gown before entering the air shower. The entering person passes into one of three, identical, shower-dressing corridors. He showers and scrubs with germicidal soap (Dial or Safeguard), and finally dresses in sterile underwear and clean-room clothing.

Clean Room Clothing

The clean-room, outer garments were fabricated from either herringbone-weave dacron or cotton "Barbac." "Barbac" is a tightly-woven, cotton fabric which retains bacteria. The suits were fabricated for CASL by the Angelica Uniform Company, St. Louis, Missouri. Further description of the suits can be found in the attached publication, "Reduction of Microbiological Shedding in Clean Rooms."

The suits being used were designed as one-piece garments with the feet covered and secure fasteners at the ankles, wrists and neckline. The suit is fastened in the front by a zipper from the neck to the abdomen. A one-piece hood extends over the shoulders and down inside the clean-room suit and leaves only the eyes, nose and mouth exposed. For routine, clean-room use, the Dacron and Barbac suits as well as all clothing worn in the clean room are sterilized prior to use with ethylene-oxide. A technician clothed to enter the clean room would don garments as follows: rayon knit shorts and cotton tee-shirt, socks and tennis shoes, suit with hood, dacron shoe covers over the feet of the suit, surgical gloves, and a face mask.

The practical results from the use of this method of suiting have been excellent. There has been essentially no contamination in the large number of samples cultured in the clean room with personnel gowned as described. To further test the efficacy of the suiting procedures then in use in the clean room and to examine others, possible methods of suiting to insure the control both particulate and bacterial contamination were examined.

Dressing-Shower Area and Clean Room Main Entrance

The dressing and shower areas and corridors leading to the clean room have no floor finish. They are maintained regularly by wet-mopping followed by wet-vacuuming. The clear-room walls and floors were surface sampled for the presence of microorganisms with Rodac plates before the room was disassembled for cleaning and sanitization. The entire floor, consisting of aluminum grills held on a raised frame, was taken up and washed in nonionic detergent before sterilization in ethylene oxide. In addition, all clean room tables and chairs were sterilized at the same time as the floor. The walls, floors and floor frame were washed with 0.5 (Chlorox 1:10) sodium hypochlorite solution and thoroughly rinsed twice with clear water to reduce the microbiological population. The room was then re-assembled using aseptic technique (sterile clothing, gloves, and shoes). Rodac plates taken after or during reassembly confirmed the effectiveness of the clean-up procedure. Walls and floors which were previously highly contaminated with Bacillus subtilis spores showed few or no viable microorganisms. After the decontamination the clean room air was sampled with a Litton High Volume Sampler operating at 40 foot^3 for 18 hrs. Only one viable microorganism was recovered during this sampling interval. No personnel were present in the room during the sampling period.

All articles passed into the clean room have been previously sterilized or wiped down with 2.5% sodium hypochlorite (50% Chlorox solution)

The clean room tables (wide mesh aluminum top) are covered with a sterile plastic mesh material prior to each work session to assure a sterile work surface. All trash is accumulated in sterile polyethylene bags and removed daily. These procedures reduce the possibility of adding any unnecessary microbiological burden to the room.

STERILIZERS

Sterilizer Modification and Operation

The large 48 x 54 x 84 in. double-door, dry-heat or ethylene-oxide sterilizer was modified by the addition of internal-external thermocouple jacks which allowed sixteen points inside to be monitored simultaneously.

A temperature monitoring experiment was performed in the large dry-heat sterilizer to determine the temperature gradients within the chamber during a 24 hr.-135 C cycle. The large steel doors lagged significantly in heating up and never exceeded 125 C. Very few portions of the chamber actually reached 135C. Only thermocouples attached to the center portions of the plenum reached 135C.

The large dry-heat-ethylene-oxide (ETO) unit has been used successfully for ETO sterilization. There has been some problem in controlling the amount of moisture added during the vacuum portion of conditioning phase of the cycle. Excessive moisture has caused a considerable condensation on the walls and doors of the unit. The cause of condensation is in the adjustment of the cycle which is the same cycle as routinely supplied on sterilizers in use for hospital supplies or industrial disposable items.

The loads which have been subjected to ETO have been relatively nonabsorbent and this has contributed to the condensation problem. The circulation fan within the unit will not funtion efficiently due to the heavy load exerted by gas when unit is charged with 800-1000 mg/liter ETO. This may be due to density of gas; however, increased circulation of the ETO did not seem to be critical to the sterilization process.

Difficulties have been encountered with the controls as a result of a variety of mechanical failures. One persistent difficulty was from dirty tanks of ETO supplied by the contractor. It was necessary to change to a different gas supplier, Pennsylvania Engineering in Philadelphia, Pa. Clogging and control problems resulting from dirty gas and polymer were greatly reduced as a result. (Penn Gas is sold only for sterilization use and is guaranteed to be trouble free).

The smaller, dry-heat sterilizer was also modified by the addition of 8 internal thermocouple leads. No great variations in temperature were noted when eight different points were monitored simultaneously. As is usual, the doors lagged in reaching temperature, but the process temperature was reached after several hours. The small, ethylene-oxide sterilizer was also similarly modified by the addition of 4 internal thermocouple jacks.

All sterilizers are functional, but many repairs and adjustments were necessary during start-up and operation. Constant attention to maintenance and operating condition is required if all sterilizers are to remain operable on a day-to-day basis.

LABORATORY FLOORS

Maintenance

Floor maintenance in clean-room areas is an essential requirement to reduce particle and microbial contamination. Paste wax routinely applied to vinyl asbestos tile by steel wool buffing as regular facility maintenance was found to be unsatisfactory in working areas adjacent to the Class 100 clean room. The paste wax had no longevity as a finish, wore off readily in the form of a fine dust which easily adhered to shoes and was tracked about by personnel. This dust created housekeeping and contamination problems in the lounge and dressing areas supporting the clean room. Buffing with steel wool served only to again distribute fine particles of the steel wool into the work areas. Floor maintenance personnel seldom completely strip old, dirty wax from the floor before applying more wax, thus, any dirt mixed with the wax was only redistributed.

To alleviate this problem, the paste-waxed floors were completely stripped with a nonionic, liquid detergent solution* applied to the floor before scrubbing with an abrasive pad mounted on a heavy floor machine. Scrubbing was followed by a wet-vacuuming to lift and remove the soil. The floor was then mopped with a clear rinse and vacuumed again. When dry, two coats of an acrylic floor-sealing material** were applied with a rayon mop. After the sealer was allowed to dry thoroughly, two coats of an acrylic floor finish*** were applied. Upon drying, the acrylic finish was lightly buffed to improve the gloss.

This acrylic finish has been quite easy to maintain. It wears very well in the high traffic areas and is not affected by spilled water or other materials common in microbiological preparation areas.

The floors were maintained daily by one pass of a chemically treated dust mop* (Di-isobutylphenoxy ethoxyethyl dimethyl benzyl ammonium chloride) over all open floor areas. This mopping removed most of the dust and fine soil grit contributed by traffic. Depending on use, the floors were wet-mopped with a weak nonionic detergent solution and wet-vacuumed every two to three weeks

to remove ground-in soil. A light mopping with acrylic floor finish in the high traffic areas restored the original gloss. In addition to the daily maintenance, a shoe cleaning apparatus and treated rugs were used at the entrance. Treated rugs were also used in the lounge areas to prevent floor dust from being carried into the air shower and dressing areas.

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* Blue Pearl synthetic detergent)Barrier Chemical Co.
** Tile-tite floor seal)Vernon, New Jersey
*** Showdown floor finish)Vernon, New Jersey
* "KEX" Eastern Overall Cleaning Co. of Md., Inc., Baltimore, Md.

BLICKMAN ISOLATOR

Modification and Operation

To be useful for absolute maintenance of sterility, the Blickman isolator was converted to work under positive pressure. This was accomplished by placing two filter canisters on each chamber. Each canister contained a neoprene-rubber seal "butterfly" valve on each side of the filter canister which allowed an absolute seal as well as allowing the sterilization of the filter, the chamber, or both, with ethylene oxide. The canisters contained either HEPA filters or many layers of glass filter media. The chambers were plumbed with stainless steel lines to provide flexibility so that each chamber could be sterilized separately, or at the same time. The AMSCO steam sterilizer attached to the Blickman unit was also plumbed to the gas system making it usable as an ETO sterilizer. A heat exchanger was placed between the ETO gas bottle and the chambers to condition the gas and prevent layering during use of the gas.

New one-piece window gaskets cut from 3/8 silicone foam rubber were installed. The chamber was leak-tested with Freon 12. Additional caulking with RVT silicone rubber improved the containment of the gas mixture sufficiently to bring about sterilization. The gas was supplied slowly into the chambers from the bottom, causing the air to be slowly displaced within the chambers. This maximized the ETO concentration which could be contained within the load.

Spore strips (50) containing 1×10^6 spores of Bacillus subtilis var. niger were used as the index for sterilization efficiency. No positive spore strips were found upon culture after 48 hours of exposure to ethylene oxide in the sterilization procedure for the Blickman hood. This sterilization procedure has been repeated successfully three times. The observed slow leakage to the exterior seemes to be the result of gas diffusion through the gloves and is to be expected.

RESEARCH

Dry Heat Resistance Studies

For "split seam" entry technique to be applied to spacecraft repair or assembly systems, the sterility and reliability of the original, sealed seam must be assured. This commonly used gnotobiological method for sterile entry from the outside has numerous other possibilities. A seam is formed on the outside of a flexible isolator system by attaching another sterile, flexible bag aganist the isolator and sealing a circular port. The seam can then be split in half and access is gained easily. Pre-sterilized tools and materials can be included in the bag attached to the outside and thereby introduced into the isolator without using cumbersome, risky ports. A variety of materials can be selected for this technique.

Two studies on the dry heat resistance of spores were performed in the laboratory to have some basis for decision concerning the sterility of seams formed under given time - temperature conditions.

In order to finally calculate what can be considered a process time for a hypothetical load, the basic heat resistance characteristics must be determined.

As in all determinations of this type, the most heat resistant spores are used for process determination.

Bacillus subtilis var. niger, a resistant aerobe and Clostridium sporogenes strain Vera as representative anaerobe were studied.

The essential details of the research are included in the reprint and copy of the papers included in the Appendix. Since B. subtilis spores are more dry heat resistant than the anaerobic ones, the F (total process time) value is calculated on the basis of the dry heat resistance of B. subtilis spores. F value calculations give the following results: $F_{250^\circ C}$ = 7.9 sec; $F_{260^\circ C}$ = 7.1 sec; $F_{270^\circ C}$ = 5.68 sec. The sealing temperature range of teflon film is 260 to 280 C. At these times and temperatures a reliable, sterile seam can be formed. The results with C. sporogenes spores can be found in the paper included in the Appendix.

Shedding Studies

The shedding studies were undertaken in an attempt to evaluate the effectiveness of clean room garments in reducing the microbial load produced as a result of skin shedding. Newly designed clean-room garments were fashioned to minimize the escape of shed skin particles containing viable microorganisms. Three types of garments have been evaluated for their effectiveness in the retention of shed microorganisms. These garments, fabricated from different materials, were:

1. tightly woven dacron.
2. tightly woven cotton-"Barbac."
3. stretch nylon.

The cotton and dacron suits demonstrated an almost equal retention of microorganisms from the skin. These suits consisted of a one piece overall with the boots sewn on and a separate hood which tucked inside the coverall. The stretch suit consisted of a leotard bottom with feet, long sleeve top and hood of the same fabric. The tight-fitting hood was open around the eyes and nose. The study demonstrated that the number of microorganisms shed could be greatly reduced when the black nylon stretch hood was worn beneath the hood of either the cotton or dacron suit. This combination of garments could reduce the number of microorganisms shed in one half hour from 5×10^6 (naked shedding) to less than 1×10^3. All garments were worn with a disposable surgical masks beneath the hoods.

Surveys of Goddard personnel were conducted to find individuals who shed extremely high numbers of microorganisms. The shedders who consistently shed 1×10^9 organisms per half hour when naked can be compared with most individuals who shed at a considerably lower rate, $1 \times 10^3 - 1 \times 10^5$.

All shedding studies were conducted in a shedding chamber operated within the Class 100 clean room. The shed microorganisms were collected from the chamber by the Litton High Volume Air Sampler which

operated at 40 ft^3/min., a volume sufficient to collect all organisms shed when used in conjunction with the clean room suits, hoods and disposable masks. The use of cosmetics on the skin, such as vaseline and iso-propyl-myristate further reduced the number of recovered microorganisms. This work has been published as Reduction of Microbiological Shedding in Clean Rooms in Developments in Industrial Microbiology, 1969, Volume 10, Chapter 32. (See Appendix)

The shedding chamber techniques were also applied to a study conducted for Langley Research Center. This study was run to determine extent of biological shedding while wearing the BISS (Bio-Isolator Suit System) undergarment with associated life support system (This study is discussed below).

SHEDDING STUDIES WITH FOAM HOOD AND FOAM SUIT

As a result of an attempt to design a face mask which could be comfortably worn by clean-room personnel, a hood constructed from an open-pore (100 pore/sq. in.) polyurethane foam. Tests were performed using the head chamber (See Appendix) in the shedding tank arrangement developed in the CASL laboratory. The hood proved to be very comfortable to wear and quite effective in reducting the number of microorganisms shed from the head region.

Carrying this work forward, a foam suit was fabricated and tested. Other designs and means of construction should be tested but the results indicate that this may be a very effective mean of reducing very greatly the material shed from personnel whether it be in clean room areas, hospitals or factories.

Garments which can increase the level of organisms retained and still be comfortable for personnel provide an advance in clean-room clothing development.

Shedding Studies With the BISS Undersuit

The Bio-Isolator-Suit-System (BISS) includes an undersuit beneath an exterior garment. The BISS suit is part of the MAST system (Model Assembly Sterilizer Technique) developed for spacecraft sterile assembly and repair work at Langley Research Center (LRC).

Since CASL had developed the capability of performing shedding studies, a series of shedding tests were conducted for LRC. The studies were designed to determine what microbial load might be expected to be contributed as a challenge to the outer suit from the individual wearing the undersuit. A program of exercises was included to simulate reasonable activity.

In the first series of one-hour runs, without a shower or scrub prior to entry, the support system did not have an air-line filter. The high level of mold recovered was probably contributed by the life support system and specifically by the air-conditioning system. These studies with a Langley technician wearing the suit gave levels ranging from $1\text{-}20 \times 10^3$ organisms.

For the second series of experiments, an absolute filter was placed in the air line. In order to establish a consistent base-line, a thorough shower with a germicidal soap was performed. With a CASL technician wearing the suit in the second series, counts ranges from $1 - 10 \times 10^3$ organisms. (A more complete description of the work can be found in the Appendix).

The shedding characterisics of any given individual vary greatly from day-to-day, without known cause. Screening and surveillance of individuals wearing clothing where the organisms shed to the environment is of critical concern should be made.

Preparation of Acrobee 350 Air Sampler Experiment

For an experiment to collect microorganisms from the upper atmosphere, an Acrobee Collector (film winding type) was modified by the removal of the film winder and the addition of a polyurethane foam collector. All the procedures for the sterilization of the collector prior to flight and all methodology for sanitizing the collector's surface prior to aseptic recovery of microorganisms from the flown-foam collection material were worked out in great detail. This effort involved the use of most of CASL's major equipment. The Blickman isolator was converted to a positive pressure containment apparatus which could be sterilized with ethylene oxide. All recovery work (removal, culturing and incubation) can be done inside the Blickman. Sanitization of samplers prior to opening can be done in Class 100 clean room. Dry-heat sterilization of the samplers in flowing nitrogen can be carried out in the small dry heat sterilizer. All recovery techniques have been developed and the samplers are ready for flight.

Aerobee 350 Air Sampler Experiment

An adequate method for the sterilization and maintenance of sterility for the air sampling hardware was developed. The recovery procedures for microorganisms collected in the flown air sampler were also developed and thoroughly tested. This effort utilized most previously developed CASL technology. The following is a description and review of all preparation and research for the flight of Aerobee experiments.

Description of Air Sampler Hardware

The air samplers to be flown in the payload of the Aerobee 350 17.05 are essentially the same as those flown previously with a few modifications.

The Kapton film and winding mechanism has been replaced with a sleeve of 100 pore/in polyurethane foam supported by 5 mil Teflon FEP film backing. This sleeve was stretched over the three internal supporting posts. The samplers have been further modified by the replacement of original filter vents with Millipore stainless steel gas-line filter holders and 0.2 micron silver membrane filters. The "O" ring seals have all been replaced with new Viton seals which can easily withstand the conditions of dry-heat sterilization. Protective aluminum covers have been fabricated for each sampler. These covers will be sealed onto the sampler faces prior to sterilization. The cover maintains sterile conditions within the sampler during the checkout phases which require opening and closing of the sterile sampler. Upon completion of exercising of the samplers during the horizontal checkout, the covers are removed. The exposed sampler surfaces and surfaces immediate to the sampler will be sanitized with a dilute solution of sodium hypochlorite (chlorox) and covered with polyethylene film to maintain its sanitized state until flight. Just prior to flight the film will be stripped away exposing the exterior surfaces of the sampler. This sanitization and covering are necessary to maintain a low bio-load on the exterior surfaces of the samplers and rocket surface surrounding the samplers. Sanitization and covering lessen the chances of contaminating the sampler with soil-borne microorganisms. A soil sample taken from the White Sands launch area was assayed to determine the number and types of microorganisms (Table 1). A large number and variety of spore forming microorganisms were found in the sample. This further indicated the necessity to protect the sanitized samplers from soil contamination prior to launch.

Air Sampler Sterilization

The modified air sampler was heated at 135 C for 24 hours in flowing nitrogen to accomplish sterilization. As a check on the sterilization procedure, the three foam collection-media sufaces of the air sampler were

inoculated with 1×10^6 of the dry heat resistant spores of Bacillus subtilis var. niger. In addition, 12 spores strips were placed at random between the foam and its Teflon film backing before closing the samplers and sterilizing at 135 C for 24 hours. Upon culturing the foam and the spore strips, no viable microorganisms were found. This experiment was repeated four times with no viable organisms found after 72 hours of incubation at 32 C in trypticase soy agar or broth.

Recovery of Microorganisms from the Air Sampler

Thorough decontamination of the exterior surfaces of the flown sampler was necessary before the sampler could be opened to remove the collection foam for microbial recovery and enumeration. Because the probability of collecting large numbers of microorganisms in the outer atmosphere is not great, care must be taken in the decontamination of the samplers. Decontamination must be extended to the point that one can be reasonably confident that the microorganisms recovered from the foam are those collected in flight and are not contaminants from the exterior of the sampler. In order to develop an efficient decontamination procedure, it was necessary to highly contaminate the exterior surfaces of an internally sterile sampler. This was accomplished by spraying all the sampler surfaces with an aerosol of 1×10^6 Bacillus subtilis var. niger spores. This inoculation with spores simulated a severe condition of soil contamination which could result from a hard landing in the desert soil. The following decontamination procedures gave the best results:

1. The sampler was scrubbed thoroughly with a very strong nonionic detergent solution (Blud Pearl - H_2O, 1/2). Scrubbing was done with toothbrushed and cotton swabs. This scrubbing was to loosen and remove as must of the contamination as possible.
2. The scrubbed samplers were then placed in the clean room and thoroughly scrubbed again with 50/50 Chlorox - sterile water. Must attention and time was placed on scrubbing all the small crevices and screwholes. Scrubbing was done with

3. The samplers were thoroughly rinsed with sterile water.
4. Steps 2 and 3 were repeated.
5. The sanitized samplers were then checked for the presence of surviving B. Subtilis spores. This was accomplished by dividing the sampler into approximately 25 zones and swabbing each zone with swabs moistened with trypticase soy broth. The swabs were then aseptically broken off into tubes of trypticase soy broth and incubated to determine the adequacy of the decontamination procedure.
6. The sanitized sampler was then passed into the Blickman isolator which had been previously sterilized with ethylene oxide. The sampler was then manually opened and the foam collection material aseptically removed. Care was taken not to touch any of the sanitized surfaces with the foam. The foam was cut into three pieces and placed in sterile 150 x 20 mm glass petri plates for culturing and recovery.
7. Surfaces which could not be swabbed until the sampler was opened were swabbed as in part 5.

This entire procedure, as developed, was repeated ten times to insure the adequacy of the decontamination and sample removal. This rather laborious route of decontamination was preferred to total emersion in liquid sterilants or gaseous decontamination because of the possibility of penetration into the sampler, thereby jeopardizing the viability of the collected microorganisms. It is interesting to note that in ten trials all of the foam cultured was sterile, however, in a few instances some remaining B. subtilis contamination was still present on the interior of samplers. This was demonstrated by positive cultures from the swabs. These contaminated areas, which were difficult to sanitize, were in noncritical regions of the sampler; the sprocket shaft and bearing and small screw holes. The simulated contamination load was probably more extreme than any natural load which would be experienced in a hard landing and/or during the subsequent removal of the sampler from the rocket.

Recovery of Microorganisms

A method for the recovery of microorganisms from the polyurethane collection foam was developed. This method required few manipulations and could be totally performed within the Blickman isolator. Trypticase soy agar containing 0.05% Tween 80 was poured over pieces of flown collection foam. A sterile fork was used to work all the air out of the foam leaving the foam entirely embedded with agar. The embedded foam was then incubated. The foam embedded in agar, were quite transparent. Any microbial colony could be seen easily in the foam or in the agar surrounding the foam. Since the entire assay and incubation was carried out in the Blickman, there was little or no chance for contamination.

The efficiency of the culture method to recover low numbers of microorganisms was evaluated. One millileter of an assayed, aqueous or alcohol, spore suspension was distributed on 10-15 pieces of sterile foam. The microorganisms were then recovered using the agar embedding technique. All the colonies from the foam were counted and the total compared to the assay results from an equal amount of the same spore suspension (Table 2).

The data in Table 2 indicated that low numbers of microorganisms could be recovered from the polyurethane collection foam by this embedding procedure. Percentages of recovery where the total numbers are lowest, showed greater variation from experiment to experiment. This poor recovery was more the result of the microbiological assay procedures (serial dilution pipetting, etc.). Variations as great as ten-fold are quite common in low number assays. Since no variations this great were noted, this was good recovery data.

The recovery method was further tested to evaluate the recovery of microorganisms found in the air. Outside air was pulled through sterile foam collection material for varying lengths of time. Just a few minutes

was necessary to collect a grat variety of microorganisms on the foam. Many molds, yeasts and bacteria were recovered. All could easily be seen growing in the agar or agar-filled foam. Molds grew very rapidly to the surface and would spread over the entire plate in a few days. This necessitated reading the plates often during the incubation period.

Membrane Filtration and Recovery of Microorganisms from Kapton Film

Previous experiments showed that low numbers of microorganisms could be recovered from Kapton film by subjecting the film to 30 minutes of ultrasonic treatment in 50 cc of 1% peptone solution. The entire 50 cc of wash fluid was then plated. This method proved quite cumbersome because there were difficulties in getting good mixing and solidification of the agar. The high incidence of fast growing bacterial spreaders also added to the enumeration problems. In an attempt to overcome some of those difficulties, recovery of microorganisms by membrane filtration was evaluated and found promising.

The films used in these recovery experiments were sterile 2" x 3" pieces exposed outdoors for 4 to 5 days of prevailing weather, picking up at random whatever microorganisms were present in the air.

Utilization of the membrane filter technique for recovery of microorganisms had one distinct disadvantage; the tendency for colonies to rapidly overgrow making counting difficult. The advantages of this technique were the concentration of all the organisms from the 50 cc peptone solution onto a small area and the ease with which the filters containing the organisms could be cultured.

Method of culturing the filters containing the concentrated organisms was thoroughly explored in order to establish a procedure which would prevent rapid confluence, overgrowth and/or spreading of colonies on the small filters.

Methods examined for recovery from the filters included the method suggested by Millipore Filter Corporation which utilizes a small sterile plate containing an absorbent pad saturated with the trypticase soy media. The filter was placed on the wetted pad, incubated and read at 19, 26, 43, and 67 hours. Spreading of the microorganisms began after 26 hours making the plates difficult to count. Another disadvantage of the rapid spreading is that organisms cannot be easily isolated. This method was compared to culturing the filters on trypticase soy agar. Incubation and counting of colonies on the filter surfaces as metioned above at 19, 20, 43 and 67 hours

showed the organisms to be discrete, countable colonies that could be readily isolated. Spreading was not seen until 50 hours. The filters cultured on the TSA contained significantly higher numbers of organisms than the filters cultured on the media pads (Table 1).

A similar experiment was conducted using filters cultured on 1 1/2 strength TSA and single strength TSA. The colonies on 1 1/2 strength agar showed less tendency to spread. With 1 1/2 strength agar the colonies grew more slowly and did not reach the size of those grown on single strength agar. Comparisons of 90 hours of incubation showed the colonies on the 1 1/2 strength agar to be countable, while those on the single strength were spread.

Comparison of the 1 1/2 strength trypticase soy agar to normal strength with agar added to make it equivalent in agar content to the 1 1/2 strength agar were made using replicate filtrates from 10 ultrasonically-washed films. This agar concentration was found to be equivalent to retarding spreading. Recovery of microorganisms was also similar. As many as 43 colonies remained as discrete colonies on the filter after 46 hours of incubation.

An experiment was conducted to determine the nature of microorganisms isolated from Kapton film. Microorganisms isolated from Kapton film were compared to another 5 samples which were heat shocked 30 minutes at 80 C. Significant differences in number were seen when the number of surviving, heat-shocked organisms were compared to those not heat shocked. This experiment employed the membrane filter technique, using 1 1/2 strength TSA. The data indicated that most of the organisms recovered from the exposed films were vegetative (Table II) since only bacterial spore forms would survive the heat shocking process.

Membrane filter experiments using 2, 3, 5, triphenyltetrazolium chloride (TTC) to detect colonies on the membrane filter were more easily evaluated. The TTC was incorporated in a layer of TSA which was poured

over the filter and a base layer of agar or over the filter placed in the bottom of the petri plate. As the organisms grow and metabolize, the colony picked up this dye, producing a bright red colony which was easily counted at 24 hours. However, counts were variable and usually much lower than those cultured without the TTC. This technique probably has more application in the enumeration of anaerobic or microaerophlic microorganisms.

Considerable confidence in ultrasonic-membrane filter technique as a recovery method for low numbers of microorganisms has been developed. Kapton film exposed outdoors for 4 to 5 days consistently showed that 10-50 organisms could be routinely recovered. There is variation in the numbers collected on the film as it is exposed to the weather conditions outside. The Millipore Filter Corporation has just made available a new, inexpensive, enclosed, polycarbonate filter holder and flask which readily lends itself to this assay procedure. These were ordered and their use was evaluated and incorporated into the recovery procedure.

Survival of Air Dried Vegetative Microorganisms

The difficulties observed in the recovery of Serratia marcescens dried on Kapton films have been shown to be the result of rapid die-off as a result of air drying. Previous experiments used the films immediately or soon after dryness was accomplished. These experiments showed unexplainable patterns of recovery. This variation was thought to be the result of air drying. Five replicates of Serratia marcescens dried on film and stainless steel strips were evaluated for survival at 0 hours, 24 hours, and 48 hours (Table III). This experiment showed that approximately one log of organisms was lost immediately by air drying. Twenty-four - 48 hours after drying, viability was found to be very low and in many instances negative. No differences in survival were noted as a result of drying on stainless steel or film.

Stock Cultures

A series of stock cultures were established for laboratory use. The two cultures now set up are Serratia marcescens 8UK (Detrick strain) and Bacillus subtilis var. niger Detrick strain (N. R. Smith - OV 356 dry heat resistant strain), the subcultures from the original slant were used to select a number of orange variants to prepare the stock cultures. A series of 100 slants of each were prepared from the original culture.

TABLE I

Comparison of Methods for Culturing Millipore Filters containing Air-Borne Microorganisms Isolated from Kapton Film.

Media	Replicates	Numbers of Organisms at Incubation Time					
		19 hrs.	26 hrs.	43 hrs.	50 hrs.	Kapton Strips	Total
TS Agar	#1	3	22	22	spreading	1	23
	#2	2	5	7	7	0	7
	#3	10	25	25	spreading	3	28
	#4	4	8	10	spreading	1	11
	#5	6	12	15	15	0	15
TS Broth on Absorbant Pads	#1	9	13 and spreading	13 badly spread	not countable	1	14
	#2	9	9"	9"	"	1	10
	#3	4	5"	5"	"	0	5
	#4	6	6"	7"	"	0	7

TABLE II

The Effect of Heat Shocking on the Recovery of Airborne Microorganisms Isolated from Kapton Film.

Media	Replicates	Numbers of Organisms			Organisms on Film Strip	Total Number
		23 hrs.	28 hrs.	90 hrs.	90 hrs.	
Heat Shocked						
1 1/2	#1	0	0	0	0	0
Strength	#2	1	2	2	0	2
TSA	#3	2	2	5	0	5
	#4	0	0	2	0	2
	#5	3	3	6	2	8
Not Heat Shocked						
1 1/2	#1	10	12	45	0	45
Strength	#2	12	13	58	0	58
TSA	#3	9	12	34	0	34
	#4	13	13	28	0	28
	#5	7	15	29	0	29

TABLE III

Die off of Serratia marcescens Dried on Kapton Film and Stainless Steel Strips

Time		Counts (replicates)				
Initial count of suspension		1 1490	2 1490	3 1490	4 1490	5 1490
0 hr	film	113	22	167	134	280
	SS Strips	23	239	12	162	249
24 hr	film	0	0	0	0	0
	SS Strips	0	0	0	0	0
48 hr	film	12	0	0	1	5
	SS Strips	0	2	6	0	4

Sterile Assembly of the Wolftrap

The Wolftrap was designed as a biological life-detection experiment for planetary probes.

The Wolftrap hardware represents a complex, sterilizable apparatus consisting of optics, electronics, moving parts, and pneumatics and therefore constitutes a good test for sterile assembly techniques. In order to best evaluate and perform the task of sterile assembly, a small assembly area within the Class 100 clean room was constructed. It consisted of a floor-to-ceiling polyethylene curtain suspended between two aluminum frames. An aluminum table with an open mesh top was fabricated for use within the assembly area. Two Statatrol air-ionizing devices were installed beneath the HEPA filters which supply laminar air to the area. These ionizing devices neutralized the static charge on the particles and surfaces within the laminar air flow facilitating the removal of any generated particles which could contaminate the sterile hardware during the assembly process. (The curtain also insured the vertical laminar air flow of the ionized air to the confined area.) The Litton High Volume Sampler was placed beneath the surface of the mesh table top in a position that enabled the air to be monitored for the biological particles generated by the workers suited in maximum clean room garb. Results indicated that very few microorganisms are liberated into the assembly area during the assembly and

disassembly period. The use of the Statatrol and complete gowning greatly reduced the charge of contamination. A sterile incubator was placed in the clean room to further decrease the chances of contaminating the recovery broths during the incubation period.

In each experiment, the Wolftrap was assembled from parts previously sterilized in flowing nitrogen for 24 hr at 135 C. The Wolftrap was then disassembled and each part cultured separately in Trypticase Soy broth for 96 hr at 35 C.

Two out of six completely sterile assembly and disassembly experiments have been performed. Those experiments (4) which showed some viable microorganisms present, indicated that level of contamination were very low. Each experiment showing contamination had only one or two small pieces out of a total of 52 showing growth when cultured.

Assembly and disassembly of the Wolftrap experiment was carried out on a horizontal laminar flow bench in a relatively clean nonlaminar-flow area. The floor of this area was mopped prior to performance of the assembly-disassembly. Three out of three of these experiments on the clean bench showed no contamination when cultured.

Sterile assembly proved successful because of the maximum level and qualities of garmenting of the personnel, critical precautions in procedure and careful training in acceptable techniques.

Broad application of experimental procedures resulting in "sterile assembly" is possible in the industrial environment as well. Much more work on techniques and statistical validation must be done.

RECOMMENDATIONS

Laboratory Design and Improved Function

The CASL contains the equipment necessary to the planeta: quarantine program as carried forward at GSFC. Several arrangeme: for communication and flow of personnel and materials are inadequate even unsafe, however. The following recommendations relate to these conditions:

1. The addition of a steam autoclave pass-through for the clean room.
2. The addition of Statatrol ionizing units to the filter band would increase the potential of the laboratory for use in sterile assembly techniques.
3. A convenient addition would involve the insertion of a window pass-through for the entrance of materials from the Bio-monitoring Laboratory into the clean room.
4. Convenience would also be increased by the addition of a walk-through area from the preparation area to the Bio-monitoring Laboratory.
5. The interlocking door system was installed but never functioned well enough to be used and was not safe if an emergency were to arise while personnel were in the shower-dressing corridor.

6. The clean-room door should have been mechanically operated so that there would be no need to touch the door with hands.

7. A second means of exiting (emergency exit) from the clean room should have been available in case of an emergency.

Equipment maintenance is of paramount importance in all CASL functions. Failure of sterile procedures in CASL could usually be traced to malfunction of sterilization equipment. Thus, maintenance services should be obtained from the manufacturer on a frequent schedule. CASL personnel should perform only a minimum, if any, maintenance of equipment.

Janitorial services needed in CASL present constant quality control problems. Senior personnel were directly involved in clean-up activities to assure high standards of cleanliness. As GSFC now has major clean areas requiring similar services, it is recommended that CASL and other "clean" facilities be served by a Center-wide professional maintenance service organized for this purpose.

Staffing

The staff for this facility, if it is to produce data with microbiological significance, should be trained, professional microbiologists with a varied background and some experience with sterilization techniques. It should be stressed that familiarity with sterilization rationale or procedures is not normally part of the educational requirements in microbiology. Associated with these microbiologists there should be a required number of technicians with laboratory experience. This is a requirement for at least one technician with electronics and mechanical experience to assist in the maintenance of equipment.

The personnel selected for the laboratory were required to have some variety in their experience as the requirements demanded the ability to adapt known techniques to a variety of new materials and procedures. Some time was required in training the personnel for the procedures in laboratory technique, cleaning and maintenance procedures and the recording of data.

The personnel staffing the facility developed well. Many rather unusual requirements demanded of them were performed willingly because of their background in biological sciences. The possession of flexibility and imagination proved to be very rewarding qualities in the personnel working in CASL.

To serve GSFC and the experimenter community adquately, a high level of professional performance by CASL personnel must be maintained. One way to accomplish this is to require the contractor to provide, in addition to operating and research services, consulting and study services to the GSFC Planetary Quarantine Program. This would involve CASL personnel with the mainstream of PQ activities in NASA, allowing more efficient research planning in CASL and wider distribution of current CASL findings. The critique of CASL's program and action by non-GSFC personnel working in planetary quarantine is an essential aspect of maintaining professional attitudes.

APPENDIX

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Dry Heat Resistance of Spores of *Bacillus subtilis* var. *niger* on Kapton and Teflon Film at High Temperatures

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To determine parameters that would assure sterility of a sealed seam of film for application in "split-seam entry," spores of *Bacillus subtilis* var. *niger* were sprayed onto pieces of Kapton and Teflon film. Short-time, high-temperature (200 to 270 C) exposures were made with film pieces between aluminum blocks in a hot-air oven, and the *D* and *z* values were determined after subculture of surviving spores. The use of Kapton film allowed the study of high temperatures, since it is not heat sealable and could be used to make thin packages for heat treatment. Spores on Teflon were dry-heat treated in a package designed to simulate an actual seam to be sealed. The *z* values of 29.1 C (52.4 F) for spores on Kapton and 139 C (250.4 F) for spores on Teflon were calculated.

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Relatively little attention has been given to the definition of high-temperature—(short-time parameters in the thermal destruction of microorganisms.

With the development of techniques for gnotobiology, means of entry or the transfer of sterile materials without violation of the sterility of the flexible film chamber housing the gnotobiotic animals has resulted. This technique is known as "split-seam" entry (10, 17, 18). With this technique, a sterile sealing tool (hot wire, etc.) and the sterile items to be transferred are placed in an internally sterile plastic bag. This bag is sealed onto a flexible film isolator, and its contents can be inserted into the isolator or film system without violation of sterility. The interior of the passageway formed by cutting or splitting of the sealed seam could contain some film not previously sterilized. Since a minute portion of the seam will be exposed to the interior surface of the system being entered, the time-temperature parameters involved in producing a physically acceptable seam must also kill all microorganisms trapped therein.

This method of entry or insertion has numerous applications, among these are uses in gnotobiology, sterile fill operations, and sterile assembly of spacecraft parts. Of the numerous films available, one which has acceptable properties and could be used in the assembly of spacecraft parts is Teflon FEP film (Teflon is a proprietary product of the E. I. Dupont De Nemours Co., Inc., Wilmington, Del.).

The existing literature contains only a few references to dry-heat resistance at elevated temperatures or in the sealing temperature range of Teflon film, 500 to 536 F (260 to 280 C). Currently, estimates of thermal kill cycles at the higher temperatures are determined by extrapolation from the existing thermal death time data. Wang et al. (19) in experiments with moist heat showed that this type of extrapolation can be in serious error. They showed a nearly 160% disagreement between experimental results and those found from extrapolation of the thermal death time curve over a range of 20 C to the highest temperature tested (295 F or 143 C).

Some early study in thermal resistance at high temperatures has been reported by Bourdillon et al. (2) in the sterilization of air with different types of furnaces. They concluded that a temperature of 482 to 572 F (250 to 300 C) was required for complete air sterilization. Decker et al. (5), also testing an air sterilizer, worked in a temperature range which more nearly coincides with the temperature range of interest in this investigation. These experiments with spores of *Bacillus subtilis* var. *niger* were designed to test the performance and biological reliability of a commercial electric-grid, hot-air sterilizer. Their results were reported in terms of per cent reduction of the original population and also the retention time at a specified temperature in the range 425 to 625 F. The time for a 6 log reduction was 24 sec at 425 F (218 C) and 3 sec at 575 F (302 C).

Again, in an attempt to determine some pa-

rameters for hot-air sterilization of laboratory material, Francis (6) heated a dried-spore inoculum of 4×10^7 organisms from hay dust in aluminum weighing dishes heated with aluminum blocks and obtained sterility at 428 F (220 C) in 10 sec

A recent investigation of the efficiency of a hot-air sterilizer was pursued by Quesnel et al (14) at 392 F (200 C) At this temperature and with an inoculum of mixed species of *B subtilis* as an air-dried film, they achieved sterilization of 20 stainless-steel strips weighing 4 g each in an overall cycle of 20 min, whereas six sputum cups with a total weight of 2 kg required a cycle of 38 min Glass jars of equivalent weight to the steel sputum mugs required a longer cycle in the sterilizer Their interpretation of the data related the surface to mass ratio of the materials carrying the inoculum to the time to sterilize at this temperature

Materials and Methods

Test organism The organism used in this study was *B. subtilis* var *niger* (Detrick strain) The culture was obtained from Charles R Phillips of the U S Army Biological Laboratories, Fort Detrick, Frederick, Md

Sporulation medium The sporulation medium was prepared from commercial dehydrated material In this case, the medium was Nutrient Agar (BBL), prepared with tap water with 10 μg of $MnSO_4$ and 80 μg of $CaCl_2$ per liter added The inoculum buildup medium was the same, without agar added

Subculture medium (spore viability medium) Dehydrated Trypticase Soy Agar (BBL) was used to determine the viability of heat-treated spores Medium was dispensed in 25-ml portions into disposable glass test tubes (50 by 150 mm) and covered with polypropylene drop caps Wide-mouth tubes were used to accommodate the pieces of film All tubes of medium were incubated for 48 hr at 32 C prior to use, and any positive tubes were discarded

Assay medium Trypticase Soy Agar was used in the assay procedure Sterile, distilled water with 0.05% Tween 80 (Atlas Chemical Industries, Inc , Wilmington, Del) was used as a wash solution in the assay of untreated, sprayed pieces of film

Preparation of spores A stock culture slant which was the first passage from the original stock culture was washed with 5 ml of sterile distilled water The suspension was decanted into a sterile, screw-cap tube and heated at 65 C for 15 min to kill any vegetative cells A 1-ml amount of this suspension was used to inoculate each 500-ml shake flask containing 50 ml of inoculum buildup medium The flasks were placed on an incubated shaker at 32 C for 16 to 18 hr A 2-ml amount of this culture was inoculated into 1-liter Erlenmeyer flasks containing 200 ml of inoculum buildup medium and incubated in a shaker at 32 C for 12 to 14 hr The inoculum was checked for vegetative growth under phase microscopy before use Glass petri dishes (150 by 25 mm) were prepared with 100 ml each of solidified sporulation agar and preincubated for 24 hr at 32 C A 2-ml amount of inoculum was spread onto the agar surface of each plate with glass spreaders, and the plates were incubated at 32 C Sporulation was checked frequently under phase microscopy Maximal sporulation (99%) was at 48 hr Plates were refrigerated overnight and then harvested Each plate was rinsed twice with approximately 10 ml of cold, sterile, distilled water while the plate was revolved on a turntable and the surface was gently agitated with a glass spreader to facilitate the removal of the growth The accumulated suspension was centrifuged in a refrigerated centrifuge at 10,400 $\times$ *g* for 20 min and decanted The spores were washed five times in sterile, distilled water, and, after the final wash, the spores were suspended in absolute ethyl alcohol All procedures of transfer, inoculation, and harvesting in the preparation of the spore suspension were performed under laminar flow conditions

Preparation of sprayed test pieces Two types of film were used in these studies, Kapton and Teflon Teflon was selected as a representative film on which to study time-temperature parameters for heat sealing, utilizing a gauge which would have practical application, whereas the Kapton was selected because of its high electrostatic charge and was chosen more to look at the heat resistance of the spores at heat-sealing temperatures on a film which would withstand the sealing temperatures of Teflon without sealing Kapton (Dupont registered trademark, E I Dupont de Nemours & Co , Inc , Wilmington, Del) is a polyamide film which maintains its physical, electrical, and mechanical properties over a wide temperature range (from -269 to 400 C) Kapton chars above 800 C, is not heat sealable, and is not soluble in any known organic solvent Kapton type H film of 1 mil thickness (0.001 inch) was used At room temperature Kapton and Mylar, a polyester film, are similar. As the temperature varies, the properties of Kapton are less affected than Mylar Mylar and Kapton are frequently used materials in the space effort Teflon FEP is a fluorocarbon film It is a transparent, thermoplastic film which can be heat sealed, thermoformed, laminated, or metalized It is virtually inert to chemicals and solvents It can be used continuously at 200 C and has good resistance to impact and tearing Teflon is widely used in the electronics and electrical industries Five mil (0.005 inch), type A Teflon was used The heat-sealing range for this film is 260 to 280 C

Large sheets of sterile film of either type were suspended in a clothesline fashion The receptacle bottle of a chromatography sprayer was filled with spore suspension in ethyl alcohol Sheets of film were sprayed at a distance of approximately 3 feet The spore film dried instantly Kapton was sprayed on both sides and Teflon on one The level of inoculation was 10^6 on each 1-inch (2.54 cm) square

Assay of nonheated, sprayed film Squares (1 inch) of film were used for assay Because of difficulty in removing spores from the Kapton for assay, the pieces of Kapton film were soaked overnight in the wash solution before assay. For both Kapton and Teflon, a square of sprayed film was placed in a screw-cap, polypropylene bottle with 50 ml of wash solution and

exposed to ultrasonic treatment at 35,000 kc for 30 min. The wash solution was routinely, serially diluted and plated with Trypticase Soy Agar and incubated for 48 hr at 32 C. Each piece of film was washed twice, and the counts from each wash were added. Initially each piece of film was also plated to detect any spores remaining on the surface, but this was discontinued since nothing significant remained.

Preparation of inoculated film packets. The Kapton packet (Fig. 1) contained 25 squares (1 inch) of Kapton film spray-inoculated on both sides. These squares were evenly spaced and maintained between two 8 by 8 inch sheets (20.3 by 20.3 cm) of Kapton. This 8 by 8 inch lamination was then placed between two 10 by 12 inch (25.4 by 30.5 cm) sheets of Kapton, and the edge was taped. The total thickness of the Kapton packet was 5 mil (0.005 inch). The high, natural electrostatic charge of Kapton film effectively held the inoculated film squares firmly in place. The packets containing the inoculated Teflon were similar (Fig. 2). They consisted of two layers of 5-mil Teflon with the inoculated sides facing together (simulating a seam to be sealed). The inoculated sheets of Teflon were heat sealed on one edge and cut to the seal, forming five 1-inch fingers of film. A Kapton separator (1 mil) was placed between the inoculated surfaces so that the heat treatment would not fuse the contaminated surfaces together. Fusion would have occluded the test organisms and prevented recovery of survivors.

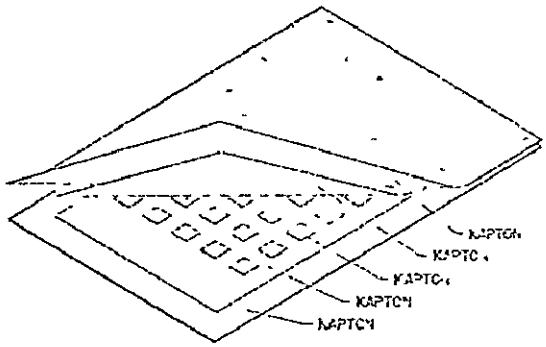

FIG. 1. *Diagrammatic representation showing the method of preparation of the Kapton test pieces.*

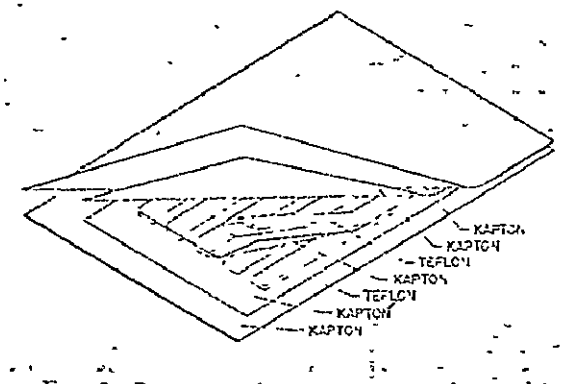

FIG. 2. *Diagrammatic representation showing the method of preparation of the Teflon test pieces.*

Dry-heat treatment of packets. The Kapton and Teflon packets were heated by placing them between two aluminum blocks. The blocks (8 inches (20.3 cm) wide by 12 inches (30.4 cm) long by 1 inch (2.5 cm) deep) were hinged on one end so that they could be rapidly opened and closed. The packet containing the inoculated film was pressed firmly between the blocks for the time interval of the heat treatment. The packets were cooled instantaneously by placing them between an identical set of cold (0 C) aluminum blocks. The heated blocks were maintained at the desired temperature by holding them in a forced-draft, hot-air oven (Hotpack Corp., Philadelphia, Pa.). The temperature of the blocks was continuously monitored by copper-constantan thermocouples attached to the blocks and to a multipoint recorder (Minneapolis-Honeywell, Philadelphia, Pa.). Each heat treatment was timed from the closing to the opening of the heated blocks. The come-up time for the Kapton packets was less than 1 sec, whereas the come-up for the Teflon packet due to its thickness was approximately 3 sec.

Subculture of heat-treated squares of film. Samples (100) were heated for each time-temperature designation. All subculturing techniques were performed in a vertical laminar flow room (20 feet (616 cm) wide by 20 feet (616 cm) long by 10 feet (308 cm) high) with personnel gowned in sterile garments completely covering their bodies including a face mask for the nose. All materials were wiped with disinfectant before being admitted to the clean room. Each heat-treated piece of film was transferred with sterile forceps (using a new pair for each transfer) into the tubes containing the subculture medium and incubated at 32 C for 3 weeks.

Analysis of data. D values (in this case, seconds required at a given heating temperature to reduce the spore population 10-fold) were calculated by the method of Stumbo as described by Schmidt (15) and are given by the formula $D = (U \text{ or } t)/(\log A - \log B)$, where A is the total number of samples heated multiplied by the number of spores per sample, B is calculated assuming one surviving spore per container when less than the total number of containers showed survival, U or t is exposure time at a given temperature. In addition, the calculations were also done by the method of Stumbo, Murphy, and Cochrane (16) as described by Pflug and Schmidt (13) which used basically the same formula described above, but the number of survivors described by B is calculated by the most probable number of Halvorson and Ziegler (8) by using the formula $U/(N_0 - N_u)$. The z value was determined from the plot of the phantom thermal death curve or thermal resistance curve and from the regression coefficient derived from the statistical analysis. The z value can be defined as the number of degrees fahrenheit for the death time curve to pass over 1-log cycle. The temperature coefficient for the change in rate of destruction with temperature can also be expressed by the Q_{10} value with the relationship $z = 18/\log Q_{10}$ for degrees F and $z = (0.555 \times 18)/\log Q_{10}$ for degrees C. F value (F_0 equals sterilization

time in minutes with respect to a given organism and has been traditionally given at 250 F) can be interconverted from D values by using the following relationship: $I = D$ (log $A \div 2$) The value of log A is from Stumbo's equation The initial population then is reduced to 1 during an exposure to heat at a given temperature for the time period of $D \times$ number of logs of the initial population, after which the area of probability of a survivor is entered Activation energy can be calculated from the simplified Arrhenius equation shown by Schmidt (15) as follows:

$$E = 4\,58\,(T_2 \times T_1)/(T_2 - T_1) \times k_2/k_1$$

where T is absolute temperature, k is the reaction rate constant and was calculated from D by $k = 2.3/D$, and 4 58 is a constant Pflug and Schmidt (13) point out that calculations of activation energies perhaps have more applicability with dry heat than with wet heat

Statistical analysis A regression analysis of the data and the least-squares regression line were calculated by using a program on an IBM 360 computer

Results and Discussion

Table 1 presents the D values obtained for *B subtilis* var *niger* spores heated on both Kapton and Teflon film at various temperatures The regression lines indicating the z values for spores on both films are shown in Fig 3–4

Much recent study (1, 9) on dry heat and particularly that of Murrell and Scott (11) have shown that the water activity, a_w (equilibrium relative humidity), of the spores themselves and of the environment in which the spores are heated affects the heat resistance In our investigation, the spores were sprayed onto the films from an ethyl alcohol suspension and were essentially dry. It is probable, however, that in the time required to form the packets for heat treatment, the spores equilibrated to something close to the equilibrium relative humidity of the environment However, as has been shown by Gilbert et al (7), spores equilibrated after desiccation will not equilibrate

TABLE 1. *Comparison of D values with data in the literature*[a]

Temp F (temp C)	D value in seconds		
	Teflon	Kapton	Dock et al
392 (200)		2 15	
410 (210)	2	0 99	
419 (215)		0 54	
425 (218 3)			3 4
428 (220)		0 46	
464 (240)	1.18		
475 (246 1)			1.4
482 (250)	0 99		
500 (260)	0 89		
518 (270)	0 71		
525 (273 9)			0 7
575 (301 6)			0 4

[a] Average D, end point calculation

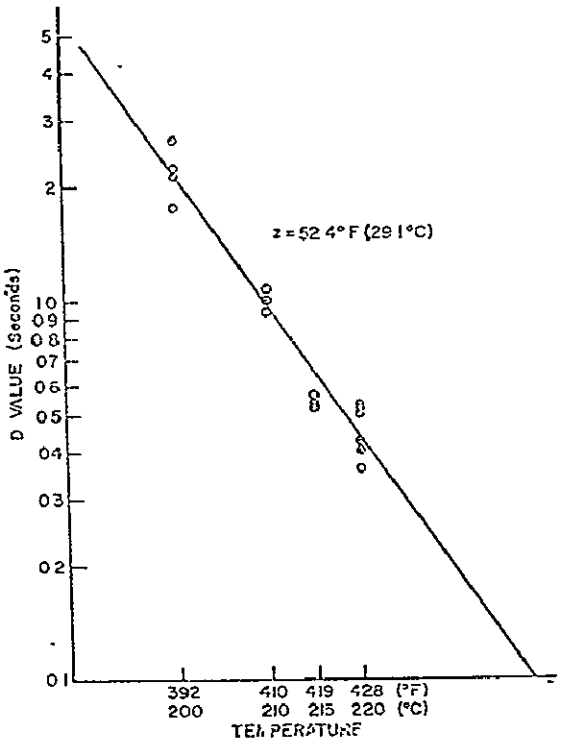

FIG 3 *The least-square regression line with the slope showing the z value and the average D values represented as original data points for spores on Kapton film*

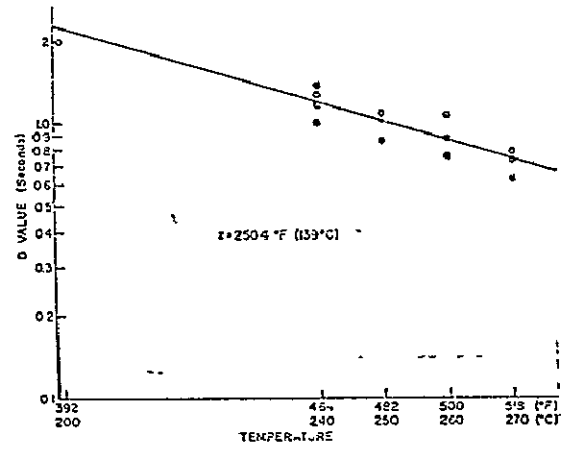

FIG 4 *The least-squares regression line with the slope showing the z value and the average D values represented as original data points for spores on Teflon film*

the same final a_w as spores that have never been desiccated. This results in a final, equilibrated a_w which is lower than for spores never desiccated. Our investigation was carried out essentially so that the results could be practically applied in the heat sealing of Teflon film. In actual experience, the spores on a given piece of film would have an undetermined water content but would probably be in a relatively desiccated state. The environment in which these packets were prepared was at approximately 50% relative humidity. The equilibrated a_w of spores showing the greatest heat resistance is in the range 0.2 to 0.6. Considering the fact that the spores were previously desiccated, it can be assumed that they were probably not at an a_w range of minimal heat resistance.

Partial explanation of the differences in z and D values of the spores heated on the two types of film can be found in the examination of the packet and the permeability of the films to water vapor. The thinner Kapton packet (total of 5 mil) is made from film permeable to water vapor [5.4 g/(100 inches^2) (24 hr),/mil] and has measurable moisture absorption capacity (1.3 at 50% relative humidity at 23.5 C). The water vapor in the spore and in the environment can be easily lost during heating, thus heating spores which are dry and which should show lower heat resistance. On the other hand, the Teflon packet is thicker (total of 15 mil) and contains a piece of Kapton inserted between two pieces of Teflon. Teflon is impermeable to water vapor, and thus the water vapor content of the spores and that released from the Kapton film are retained in the immediate environment of the spore. The spores are then heated at an intermediate a_w giving higher heat resistance. This factor of penetration of water vapor combined with the longer come-up time for the thicker packet and consideration of the surface area to mass ratio for the Teflon packets may provide some reasons for the divergence in z values.

The come-up time for the Kapton film package was less than 1 sec, so that the calculated z probably represents a more accurate value for the spores than the thicker Teflon package with its longer come-up time. The Kapton system was chosen so that spore destruction at elevated temperatures could be studied without the film sealing. The thinner Kapton packet (total 5 mil) also offered a spore-carrier system with less mass.

The D values reported show a general agreement with the data of Decker et al. (5) with which they are compared in Table 1. Analysis of the data of Decker et al. (5) showed a straight-line Arrhenius plot. Arrhenius plots with our data were drawn and also produced straight lines. The results of calculations of activation energy are given in Table 2 and are compared to the data of Decker et al. (5) and some other values in the literature. The lower activation energies obtained at these high temperatures compared with values obtained for dry-heat resistance at lower temperatures (3) might indicate some different lethal reaction. Calculated activation energy from the investigation of Decker et al. (5) associated with the death reaction would be approximately 12,600 cal/g-mole.

TABLE 2. *Activation energies and Q_{10} values compared with values in the literature*

Source	Activation energy (cal/g-mole)	Q_{10} value
Kapton (this study)	35,500	2.1
Teflon (this study)	9,500	1.2
Wave dry heat spores of *B. subtilis* [a]	40,200	
C-N bond energy [a]	49,000	
Decker et al. (5), spores of *B. subtilis*	12,600	

[a] R. G. Wax, Ph.D. Thesis, Pennsylvania State University, 1963.

As has been pointed out by Quesnel et al. (14), the ratio of surface area to mass could be a significant factor in dry-heat sterilization. Since the value for specific heat and the coefficient of thermal conductivity are essentially the same for Kapton and Teflon, the difference in z and D values between the two films may reflect the greater surface area to mass ratio with the Teflon packets.

It would seem that regardless of the explanations given, the common practice of extrapolating the z curve to higher temperatures cannot be defended. The lower z values for dry heat (30 to 40 F range, 1, 4, 12) obtained at lower temperatures (135 C) would show considerable error if arbitrarily extrapolated to the 200 to 275 C range.

In the application of the data derived from this study to actual heat-sealing practice, it is necessary to determine the time required at a given sealing temperature (using average D values) with a hypothetical load plus a safety factor, which is the probability of one viable spore in any given number.

A total F calculation (heat-sealing time at given temperature to assure sterilization) from the data presented would indicate a time for F_{250C} = 7.9 sec, F_{260C} = 7.1 sec, F_{270C} = 5.68 sec, assuming a maximal load on the film of 10^5 spores plus a probability of one viable

spore in 10^1. Our investigation has shown that a normal load on unsterilized Teflon is about 10 to 20 organisms per square inch.

ACKNOWLEDGMENTS

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DRY HEAT RESISTANCE OF SPORES OF CLOSTRIDIUM SPOROGENES ON KAPTON AND TEFLON FILM AT HIGH TEMPERATURES

by

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As was discussed in our recent publication concerning the high-temperature dry heat resistance of Bacillus subtilis var niger, little attention has been given to dry heat resistances at higher temperatures. This is particularly true when discussing anaerobic spores. This work and the aforementioned work with B. subtilis var. niger were undertaken in the examination of the feasibility and reliability of the gnotobiotic technique of "split-seam" entry.

A survey of the few references on high-temperature, dry-heat resistance can be found in our previous publication (in the range 320-410 F (160-210 C). It was also pointed out that discrepancies can exist where thermal resistance curves are extrapolated to higher temperatures (Wang et al.,). The organism used for this study was selected since there is some indication of the dry heat resistance of the spores in the literature (Bruch et al.,).

Materials and Methods

Test Organism The organism used in this study was Clostridium sporogenes strain Vera and was originally obtained from Dr. Harietle Vera of the Becton-Dickinson Company, Baltimore, Maryland.

Sporulation medium The sporulation medium was 10% trypticase plus 0.1% Na thioglycollate. The inoculum build-up medium was the same.

Subculture medium (spore viability medium). Dehydrated Reinforced Clostridial Medium (Oxoid-Colab) was used to determine the viability of heat-treated spores. Medium was dispensed into disposable glass test tubes (50 by 150 mm) in 25-ml quantities, overlayed with stratifying agar (5-10 ml) and covered with polypropylene drop caps. Wide-mouth tubes were used to accommodate the pieces of film. All tubes of medium were incubated for 48 hr at 32 C prior to use. Any positive tubes were discarded.

Stratifying medium Stratifying medium was 3% agar with 0.1% sodium thioglycollate added.

Assay medium Reinforced Clostridial Medium was used in the assay procedure.

Preparation of spores A stock-culture slant, which was the first passage from the original stock culture was washed with 5 ml of sterile distilled water. The suspension was decanted into a sterile, screw-cap tube and heated at 65 C for 15 min. to kill any vegetative cells. This inoculum was used to build up a high cells-per-volume-of-liquid inoculum in tubes and/or flasks. A

constant check, using phase microscopy was made to determine the state of the culture. Harvest was begun at estimation of maximum sporulation. Maximum sporulation was approximately 60-70%. Flasks were chilled overnight in the refrigerator. Harvest was accomplished with a continuous flow attachment in a refrigerated centrifuge at 10,400 X g. The spores were washed five times in sterile distilled water. After the final wash, the spores were suspended in absolute ethyl alcohol. All procedures in culturing were performed under laminar flow conditions.

Preparation of sprayed test pieces Two types of film were used in these studies, Kapton and Teflon. Teflon was selected as a representative film on which to study time-temperature parameters for heat sealing, utilizing a gauge which would have practical application, whereas the Kapton was selected because of its high electrostatic charge and was chosen more to look at the heat resistance of the spores at heat-sealing temperatures on a film which would withstand the sealing temperatures of Teflon without actually sealing. Kapton (Dupont registered trademark, E. I. Dupont de Nemours & Co., Inc., Wilmington, Del.) is a polyamide film which maintains its physical, electrical, and mechanical properties over a wide temperature range (from -269 to 400 C). Kapton chars above 800 C, is not heat sealable, and is not soluble in any known organic solvent. Kapton type H film of 1 mil thickness (0.001 inch) was used. At room temperature Kapton and Mylar, a polyester film, are similar. As the temperature varies, the properties of Kapton are less affected than Mylar. Mylar and Kapton are

frequently used materials in the space effort. Teflon FEP is a fluorocarbon film. It is a transparent, thermoplastic film which can be heat sealed, thermoformed, laminated, or metalized. It is virtually inert to chemicals and solvents. It can be used continuously at 200 C and has good resistance to impact and tearing. Teflon is widely used in the electronics and electrical industries. Five mil used in the electronics and electrical industries. Five mil (0.005 inch), type A Teflon was used. The heat-sealing range for this film is 260 to 280 C.

Large sheets of sterile film of either type were suspended in a clothesline fashion. The receptacle bottle of a chromatography sprayer was filled with spore suspension in ethyl alcohol. Sheets of film were sprayed at a distance of approximately 3 feet. The spore film dried instantly. Kapton was sprayed on both sides and Teflon on one. The level of inoculation was 10^5 on each 1-inch (2.54 cm) square.

Assay of nonheated sprayed film Square (1 inch) of film were used for assay of spores on both Kapton and Teflon film. Each sprayed square of film was soaked in 50 ml of wash solution. The wash solution was constituted of 1% peptone and 0.1% Na thioglycollate. The solution and piece of film, contained in a screw-cap polypropylene bottle was insonated at 35,000 kc for 15 min. The was solution was routinely, serially diluted and assayed in stratified deep agar tubes of Reinforced Clostridial Medium and incubated at 32 C for 24-48 hours. Each piece of film was washed twice, and the counts from each wash were added.

Preparation of inoculated film packets The Kapton packet (Fig. 1) contained 25 squares (1 inch) of Kapton film spray-inoculated on both sides. These squares were evenly spaced and maintained between two 8 by 8 inch sheets (20.3 by 20.3 cm) of Kapton. This 8 by 8 inch lamination was then placed between two 10 by 12 inch (25.4 by 30.5 cm) sheets of Kapton, and the edge was taped. The total thickness of the Kapton packet was 5 mil (0.005 inch). The high, natural electrostatic charge of Kapton film effectively held the inoculated film squares firmly in place. The packets containing the inoculated Teflon was similar (Fig. 2). They consisted of two layers of 5-mil Teflon with the inoculated sides facing together (simulating a seam to be sealed). The inoculated sheets of Teflon were heat sealed on one edge and cut to the seal, forming five 1-inch fingers of film. A Kapton separator (1 mil) was placed between the inoculated surfaces so that the heat treatment would not fuse the contaminated surfaces together. Fusion would have occluded the test organisms and prevented recovery of survivors.

Dry-heat treatment of packets The Kapton and Teflon packets were heated by placing them between two aluminum blocks. The blocks (8 inches (20.3 cm) wide by 12 inches (30.4 cm) long by 1 inch (2.5 cm) deep) were hinged on one end so that they could be rapidly opened and closed. The packet containing the inoculated film was pressed firmly between the blocks for the time interval of the heat treatment. The packets were cooled instantaneously by placing them between an identical set of cold (0 C) aluminum blocks. The heated

blocks were maintained at the desired temperature by holding them in a forced-draft, hot-air oven (Hotpack Corp., Philadelphia, Pa.). The temperature of the blocks was continuously monitored by copper-constantan thermocouples attached to the blocks and to a multipoint recorder (Minneapolis-Honeywell, Philadelphia, Pa.). Each heat treatment was timed from the closing to the opening of the heated blocks. The come-up time for the Kapton packets was less than 1 sec, whereas the come-up for the Teflon packet due to its thickness was approximately 3 sec.

Subculture of heat-treated squares of film Samples (100) were heated for each time-temperature designation. All subculturing techniques were performed in a vertical laminar room (20 feet (616 cm) wide by 20 feet (616 cm) long by 10 feet (308 cm) high) with personnel gowned in sterile garments completely covering their bodies including a face mask over the mouth and nose. All materials were wiped with disinfectant before being admitted to the clean room. Each heat-treated piece of film was transferred with sterile forceps (using a new pair for each transfer) into the tubes containing the subculture medium and incubated at 32 C for 3 weeks.

Analysis of data D values (in this case, seconds required at a given heating temperature to reduce the spore population 10 fold) were calculated by the method of Stumbo as described by Schmidt ( ) and are given by the formula: $D=(U \text{ or } t)/(\log A - \log B)$, where A is the total number of samples heated multiplied by the number of spores per sample; B is calculated assuming one surviving spore per container when less than the total number of containers showed survival; U or t is exposure time at a given temperature.

The z values were determined from the plot of the phantom thermal death curve or thermal resistance curve and from the regression coefficient derived from the statistical analysis. The z value can be defined as the number of degrees fahrenheit for the death time curve to pass over 1 log-cycle. The temperature coefficient for the change in rate of destruction with temperature can also be expressed by the Q_{10} value with the relationship $x = 18 \log Q_{10}$ for degrees F and $z = (0.555 \times 18)/\log Q_{10}$ for degrees C. F value (F_o equals sterilization time in minutes with respect to a given organism and has been traditionally given at 250 F) can be interconverted from D values by using the following relationship: $F = D (\log A + 2)$. The value of log A is from Stumbo's equation. The initial population then is reduced to 1 during an exposure to heat at a given temperature for the time period of D x number of logs of the initial population; after which the area of probability of a survivor is entered. Activation energy can be calculated from the simplified Arrhenius equation shown by Schmidt (15) as follows:

$$E = 4.58 (T_2 \times T_1)/(T_2 - T_1) \times \log k_2/k_1$$

where T is absolute temperature, k is the reaction rate constant and was calculated from D by $k = 2.3/D$, and 4.58 is a constant. Pflug and Schmidt ( ) point out that calculations of activation energies perhaps have more applicability with dry heat than with wet heat.

Statistical analysis A regression analysis of the data and the least-squares regression line were calculated by using a program on an IBM 360 computer.

Results and Discussions

Table 1 presents the D values obtained for C. sporogenes spores heated on both Kapton and Teflon film at various temperatures. The regression lines indicating the z values are shown in Figs. 3-4.

The relationship of a_w of the spores themselves and the relative humidity of the environment surrounding the spores during heat treatment was discussed in our previous paper (Bruch and Smith,) dealing with Bacillus subtilis var niger. It was pointed out that the differential permeability or impermeability of the films to water vapor may have influenced the resistance of the spores on a specific film. The large z value for spores on teflon film can be partially explained by the fact that teflon film is impermeable to water vapor coupled with the increased ratio of surface area to mass (Quesnel et al.), contributed by the thicker teflon packet. The values shown for spores on Kapton film probably represents the more accurate results for the dry heat resistance of the spores. Since the results were to be practically applied to the heat sealing of films for sterile entry techniques, the teflon work was also performed. This was done to simulate a seam to be actually sealed using film of a thickness which could be feasibly utilized.

F calculations were made so as to give a parameter when performing heat sealing techniques to insure a sterile seam. Assuming a load of 10^3 on the film plus the probability of one survivor in 10^3 the F for teflon film at 200 and 210 would be:

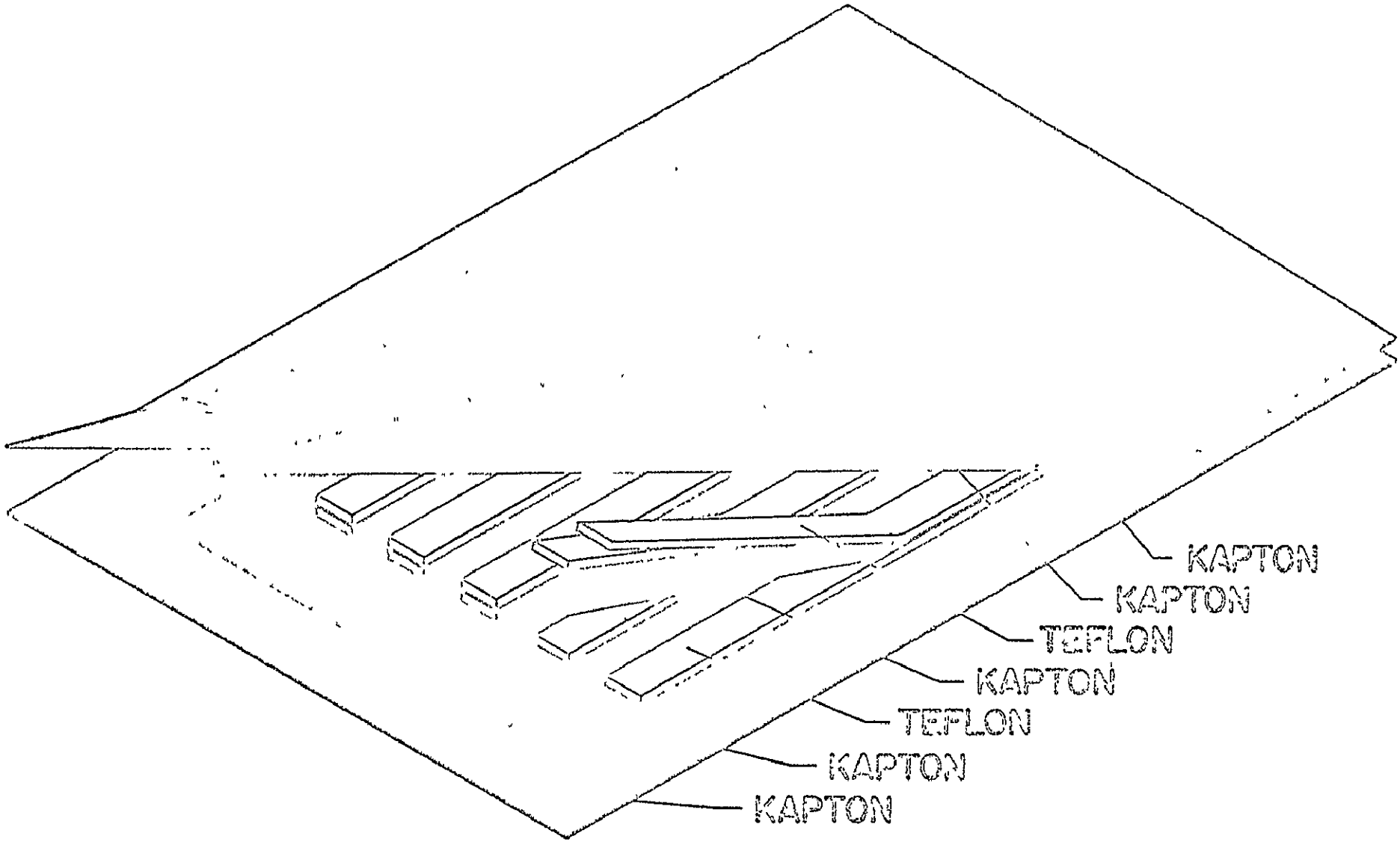
F_{200} = 17.7 sec. : F_{210} = 11.5 sec.

Since B. subtilis var niger has the higher heat resistance, the basic process calculation would be made on that basis if a sterile seam were to be produced.

Table 1. D values for Clostridium sporogenes on Kapton and Teflon film

	D value* in seconds	
Temp F (Temp C)	Teflon	Kapton
300 (149)		6.8
320 (160)		3
338 (170)		1.3
356 (180)	3.3	.68
374 (190)	2.5	
392 (200)	2.0	
410 (210)	1.3	

*Average D values.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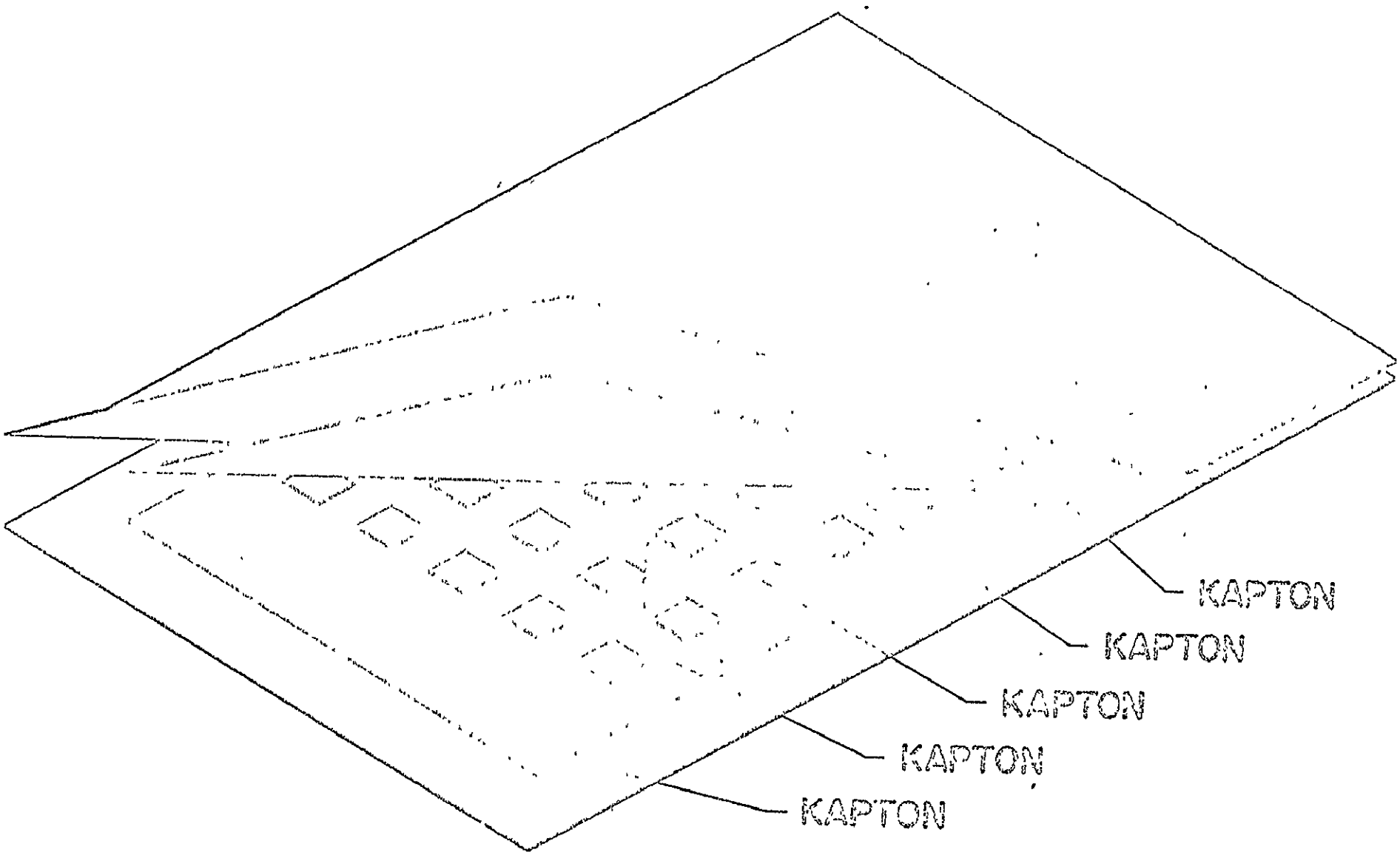
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Reduction of Microbiological Shedding in Clean Rooms

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Operation of Class 100 clean rooms as an adjunct to microbiological research and as an environment for particle clean and/or sterile assembly required that studies be conducted to find means for reducing the biological burden contributed by clean room personnel. Studies were conducted using some newly designed clean room clothing made from dacron, stretch nylon, and a proprietary, tightly woven, cotton material (Barbee). These garments were compared for their ability to reduce by containment the number of microorganisms normally shed from the human skin. Two types of shedding chambers were fabricated for this investigation. The HEPA-filtered air (40 cfm) in the Class 100 clean room was used to flush the shed microorganisms into the Litton High Volume Air Sampler for collection and enumeration. Some ancillary factors which contribute to or reduce microbiological shedding are reported.

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CHAPTER 32

Reduction of Microbiological Shedding in Clean Rooms

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Operation of Class 100 clean rooms as an adjunct to microbiological research and as an environment for particle clean and/or sterile assembly required that studies be conducted to find means for reducing the biological burden contributed by clean room personnel Studies were conducted using some newly designed clean room clothing made from dacron, stretch nylon, and a proprietary, tightly woven, cotton material (Barbie) These garments were compared for their ability to reduce by containment the number of microorganisms normally shed from the human skin Two types of shedding chambers were fabricated for this investigation The HEPA-filtered air (40 cfm) in the Class 100 clean room was used to flush the shed microorganisms into the Litton High Volume Air Sampler for collection and enumeration Some ancillary factors which contribute to or reduce microbiological shedding are reported

Much attention is now being focused on requirements for the maintenance of extremely clean environments These clean environments are being extensively utilized in the spacecraft and electronic industries, as well as in current medical research and practice. These extremely clean areas, i e , laminar flow clean rooms, surgical areas, pharmaceutical assembly lines, and life islands are maintained in their clean state by the flow of air through highly efficient filters This air is filtered to near particle-free state by high efficiency particulate air filters (HEPA) The air forced through these filters forms a flowing laminar curtain of air which sweeps away generated particles to maintain these environments in a nearly particle-free state Presently, the most efficient of the clean environments, the Class 100 clean room, maintains a curtain of nonturbulent, flowing air containing less than 100 particles, 0 5 μ or less, per cubic foot. Addition of people to these areas always raises the viable and nonviable particulate level The manner in which the people are clothed plays an important role in the maintenance of these extremely clean conditions.

The concept of reducing the dissemination of infectious particles through the use of sterile gowns and other encapsulating garments first found application in surgical practices It was found that the conventional clothing worn by personnel in the operating theater did not prevent the liberation of particle-born bacteria from the body and that special encapsulating garments were necessary to significantly reduce the numbers of viable particles disseminated (Dugnid and Wallace, 1948) This dispersal of desquamated particles of skin, referred to as scale, is one of the prime sources of particulate matter contributed by clean room personnel In biological or medical application of clean room techniques, it is necessary that the dispersal of scale, bearing viable microorganisms, be controlled and held to an absolute minimum The mean diameter of airborne scale particles carrying microorganisms such as staphylococci was shown by Noble (1961) to be 13 5 μ Bernard et al (1965a) demonstrated that suits fabricated from impermeable substances, such as polyethylene, would virtually eliminate the shed-

ding of skin bacteria into the environment Suits of closely woven cotton fabric with an average pore size of 10 μ also substantially reduced the liberation of skin bacteria by the containment of the skin particles

This investigation was undertaken to evaluate and possibly improve the type of garments presently being used for maximum particle containment and to study other factors which may influence the shedding or containment

Materials and Methods

All shedding experiments were conducted in a Class 100 vertical down-flow clean room* which was provided with an air source essentially free from microbiological contamination The room was operated at 68-70 F with a relative humidity of 45-50%

* Federal Standard No 209a, 1966 Clean Room Work Station Requirements, Controlled Environments

FIG 1. Aluminum shedding chamber connected to the Litton High Volume Air Sampler.

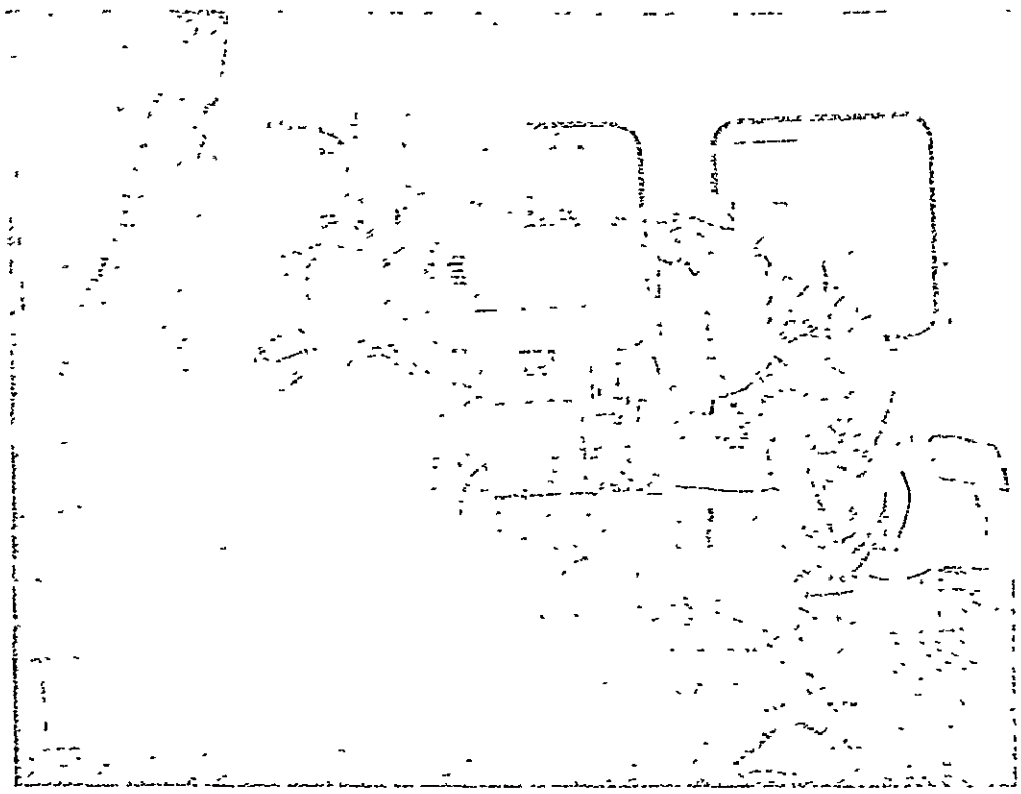

FIG. 2 Collection of shed microorganisms using the small plexiglass shedding chamber and Litton High Volume Air Sampler

An air-ionizing device° was installed under a portion of the HEPA filter bank to supply a flow of positive and negative ions. These ions neutralized any static charges which could cause the shed particles to be attracted to the shedding chambers, hoses, or air sampler. This clean, ionized air was pulled through the collection chambers by a Litton High Volume Air Sampler°° at a rate of 40 ft³/min. The Litton High Volume Air Sampler is a high voltage device which creates a corona discharge capable of efficiently precipitating small particles carried in the air stream. The precipitated particles were continuously collected in half-strength, Trypticase Soy Broth (BBL) and flushed into an ice-cooled collection flask. Two different shedding chambers were employed in this study. One was a cylindrical, welded aluminum chamber 7 × 3 ft (49.45 ft³) which comfortably held a standing subject (Fig. 1). A 4-inch opening in the top of the chamber allowed the entrance of air from the ceiling of HEPA filters. Another 4-inch opening at the bottom of one side of the chamber provided an exit for air being drawn into the sampler. A close-fitting door sealed the chamber and prevented any possible air leaks. The floor of the chamber held a 3-ft stainless steel pan which collected any of the heavier or larger particles not swept into the air sampler. A smaller, plexiglass chamber (18 × 12 × 12 inches), which fit over a subject's head, was used to collect particles dispersed from the head and neck region (Fig. 2). To enter the chamber the shedder's head was placed through a snug-fitting, rubber diaphram in the

° Statitrol Corp., Denver, Colo.
°° Litton Industries, Minneapolis, Minn.

bottom of the chamber This diaphram isolated the head and neck from the rest of the body. The chamber was then supported by the shoulders during the shedding period A sterile, 4-inch flexible rubber hose carried clean air from the ceiling, HEPA filter bank to the chamber entrance A 4-inch opening in the front of the chamber allowed the entering air to strike the subject's face Another 4-inch opening in the rear of the chamber allowed the exiting air to flow into the high volume sampler

Collection and Culture Methods

The total number of viable, aerobic microorganisms collected from the aluminum chamber during a 30-min shedding period was obtained by assaying the collection broths from the 30-min shedding period, the ensuing 30-min flush period, and by culturing the 3-ft removable stainless steel pan (Fig 3) which covered the chamber floor Trypticase Soy Agar was utilized for all assay procedures Five replicate plates were used for all dilutions assayed Experiments in which low numbers of microorganisms were recovered required the plating of the total volume of the collected broth samples The large stainless steel floor plate, which was the bottom portion of a giant, stainless steel, petri plate, was poured with a thin layer of agar and covered with its complementary, stainless steel lid All samples were incubated 48 hours at 32 C and counted. Similarly, samples from the small plexiglass chamber, collected during the 30-min shedding period and the following 15-min flush period, were cultured A chamber and air-sampler sterility control were taken before each shedding The controls consisted of

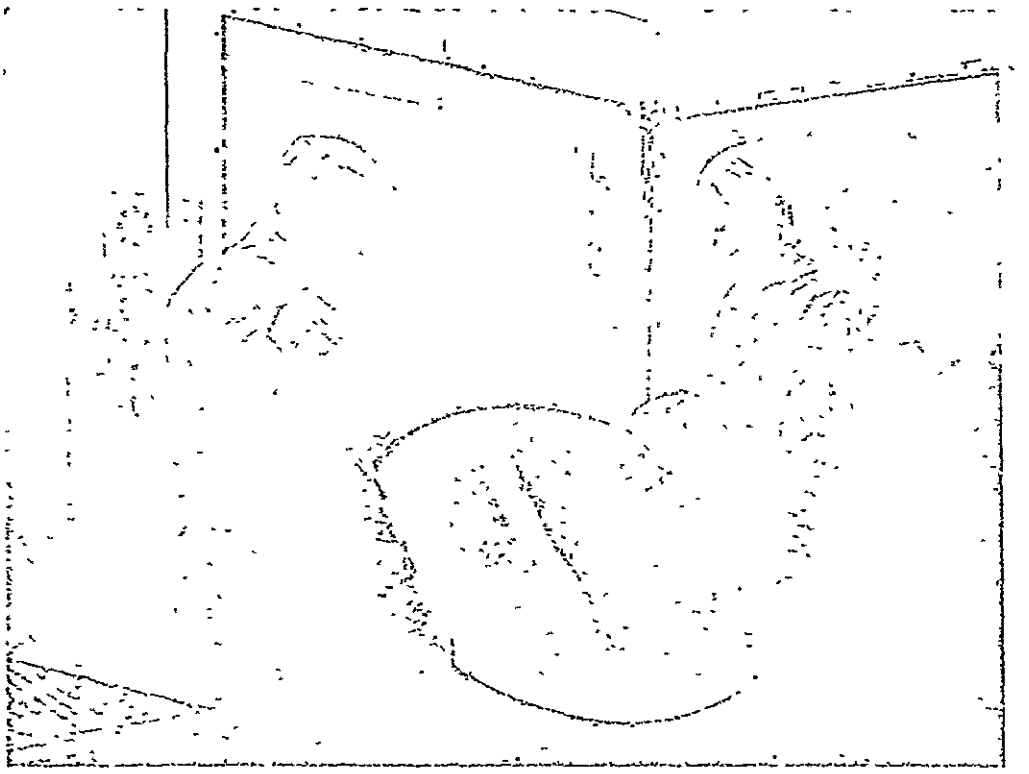

FIG. 3 Clean room technicians hold the agar poured stainless steel floor pan from the shedding chamber

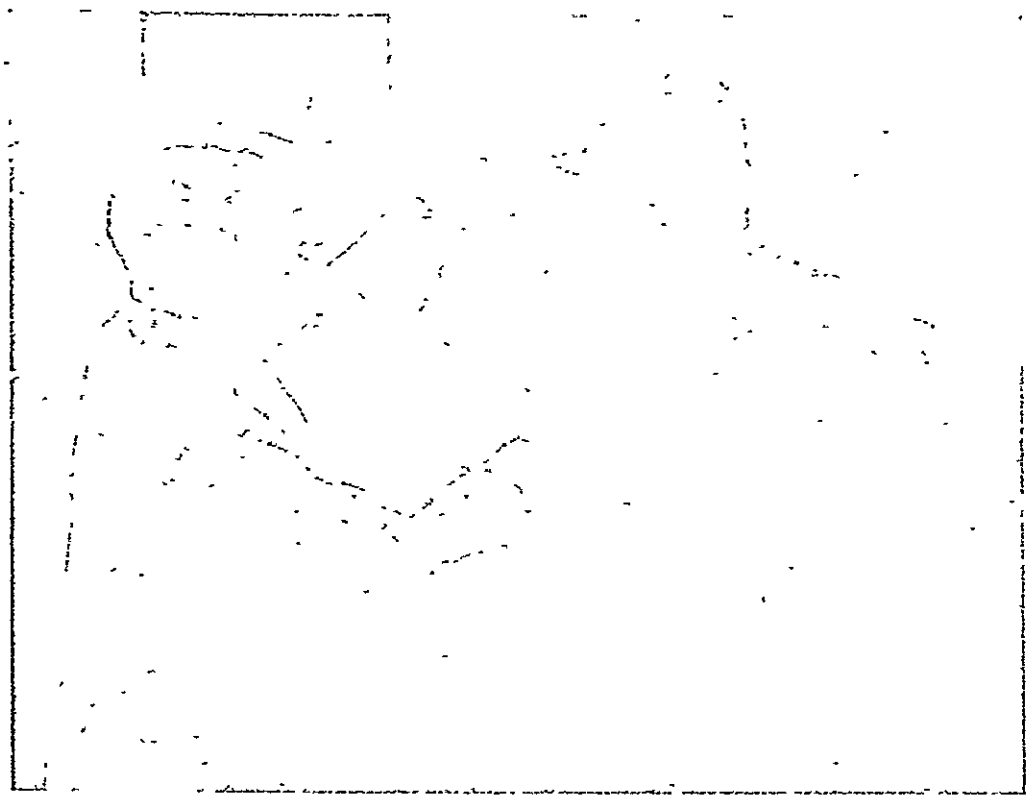

FIG. 4 Left, a two piece dacron suit Right, a two piece cotton suit with the hood outside to show configuration of hood

broths collected from a 15-min flush of the sampler alone and a 15-min flush of the sampler and chamber The broths were each filtered through a 0 22 μ membrane filter The filters were also incubated at 32 C for 48 hours on Trypticase Soy Agar plates The air-sampler control demonstrated the sterility of the air sampler, while the second control demonstrated the cleanliness of the shedding chamber. Controls were run before each shedding experiment

Clean Room Clothing

The clothing worn in these evaluations consisted of two types. one, the clean room coverall or bunny suit with hood and bootees, the other, a nylon body stocking and hood The bunny suits were modified slightly from the conventional garment Instead of separate bootees, which are normally tied around and over the legs of the suit, the bootees were sewn onto the legs making each suit two pieces instead of four Suits made of the conventional herringbone-twill (100% dacron polyester, continuous filament, high tenacity, 220 denier yarn in both directions) fabric° or a tightly woven, cotton fabric°° were worn in the study (Fig. 4) The nylon body stocking was a leotard† with feet and a long-sleeved, turtle-neck shirt and a hood made from the same stretch material The hood covered the head and neck entirely, except for the eye open-

° Dacron Fabric, Angelica Uniform Co , St Louis, Mo.
°° Barbac, Angelica Uniform Co , St Louis, Mo
† Danskin Corp., New York, N Y

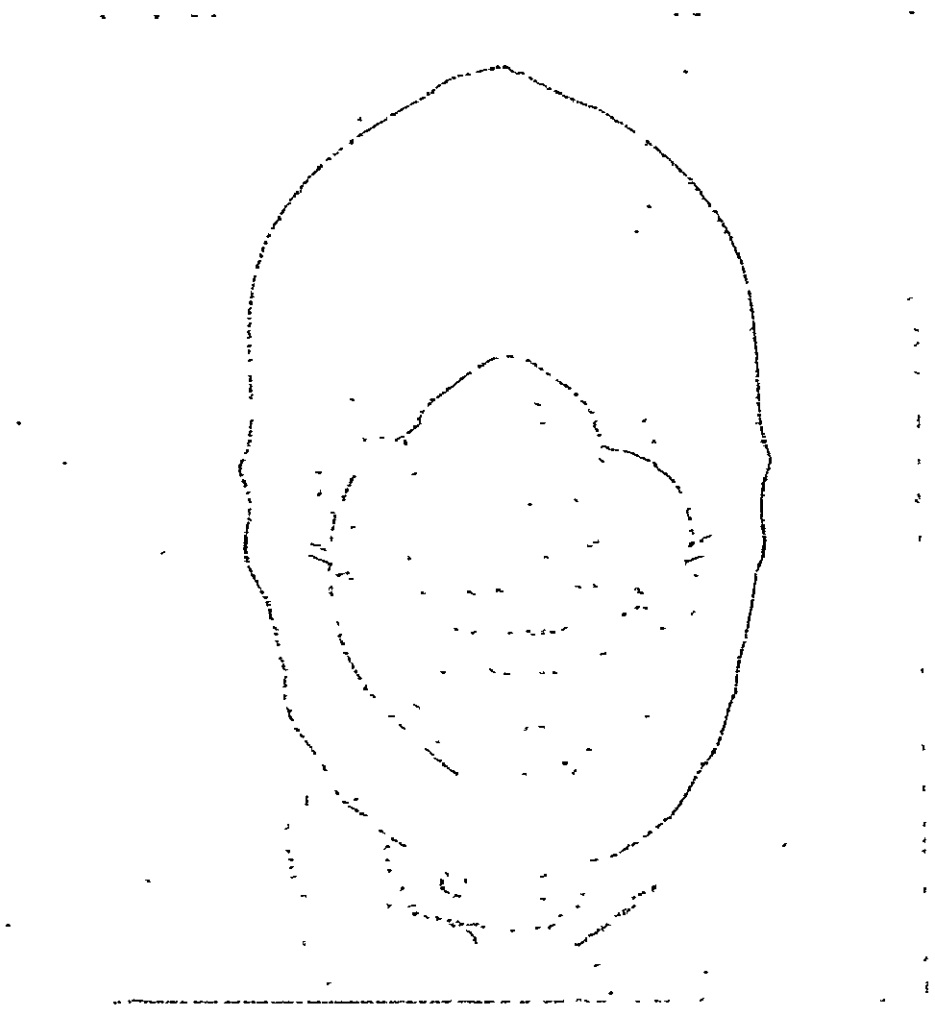

FIG. 5 Stretch nylon hood and disposable surgical mask which were worn under the dacron or nylon hoods

ings A piece of ⅛-inch polyurethane foam (80-pore) was sewn inside, as a mask, to filter particles generated by the nasopharangeal areas Another stretch-nylon hood design was also evaluated in conjunction with the bunny suits (Fig 5) Like the other, it entirely covered the head and neck, but had one, larger opening which exposed the subject's eyes, nose, and mouth This hood and the conventional dacron or cotton clean-room hoods were worn with a disposable surgical mask * This mask tightly covered the face from the bridge of the nose to the chin Latex surgeons' gloves were worn in all shedding experiments where clothing was worn

All clothing, gloves, and masks were wrapped and sterilized before use. Dressing and other preparations for entrance into the clean room and shedding tanks were carried out in one of three clean shower and dressing areas adjacent to the clean room Sterile shoe covers were placed over the feet of the shedding suits to prevent microbiological contamination The slipper-like shoe covers were carefully removed upon entering the shedding chamber.

* Aseptex Surgical Mask, 3M Company, Minneapolis, Minn.

Cosmetics and Toiletries

A germicidal soap° containing 0.75% hexachlorophene and 0.75% trichlorocarbonalide, a shampoo containing hexachlorophene,°° skin conditioner† containing isopropylmyristate, and sterile vaseline were utilized in selected experiments

Results and Discussion

In order to evaluate and improve the reduction and containment of shed particles, it was necessary to find some subjects who routinely shed high numbers of microorganisms. In order to screen for possible subjects, the foreheads and cheek angles of approximately 100 persons were sampled with Rodac plates‡ containing Trypticase Soy Agar (BBL). This screening procedure allowed us to find the few subjects who would consistently have high numbers of organisms on their skin. These individuals showing high numbers of microorganisms on the skin were used throughout the study to provide difficult situations (worst case) for containment and control. To obtain some order of magnitude for the number of particles an individual might shed, five subjects, including those with highly contaminated skins, refrained from bathing for 24 hours prior to shedding (Table 1). After the initial 30-min shedding period, the subjects showered with germicidal soap, thoroughly washing with special attention to hair, face, and perianal area. The perianal area is reportedly one of the most significant sources of shed microorganisms (Bethune et al., 1965). A 30-min sampling period following the shower caused more than a tenfold reduction in the number of recoverable organisms. This finding is in disagreement with Bernard et al. (1965b), who found an immediate increase after showering. However, in their study no germicidal soap was used for bathing.

The effects of containment on the shedding of viable particles was significant (Table 2). All subjects refrained from bathing for 24 hours before shedding naked for 30 min. This was immediately followed by another 30-min shedding period while the subjects were dressed in the sterile, cotton bunny suit. No underwear was worn under the suits during the shedding period. The cotton suits reduced the number of microorganisms shed by as much as a thousandfold. The tightly-woven, water-repellent, cotton suits were chosen for this evaluation because this fabric has been shown previously to be an effective barrier to the transmission of fluids and skin particles, while still permitting the transfer of water vapor necessary for the comfort of the wearer (Bernard et al., 1967). It must be noted that garments made of this particular fabric could find acceptability in applications where biological cleanliness only is of prime importance. However, cotton would not be acceptable where freedom from all types of particles is of equal or greater importance, i.e., space craft and/or electronic assembly areas. Cotton fabrics tend to shed small linters and are less desirable from the viewpoint of total particles than are nonshedding fabrics manufactured from continuous filaments. It should be noted that the shedding experiments (Table 2) were not performed using the air ionizer. The containment data in Table 2 are similar to the data shown in Table 3 where the ionized air was supplied. The effects of ionized air was most obvious when the chamber floor counts were compared. Significantly fewer bacterial colonies were

° Dial Soap, Armour Grocery Products Co., Chicago, Ill.
°° Dial Shampoo, Armour Grocery Products Co., Chicago, Ill.
† Skin-So-Soft, Avon Products Inc., New York, N. Y.
‡ Falcon Plastics, Los Angeles, Calif.

TABLE 1 *Comparison of naked shedding before and after showering*

Subject	Microorganisms Collected from 30 Min of Shedding	
	Before Showering	After Showering
A	6,598,000	714,000
B	3,125,000	151,570
C	313,805	82,143
D	1,488,000	387,660
E	2,513,000	179,871

TABLE 2. *Effect of containment on shedding naked compared to a tightly woven cotton suit*

Subject	Microorganisms Collected from 30 Min of Shedding	
	Naked	Cotton Suit[a]
1.	92,910	252
2	478,242	860
3.	2,147,000	1,692
4.	58,038	688
5.	39,971	2,366
6.	361,500	2,738

[a] Barbae, Angelica Uniform Company, St Louis, Mo

TABLE 3 *Comparison of shedding wearing dacron or cotton suits*

Subject	Microorganisms Collected from 30 Min of Shedding	
	Dacron Suit	Cotton Suit
A	1,981	1,800
	1,496	1,914
B	12,986	5,024
C	333	589
D	553	1,468
E	1,979	1,622
F	347	1,697

counted on the floor plates when the ionizer was used. This indicated that the neutralization of the static charge was effective in keeping more particles suspended in the air until drawn through the sampler

Table 3 compares the containment effects of the dacron and cotton suits. No attempt was made to control the personal hygiene regimen of the subjects Each wore his regular underwear The suits were worn in a random order so that no particular fabric was always worn first In most instances, the dacron suit demonstrated equal or slightly better containment of shed microorganisms than the tightly woven cotton suit These data compared favorably with the data of Riemensnider (1967) who used a total collection technique .The total number of recoverable microorganisms for a given shedding time was found to be slightly lower than that shown by Riemensnider (1967). However, he did not use surgical masks in his evaluation of the dacron bunny suit

Subjects A, B, and E were consistently high shedders (naked) who released in excess of 2 million particles in 30 min. These three subjects had been selected from the screening of the 100 individuals.

TABLE 4 *Comparison of reduction of shedding by nylon body stocking and dacron suit*

Subject	Microorganisms Collected from 30 Min of Shedding		
	Naked	Nylon Body Stocking	Body Stocking Plus Dacron Suit
A	—	60,883	3,774
	—	45,091	387
B	—	65,480	485
	—	33,095	761
A	2,505,000	14,900	—
B	5,695,860	7,600	—

In an attempt to further reduce and prevent the shedding of microorganisms, subjects A and B were chosen for the study (Table 4). No particular hygiene regimen was used. Both subjects shed naked and wearing the nylon body stocking which included the hood-mask combination. The body stocking significantly reduced the number of shed microorganisms. Other trials in which the body stocking was worn separately and in combination with the dacron suit proved that by itself it did not equal the containment properties of either the dacron or cotton bunny suit. However, in most cases the combination of the body stocking and bunny suit was superior to either the dacron or cotton suit worn singly (Table 3).

Shedding from neck and head areas was investigated because it was the only area where the skin was not completely covered. Naked areas of the face not covered by the hood or surgical mask were thought to be a probable source of shed microorganisms when the subjects were dressed in the bunny suits. The small plexiglass shedding chamber was used to collect microorganisms shed from the region of the neck and head. Table 5 shows the numbers of microorganisms recovered in 30 min when the subjects wore dacron hoods and surgical masks or cotton hoods and surgical masks in the small chamber. The total organisms shed in a 30-min period in the large aluminum chamber by the suited subjects were in the same range as those shed in the small plexiglass chamber. This was indicative that most of the shed microorganisms were coming from the uncovered areas around the eyes and the mouth. This opening allowed the escape of particles which were probably generated by the movement of the head against the hood or particles shed from the eyebrow or other areas not covered by the surgical mask.

Because the nylon body stocking when worn with the dacron suit decreased the number of microorganisms shed (Table 4), a less restrictive, open-faced, nylon hood was chosen to be worn under the conventional dacron and cotton hoods. The effect of

TABLE 5 *Effect of containment on shedding: comparison of head and neck region while wearing dacron or cotton hoods*

Subject	Microorganisms Collected from 30 Min of Shedding	
	Dacron Hood	Cotton Hood
A	1,994	1,715
B	491	701
C	24	299
D	3,285	933
E	87	66
F	29	40

TABLE 6. *Effect of containment on shedding comparisons of shedding wearing the nylon hood plus the dacron or cotton hood*

Subject	Microorganisms Collected from 30 Min of Shedding	
	Nylon Plus Dacron Hoods	Nylon Plus Cotton Hoods
A	233	197
B	838	852
F	66	87

TABLE 7. *Comparison of shedding using skin cosmetics with bunny suits and nylon hoods*

	Microorganisms Collected from 30 Min of Shedding								
	Head and Neck				Entire Body				
	Shower and Skin Conditioner		Shower and Vaseline		Shower and Skin Conditioner		Shower and Vaseline		
Subject	Dacron Suit	Cotton Suit	Dacron Suit	Cotton Suit	Dacron Suit	Cotton Suit	Dacron Suit	Cotton Suit	Naked
A	103	190	24	48	580	391	136	561	6,598,000
B	713	—	96	—	341	510	122	604	5,695,000
F	39	65	20	40	24	51	38	72	39,971
G	16	40	20	161	136	756	95	136	35,382
H	205	71	69	73	75	256	550	130	1,457,680

wearing the tight-fitting nylon hood under the other hoods is shown in Table 6. Subjects A and B were again the worst case individuals and subject F was a moderate shedder. This combination of hoods was found to be reasonably comfortable, in addition to being effective in further reducing the number of viable particles shed.

Since the addition of the tight-fitting nylon hood improved the containment of skin microorganisms and showering with germicidal soap also produced a reduction effect (Table 1), a combination of the two seemed a logical step toward further reduction.

Conditioning of the skin to retard the dispersal of scale by the application of cosmetics was investigated as a method for reducing the shedding rate. Table 7 shows the effect of cosmetics, showering, and maximum containment by garments on the reduction of shed microorganisms. Two distinct types of cosmetics were evaluated. Skin-So-Soft, a skin conditioner containing isopropyl-myristate, was applied in a liberal manner to the perianal area, torso, arms, neck, face and hair, after showering. A thorough hair-wash with a germicidal shampoo was included with the showering procedure. Sterile vaseline was similarly applied after the shower. Both the sterile vaseline and the Skin-So-Soft were applied with sterile gloves. Again subjects A, B, and F were compared. When shedding was measured in both chambers, the combination of showering, sterile vaseline, tight nylon hood, conventional dacron hood and suit showed the best overall containment of microorganisms.

ACKNOWLEDGMENTS

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Improved Method for Pouring Rodac Plates

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Received for publication 18 June 1968

A rapid method for the preparation of quality sterile Roaac plates was devised. A brass plate was fabricated to fit a laminar flow work station. To back of the plate was soldered a continuous copper coil. The plate was placed in the clean bench soil side down and cold tap water circulated through the coil. The water cooled tha plate and acted as heat exchanger. Empty sterile rodac plates were placed on the cold plate and 17 ml. of media (2% agar) was poured into each plate easily forming a meniscus. The agar hardened rapidly maintaining the agar meniscus whichwas crutial for Rodac plate preparation.

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Improved Method for Pouring Rodac Plates

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The Rodac plate (Falcon Plastics, Los Angeles, Calif.) was originally described by Hall and Hartnett (2) for use in direct contact sampling for surface contamination. The Rodac plate has found wide acceptance and use in a variety of areas where sanitation and contamination level are important, particularly in institutional areas, such as hospitals and food production and service facilities (1; R. G. Bond et al., *unpublished data*). The use of Rodac plates for surface sampling can be valuable in the field because of its simplicity, reliability, and transportability, particularly in estimating the bacterial contamination on flat surfaces. Constant use of Rodac plates has been required in the Clean Assembly and Sterilization Laboratory in determining the contamination level and housekeeping reliability in a laminar flow room and adjacent service areas.

This method of preparation is facilitated by utilization of laminar flow conditions. A brass plate measuring 21 by 46 inches (53 × 117 cm) was fabricated to exactly fit a laminar flow bench. A copper coil was firmly soldered onto one side of the plate. Cold water was supplied to the coil by easily detachable, flexible plumbing (Fig. 1).

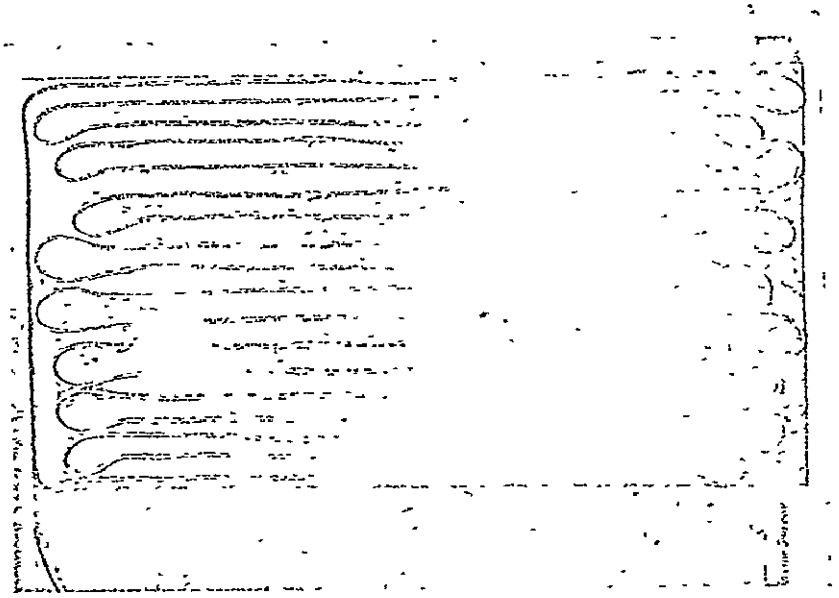

FIG. 1. *View of the bottom of the brass plate, showing the attachment of the copper coil.*

Prior to pouring, the plate was placed onto the work surface of the laminar flow bench and sanitized. Cold tap water was circulated through the copper coil to chill the plate. A measured amount (17 ml, final volume) of the selected agar fortified with 0.5% additional agar (2% total agar) was sterilized in individual test tubes. When tempered to 45 to 47 C, the agar from a single tube was poured carefully into an open Rodac plate placed on the cold surface (Fig. 2). Very rapid solidification enhanced the formation of the meniscus. After solidification, the tops of the dishes were carefully dropped in place. A large number of plates can be prepared quickly and easily by this method.

Use of the laminar flow bench essentially eliminates concern about contamination. An automatic pipetter is commonly used in the preparation of Rodac plates. Prior apportionment, sterilization, and tempering of the agar eliminates the difficulties encountered with automatic pipetting. In addition, this method will allow flexibility in the use of a variety of media at one time. The meniscus of each Rodac plate should be examined, defective ones discarded, and the re-

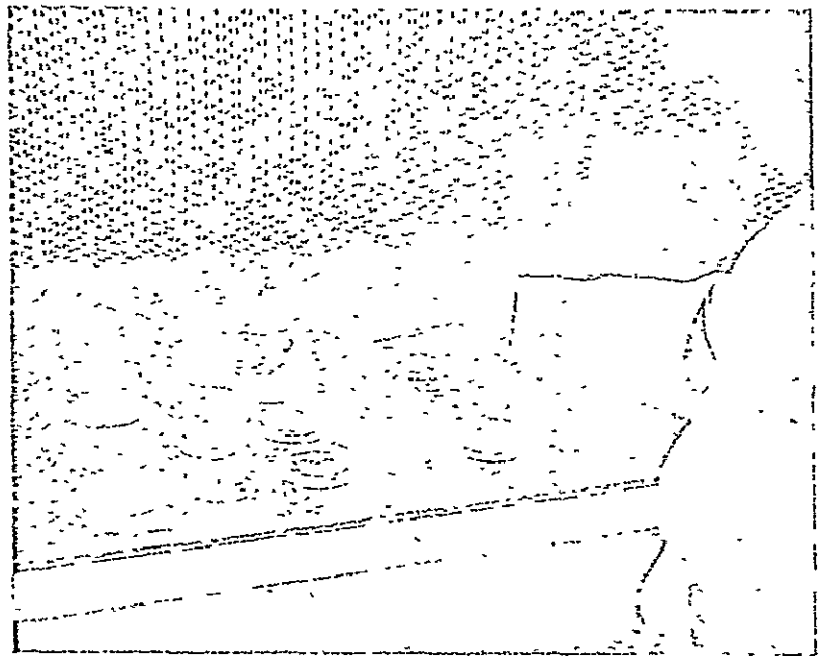

FIG 2 *The brass plate in use in a laminar flow bench during the preparation of Rodac plates*

mainder incubated prior to use to detect any adventitious contamination

This work was supported by NASA/GSFC contract NAS 5-9245

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Microbiological Shedding Study-BISS Undersuit

Clean Assembly and Sterilization Laboratory
Goddard Space Flight Center
Greenbelt, Maryland

A shedding study was run to determine the extent of biological shedding while wearing the BISS undersuit. All shedding experiments were performed in the shedding chamber with the undersuits life support system in operation. Cotton long underwear was worn under the undersuit. White cotton gloves were also worn. No attempt was made to cover the head during shedding. The subject Mr. A. Jessup from the Langley Research Center did not shower before shedding, and took no extra hygenic precautions such as the use of germicidal soap, etc. Sterile shoe covers were worn on the feet to prevent intimate contact with shedding tank floor.

The shedding chamber was placed in the Class 100 downflow clean room under a Statatrol device which provided a flow of ionized air into the chamber. The ionized air aided the flow of shed particles into the Litton high volume air sampler operated at 40 ft^3 per minute. A plastic curtain was placed around the chamber to contain and direct the flow of ionized air around the shedding chamber. The first four collection intervals (15 min. each) corresponded to a program of exercises:

Collection Intervals	Exercises	
#1	Deep knee bends	15/15 min.
#2	Arm reach (both hands)	15/15 min.
#3	Toe touch (both hands)	15/15 min.
#4	Walk in Place (90 steps/min)	5 min.
	Flex hands (both hands)	5 min.
	Walk in Place (90 steps/min)	5 min.

The two flush periods represented the microorganisms remaining in the chamber after the subject had exited. All the microorganisms collected during four sampling periods, two flush periods and the microorganisms remaining on the floor comprised the total sample for a one hour shedding period.

Before any shedding was performed, the BISS undersuit and life support hose were wiped with a mild detergent solution to remove as much dust as possible. Prior to wearing the suit, it was hung in the flowing ionized air with the life support system running at full volume. This was an attempt to flush out or sweep away as much contamination as possible.

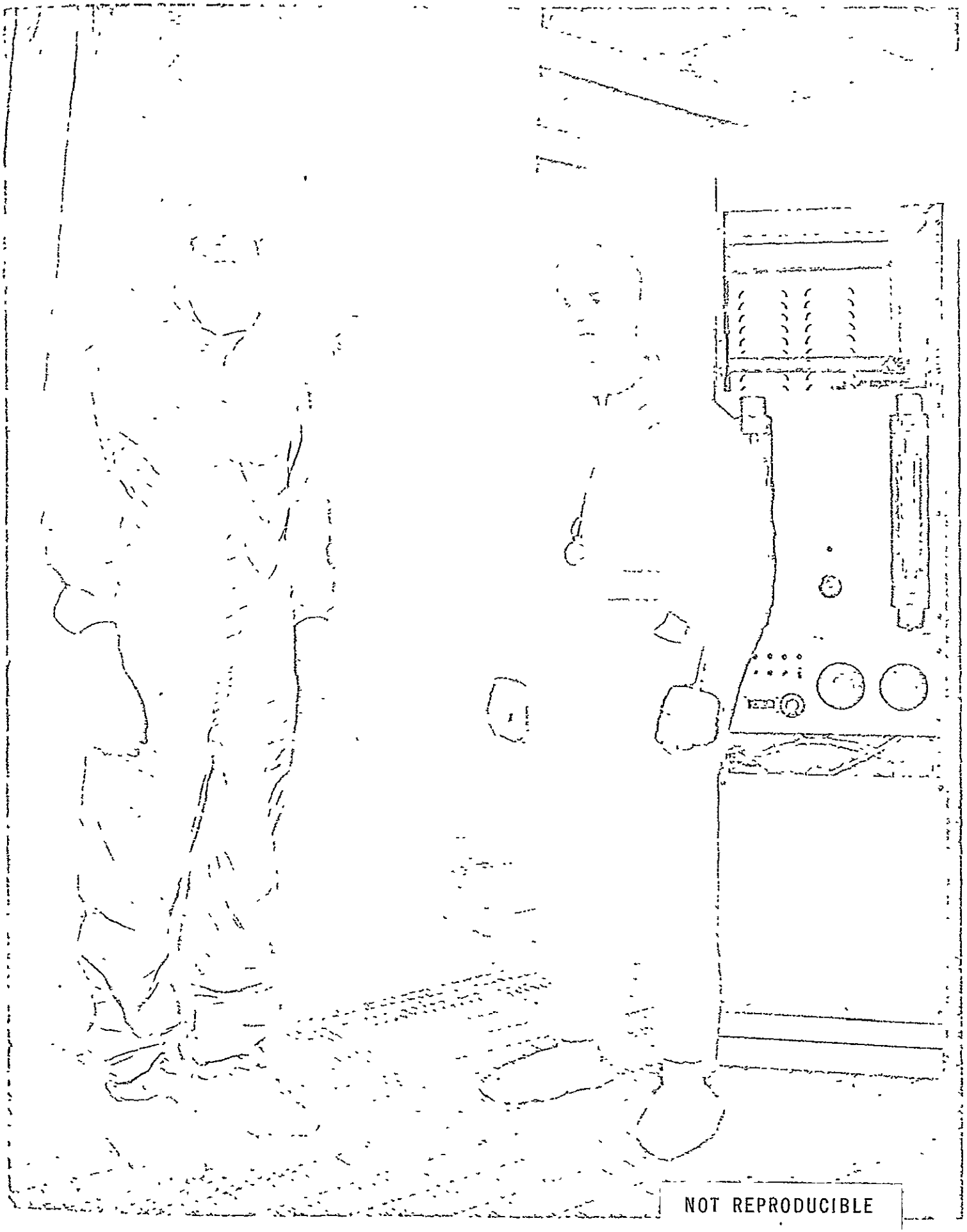
The culture plates from the assays revealed an unusually large number of fungi, many more than one would expect from shedding skin Possible sources of the fungi are the life support system or the undersuit. It is suggested that these sources be checked out before any more shedding experiments are conducted.

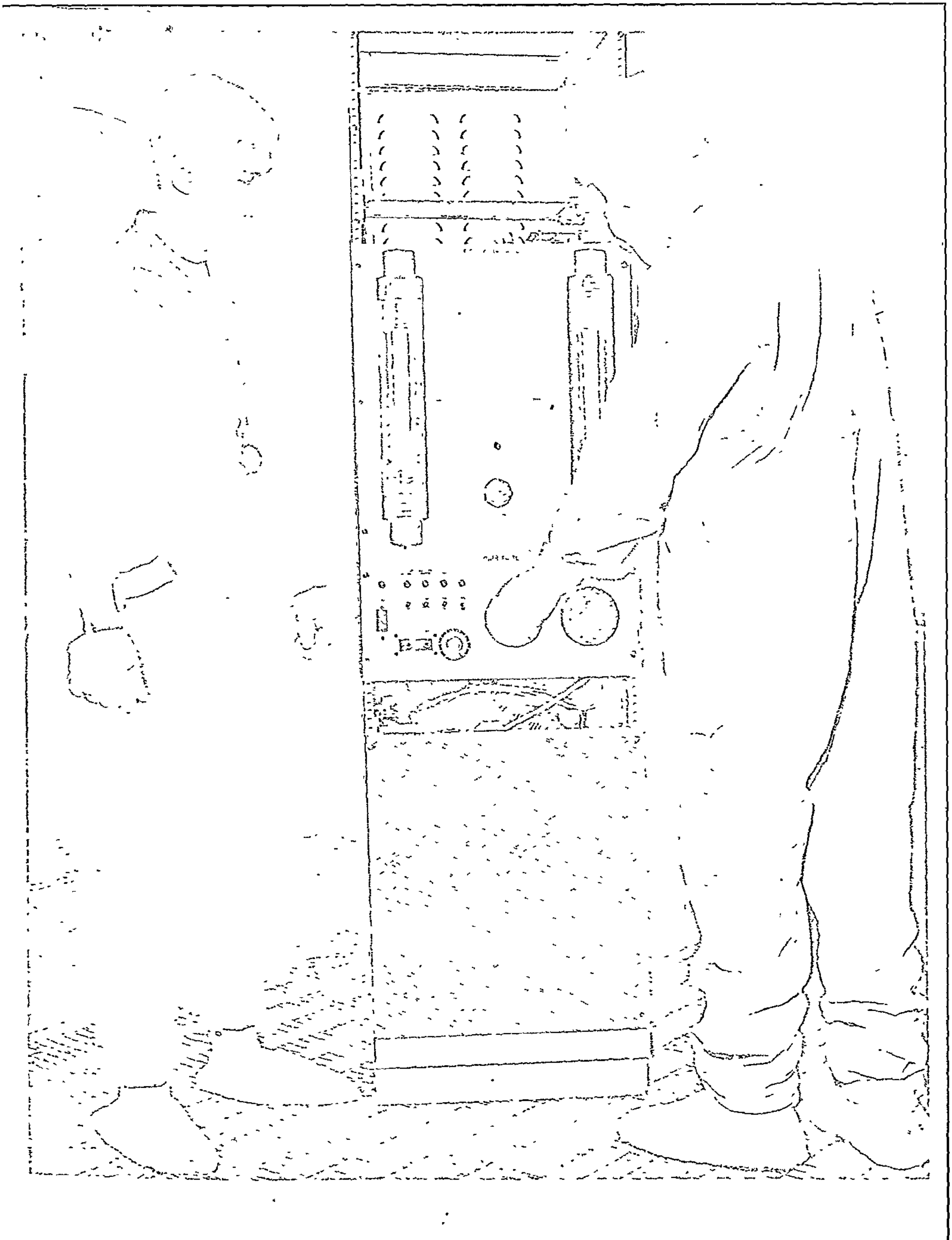
The microbiological data from the five shedding runs are shown in Table I.

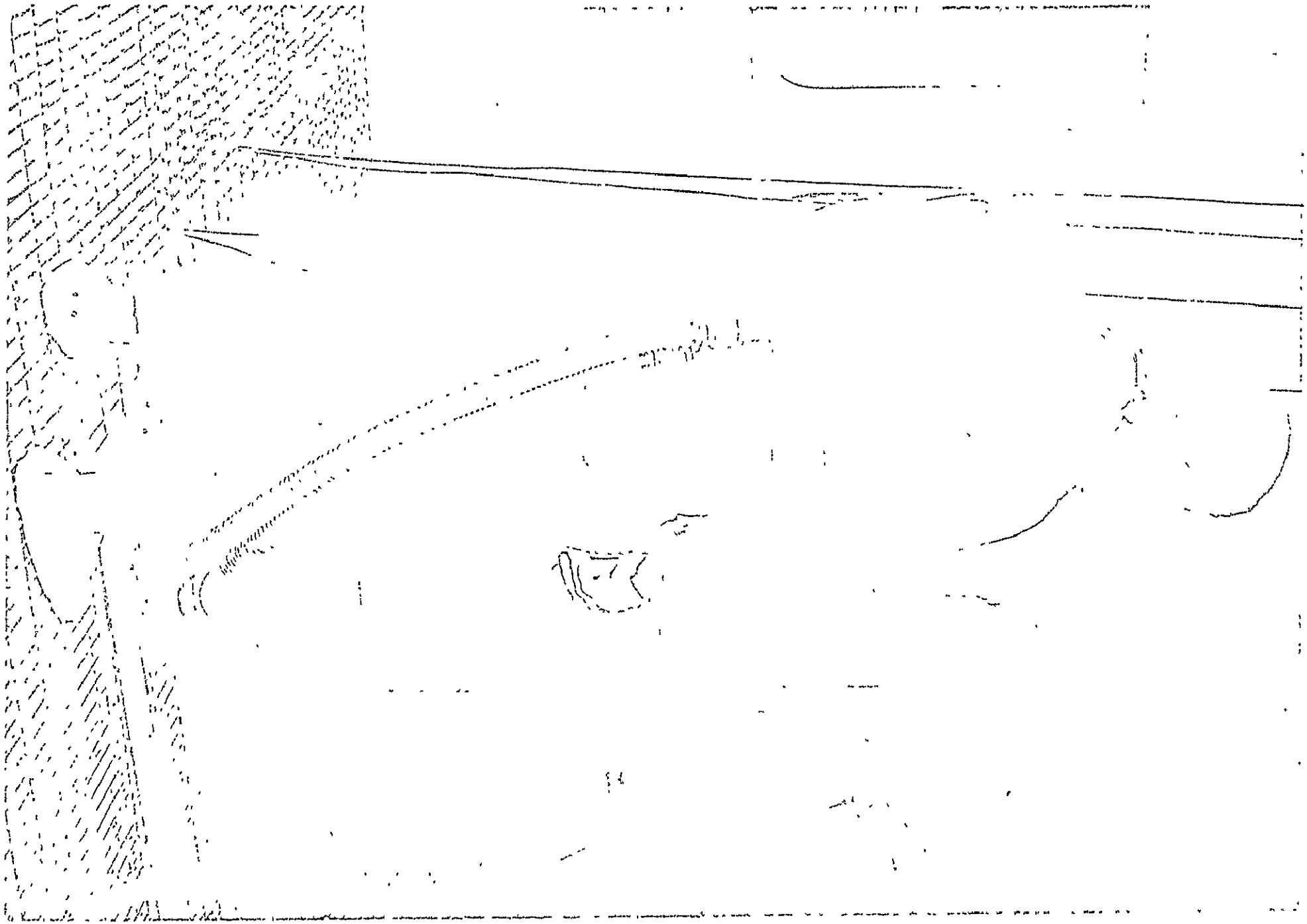
TABLE I

Microbiological Shedding Counts-BISS Undersuit

Sample Intervals (15 Min)	Shedding Runs				
	June 16 PM	June 17 AM	June 17 PM	June 18 AM	June 18 PM
#1	1370	1515	1176	690	3468
#2	1880	376	1087	504	1222
#3	4646	265	1723	478	1001
#4	13290	1060	4240	1086	908
Flush #1	3432	467	970	3475	1379
Flush #2	1228	136	446	1635	466
Chamber Floor Count	823	70	904	585	488
Total	26,669	3889	10,546	8,453	8,932







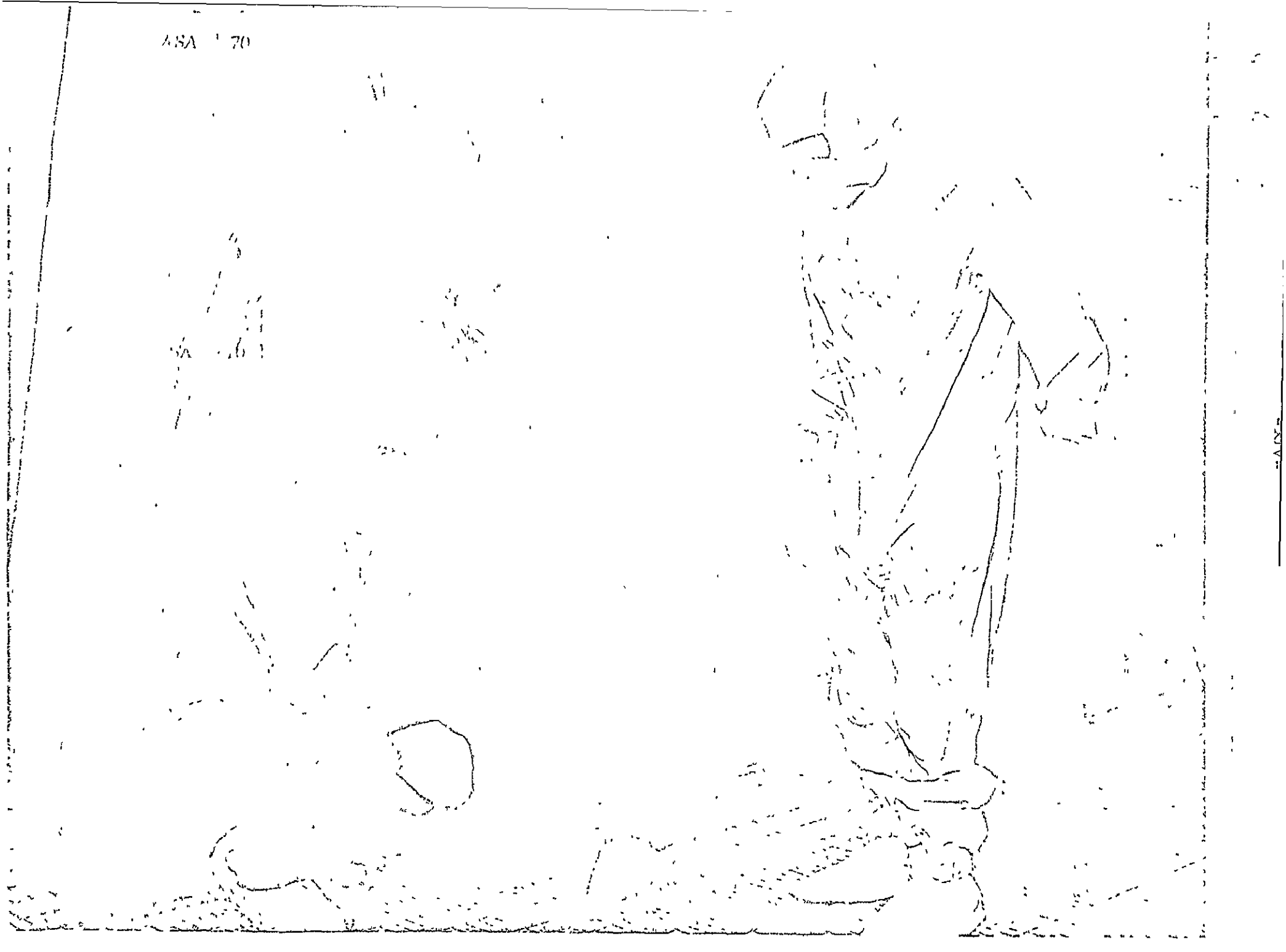
NOT REPRODUCIBLE

FOAM SUIT PHOTOGRAPHS

1. Test of foam hood in Class 100 clean room using shedding chamber for the head and Litton High Volume air sampler.

2. Foam suit compared to standard dacron suit and "Barbac" suit.

NASA G-70- 156



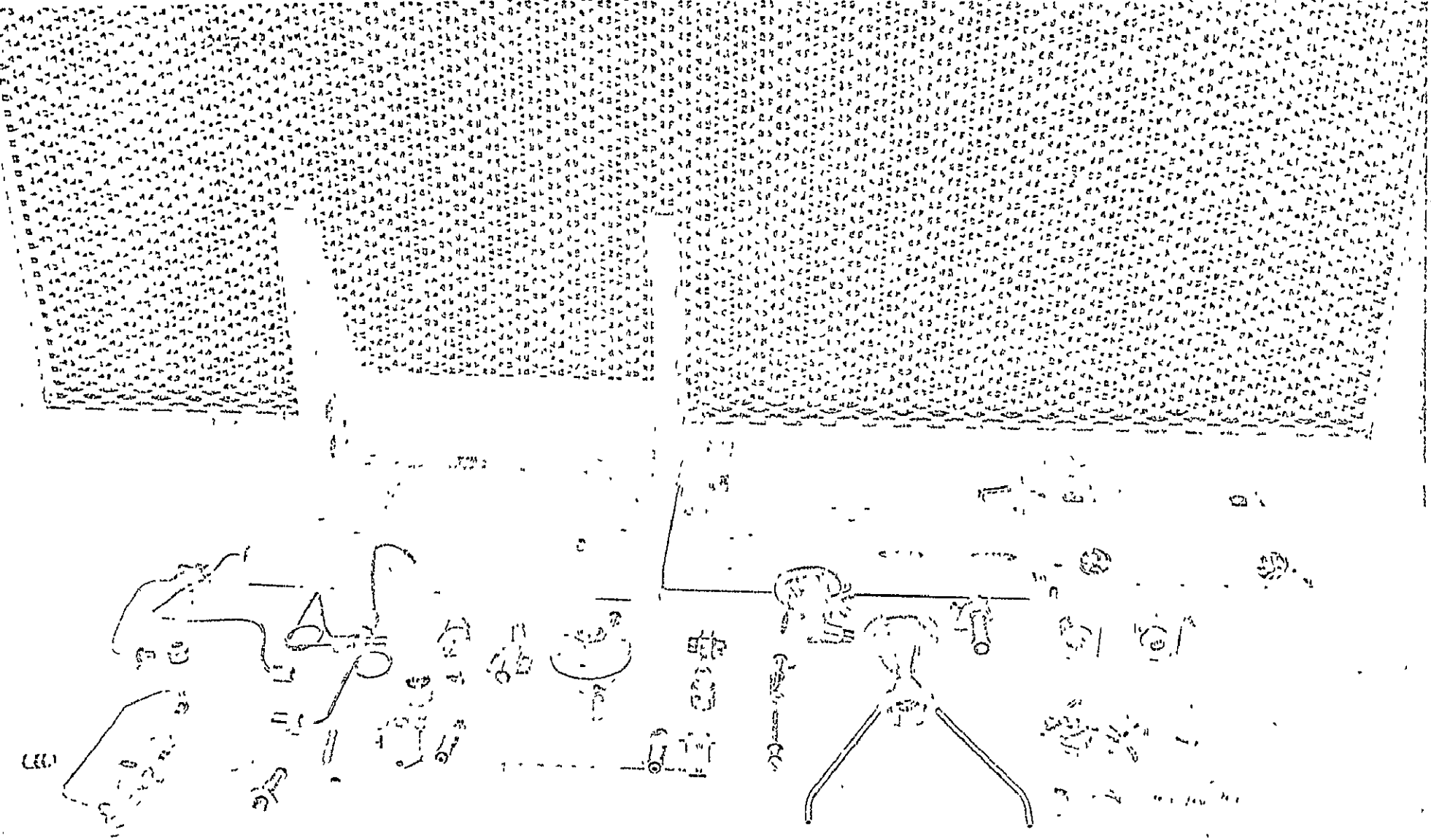
NASA G-70- 224

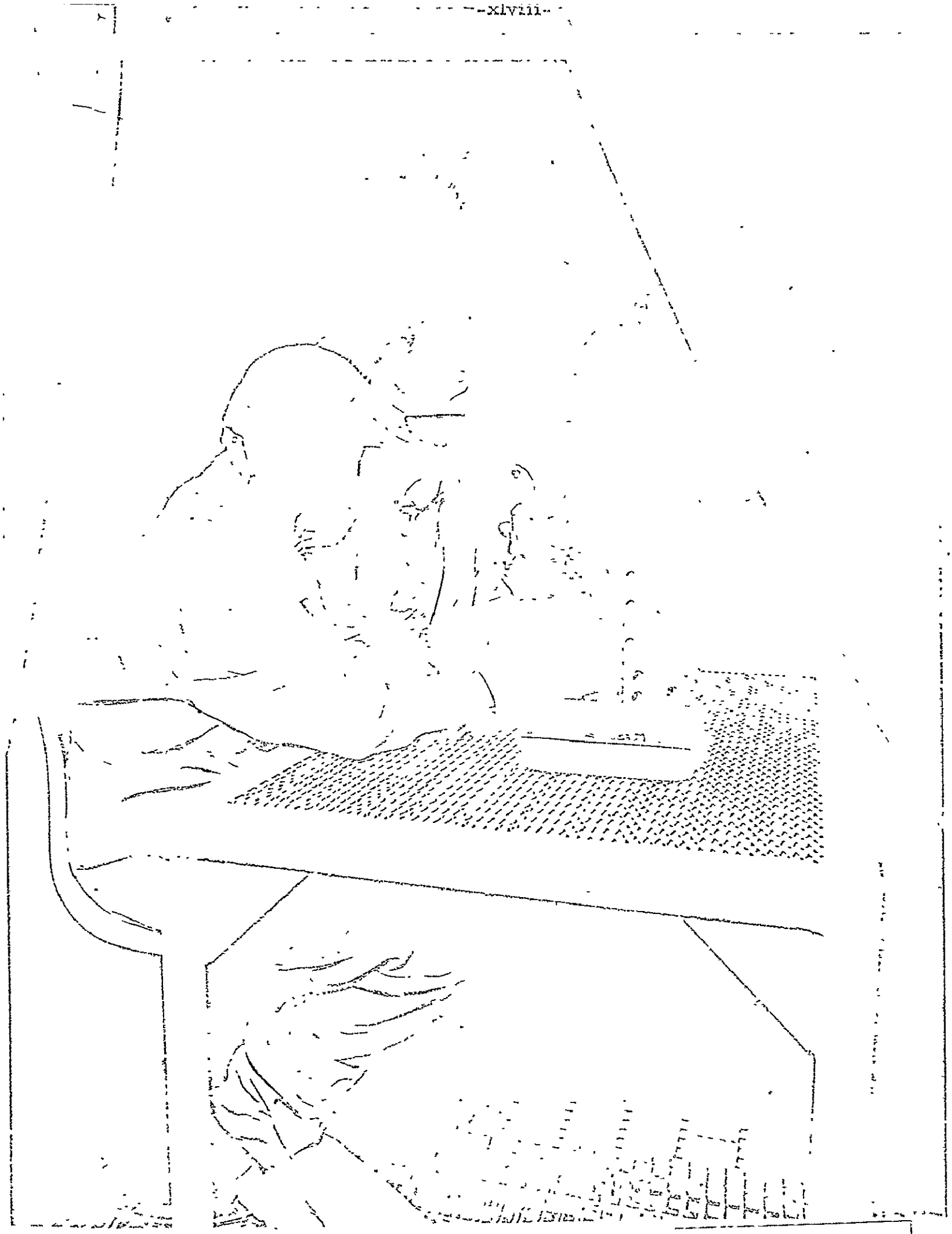
WOLFTRAP PHOTOGRAPHS

1. Parts and pieces of Wolftrap before assembly.

2. Assembly of Wolftrap in Class 100 clean room showing table, curtain and garmented workers.

3. Assembly of Wolftrap in room area outside clean room on a horizontal-flow bench showing garmented workers.



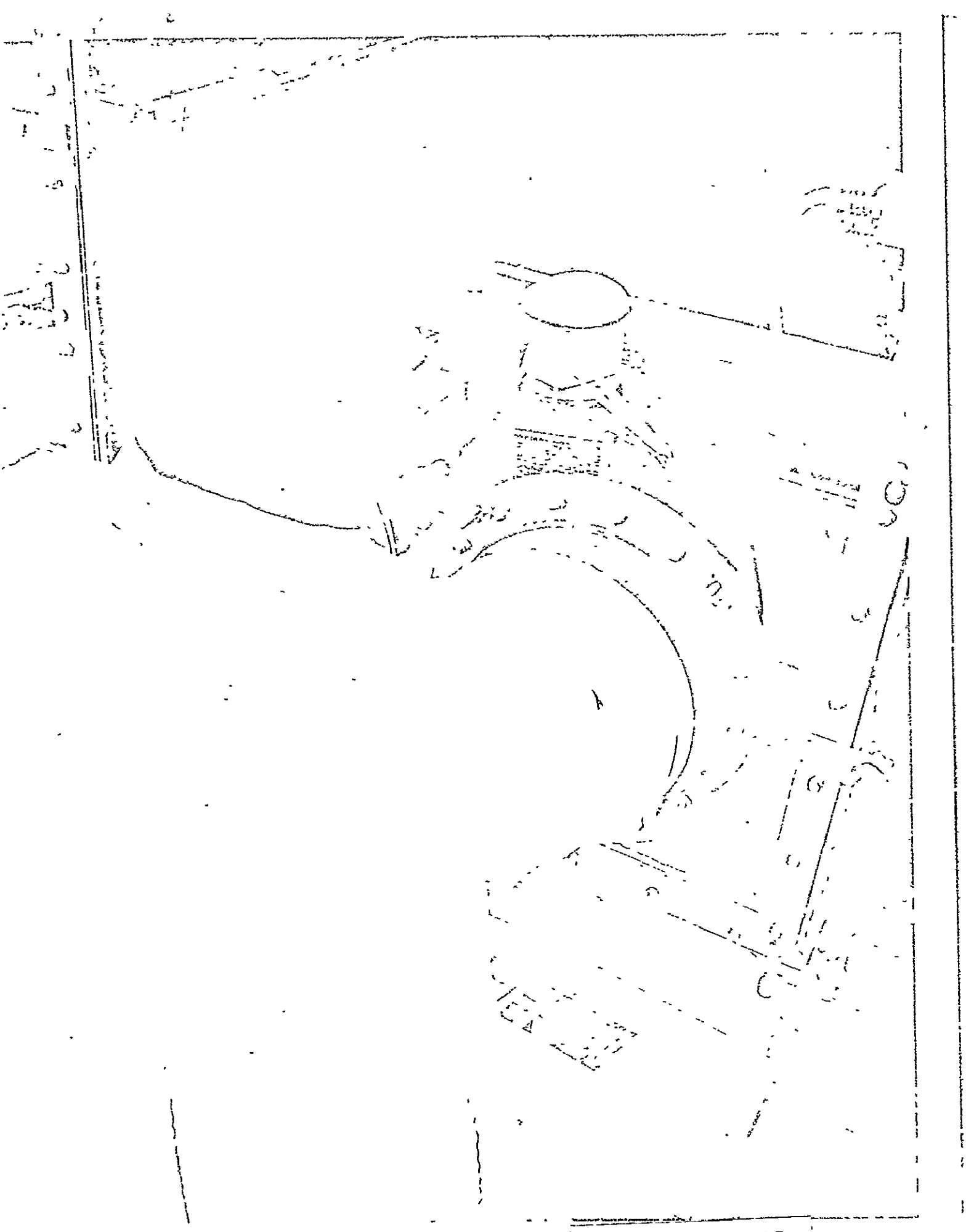


NASA G-70- 1561

NASA G-70- 1563

NASA

Aerobee 350 Air Sampling Experiment
View of Microbilogical Recovery and Assay being
Performed within the Sterile Blickman Isolator
(Distribution of Foam Collection Material into Petri Plates)



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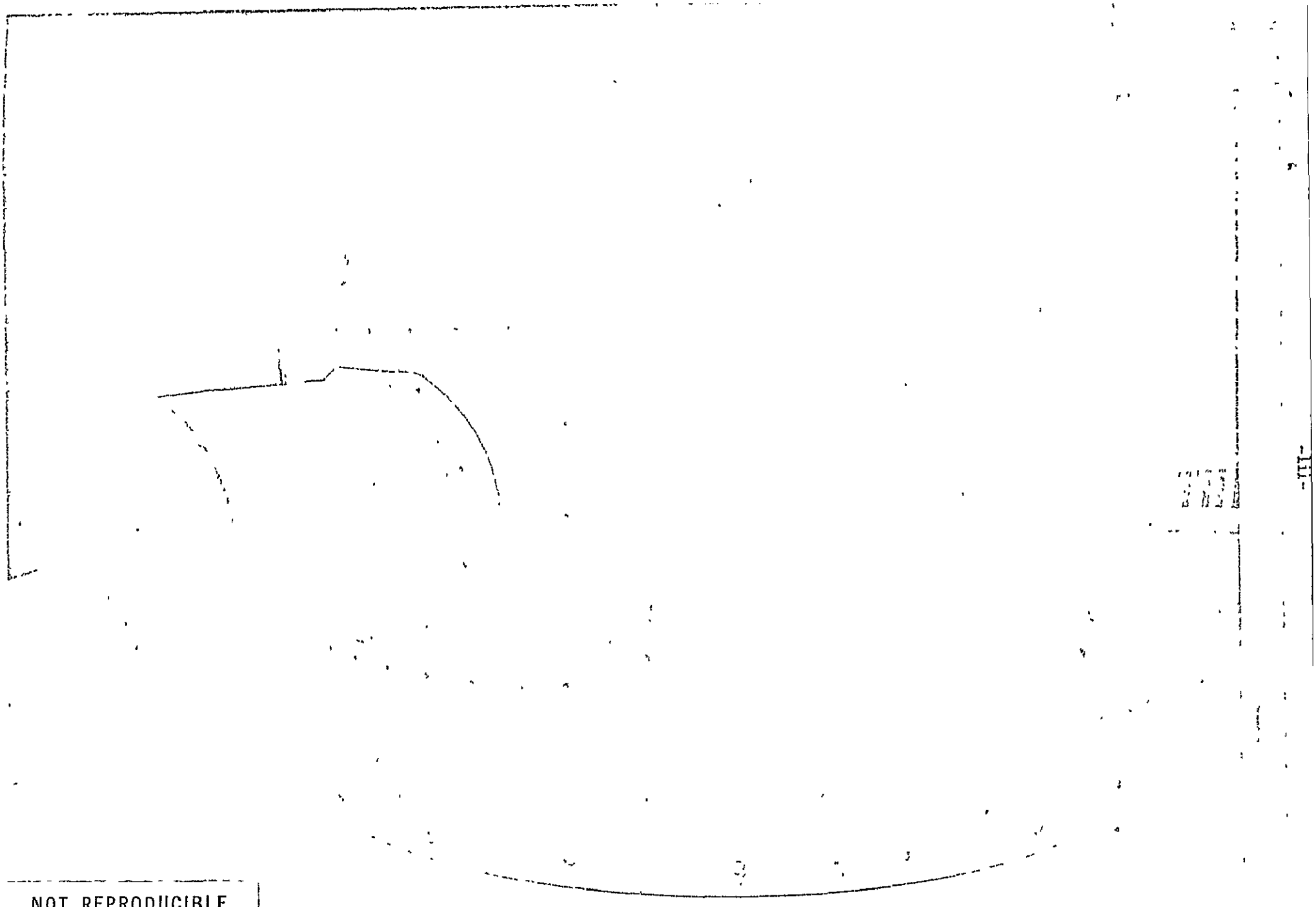
Aerobee 350 Air Sampling Experiment



NOT REPRODUCIBLE

Aerobee 350 Air Sampling Experiment
View Showing Protective Exercise Boot
Over the Air Sampler

NASA C-6[illegible]

Aerobee 350 Air Sampling Experiment
View Showing Extension of Microbiological
Collectors from the Rocket Section