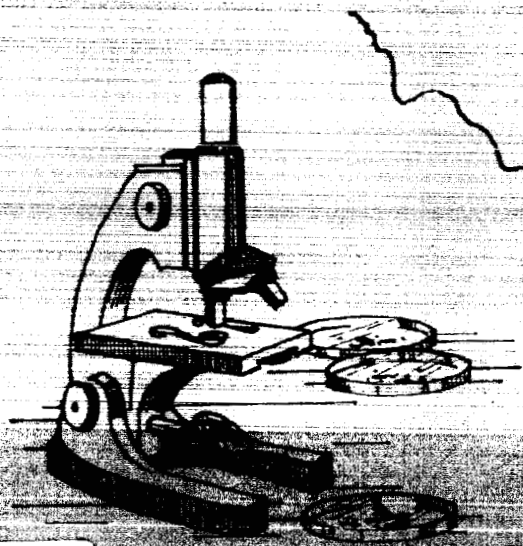
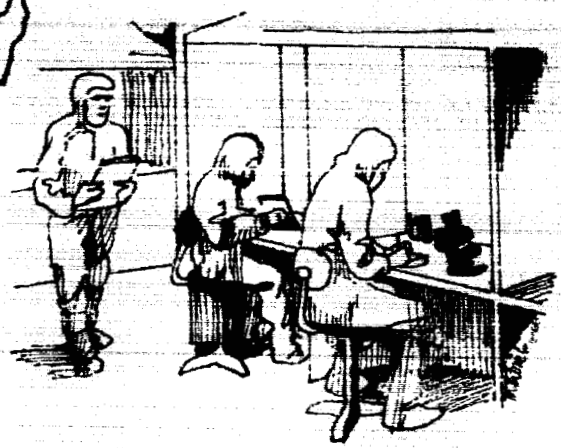
THE BACTERIOLOGY OF CLEAN ROOMS





SCHOOL OF PUBLIC HEALTH
UNIVERSITY OF MINNESOTA
MINNEAPOLIS, MINNESOTA

FINAL REPORT
GRANT NO. NSG-643
N.A.S.A.

JULY, 1966

The Bacteriology of "Clean Rooms"

by

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Final Report

under

Grant No. NSG-643

from the

National Aeronautics and Space Administration

School of Public Health
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Minneapolis, Minnesota 55455

July, 1966

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ACKNOWLEDGEMENTS

During the various phases of this wide-ranging research project, it has been gratifying to note the excellent cooperation and generous assistance rendered by numerous individuals and organizations.

Appreciation is expressed to the management officials of the Ordnance Division, Honeywell, Inc. and the Univac Division of Sperry Rand, Inc. for permission to conduct studies in their Twin Cities plants. For continued help in arrangements for field work, special thanks are extended to the following representatives of these firms:

Mr. Ward Crago, Production Engineer, Honeywell

Mr. Tom Cameron, Senior Contamination Analyst, Honeywell

Mr. Richard Newman, Manager, Plant Engineering Department, Univac

Mr. James Clack, Senior Plant Engineer, Univac

Mr. Don Jorgenson, Component Assembly Supervisor, Univac

The assistance and counsel of Dr. Jacob Bearman, Department of Biostatistics, University of Minnesota, and the guidance and suggestions of Mr. Lawrence B. Hall, Planetary Quarantine Officer, N.A.S.A. are gratefully acknowledged.

Sincere appreciation is also extended to Mrs. Mary Halbert and Mrs. Geraldine Smith, Associate Scientists, School of Public Health, University of Minnesota, for their fine assistance in the field investigations and throughout the laboratory analyses.

CHAPTER I

INTRODUCTION

A. Historical Review

On October 4, 1957, the Soviet Union succeeded in placing the first man-made satellite into orbit around the Earth. That date marked the emergence of man's dreams of the conquest of outer space from the realm of science fiction to reality. It was very shortly thereafter that the scientific community recognized the potential significance of extraterrestrial bodies as "biological preserves". In the ensuing years a monumental effort has been generated with the objective of ensuring the protection of these "biological preserves" at least until they can be adequately investigated.

The crux of this program is the sterilization of space hardware to assure that no man-made object impacting on a potential "biological preserve" in space will introduce biota of earthly origin. To do so would be to obscure forever the true significance of any subsequent biological discovery on that body. The historical development of this concept of "Planetary Quarantine" can be traced through the literature since 1958. Lederberg's "Moondust" article (1958) is generally recognized as the pioneering publication on this subject. Subsequent articles of note which have helped to delineate the over-all program include those by CETEX (1958), Phillips and Hoffman (1960), Davies and Communtzis (1960), Hobby (1962), the National Academy of Sciences (1962), and Jaffe (1963).

It was recognized early that the accomplishment of sterilization of space hardware would not be easy. Indeed, initial efforts to sterilize Ranger Moon probes were frequently blamed for the failure of those probes to function satisfactorily. Moist heat was ruled out as a sterilization technic

due to its obvious deleterious effect on electronic components. Ionizing radiation, liquid chemical sterilants and gaseous sterilants were also ruled out as primary methods due to a combination of incomplete kill, failure to penetrate interiors of components and deleterious effects on some hardware. Dry heat remained as the most promising technic available. Studies by Koesterer and Bruch (1962) and Bruch, et al. (1962) indicated the approximate time and temperature relationships which would be necessary to effect dry heat sterilization.

Several broad categories of significant factors needed to be investigated in order to develop a comprehensive and workable sterilization standard. It was apparent that some space hardware would be damaged by the dry heat cycle of 135° C. for 24 hours which had emerged from available data. Thus, one avenue of investigation was obviously the development of hardware with proven capability to function subsequent to dry heat sterilization. The other approach was related to Hobby's estimate (1962) of 10^9 organisms per spacecraft as a logical starting point for the heat cycle. It was obvious that production of a spacecraft with significantly fewer contaminants might enable a time and temperature reduction in the cycle and thus increase the probability of reliable performance of the various components. For this reason, considerable emphasis has been placed on clean assembly as an important aspect of the sterilization program.

Information on microbial contamination in potential "clean assembly" areas and microbial evaluation of assembly techniques were entirely lacking. Pioneering work in this area was initiated at Fort Detrick Army Biological Laboratory (Portner, et al. 1964, Hoffman 1964 and 1965). Michaelsen and Vesley (1963) compared airborne microbial contamination in industrial clean rooms and hospital operating rooms, and several studies were soon initiated

by N.A.S.A. to provide detailed information about the microbiological aspects of clean assembly (Favero, et al. 1965, McDade, 1965, Douglas Aircraft Co., 1965, Lockheed Missiles and Space Co., 1965). It was as part of this expanded N.A.S.A. program that the research summarized in this report was funded and initiated.

Even while this work was in progress, interest developed in the new concept of laminar flow rooms (Whitfield, 1962) as a possible major innovation in a clean assembly program. The laminar flow facility which was built for and assembled at the University of Minnesota, School of Public Health laboratories is one of several which have now been subjected to microbial monitoring (Powers, 1965, McDade, et al., 1965).

A summary of applicable microbial contamination control methods was prepared by Phillips, et al. (1965), and McDade, et al. (1965) have reviewed the present status of clean room microbiology. A symposium on the sterilization program and its problems was held in Pasadena, California in November, 1965 which helped to bring together and clarify the many elements of the program, and an up-to-date discussion of spacecraft sterilization problems was prepared by Hall (1966).

B. Statement of the Problem

The University of Minnesota study on the "Bacteriology of Clean Rooms" was initiated in April, 1964 at a time when very little information on this subject was available.

The details of the various aspects of this study are reported in the ensuing chapters. The first year was devoted to a comparison of microbial levels in the environments of four different conventional industrial clean rooms located at Honeywell Ordnance Division and Univac Division of the Sperry Rand Corporation.

The rooms were selected on the basis of existing contamination control measures. These ranged from negligible to very stringent controls. An effort was made to determine the differences, if any, in contamination levels related to the specific controls in each room. The parameters studied were ambient airborne contamination levels, surface contamination on work benches and fall-out of contaminants onto stainless steel strips left in the work area for periods up to 21 weeks. Heat resistant isolates from these strips were later identified and a proportion studied for relative resistance to dry heat. The details of this phase of the project are presented in Chapter II.

The second year of the project was primarily devoted to evaluating specific factors thought to be important in minimizing the level of contaminants on space hardware. The factors investigated included: the effect of composition of materials on retention and survival of contaminants, die off of contaminants on handled strips of various materials, and heat resistance studies of contaminants on handled strips. These investigations are presented in detail in Chapter III.

The emergence of laminar flow as a potentially significant inovation led to the installation of a small vertical laminar flow room toward the end of the second year of the project. Continuing funds for "Basic Studies in Environmental Microbiology as Related to Planetary Quarantine" are supporting investigations related to personnel barriers and hand contact contamination during assembly of simulated space hardware in this facility. For the purposes of the original project, the comparison of microbial contamination levels in the laminar flow facility with levels in conventional clean rooms is of interest, and preliminary data on these levels are presented in Chapter IV.

CHAPTER II

MICROBIOLOGICAL SURVEYS OF INDUSTRIAL CLEAN ROOMS

From July 1964 through 1965, a series of surveys was conducted in selected industrial clean rooms located in the Minneapolis-St. Paul Metropolitan area. These investigations were concerned primarily with efforts to obtain basic data about the microbiological quality of the air, the levels of biological contamination on surfaces, the intensity of microbial fall-out on exposed materials and other factors which might be related to these phenomena.

Preliminary visits were made to various Twin City industrial plants in order to determine which sites might be most suitable for this study. Ultimately, the choice of rooms was made in a manner which provided a cross section of areas differing widely in air handling systems and personnel control measures. Industrial rooms selected for this study were located in plants operated by Honeywell, Inc. and by the Univac Division of Sperry Rand. During the period of the surveys, all rooms were being actively used for the assembly and testing of various electronic components.

I. DESCRIPTION OF INDIVIDUAL ROOM CHARACTERISTICS

These rooms were selected because they represented a wide range of differences in environmental controls and personnel practices which might influence the levels of particulate and microbiological contamination in the air and on surfaces of work areas. The following descriptions of individual rooms will indicate the salient features of interest for each room studied.

Univac Room E (Defense Aerospace Printed Circuit Area)

This room was an ordinary factory area with an open bar joist ceiling and about 1,000 sq. ft. of floor space. No special precautions for contamination

control were instituted in Room E.

The air supply for this room was furnished from a general, office type air conditioner with standard, two inch fiberglass, throw-away filters. Rate of air flow was about ten changes per hour.

Personnel wore ordinary street clothing, and from six to nine individuals were actively engaged in production work in this room. Ordinary janitorial service, primarily floor mopping, was performed routinely with intermittant dusting of bench tops.

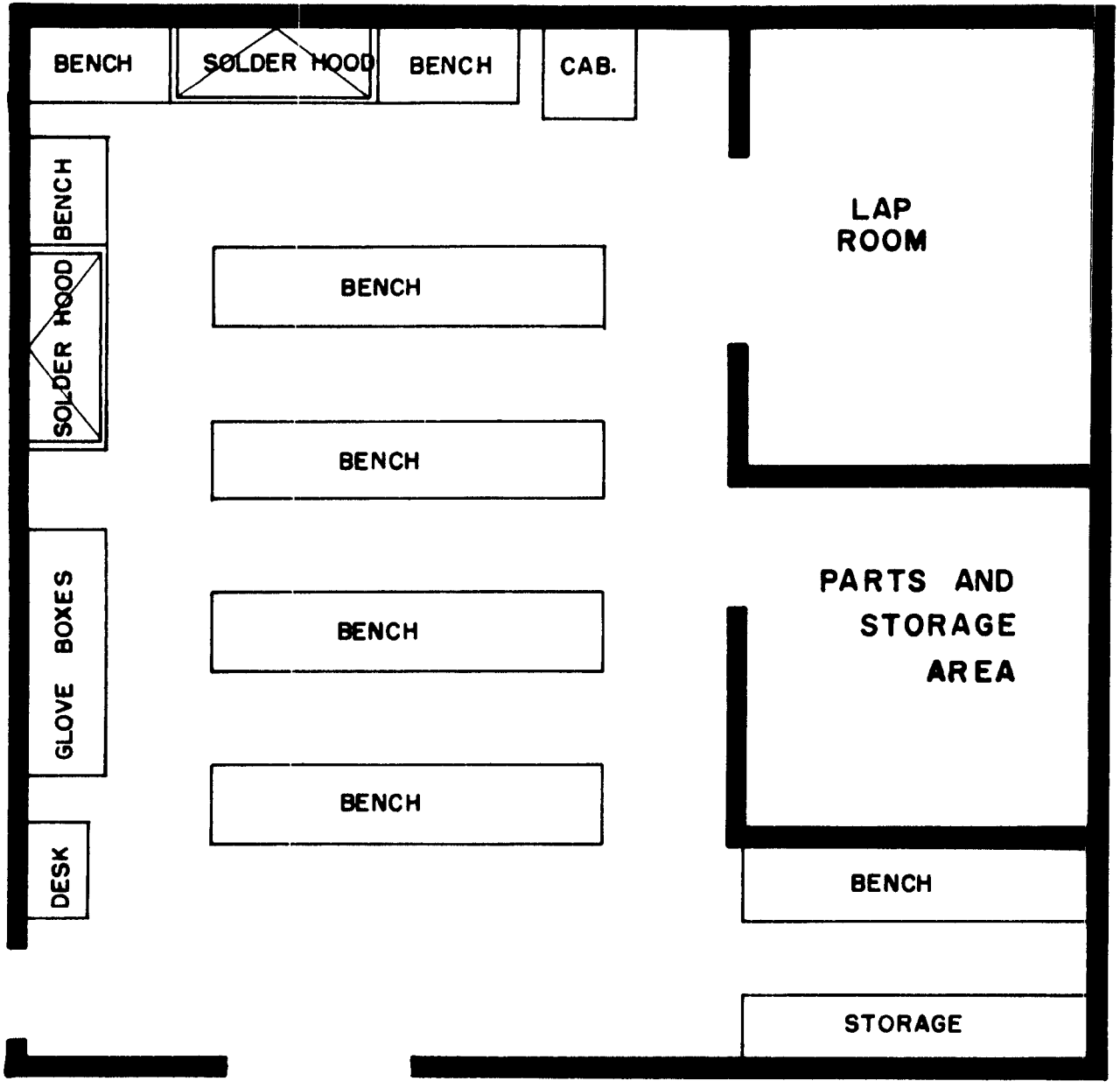
Locations of work benches and equipment in this room are shown in the floor plan of Figure 1. The area was used mainly for testing of circuits, drilling circuit boards and soldering of components.

Univac Room D (Memory Unit Manufacturing)

Certain special practices had been instituted in this area to reduce contamination by particulates. Between 25 to 28 workers were assembling components in Room D which occupied approximately 2,000 sq. ft. of floor space. Room D was the largest of the four areas sampled and also had the greatest amount of bench top surface (see floor plan of Figure 2).

The room was under positive pressure and air was supplied by a conditioning system which controlled the temperature at 75° F. $\pm 5^{\circ}$ and the relative humidity between levels of 30 to 50 per cent. Before entering the room, all air was passed through a Cambridge Aerosolve #95 mechanical filter. An "Airson" false ceiling system delivered forced air into the area at a rate of approximately 15 changes per hour.

All personnel entering this area were required to wear head covers and a smock over street clothing. An air lock dressing area was provided. Although a vacuum shoe cleaner was available, it appeared to be used only casually and infrequently. Shoe covers were not required and there were no

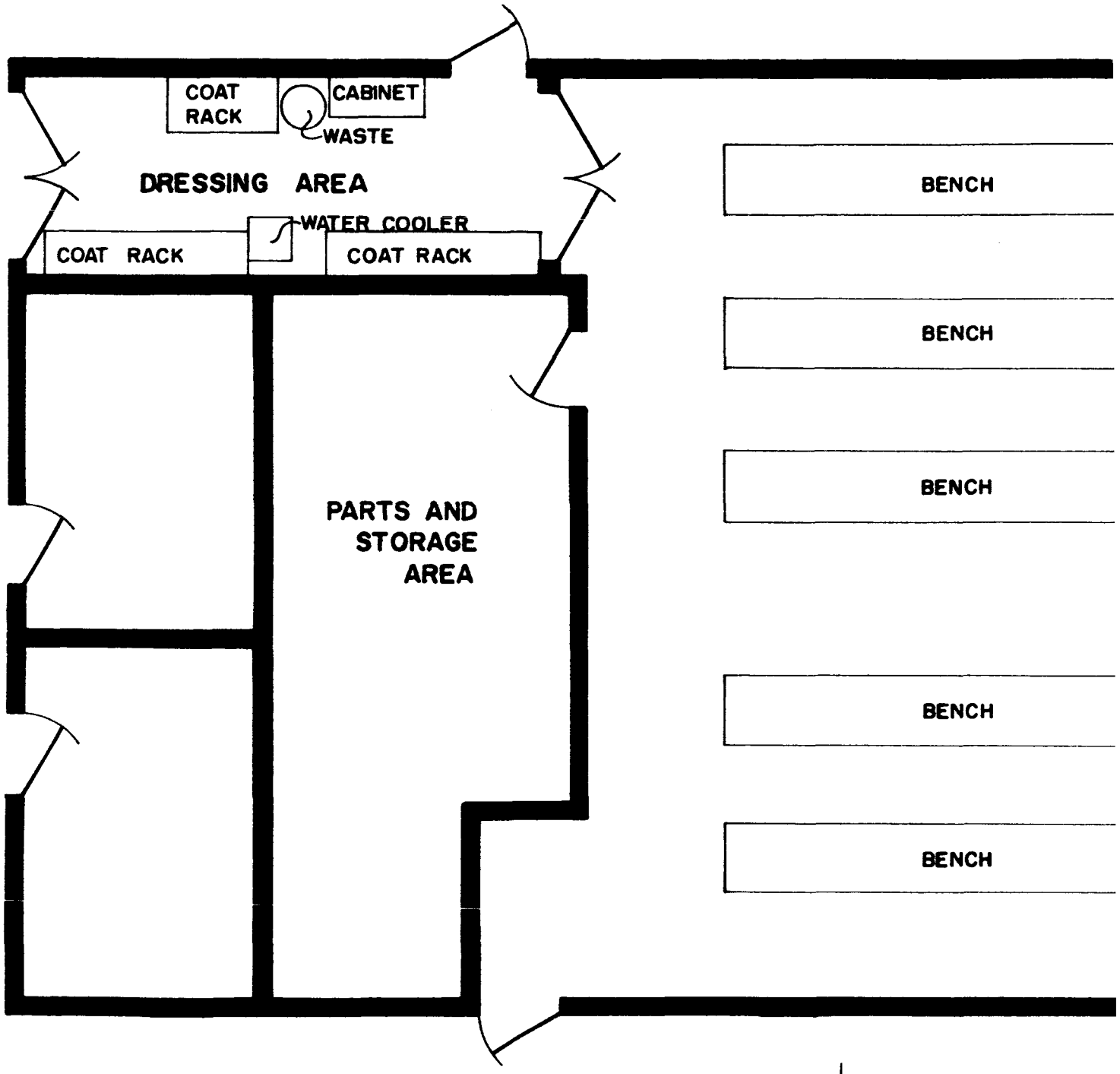


UNIVAC ROOM
E-71

DEFENSE
AEROSPACE
PRINTED
CIRCUIT
AREA

FIGURE 1

scale: 3/16" = 1'-0"



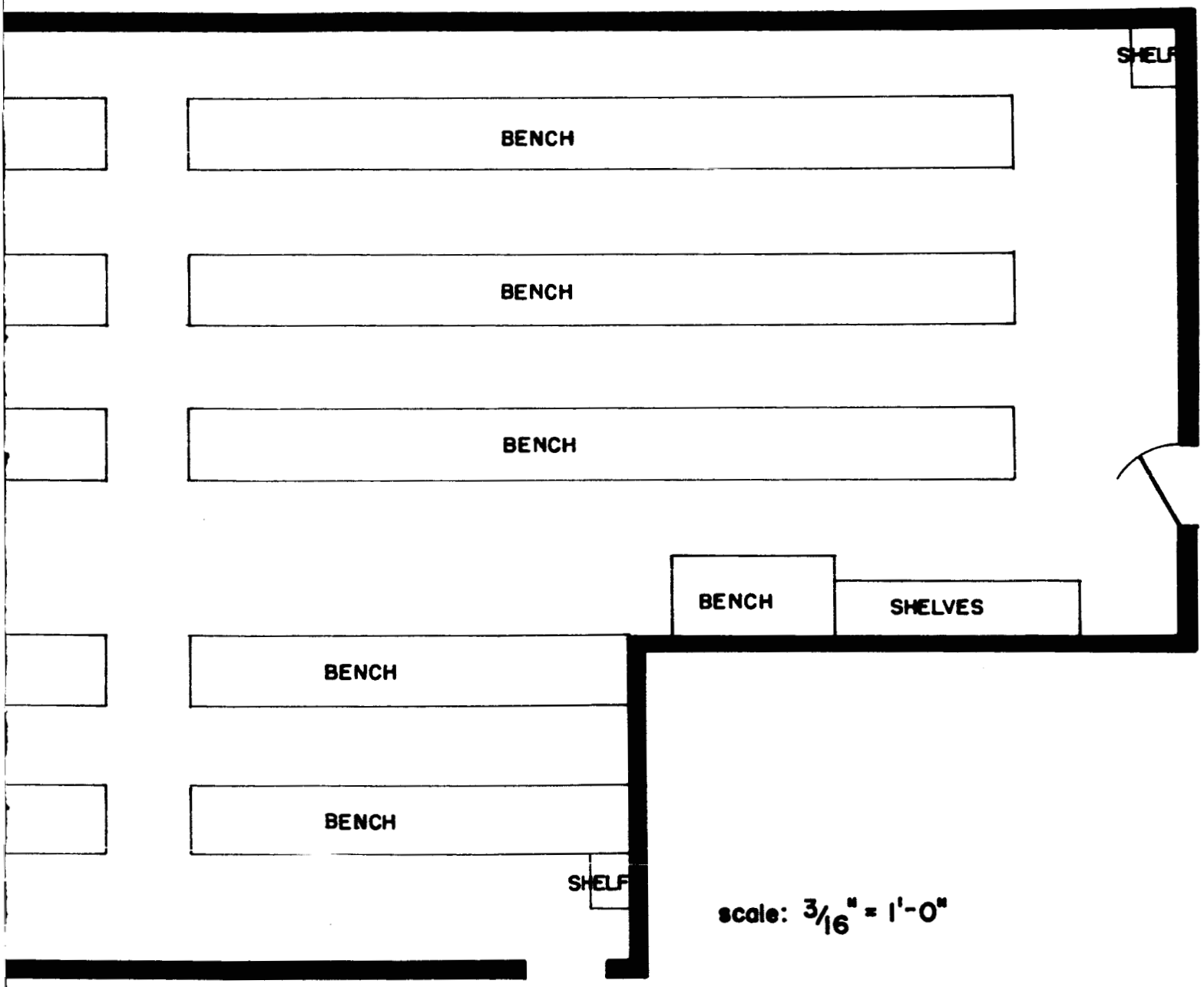
UNIVAC ROOM D-

FILM MEMORY MA

FIGURE 2

8-1

8



ACTURING CLEANROOM

8-2

special hand washing facilities in the dressing area. Figure 3 provides an interior view of Univac Room D and illustrates the type of clothing worn, as well as the general distribution of personnel in the area.

Janitorial services included a once daily vacuuming and wet mopping of floors with detergent designated for clean room use. Bench tops and legs were also vacuumed daily and were washed at one to three month intervals. Occasionally, at the discretion of the workers, they also cleaned a bench top in their immediate area if "it looked dirty".

Honeywell Room C (Component Preparation and Cleaning Area)

This 750 sq. ft. area had a number of features which suggested that it might be somewhat more efficient for prevention of particulate contamination than Room D. Work in this area was usually carried on by five to seven people and the total personnel load never exceeded ten persons.

The air supply for Room C passed through a fiberglass roughing filter, an electrostatic precipitator, an air conditioner and, ultimately, through Cambridge absolute filters. Air entered the room through ceiling louvers and was exhausted through wall vents located near the floor. Temperature was maintained at 72° F. $\pm 5^{\circ}$, relative humidity was controlled to remain below 50 per cent and the room was under positive pressure. Flow rate of the system was approximately 24 changes per hour, with approximately 60 per cent make-up air. Standards for this room required that dust counts be no higher than 400 particles $> 5\mu$ per cubic foot. The general layout of Honeywell Room C is shown in the floor plan of Figure 4.

Strict control was exercised over personnel entering the area. Except for workers assigned there, registration was required of all people. All persons had to use the shoe cleaner and wash their hands before dressing in the outer air lock. Standard dress requirements included smocks or suits,

FIGURE 3 INTERIOR VIEW, MEMORY UNIT MANUFACTURING AREA.
UNIVAC ROOM D-21

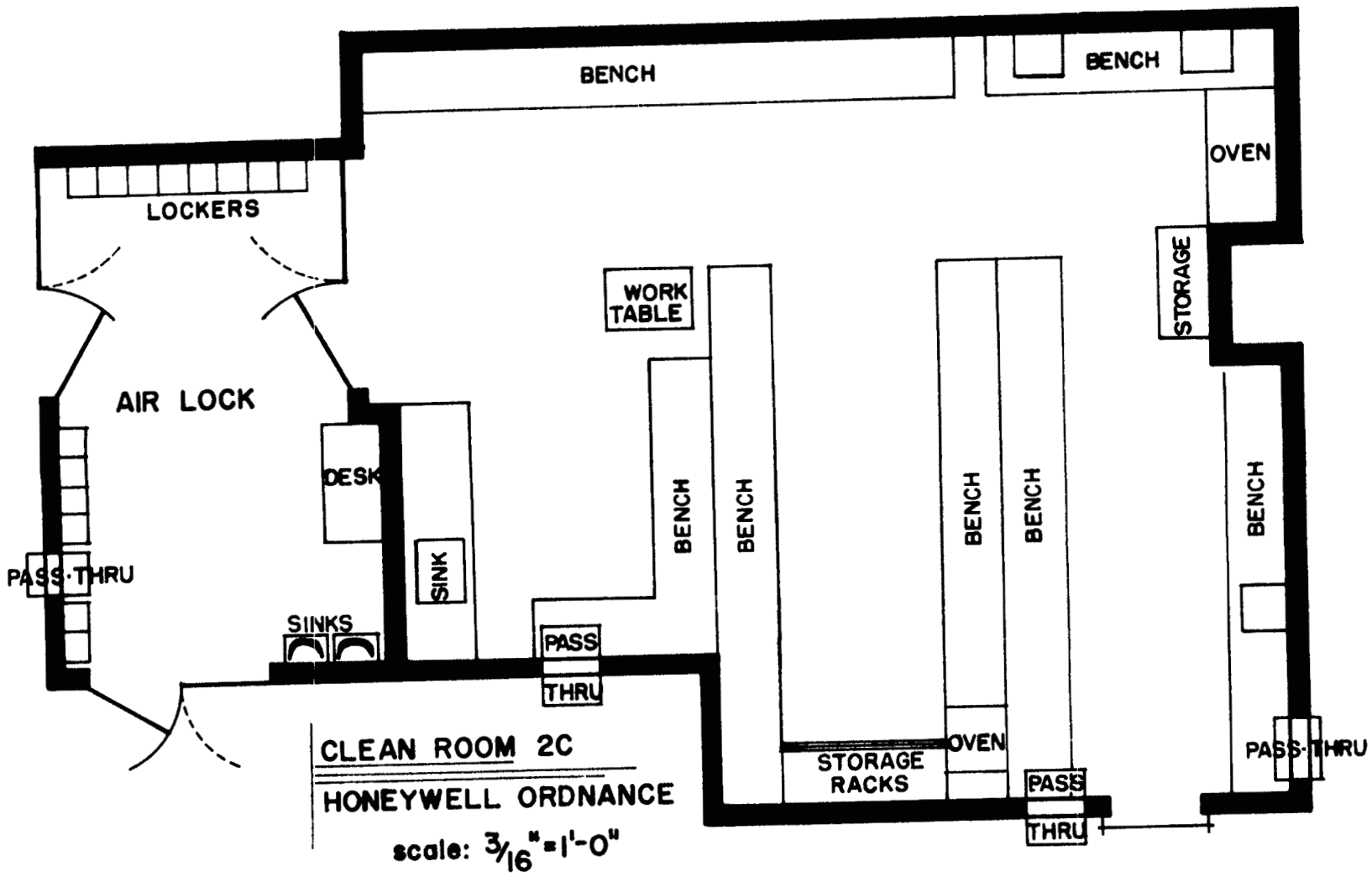

BENCH
BENCH
OVEN
LOCKERS
STORAGE
WORK TABLE
AIR LOCK
DESK
BENCH
BENCH
BENCH
BENCH
BENCH
PASS-THRU
SINK
SINKS
PASS
THRU
CLEAN ROOM 2C
HONEYWELL ORDNANCE
scale: 3/16"=1'-0"
STORAGE RACKS
OVEN
PASS
THRU
PASS-THRU

FIGURE 4

head covers and shoe covers.

Janitorial services were carried out on a rigidly maintained schedule and rigid inspections ensured compliance. During the day shift, all floors were vacuumed twice and outside passages were wet mopped. A second shift crew was responsible for another daily vacuuming of walls, fixtures and horizontal surfaces, except bench tops and shelves. The same shift also again wet mopped all floors. Assembly workers were required to cleanse bench tops each morning by swabbing with isopropyl alcohol saturated sponges.

Honeywell Room A (Component Assembly Area)

Honeywell Clean Room A appeared to be the most sophisticated of the areas studied. The floor plan for this room is illustrated in Figure 5 and an interior view of the area is shown in Figure 6. Floor space and worker load were almost identical to those described for Room C; however, the operating characteristics were somewhat more refined than those of Room C, especially as indicated by the following important additional features:

1. An air shower was required for all personnel before admittance to the dressing room air lock.

2. The air handling system was basically that of Room C, but was modified in several aspects. Among these was the addition of an aerosolve pre-filter stage to the air stream cleansing unit. Furthermore, the flow rate for this room was increased to 30 changes per hour and the supply received 33 per cent make-up air.

3. Particulate contamination standards were set at 200 particles > 5μ per cu. ft. and 25,000 particles > 0.5μ per cu. ft.

4. All materials entering this room had been cleaned previously in another area of comparable atmosphere. Parts and other materials were transferred into Room A through air lock facilities. Particulate shedding

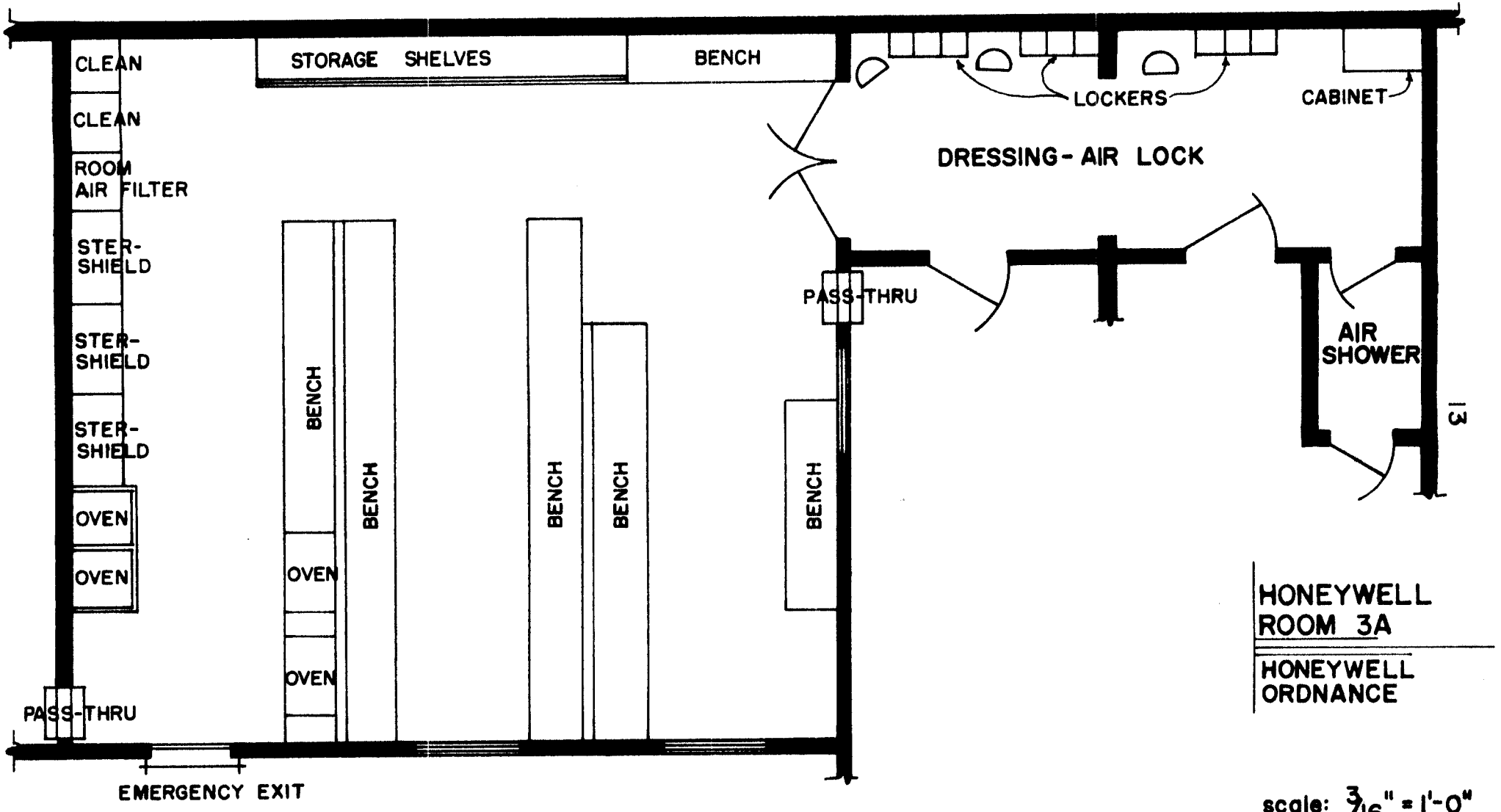

CLEAN
CLEAN
ROOM AIR FILTER
STER-SHIELD
STER-SHIELD
STER-SHIELD
OVEN
OVEN
PASS-THRU
STORAGE SHELVES
BENCH
BENCH
BENCH
OVEN
OVEN
BENCH
BENCH
BENCH
LOCKERS
CABINET
DRESSING - AIR LOCK
PASS-THRU
AIR SHOWER
EMERGENCY EXIT
HONEYWELL
ROOM 3A
HONEYWELL
ORDNANCE
scale: 3/16" = 1'-0"

FIGURE 5

FIGURE 6 INTERIOR VIEW, COMPONENT ASSEMBLY AREA.
HONEYWELL ROOM 3A

materials such as pencils, paper, etc. were excluded from the room.

II. METHODS

The routine sampling procedures for monitoring the microbiological content of air and contamination levels on surfaces are briefly summarized here. These sampling operations were repeated on eight separate occasions in each of the four rooms over an approximate period of nine months. In conjunction with air and surface sampling, prolonged exposures of stainless steel strips served as a means for assaying fall-out contamination. Details of procedures are outlined in the sections that follow.

A. Ambient Air Sampling

On each visit to a clean room, a Reyniers slit sampler (Wolf, et al., 1959) was set up at bench top levels near the center of the room's activities (see Figure 7). A series of five consecutive one-hour samples, at a rate of one cu. ft. of air per minute, was collected, making a total sampling volume of 300 cu. ft. per room for each sampling day. All microbiological samples were collected on Trypticase Soy Agar (TSA) medium and incubated for 43½ hours at 32° C. Enumerations of viable particles were made with the aid of colony counters.

B. Bench Top Surface Sampling

All sampling of surfaces was done with Rodac agar contact plates according to procedures previously described by Bond, et al. (1963). This technique is demonstrated in Figure 8. Preliminary sampling data were used to calculate the number of randomly chosen bench top sampling sites necessary to measure accurately the bench top contamination in each room. In each of Rooms A, D, and E 90 sites were sampled during the a.m. and 90 different sites during the p.m. for a total of 180 Rodac samples per room per sampling day. An unexplained greater variability in Room C required 150 sampling

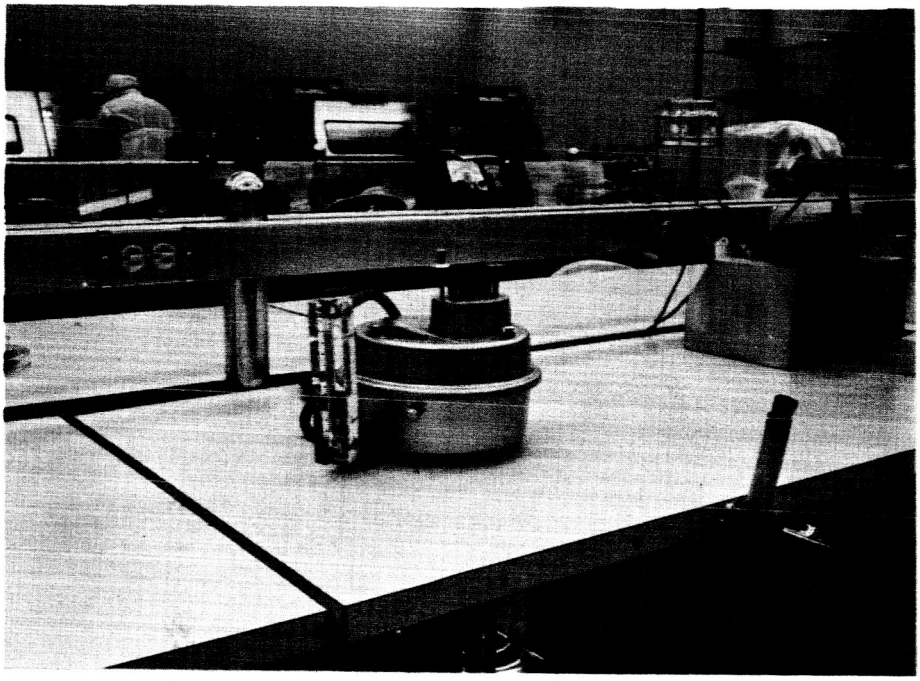


FIGURE 7 REYNIERS AIR SAMPLER

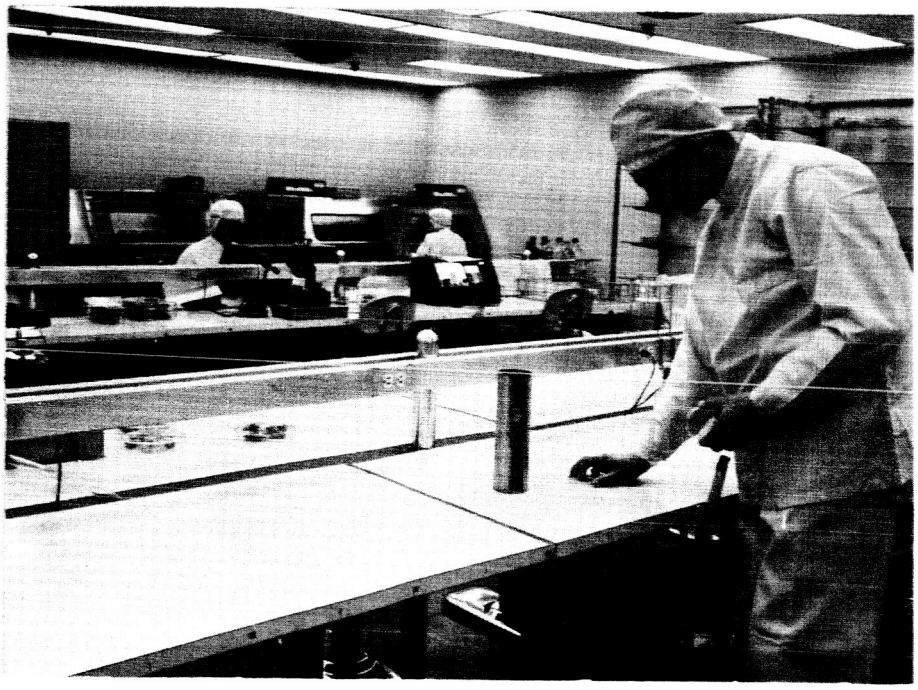


FIGURE 8 SURFACE SAMPLING WITH RODAC PLATES

sites per series or 300 samples per sampling day. Again, TSA agar was used and plates were incubated 43½ hours at 32° C. before counting.

C. Analyses of Microbial Fall-out on Strips

For the initial studies, previously cleaned, sterilized, stainless steel strips (1" x 2") were placed in separate, empty Rodac plate bottoms and exposed in Rooms A and D for evaluations of fall-out contamination. Subsequently, additional strips of other metallics (copper, aluminum, solder) and plastics (teflon, lucite, epoxy) were also exposed in Room D atmospheres.

For the studies with stainless steel alone, each series consisted of 42 strips (three blocks of 14 strips each) which were set out at three separate bench top locations within each room. Six strips from each series were assayed at three week intervals for a total of 21 weeks.

In the combined studies of metallics and plastics in Room D, a total of 196 strips were used. These were set out in four blocks of 49 strips per block at different locations in the room. All blocks contained seven strips each of seven different materials. At intervals of three weeks, four strips of each type (one from each exposure site) were covered with sterile Rodac covers and transported to the laboratory for processing. Thus, a total of 28 strips were analyzed for each collection period including four each of seven different materials.

The analysis of all strips consisted of placing each strip in a bottle with 25 cc of phosphate buffer plus 0.02% Tween 20. The bottles were shaken for five minutes in a gyrorotatory mechanical shaker; followed by plating of 5 ml aliquots in each of the following ways:

1. Five ml plated in TSA agar and incubated aerobically for 72 hours at 32° C.

2. Five ml plated in TSA agar and incubated anaerobically (in 95 per

cent N_2-5 per cent CO_2 atmospheres) for 72 hours at 32° C. With each series, cultures of Clostridium perfringens were also incubated as a check on maintenance of anaerobic conditions.

3. Five ml heat shocked (80° C. for 20 minutes), then plated in TSA agar and incubated aerobically for 72 hours at 32° C.

4. Five ml heat shocked (80° C. for 20 minutes), then plated in TSA agar and incubated anaerobically for 72 hours at 32° C.

D. Qualitative Microbial Determinations

From the routine air and bench top samples, a varying number of plates were randomly selected each week for characterization of colonies. The characterizations included a morphological study of all colonies on the plate by smears made on 15-place milk slides. The smears were Gram stained, examined microscopically and results recorded.

In the qualitative microbial analyses of stainless steel strips, only those colonies developing from heat shocked aliquots were selected and studied. These colonies were sub-cultured for purification and then subjected to detailed identification procedures. Thus, for the stainless steel strips, an attempt was made to characterize the aerobic, heat resistant organisms present (organisms surviving 80° C. for 20 minutes). Details of these methods are outlined in the following paragraphs.

Colonies isolated from the aerobic plates, previously inoculated with heat shocked aliquots, were transferred to brain heart infusion broth tubes and incubated at 32° C. After 48 hours, cultures from tubes demonstrating growth were streaked on trypticase soy agar plates. These plates were incubated for 24 hours and then colonies were transferred to TAM (Thermoacidurans Agar Modified) sporulation slants as recommended by Lechowitz and Ordal (1962). Broth tubes showing no growth were retained for one week before

being discarded as negative.

Smears were made from TAM slants after 48 hours incubation at 32° C. to check for culture purity, Gram reaction and sporulation. If no sporulation was detected, slants were re-incubated and checks for spores were made at 48 hour intervals. After eight days incubation, if no sporulation was observed, the organisms were transferred to "G" medium (Stewart and Halvorson, 1953) and the above procedure repeated. Of the isolates studied, a total of three have resisted all attempts to induce sporulation.

After sporulation was observed, isolates were identified using procedures recommended by Smith, Gordon and Clark (1952). All identifications were based on staining characteristics, microscopic morphology and biochemical reactions. No agglutinations or bacteriophage typing were attempted.

A number of the organisms did not fit precisely into the key of Smith, et al. (1952) nor into any of the four additional species described in the 7th Edition of Bergeys Manual of Determinative Bacteriology. These organisms were placed arbitrarily into the species category which they most closely resembled according to the above two taxonomic schemes.

All initial isolations were made from TSA media at 32° C.; therefore, certain bacteria such as Bacillus stearothermophilus, which requires an incubation temperature of 60° C., and Bacillus pasteurii, which requires urea or alkaline growth conditions, would not have been isolated had they been present on the strips.

E. Dry Heat Thermal Death Times of Spore Forming Isolates

Procedures used to test the susceptibility to dry heat of spore forming bacteria isolated from clean room areas were as follows:

1. All single, pure culture isolates, previously identified and held on TSA agar, were streaked on "G" agar (Stewart and Halvorson, 1953) and

incubated for 48 hours at 32° C. Spore stains were prepared to check the culture purity and degree of sporulation. If 70 per cent sporulation was not achieved, cultures were grown for an additional 72 hours. This practice assured a sufficient concentration for preparation of the test aliquots and all cultures tested have reached this degree of sporulation.

2. Two to five isolates of the same organism were transferred from "G" broth; the flask was heat shocked for 15 minutes at 80° C., placed on a mechanical shaker and incubated for 72 hours at 32° C. A spore stain was made and, if 70 per cent sporulation was not evident, an additional 48 hours of incubation was completed. If sporulation had not reached 70 per cent at this time, a new culture was prepared from the original isolate.

3. Following adequate sporulation, the temperature of the culture was reduced to 5° C. for 15 minutes; then 20 mls of the culture were transferred to a sterile screw cap centrifuge tube and centrifuged at 2,000 rpm for 60 minutes at 1° C. The supernatant was discarded and replaced with 20 mls of sterile distilled water.

4. The resulting 20 ml suspension was diluted to obtain a concentration of approximately 100 to 1,000 spores per 0.1 ml aliquot. A 10^{-5} dilution of the reconstituted spore suspension generally yielded this concentration.

5. Spore concentrations in each aliquot of the 10^{-5} dilution were determined by plating 0.01, 0.05 and 0.1 ml volumes of the spore suspension in 100 mls of Tryptone Glucose Yeast extract agar. The plates were incubated at 32° C. for 72 hours and enumerated on a Bactronic Colony Counter.

6. 0.1 ml of dilute spore suspension is deposited on a clean, sterile 1" x 2" stainless steel strip contained in a sterile Petri plate; the innoculum is spread out with a wire, and with the Petri plate covers ajar, allowed to dry in a hood equipped with an absolute filter.

7. Four groups (10 strips/group) were prepared in this manner. A positive control was run with each culture tested.

8. At 135° C. one group of ten strips was challenged to 15 minutes, one group to 30 minutes, one group to 60 minutes and one group to 24 hours of dry heat in a hot air convection oven equipped with a constant recording thermometer.

9. After heat treatment each strip was placed individually into 50 mls of Tryptone Glucose Yeast extract broth and allowed to incubate for 14 days at 32° C. All transfers were made in the hood with absolute filter.

10. The data are reported as a ratio of the number of positive strips (demonstrating growth in TGY broth) to the total number of strips tested in each series. The positive control strips had to demonstrate growth in TGY broth before the result was accepted.

11. Selected tubes of organisms from bottles showing growth, as indicated by turbidity, were verified as similar to the original culture by biochemical, morphological and other routine microbiological studies.

III. RESULTS

The 1964 and 1965 microbiological surveys, on eight separate occasions in each industrial area, provided extensive data about the relative concentrations of organisms in the air and on bench surfaces. Information in this report is based on summaries from laboratory analyses of approximately 9,600 cu. ft. of air and more than 6,700 Rodac surface samples taken in the four rooms studied.

A. Quantitative Air and Surface Microbiology

Quantitative microbial data from air and bench top sampling in each of the four industrial rooms are summarized in Table I. These results deal only with the aerobic, mesophilic, heterotrophic microbial populations. No

TABLE I

QUANTITATIVE MICROBIOLOGICAL DATA SUMMARY - HONEYWELL AND UNIVAC CLEAN ROOMS

SAMPLING DAY	HONEYWELL				UNIVAC			
	Room A		Room C		Room D		Room E	
	Air Col / Cu. Ft.	Bench Top Col / Rodac	Air Col / Cu. Ft.	Bench Top Col / Rodac	Air Col / Cu. Ft.	Bench Top Col / Rodac	Air Col / Cu. Ft.	Bench Top Col / Rodac
1	0.38	a.m. 6.8 p.m. 10.8	0.41	a.m. 20.2 p.m. 6.5	0.82	a.m. 10.1 p.m. 14.5	2.71	a.m. 33.2 p.m. 28.1
2	0.33	a.m. 4.1 p.m. 6.1	0.30	a.m. 8.4 p.m. 24.7	1.43	a.m. 16.6 p.m. 22.1	4.54	a.m. 36.3 p.m. 44.3
3	0.16	a.m. 5.7 p.m. 6.5	0.13	a.m. 21.6 p.m. 4.8	1.56	a.m. 15.1 p.m. 17.6	5.86	a.m. 34.6 p.m. 44.2
4	0.30	a.m. 3.9 p.m. 12.1	0.26	a.m. 13.4 p.m. 12.4	1.80	a.m. 22.0 p.m. 17.6	4.66	a.m. 33.6 p.m. 39.3
5	0.19	a.m. 7.7 p.m. 5.6	0.46	a.m. 8.1 p.m. 11.3	1.41	a.m. 11.6 p.m. 11.2	3.21	a.m. 27.6 p.m. 34.2
6	0.11	a.m. 7.9 p.m. 8.9	0.26	a.m. 17.3 p.m. 27.1	2.05	a.m. 17.7 p.m. 19.0	5.69	a.m. 41.6 p.m. 44.1
7	0.11	a.m. 4.7 p.m. 4.1	0.23	a.m. 54.8 p.m. 43.1	1.55	a.m. 12.3 p.m. 15.4	4.40	a.m. 38.1 p.m. 42.0
8	0.17	a.m. 6.1 p.m. 12.7	0.16	a.m. 41.7 p.m. 19.8	0.81	a.m. 17.1 p.m. 15.6	2.54	a.m. 25.6 p.m. 47.0
Mean	0.22	a.m. 5.9 p.m. 8.4 Total 7.2	0.28	a.m. 23.2 p.m. 18.7 Total 21.0	1.43	a.m. 15.3 p.m. 16.6 Total 16.0	4.20	a.m. 33.8 p.m. 40.4 Total 37.6
Median	—	3.2	—	4.0	—	11.3	—	29.5

other special groups were studied.

The microbial counts from the air of these rooms seemed to reflect the impact of differences in the degree of control measures in a very predictable manner over approximately one order of magnitude. Rooms with the most stringent contamination precautions also had the lowest number of viable particles in the air. For example, the area with greatest engineering and personnel control features (Honeywell Room A) yielded a mean count of 0.22 colonies per cu. ft. This value contrasted with Univac Room E where least control was exercised and where the mean colony counts reached 4.20 viable particles per cu. ft.

Air counts in Univac Room D, a region where control measures were more relaxed than in Room A, yielded a mean value of 1.43 viable particles per cu. ft. On all eight sampling periods microbial concentrations in air of Room D were intermediate between those of Room E and Room A. It is of special interest to note that the mean air counts of less than one colony per cu. ft. reported for Rooms A and C represent extremely low microbial levels for occupied areas compared, for instance, to critical areas of hospitals which had been obviously thought to represent the paragon of cleanliness.

Sampling data from bench tops showed that mean concentrations of microorganisms ranged from 7.2 colonies per Rodac plate in Room A to 37.6 colonies per Rodac in Room E. Mean values for Rooms C and D fell between these extremes of detected contamination. Further review of data in Table I indicates that surface sampling results did not reveal trends which were as clear cut as those for the air. An extremely odd distribution of contamination in Room C was apparently responsible for the discrepancy in the data pattern because this area yielded a mean count 21.0 colonies per Rodac plate,

but a median value of only 4.0 colonies per plate. However, if only the median values are considered, the bench top concentrations demonstrate a relationship to the degree of contamination control which appears similar to that observed for the air counts. Of additional interest is the fact that, regardless of whether means or medians are considered. Room A generally demonstrated a predominance of microbial surface counts which were clearly lower than those from the other areas.

B. Characterization of Room Air and Surface Contaminants

Over 13,500 colonies from air and bench top samples were isolated, Gram stained and examined microscopically in an attempt to determine the qualitative composition of the microflora from the industrial rooms. Morphological studies of various microbial types among the contaminants and their relative abundance indicated that some differences did occur among rooms in the same building and also among buildings. Data assembled in Tables II and III illustrate the qualitative breakdown of microbial contaminants found in air and on bench tops of the surveyed sites.

Among the interesting differences observed was the high percentage of yeasts detected on benches of Room C, although these organisms were not found in as high a proportion in the air of Room C or in other rooms sampled. Another noteworthy feature was the very high percentage of diphtheroids among the microflora of Rooms D and E as contrasted to the noticeably lower percentages recorded in Rooms A and C. Molds were barely detected or absent in Rooms A, C, and D, but comprised 2.5 per cent of colonies from air and 4.5 per cent of bench top isolates in Room E.

In addition to the observed differences in the composition of microflora from these areas, certain salient similarities were also recognizable. It would appear that the predominant biological contaminants, in all rooms, were

TABLE II

RELATIVE OCCURRENCE OF MICROBIAL TYPES

HONEYWELL SUMMARY · AIR AND BENCH TOP* SURFACES

	ROOM A		ROOM C	
	Air	Bench Top	Air	Bench Top
NUMBER OF COLONIES EXAMINED	362	1428	463	2125
GRAM + COCCI	77.3%	74.4%	74.8%	69.6%
GRAM − COCCI OR COCCOBACILLI	0.5%	0.4%	0.2%	0.1%
GRAM + BACILLI (NO SPORES)	12.2%	20.6%	14.9%	11.8%
GRAM + BACILLI (SPORES)	2.8%	1.0%	4.1%	0.5%
GRAM − BACILLI	6.1%	1.5%	0.6%	2.0%
DIPHTHEROIDS	0.8%	1.5%	5.0%	3.6%
ACTINOMYCETES	—	0.1%	—	0.1%
YEASTS	0.3%	0.5%	0.2%	12.1%
MOLDS	—	—	0.2%	0.1%

*Based on 8 sampling days (12-2-64 to 1-26-65)

TABLE III

RELATIVE OCCURRENCE OF MICROBIAL TYPES

UNIVAC SUMMARY · AIR AND BENCH TOP* SURFACES

	ROOM D		ROOM E	
	Air	Bench Top	Air	Bench Top
NUMBER OF COLONIES EXAMINED	786	2596	1927	4535
GRAM + COCCI	68.5 %	53.7 %	49.6 %	38.1 %
GRAM - COCCI OR COCCOBACILLI	0.1 %	0.3 %	0.3 %	0.1 %
GRAM + BACILLI (NO SPORES)	2.5 %	4.9 %	6.1 %	5.4 %
GRAM + BACILLI (SPORES)	3.2 %	2.6 %	2.3 %	3.2 %
GRAM - BACILLI	0.1 %	0.2 %	1.3 %	0.4 %
DIPHTHEROIDS	25.6 %	37.6 %	37.1 %	47.0 %
ACTINOMYCETES	—	0.1 %	0.6 %	0.5 %
YEASTS	—	0.4 %	0.2 %	0.8 %
MOLDS	—	0.2 %	2.5 %	4.5 %

* Based on 8 sampling days (2-2-65 to 3-23-65)

principally of human origin (Gram positive cocci and diphtheroids) as ~~approved~~ opposed to the more typical soil and dust microbes. In all areas, the cocci and diphtheroids constituted at least 70-75 per cent of the microbial types present. Another feature of great significance was the fact that these data show that the percentage of "spore forming" bacteria (those which gave visual evidence of spores in Gram stains) remained relatively low (0.5-4.1 per cent) in all four rooms.

C. Microbial Fall-out Contamination in Clean Rooms

The intensity of microbiological contamination from aerial fall-out in the clean rooms was evaluated by exposure of stainless steel strips and other aerospace materials placed at bench top heights. Most of these studies were done in Univac Room D and Honeywell Room A. Supplemental experiments to determine effects of storage on contamination levels of fall-out strips were performed in the laboratory.

1. Stainless Steel Strip Exposures

Microbiological analyses of fall-out strips over a 21 week period (November, 1964 to May, 1965) demonstrated the occurrence of fluctuations in mean contamination levels from one sampling period to the next. The accrued microbial fall-out on the strips as detected at each three week interval is shown in Figures 9 and 10. The tabulated mean plate counts per strip for each room are also summarized in Table IV.

During almost all sampling periods, the aerobic, non-heat shocked samples yielded the highest counts in both Honeywell Room A and Univac Room D. Maximum observed concentrations of aerobic, microbial fall-out were detected after three weeks exposure when mean counts of between 40 and 45 viable particles per 1" x 2" strip were reported. Following this period, several marked fluctuations between this level and lesser numbers of aerobic

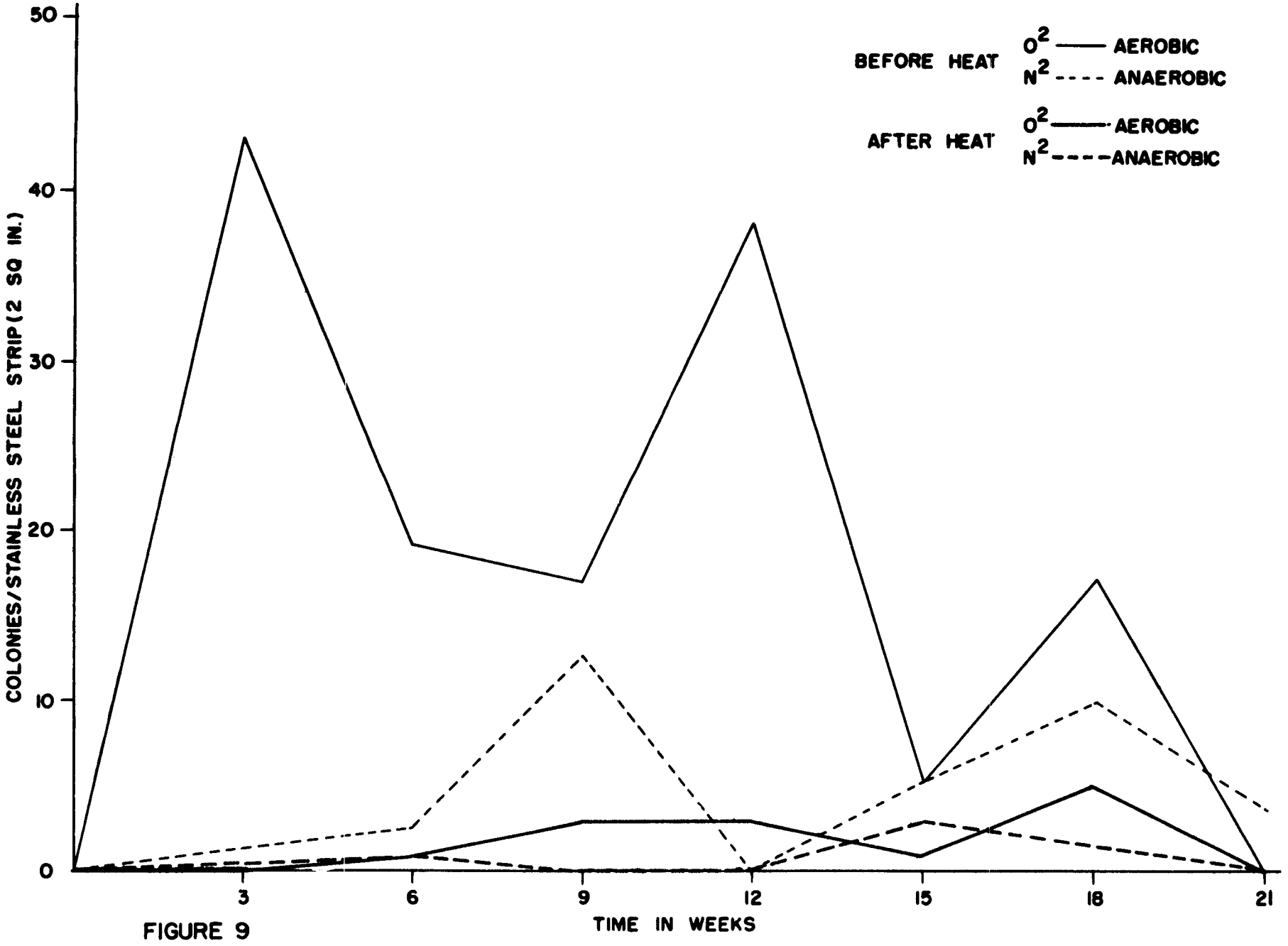


FIGURE 9

HONEYWELL STAINLESS STEEL STRIPS(21 WEEK SERIES)
MEAN COLONIES/STRIP(6-1"x2" STRIPS/TIME PERIOD)

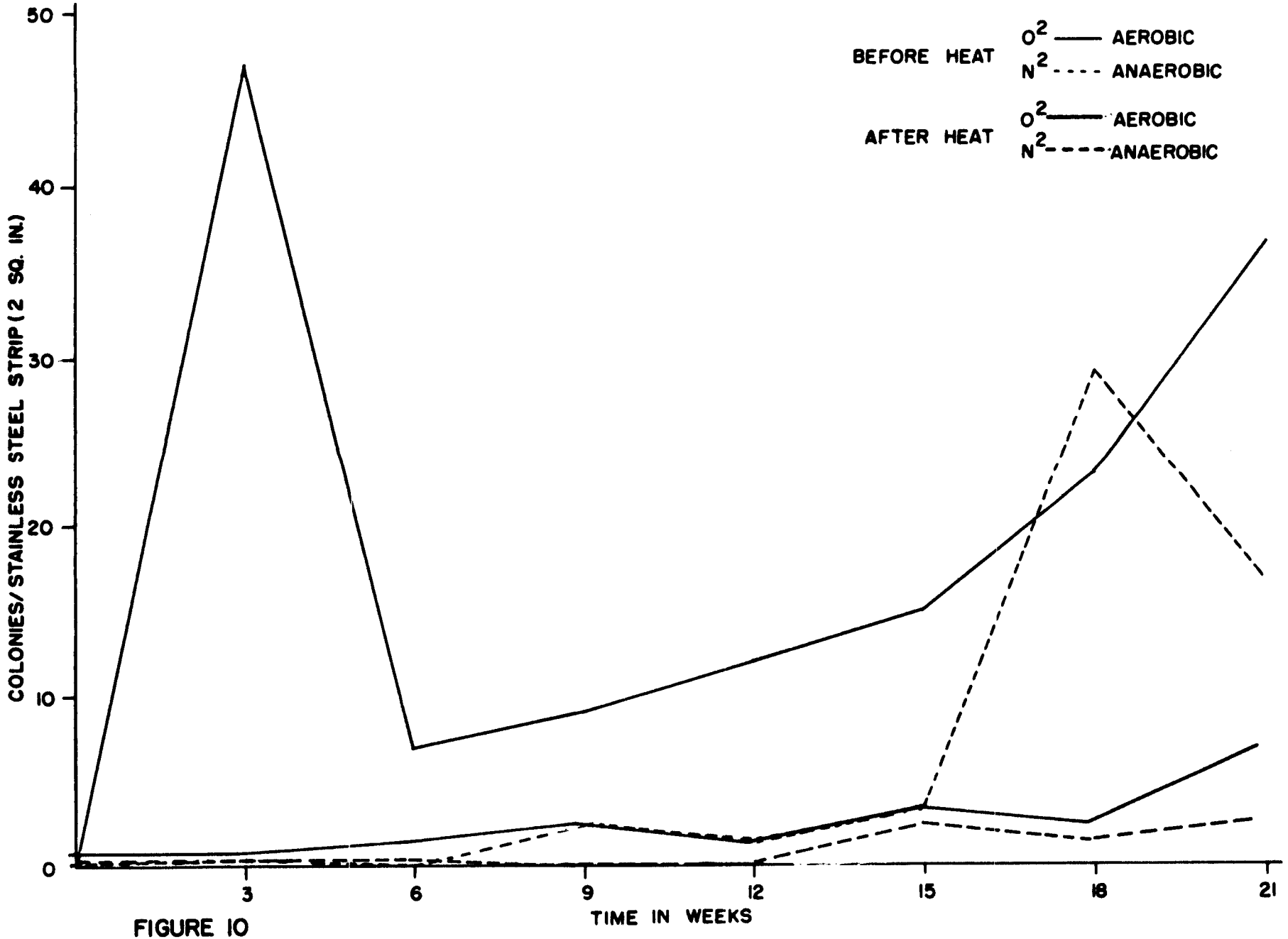


FIGURE 10

UNIVAC STAINLESS STEEL STRIPS (21 WEEK SERIES)

MEAN COLONIES/STRIP (6 - 1" x 2" STRIPS/TIME PERIOD)

TABLE IV

SUMMARY DATA— MICROBIOLOGICAL FALLOUT DETECTED ON STAINLESS STEEL STRIPS EXPOSED TO AIR IN HONEYWELL AND UNIVAC ROOMS
(colony counts per 1" X 2" strips)

WEEK	HONEYWELL (Room A)				UNIVAC (Room D)			
	Aerobic		Anaerobic		Aerobic		Anaerobic	
	Before Heat	After Heat	Before Heat	After Heat	Before Heat	After Heat	Before Heat	After Heat
3	43.3	0	—	—	46.7	0	0	0
6	19.2	0.8	2.5	0.8	6.7	1.7	0	0
9	16.7	3.3	12.5	0	9.2	3.3	2.5	0
12	38.3	3.3	0	0	12.5	1.7	0.8	0
15	4.2	1.7	4.2	3.3	15.0	3.3	3.3	2.5
18	16.7	4.2	10.0	0.7	23.3	2.5	29.2	1.7
21	0 *	0*	3.3*	0*	36.7	9.0	16.7	2.5
Mean	19.8	1.9	4.6	0.7	21.4	3.1	7.5	1.0

* Room unoccupied for 10 days prior to strip analysis.

organisms were observed on strips from the Honeywell clean room. Fluctuations were also noticeable in the anaerobic counts. However, these were not of the same magnitude and the counts were usually much lower. Somewhat analogous results were obtained in Univac Room D.

Spore forming bacteria apparently were present in relatively low numbers on the strips exposed in both rooms. This concept is substantiated by the fact that concentrations of viable particles detected in heat shocked samples never exceeded a mean count of about four organisms per strip and, usually, the value was lower.

Of great interest was the fact that these data provide evidence indicating that there was no marked, continuous build up of microbial contamination on the stainless steel strips over prolonged exposure times. This appeared to be the case even for heat resistant forms. Indeed, in Clean Room A, when the room was totally void of personnel for a period of ten days preceding the last strip analyses, the contamination levels dropped virtually to zero. The results suggest that contamination was primarily contributed by the human activity and, furthermore, that the die off and/or blow off of organisms may be a significant factor in reduction or stabilization of levels of contamination. The data tend to confirm previous experiments conducted by the Army Biological Laboratories at Fort Detrick (Portner, et al. 1964) and the investigations of Favero and coworkers (1965) who studied West Coast clean rooms. This phenomenon has come to be known as the "Plateau effect".

Another important feature demonstrated by the fall-out data was the apparent negligible differences in mean contamination levels on strips when comparisons were made for the two rooms. This is somewhat surprising in

view of the fact that the two areas differed considerably in the nature of environmental controls exercised over personnel.

2. Identification of Aerobic Spore Formers

Although the counts of aerobic, mesophilic, heterotrophic, spore forming bacteria were far less than those for other microbial types, these organisms are ultimately of extreme importance in aerospace biological contamination control. This is true because some of the aerobic spore formers are among the species most resistant to sterilization processes. Therefore, another phase of this investigation concentrated on the isolation and identification of these bacteria to species.

During the analyses of stainless steel strips from the clean rooms, 77 spore forming organisms were isolated and 76 of these were tentatively identified to species. Virtually all of the isolates appeared to be very common environmental spore forming bacteria (see Table V). The most predominant species among these isolates were Bacillus subtilis and Bacillus pumilus which together comprised more than 75 per cent of the cultures studied. Five other species of aerobic spore formers were also present among the isolates and are listed in the Table.

3. Thermal Resistance of Spore Forming Isolates

Subsequent to their isolation and identification, the predominant species of aerobic spore formers were sub-cultured and their thermal resistance to dry heat treatment was investigated. Spore preparations were meticulously produced as described in the protocol of the methods section. Heat resistance of the spores was tested on stainless steel strips heated to 135° C. for periods ranging from 15 minutes to 24 hours.

Results of these studies are summarized in Table VI. Among the species tested, Bacillus subtilis appeared to be the most resistant organism, since

TABLE V

SPECIES IDENTIFICATION OF SPORE FORMERS ISOLATED FROM STAINLESS STEEL STRIPS IN CONVENTIONAL AEROSPACE "CLEAN ROOMS"

SPECIES	NUMBER OF ISOLATES
B. subtilis	33
B. pumilus	28
B. megaterium	6
B. cereus	6
B. pantothenticus	1
B. circulans	1
B. laterosporus	1
Actinomycetales*	1
Total	77

*This organism identified only to order, not genus and species.

TABLE VI

DRY HEAT RESISTANCE OF SPORE FORMING BACTERIA
ISOLATED FROM CONVENTIONAL AEROSPACE "CLEAN ROOMS"

Species			Ratio of positive strips to total strips following heat shock at 135° C.			
	No. of isolates heat shocked	Mean spores per strip	15 min.	30 min.	60 min.	24 hrs.
Bacillus subtilus	14	437	114 / 140	22 / 140	2 / 140	0 / 140
Bacillus megaterium	2	265	1 / 15	0 / 15	0 / 15	0 / 15
Bacillus pumilus	15	774	105 / 146	18 / 144	0 / 145	0 / 145
Bacillus cereus	4	468	4 / 40	0 / 40	0 / 40	0 / 40
Actinomycetales*	1	3217	2 / 10	0 / 10	0 / 10	0 / 10
Total	36	—	226 / 351	40 / 349	2 / 350	0 / 350

*This organism identifed only to order, not to genus and species.

two out of 140 preparations survived 135° C. for 60 minutes. Both Bacillus subtilis and Bacillus pumilis demonstrated marked resistance to dry heat treatment over 30 minutes exposure, but none of the other species tested were able to survive this treatment. Perhaps of greatest significance is the fact that, out of 350 spore preparations subjected to thermal testing, none survived the 135° C. temperature for 24 hours. However, it must be borne in mind that these were cleaned spore preparations from pure cultures. It is entirely possible that susceptibility to heat treatment may be less marked when spores are coated or insulated by extraneous organic matter, soil, dust or are embedded in materials.

4. Fall-out on Plastics and Metallic Strips

Because various aerospace materials differs widely in composition and surface properties, interest was expressed in whether or not they might also differ in their affinity for attracting cumulative microbial contamination from fall-out in clean rooms. In order to investigate this question, a combination of plastic and metallic materials were exposed to fall-out in Univac Room D from June to November, 1965. A total of 196 strips were exposed initially and at three week intervals strips were analyzed for aerobic, anaerobic and heat resistant forms as described in the section dealing with methodology.

Results of these experiments are illustrated in graphs of Figures 11 and 12 which show the fluctuations in levels of microbial contamination as detected at each three week sampling interval. A summary of the aggregate mean counts as calculated for all materials, all plastics and all metals, by groups, is presented in Table VII.

For all materials studied throughout this experiment, the greatest proportion of fall-out contamination was from organisms that grow under

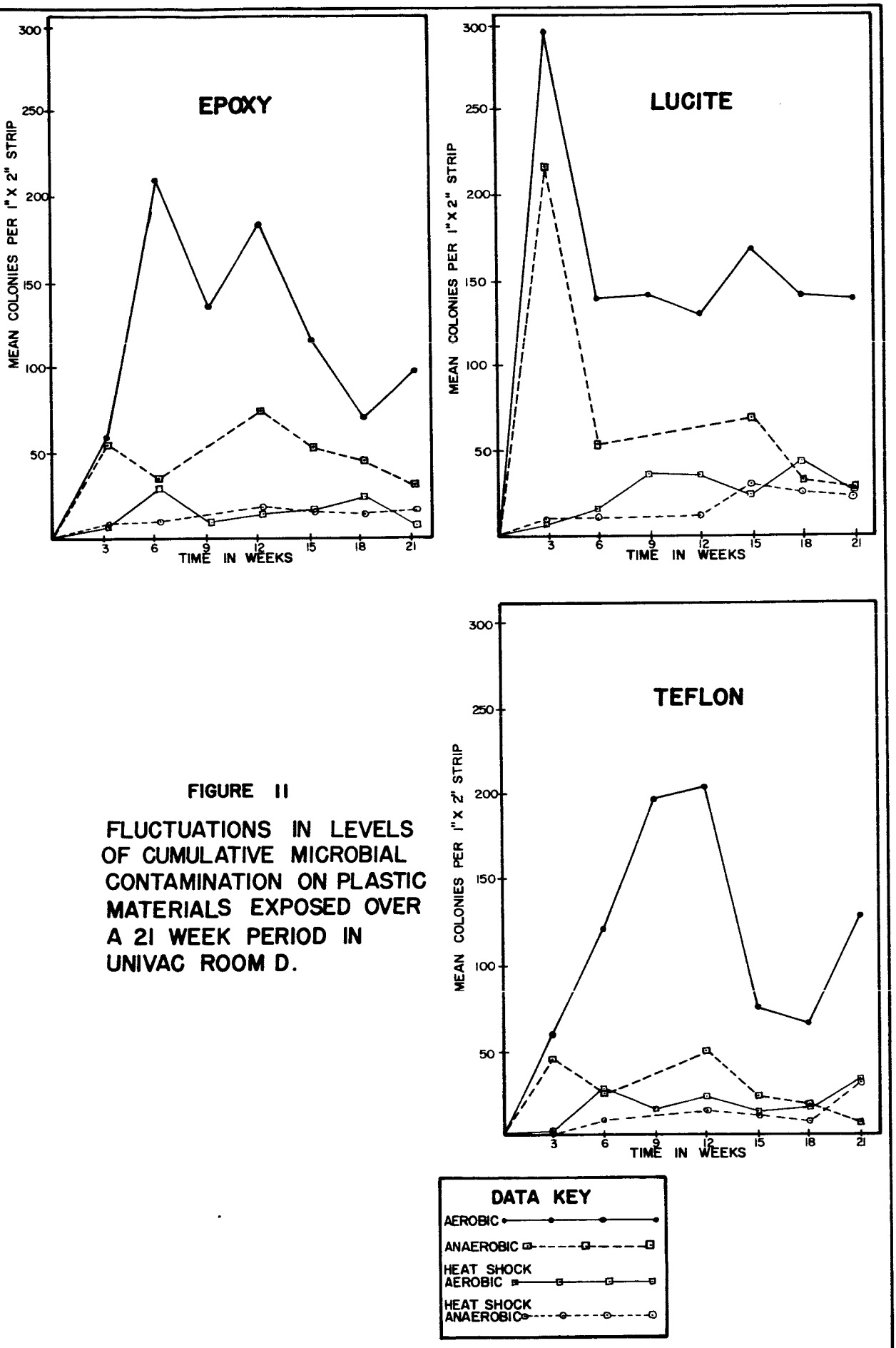


FIGURE 11

FLUCTUATIONS IN LEVELS OF CUMULATIVE MICROBIAL CONTAMINATION ON PLASTIC MATERIALS EXPOSED OVER A 21 WEEK PERIOD IN UNIVAC ROOM D.

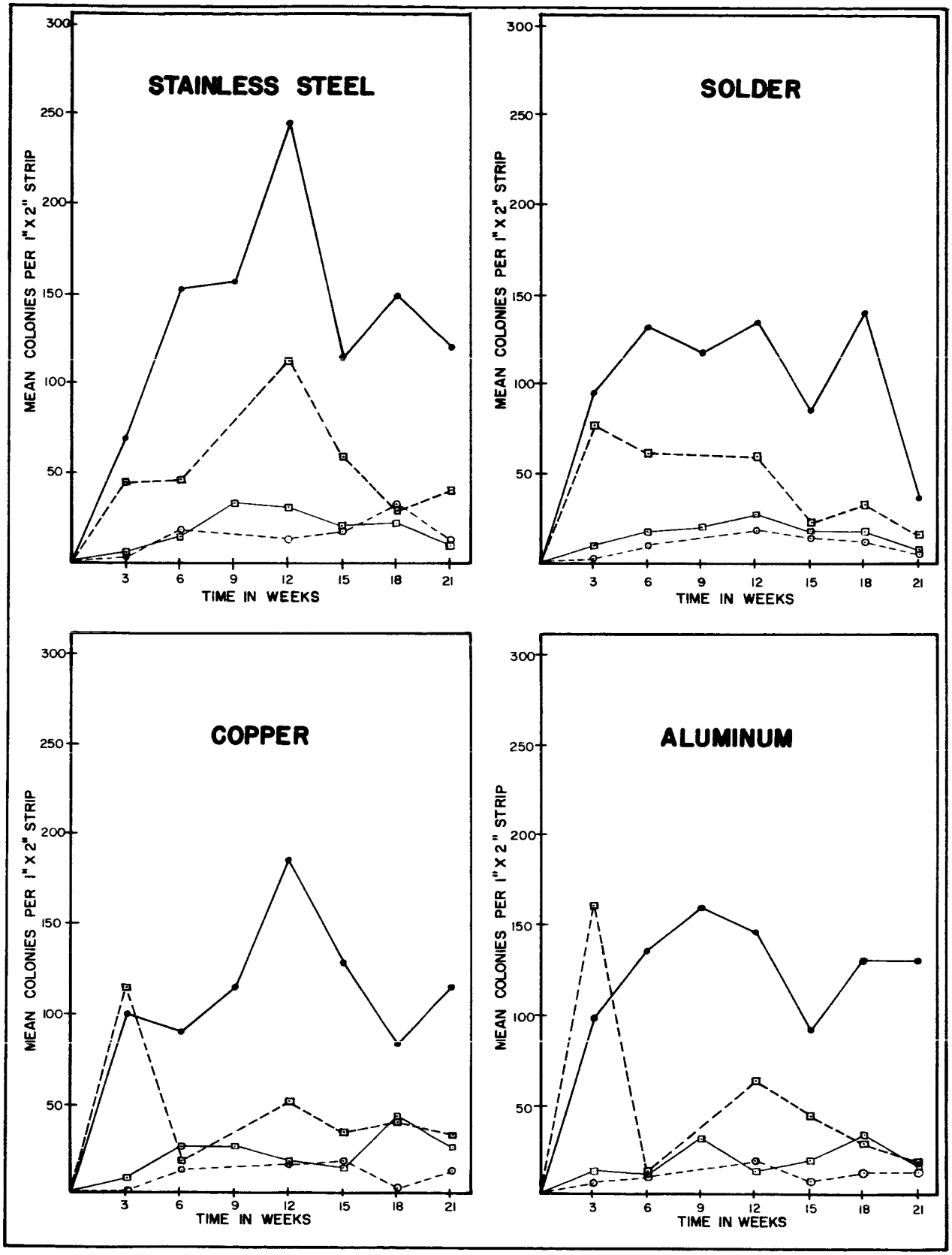


FIGURE 12

FLUCTUATIONS IN LEVELS OF CUMULATIVE MICROBIAL CONTAMINATION ON METALLIC MATERIALS EXPOSED OVER A 21 WEEK PERIOD IN UNIVAC ROOM D.

TABLE VII

SUMMARY OF MICROBIAL CONTAMINATION LEVELS DETECTED ON STRIPS EXPOSED TO AERIAL FALLOUT IN UNIVAC ROOM D OVER A 21 WEEK, JUNE—NOV. 1965

CONDITIONS OF CULTURE AND STRIP TYPES*	MEAN PLATE COUNT PER 1" X 2" STRIP						
	3 wks.	6 wks.	9 wks.	12 wks.	15 wks.	18 wks.	21 wks.
AEROBIC COUNTS							
ALL 7 MATERIALS	110.7	140.4	146.4	336.3	111.8	112.1	115.0
ALL PLASTICS	138.8	157.1	158.8	546.7	120.0	93.3	124.5
ALL METALS	89.7	127.8	137.2	178.4	105.6	126.3	108.8
ANAEROBIC COUNTS							
ALL 7 MATERIALS	102.7	36.8	**	69.8	45.7	33.8	26.1
ALL PLASTICS	106.3	38.8	**	63.8	51.7	33.4	22.7
ALL METALS	99.9	35.3	**	72.8	41.3	34.1	28.2
HEAT SHOCKED AEROBIC COUNTS							
ALL 7 MATERIALS	8.4	21.5	25.4	24.1	19.3	30.4	20.5
ALL PLASTICS	6.3	25.0	21.7	25.0	19.2	30.0	25.5
ALL METALS	10.0	18.0	28.1	23.4	19.4	30.6	17.4
HEAT SHOCKED ANAEROBIC COUNTS							
ALL 7 MATERIALS	5.4	12.9	**	17.1	19.1	16.8	18.0
ALL PLASTICS	7.1	10.8	**	15.8	20.4	17.5	25.9
ALL METALS	4.1	14.4	**	18.1	18.1	16.3	12.9

* Included three plastics (Teflon, Lucite & epoxy) and four metals (stainless steel, copper, aluminum & solder)

** Malfunction of anaerobic chamber precluded collection of data

conditions of aerobic culture. With only two exceptions (copper and aluminum in the first sampling period), the aerobic counts were persistently higher than those for other groups. Maximum mean values for aerobic colonies from different materials ranged from approximately 140 to 300 viable particles per 1" x 2" strip. Peak values were observed on both plastics and metals, but these maximum counts did not occur simultaneously on all materials. Highest concentrations were detected on the plastics and stainless steel where mean counts, on occasion, were greater than 200 viable particles per strip. However, considerable fluctuation in counts were observed throughout the time of this study and this phenomenon occurred on most materials.

Counts of microorganisms capable of growth under anaerobic conditions were generally much lower than those for aerobic types. However, on a few sampling periods, the anaerobic colony counts exceeded 100 viable particles per strip (see Lucite, aluminum, copper).

Lowest counts for the microbial groups studied were those for the spore forming bacteria. The data for heat shocked aerobic and anaerobic cultures demonstrate that levels of detectable spore formers generally ranged between mean counts of approximately 10 to 40 organisms per strip. Although fluctuations were also noticeable in these counts, they did not vary widely from one sampling period to the next.

A survey of the graphs (Figures 11 and 12) from this experiment does not reveal any striking differences in levels of cumulative fall-out which might be attributed to differences in materials. For certain sampling periods, the plastics yielded higher microbial concentrations than metals such as aluminum, solder or copper. However, this trend was not sustained throughout the experiment. In fact, at the end of the 21 week period, no significant differences could be discerned among the various materials exposed

to fall-out effects (see Table VII).

An important feature of this study was the fact that, again, there was no evidence which suggested any continuous build up of microorganisms over prolonged exposure times. This situation prevailed for all types of materials tested. Thus, these data provide additional supporting evidence for the statements by other investigators who have suggested the occurrence of the "plateau phenomenon" in clean room fall-out studies. Apparently this situation arises from an equilibrium between rates of deposition of new contaminants and rates of loss through die-off and other mechanisms causing a decrease in residual numbers on the strips.

Another noteworthy aspect of this experiment was the fact that the microbial counts on stainless steel in this series (Figure 12) greatly exceeded previous levels attained in Room D during an earlier study shown in Figure 10. Mean aerobic counts of no greater than 40 viable particles per strip were detected during the earlier analyses done over a winter-spring period. This is in marked contrast to the peak counts of 150 to 250 organisms during the summer-fall studies shown in Figure 12. Whether this phenomenon is a reflection of seasonal influences, with attendant changes in air mass characteristics, caused by differences in personnel activity, or by differences in personnel physiology, etc. remains unknown. Further investigations of seasonal effects and other aspects of human shedding phenomena might assist in clarifying this question.

D. Laboratory Tests of Microbial Fall-out and Die-off

Following the investigations of cumulative fall-out on strips in clean rooms, the question arose as to whether die-off was a major contributing factor to the observed retardation in microbial build up or the equilibrium phenomena. There was further speculation about the effects of air currents

which might sweep organisms off strips after initial deposition. For this reason a supplemental experiment was run to investigate the possible effects of microbial die-off as contrasted with blow off on exposed strips.

For the experiment, a total of 168 sterile stainless steel strips, individually placed in bottom halves of Rodac plates, were set out at three different sites (56 strips per site) on the open laboratory benches. Over a 14 day period, 4 strips were collected daily from each site (a total of 12 per day) and processed. From each day's total of 12 strips, half of these were analyzed immediately, while the other six were covered with sterile Rodac tops, stored at ambient room conditions for two weeks, and then plated for counts of viable particles. The protocol for microbiological analysis was as described previously, except that only aerobic cultures were done.

Thus, this experiment attempted to follow the cumulative build up of total aerobic counts and aerobic heat resistant forms on a day-by-day basis over the two week period. Simultaneously, it was expected that a comparison of data from strips plated immediately to those covered and stored for two weeks might provide a means for evaluation of the die-off patterns.

Ranges of the aerobic total counts varied widely as may be noted in the graphs of Figures 13 and 14. This was especially true in the early phases of the sampling period. It is possible that these data may be a reflection of variations in the intensity of fall-out contamination in certain local exposure sites.

Mean values for both the strips plated immediately after exposure and those stored for two weeks showed day-to-day fluctuations. The highest mean concentration of contamination on strips plated immediately following exposure was approximately 40 to 50 viable particles per strip. Beyond eight days of exposure time, the mean values for these strips remained at

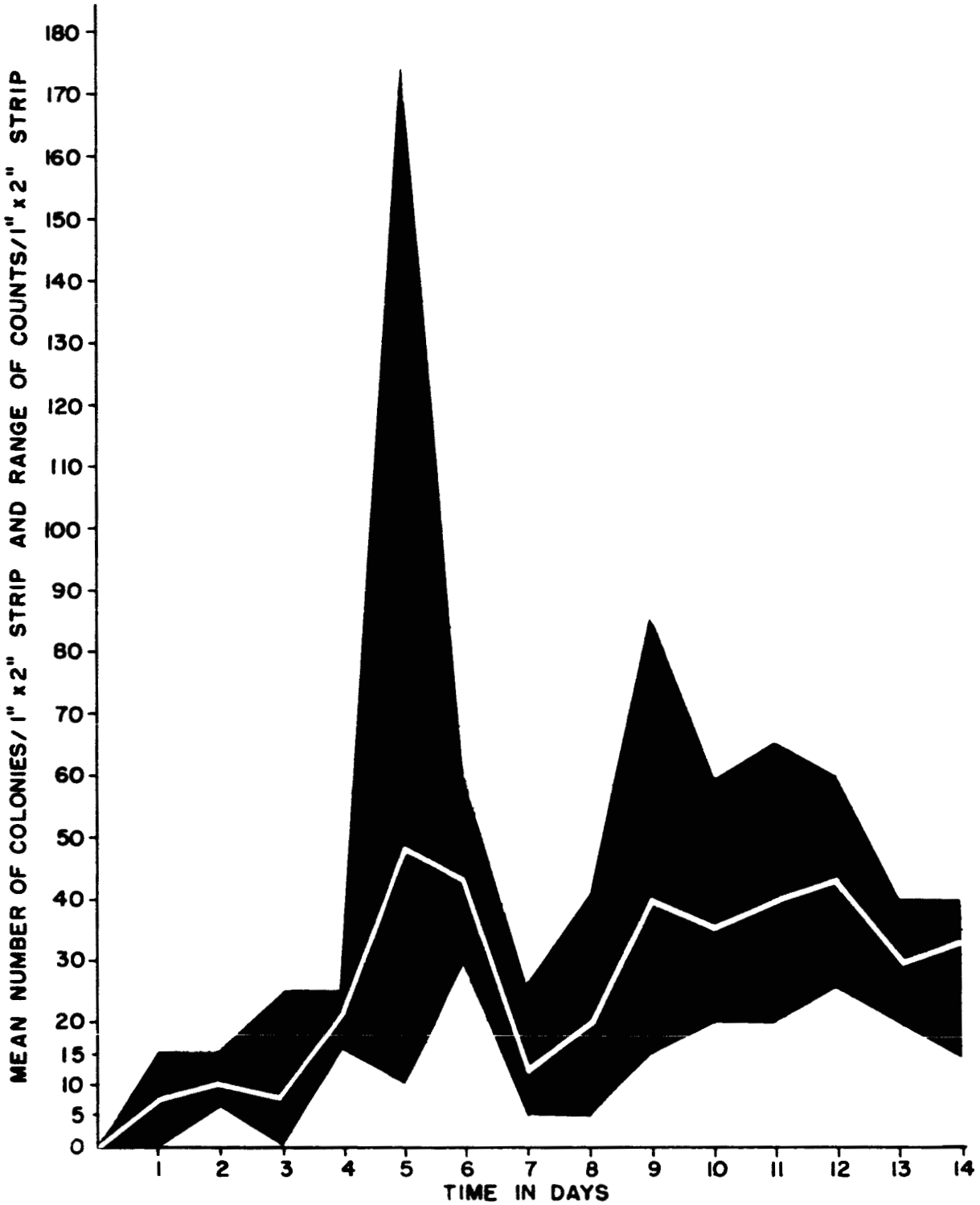


FIGURE 13

THE MEAN AND RANGE OF TOTAL COUNTS PER 1" x 2" STAINLESS STEEL STRIP EXPOSED IN LAB I TO 14 DAYS AND PLATED IMMEDIATELY AFTER EXPOSURE.

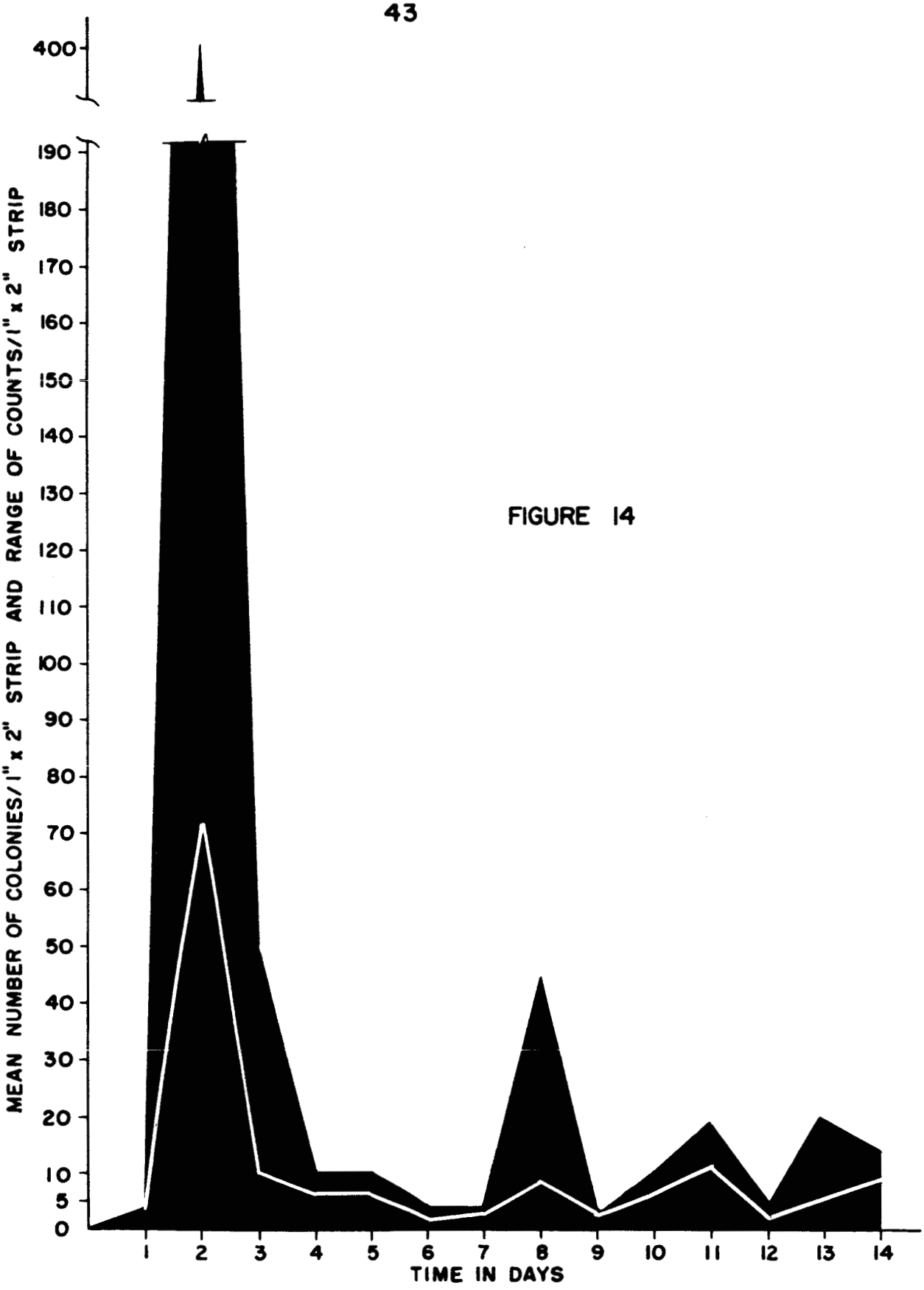


FIGURE 14

THE MEAN AND RANGE OF TOTAL COUNTS PER 1" x 2" STAINLESS STEEL STRIP EXPOSED IN LAB I TO 14 DAYS, COVERED TWO WEEKS, AND THEN PLATED

levels of about 30 to 40 colonies per strip, apparently reaching a plateau during this time without any further build up being detected.

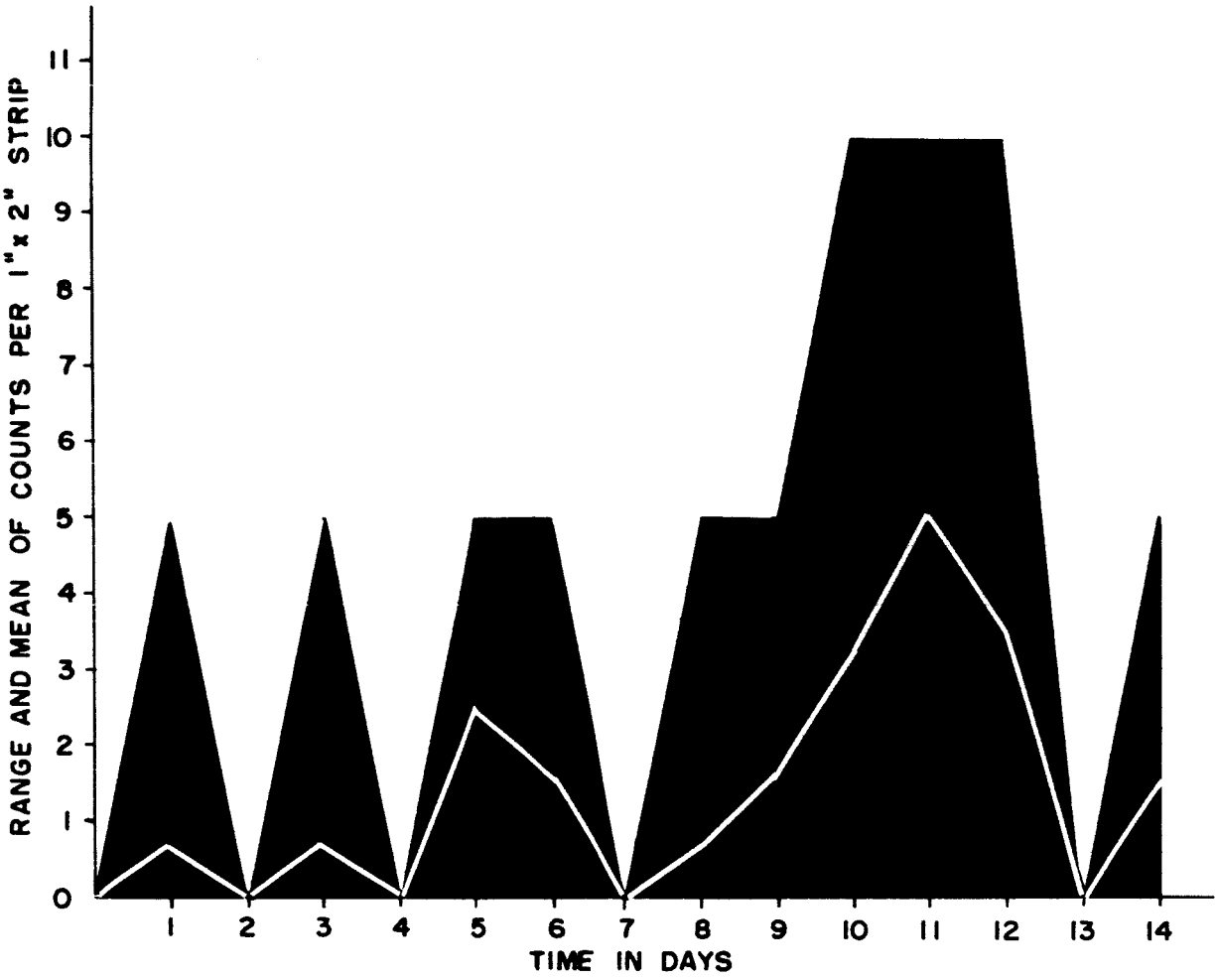
With the exception of counts for the second day's exposure, the stored strips yielded daily mean values considerably lower than those for strips plated immediately. As shown in Figure 14, the mean colony counts for the stored strips never exceeded 10 colonies per strip throughout the last 12 days of the study. Often the mean values were less than 10 colonies per strip.

Since the stored plates were covered until analyzed, it is doubtful that air currents could have been responsible for the reduction in counts of viable particulates. These data indicate that the die-off of deposited microflora was rather extensive under these simple conditions of storage and protection from further fall-out. On the basis of these preliminary data, it appears that die-off through a two week storage period effected a reduction of from 60 to 75 per cent of the common aerobic microflora deposited on the stainless steel strips.

Results from the counts of heat resistant microorganisms under similar conditions of treatment are extremely difficult to interpret. These data are presented in Figures 15 and 16. Wide fluctuations were observed in both the ranges and mean counts per strip. Because of the variations in counts, apparent spotty distribution, and very low levels of spore formers, no extensive conclusions regarding cumulative build up or die-off can be drawn from these limited observations. Additional studies, perhaps in areas with a greater intensity of spore deposition or with more prolonged exposure times, might furnish useful information concerning the spore formers and their build up and survival times on surfaces of interest.

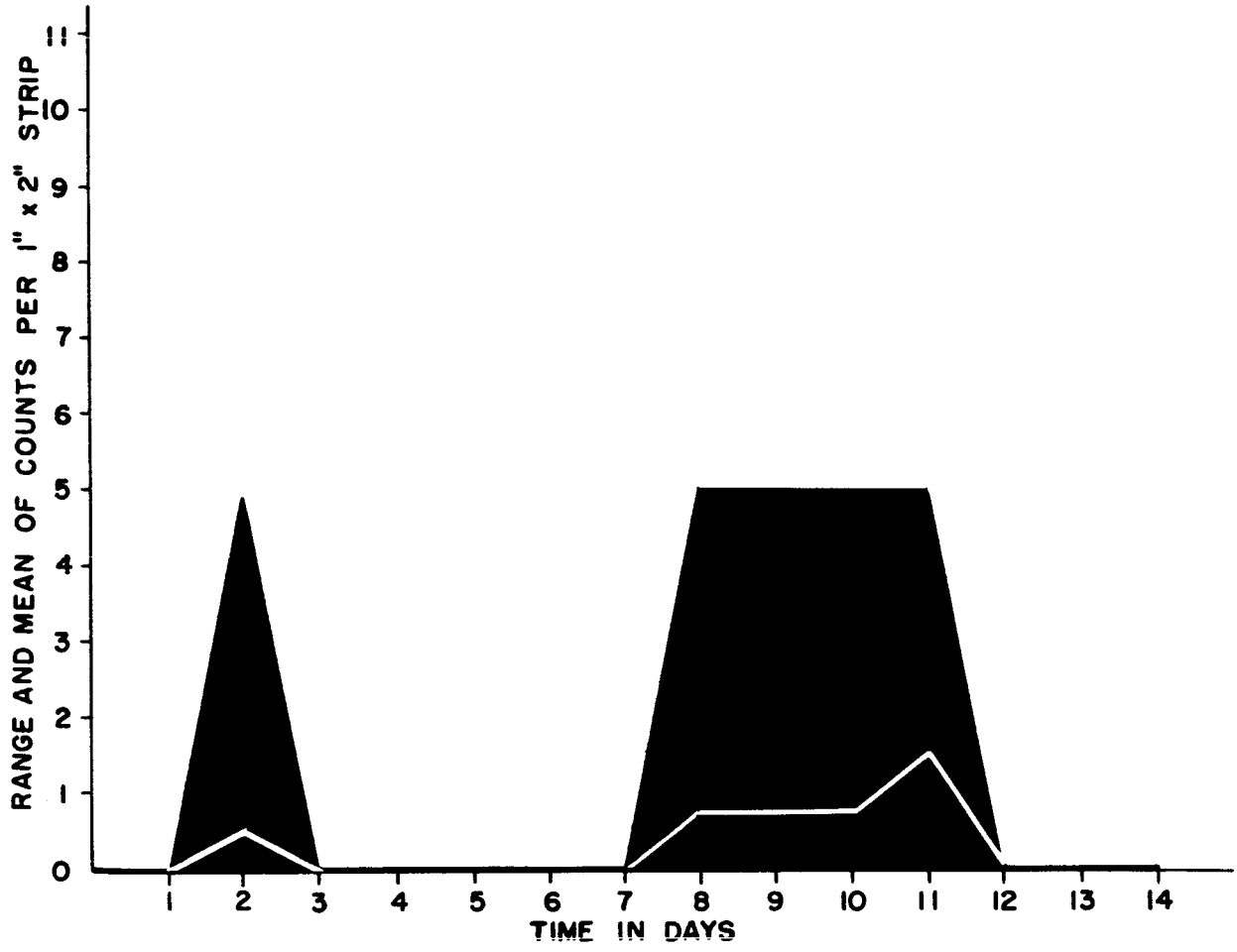
E. Statistical Evaluations of Clean Room Data

Analyses of variance were computed to confirm the over-all impressions concerning statistically significant differences in contamination levels between rooms with varying control procedures (as measured by surface contamination levels). As was expected, there was a significant difference at the 95 per cent confidence level between the relatively well controlled Honeywell rooms and the relatively uncontrolled Univac rooms. However, from a practical point of view the actual difference of no more than one order of magnitude suggested that the use of conventional clean room facilities as such, would not be justified for space hardware assembly from a microbiological contamination reduction view point.



THE MEAN AND RANGE OF HEAT RESISTANT COUNTS PER 1" x 2" STAINLESS STEEL STRIP EXPOSED IN LAB 1 TO 14 DAYS AND PLATED IMMEDIATELY AFTER EXPOSURE

FIGURE 15



THE MEAN AND RANGE OF HEAT RESISTANT COUNTS PER 1" x 2" STAINLESS STEEL STRIP EXPOSED IN LAB 1 TO 14 DAYS, COVERED TWO WEEKS, AND THEN PLATED

FIGURE 16

CHAPTER III

EXPERIMENTS CONCERNED WITH HUMAN CONTACT AS A SOURCE OF MICROBIAL CONTAMINATION ON AEROSPACE MATERIALS

I. BACKGROUND INFORMATION

Previous research has accumulated evidence that human beings are a principal source of microbial contaminants in clean room environments. This appears to be the case for special hospital facilities as well as industrial areas. Therefore, information concerned with contamination levels transferred by workers in clean room assembly processes, as well as the efficacy of control procedures, are obviously of interest to the aerospace program.

Hand contact appears to be one of the important mechanisms for transmission of indigenous and transient microflora from the assembly line technician to his immediate surroundings. Because they come into intimate contact with the nose, mouth, body surfaces, and clothing as well as dust, soil, and numerous contaminated objects, the worker's hands are especially vulnerable to the accumulation of heavy contamination.

For this reason a series of experiments were conducted in the laboratory in order to explore several facets of this problem. These investigations were directed primarily toward analyses of the extent, persistence and control of microbial contamination ordinarily transferred to materials by hand contact. During these investigations, major emphasis was focused on the following:

Study A - An analysis of the degree of contamination deposited on different component materials by contact and manipulation with hands and fingers.

Study B - A determination of die-off rates of natural, heterogeneous microbial populations deposited on surfaces of various

materials by human handling. And a microscopic and morphological characterization of the survivors following prolonged storage.

Study C - An analysis of heat susceptibility of heterogeneous microflora deposited on stainless steel by hand contact.

Study D - Investigations concerned with the effectiveness of different control measures for reducing microbial contact contamination in simulated small component assembly processes.

II. METHODS

Materials used in these experiments were selected as being representative of some typical, common types found in aerospace components. Tests were conducted on 1" x 2" strips of the following seven plastics and metals: (a) 28 gauge stainless steel with a No. 5 or 6 finish; (b) 1/32" aluminum; (c) 1/32" epoxy glass laminate; (d) 1/16" copper covered epoxy laminate; (e) 2 oz. rolled copper plated with lead-tin solder; (f) 1/16" lucite; (g) 1/32" teflon. Simulated component assembly trials were done with 1/4" steel machine screws, washers and nuts.

All materials were thoroughly cleansed by washing, in turn, with hot detergent solution, hot tap water, hot distilled water, isopropyl alcohol, and then ether rinsed and drained dry. Dry heat sterilization at 180° C. for 90 minutes was used to kill any remaining microorganisms (except for heat labile materials which were steam autoclaved at 110° C. for 30 minutes).

Levels of contamination resulting from human handling (Study A) were determined using the 1" x 2" strips of different materials. All seven types of material were contaminated simultaneously. The sterile strips were handled according to a statistically designed, randomized protocol. A group of four laboratory workers (two males and two females) handled the

strips in each trial. By having each strip handled by four persons, it was hoped that much of the expected individual variability could be counteracted. The workers were positioned along a laboratory bench to simulate an assembly line. The first person of the group picked up the strip, manipulated it with fingers, turned it over once and then passed it on to the next individual in line. Strips were ultimately collected in sterile Petri dishes and analyzed. A total of four trials were conducted, each consisting of 25 strips of each material.

Aseptic microbiological techniques were scrupulously observed in the analyses. In randomized order, each strip was placed in 25 ml of warmed (48° C.), M/15 phosphate buffer, pH 7, with 0.02% Tween "20", and shaken on a shaking machine for five minutes. Then strips were removed, embedded in Petri dishes containing 15 ml of molten trypticase soy agar (TSA), and incubated at 32° C. for 72 hours after which a colony count was made.

The total 25 ml buffer eluate for each strip was poured into a 150 mm Petri dish containing a previously prepared 2 mm solidified TSA agar base. The shaker bottle was rinsed with approximately 25 ml of molten TSA agar which was added to the eluate plate. Lastly, an additional 100 ml of molten agar was added to the dish to complete the pour plate. All plating was done in special cabinets equipped with absolute filters. After 72 hours of aerobic incubation at 32° C., colonies were counted with a Bactronic Colony Counter.

Total counts of viable particles for each strip comprise the eluate counts plus the count of residual colonies detected on the embedded strip. Data are reported as viable colonies per strip. With each series, sterile strips were processed as controls to check on the techniques in plating.

The analyses of microbial die-off rates (Study "B") were also performed on all seven types of strip materials. However, in this series, each type of

material was assayed in a separate trial. Groups of four persons contaminated these strips by manipulation as outlined for Study "A".

Sets of 50 strips were analyzed immediately after contamination. Similar sets of contaminated strips (50 per set for periods through 1 week, 25 per set for longer time intervals) were stored in sterile Petri dishes at 72 $\pm$ 4° F. and 60 $\pm$ 10% R.H. Counts of microorganisms surviving storage were made after 1, 2 and 3 days and after 1, 2, 6, 12 and 20 weeks following contamination.

Quantitative microbiological analyses of these strips followed the protocol described for Study "A". In addition to the determination of viable particulates on the stored strips, examinations of the long term survivors were also made. Gram stains of the isolates from colonies developed in plated eluates of 12 and 20 week strips were studied and the microscopic morphology was used to characterize the predominant microbial types among survivors.

Thermal destruction times for heterogeneous contamination, naturally deposited by handling, were studied using stainless steel (Study "C"). Approximately 260 1" x 2" steel strips were thoroughly cleansed with a detergent-water-alcohol treatment and then sterilized in Petri dishes at 330° F. for two hours.

Following sterilization, all strips were subjected to extensive contamination from fingers and hands of four operators who handled each strip separately in manipulations similar to those described for Study "A". Contaminated strips were collected in five sterile 150 mm x 25 mm Petri dishes (in lots of 50 per dish).

Fifty strips (ten from each dish were analyzed immediately for an initial count of viable particles per strip in order to determine the mean

load of the deposited contamination. With a sterile forceps, the remaining 200 strips were placed into individual sterile Petri dishes and sorted into four blocks of 50 strips per block. Each block was then subjected to a predetermined exposure time interval at a constant temperature of 135° C. in a dry heat convection oven equipped with a 24 hour recording thermometer. Exposure times were 15 minutes, 30 minutes, 60 minutes and 24 hours.

Following the heat treatment, sterile forceps were used to transfer each strip into a screw cap bottle containing 50 mls of tryptone glucose yeast extract broth. The bottles were incubated for 14 days at 32° C. and then checked visually for growth as indicated by turbidity.

Effects of special contamination control procedures (Study "D") were evaluated by simulating component production operations through the assembly of previously sterilized 1/4" steel machine screws, washer and nut combinations. Four operators, two male and two female, each assembled 20 units which were collected in sterile Petri dishes labelled for each person. Benches where assembly was done were previously cleansed with 70% ethanol.

After all "components" were completed, groups of five units from each individual's assemblies were placed in bottles of buffer solution and analyzed by the method described in Study "A". With this technique comparative studies were made of the following:

1. Open laboratory assembly with
    a. no hand care
    b. two minute Ivory soap wash
    c. two minute Phisohex wash
2. Assembly in a special cabinet purged by an absolute filtered air stream. Units were assembled with hand care similar to open laboratory combinations and an additional experiment using a two minute Phisohex wash

plus sterile gloves.

III. RESULTS

All data obtained from this investigation deal only with the aerobic, mesophilic, heterotrophic microorganisms. No attempt was made to enumerate other physiological forms. Therefore, results discussed in this section refer only to a fraction of the total biological contamination and obviously do not represent a total microbial count. However, the group of microorganisms which were studied appear to be among the most frequently occurring forms and thus serve as useful indicators of contamination.

Data in Table VIII indicate the differences in average numbers of microorganisms which were deposited on component materials when handled by groups of individuals. These results show that observed mean concentrations of microbial contamination differed from one group to the next, and apparently reflected the influence of large individual variability. Furthermore, the mean levels of contamination also varied with the type of material tested.

With the exception of solder strips, the mean levels of contamination deposited by persons in Group A were consistently higher than those of Group B. Data for Groups C and D indicate that contamination from these groups was of intermediate levels. Under the conditions of generally more intense microbial deposition from Group A, the greatest accumulated mean contamination occurred on lucite and epoxy laminate, with approximately 63 and 33 viable particles per strip, respectively. Scrutiny of combined means for all groups provides evidence which suggests that greater numbers of viable particles are retained on non-metallic materials than on metallic materials under similar conditions of contamination.

There is reason to suspect that some of the variability observed in contact contamination is caused by physiological differences among personnel.

TABLE VIII

MICROBIAL CONTAMINATION DETECTED ON COMPONENT MATERIALS AFTER HANDLING BY GROUPS OF FOUR PERSONS

Material	Mean microbial plate count* per 1" X 2" strip				
	group A	group B	group C	group D	material mean**
A. METALLICS					
Stainless steel	14.6	7.4	2.2	8.4	8.2
Copper	16.9	3.3	3.8	6.3	7.6
Aluminum	17.1	3.1	6.3	10.9	9.4
Solder	10.2	18.9	2.4	3.8	8.8
All metallics	—	—	—	—	8.5
B. NON-METALLICS					
Epoxy laminate	33.0	9.4	16.7	18.8	19.5
Lucite	63.4	10.8	25.9	17.2	29.3
Teflon	17.0	6.5	18.4	16.2	14.7
All non-metallics	—	—	—	—	21.1
Group mean	24.6	8.5	10.8	11.6	—

*represents mean count for 25 strips of each material per group.

**represents mean count averaged over all groups, a total of 100 strips per material.

Skin conditions, individuals recovering from colds, previous contact with other contaminated areas, etc. may all influence levels of deposition from time to time. At the present time, the extent of these influences have not been evaluated.

The experimental results obtained from analyses of microbial survival on the seven different materials demonstrated interesting, recognizable differences (see Figures 17 through 22). These graphs show that the mean concentrations of microorganisms initially deposited by handling of the materials varied widely. For example, mean values of 240 viable particles were detected on stainless steel, while a mean level of only 17.7 viable particles per strip occurred on aluminum. Presumably these variations resulted from differences in cleanliness of hands, or in shedding from hands, of the persons participating in these studies. Since these materials were handled by different groups of individuals on different days, such variation in concentrations of initial contamination might be expected.

Information collected during these experimental trials demonstrated certain trends in microbial survival on the materials studied. Stainless steel, aluminum and teflon (Figures 17, 18 and 22) yielded fairly clear cut patterns suggesting a progressive die-off of microflora. A similar trend was noticeable on the solder strips as shown in Figure 19. An exceptionally high count obtained on one of the seven day solder strips was responsible for the high mean number of survivors calculated for that day. Since there was a possibility that extraneous contamination had occurred in this sample, the dotted bar was used on the graph.

The patterns of reduction in microflora counts on storage of copper, epoxy and lucite materials showed considerable variations from any progressive die-off trend. As indicated by Figures 20, 21 and 23, marked fluctuations in

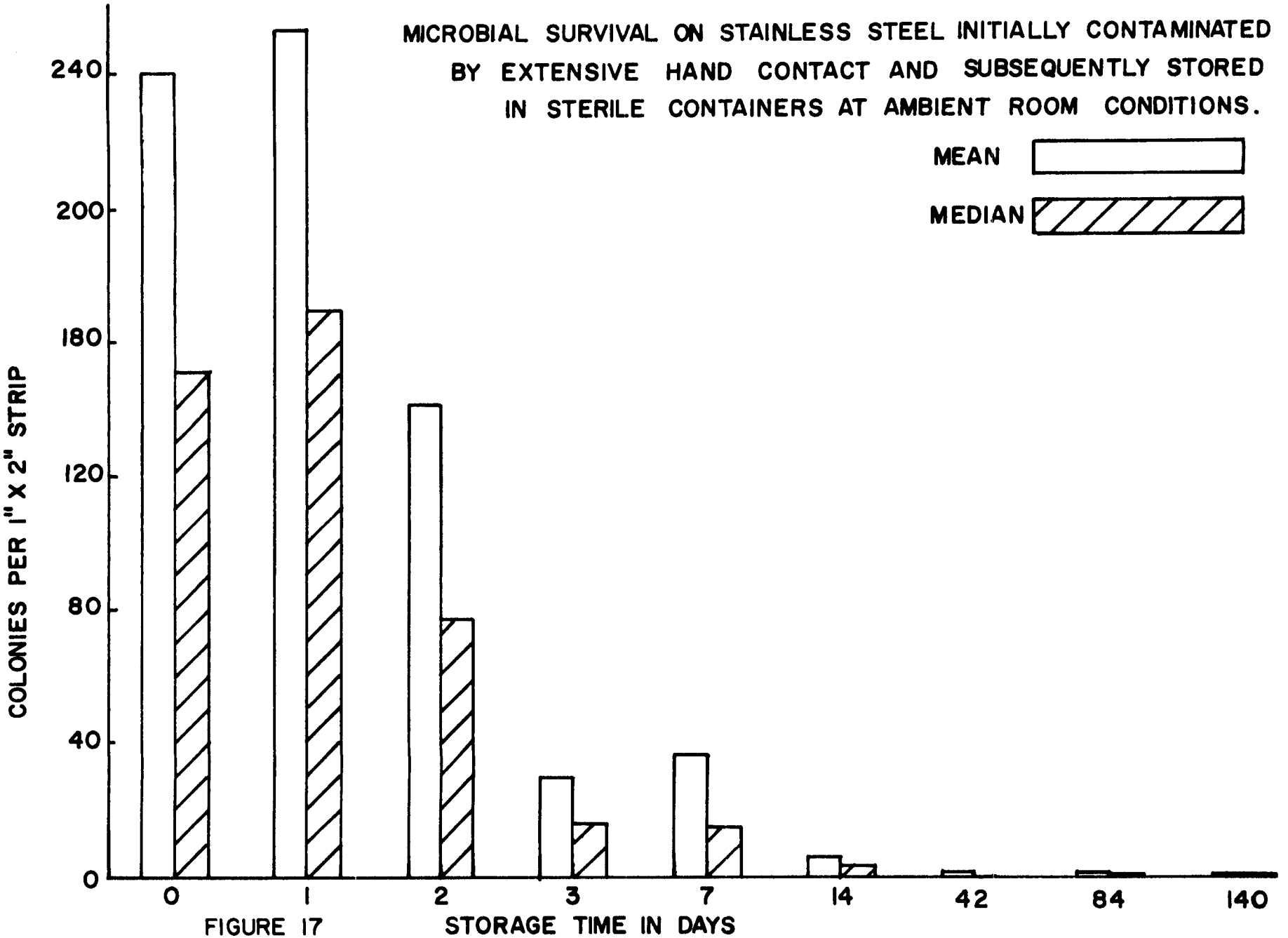

MICROBIAL SURVIVAL ON STAINLESS STEEL INITIALLY CONTAMINATED
BY EXTENSIVE HAND CONTACT AND SUBSEQUENTLY STORED
IN STERILE CONTAINERS AT AMBIENT ROOM CONDITIONS.
MEAN
MEDIAN
COLONIES PER 1" X 2" STRIP
240
200
180
120
80
40
0
0
1
2
3
7
14
42
84
140
STORAGE TIME IN DAYS

FIGURE 17

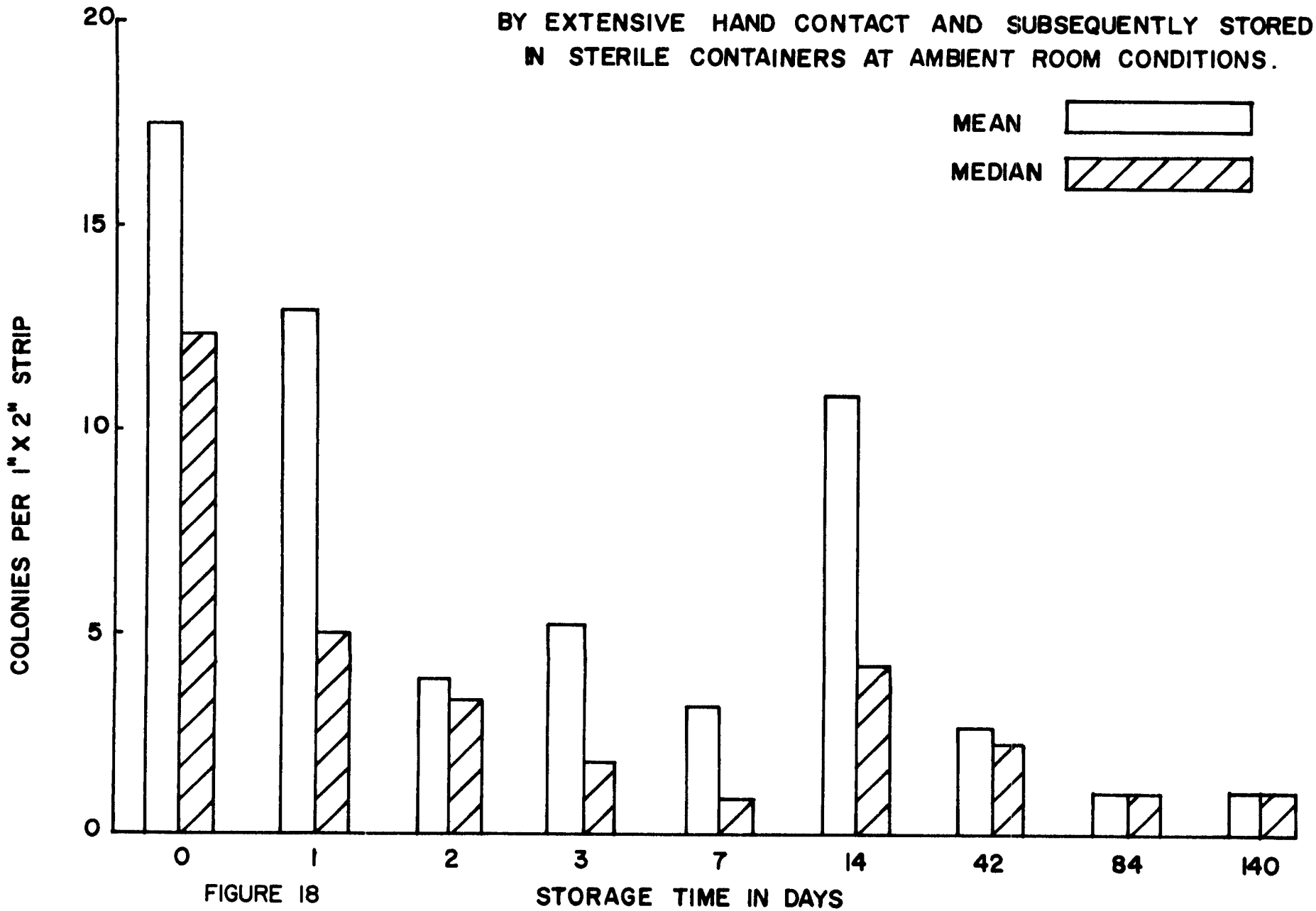

MICROBIAL SURVIVAL ON ALUMINUM INITIALLY CONTAMINATED
BY EXTENSIVE HAND CONTACT AND SUBSEQUENTLY STORED
IN STERILE CONTAINERS AT AMBIENT ROOM CONDITIONS.
MEAN
MEDIAN
COLONIES PER 1" X 2" STRIP
20
15
10
5
0
0
1
2
3
7
14
42
84
140
STORAGE TIME IN DAYS

FIGURE 18

MICROBIAL SURVIVAL ON SOLDER INITIALLY CONTAMINATED BY EXTENSIVE HAND CONTACT AND SUBSEQUENTLY STORED IN STERILE CONTAINERS AT AMBIENT ROOM CONDITIONS.

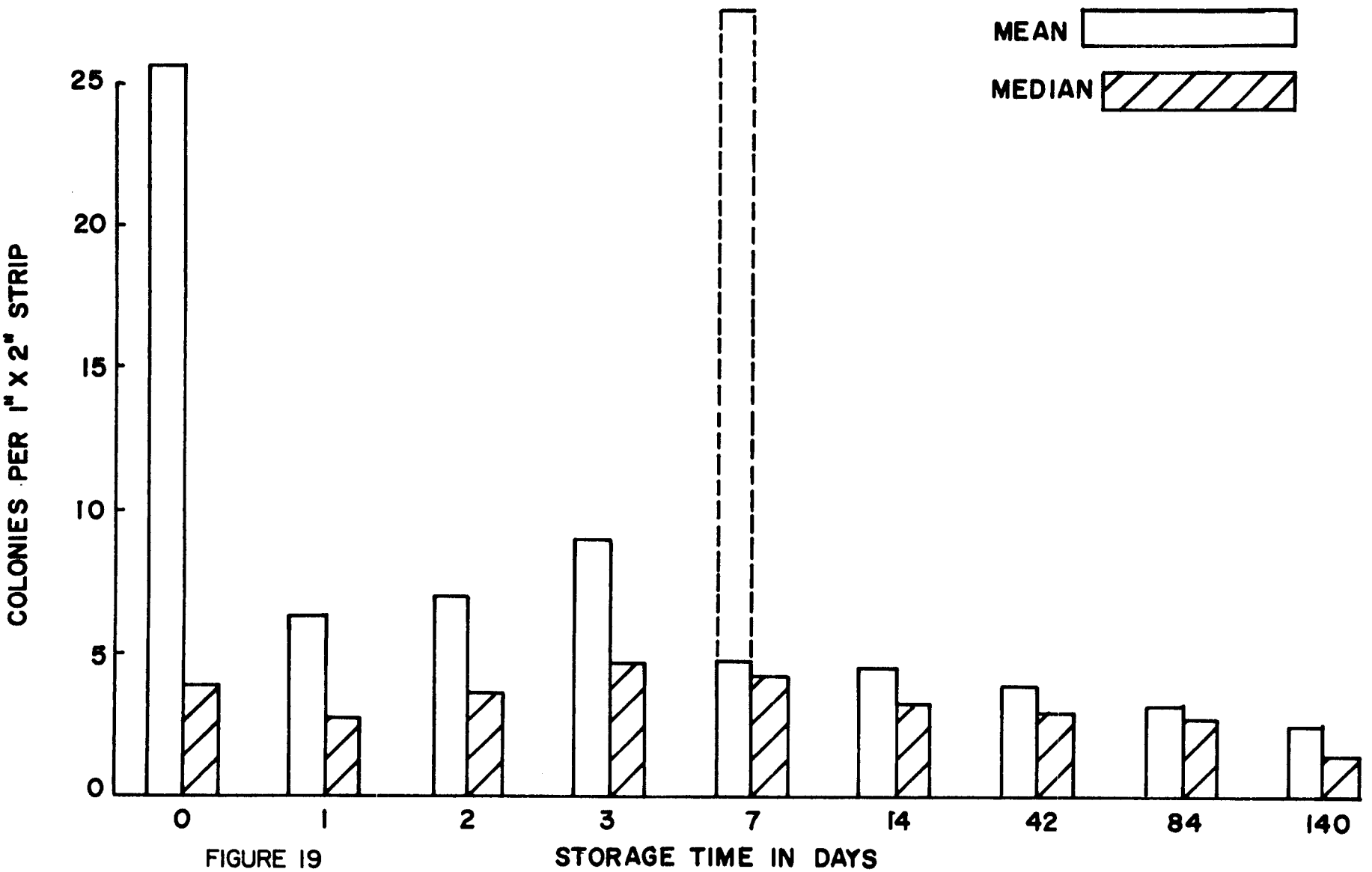


FIGURE 19

MICROBIAL SURVIVAL ON COPPER INITIALLY CONTAMINATED BY EXTENSIVE HAND CONTACT AND SUBSEQUENTLY STORED IN STERILE CONTAINERS AT AMBIENT ROOM CONDITIONS.

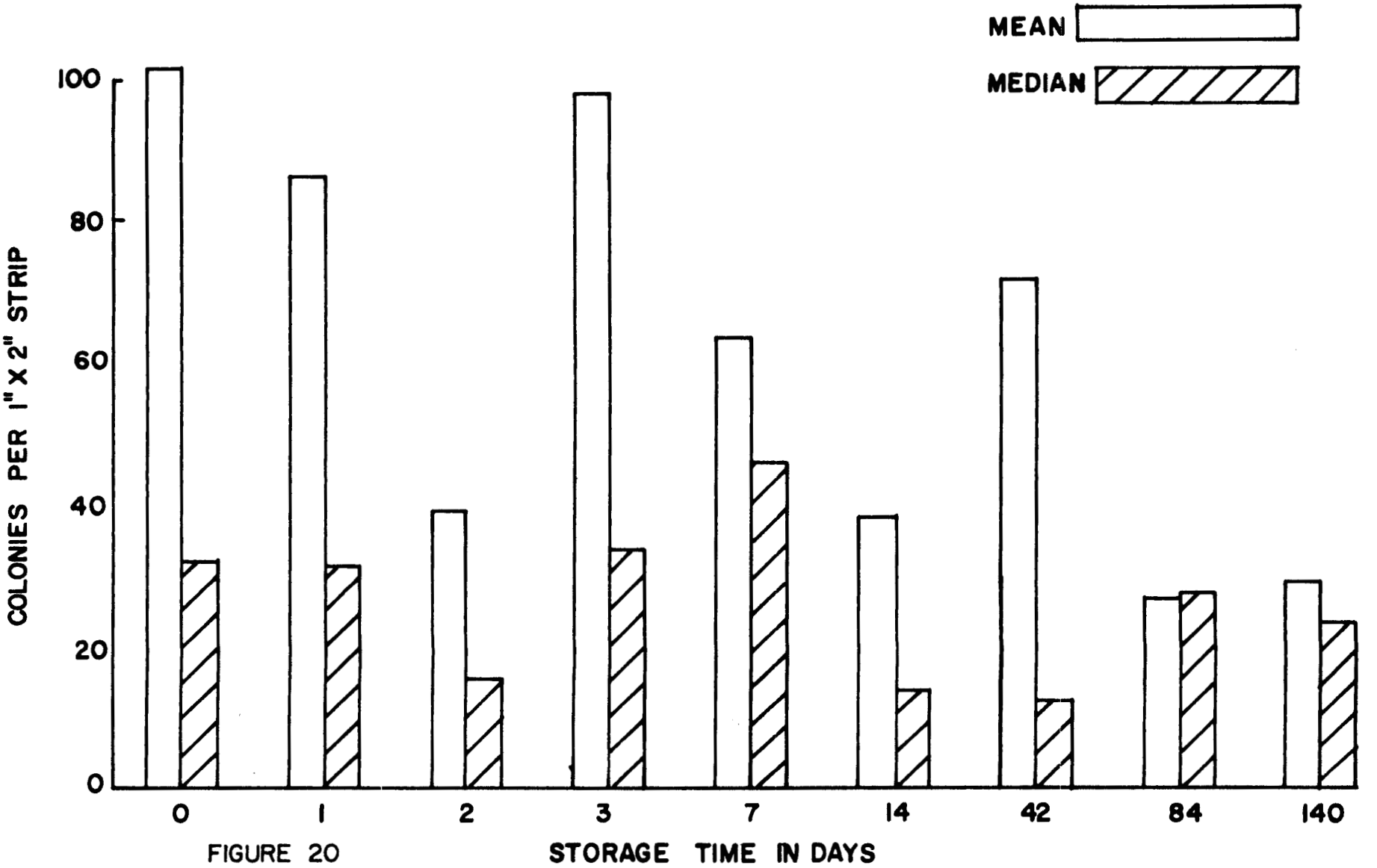


FIGURE 20

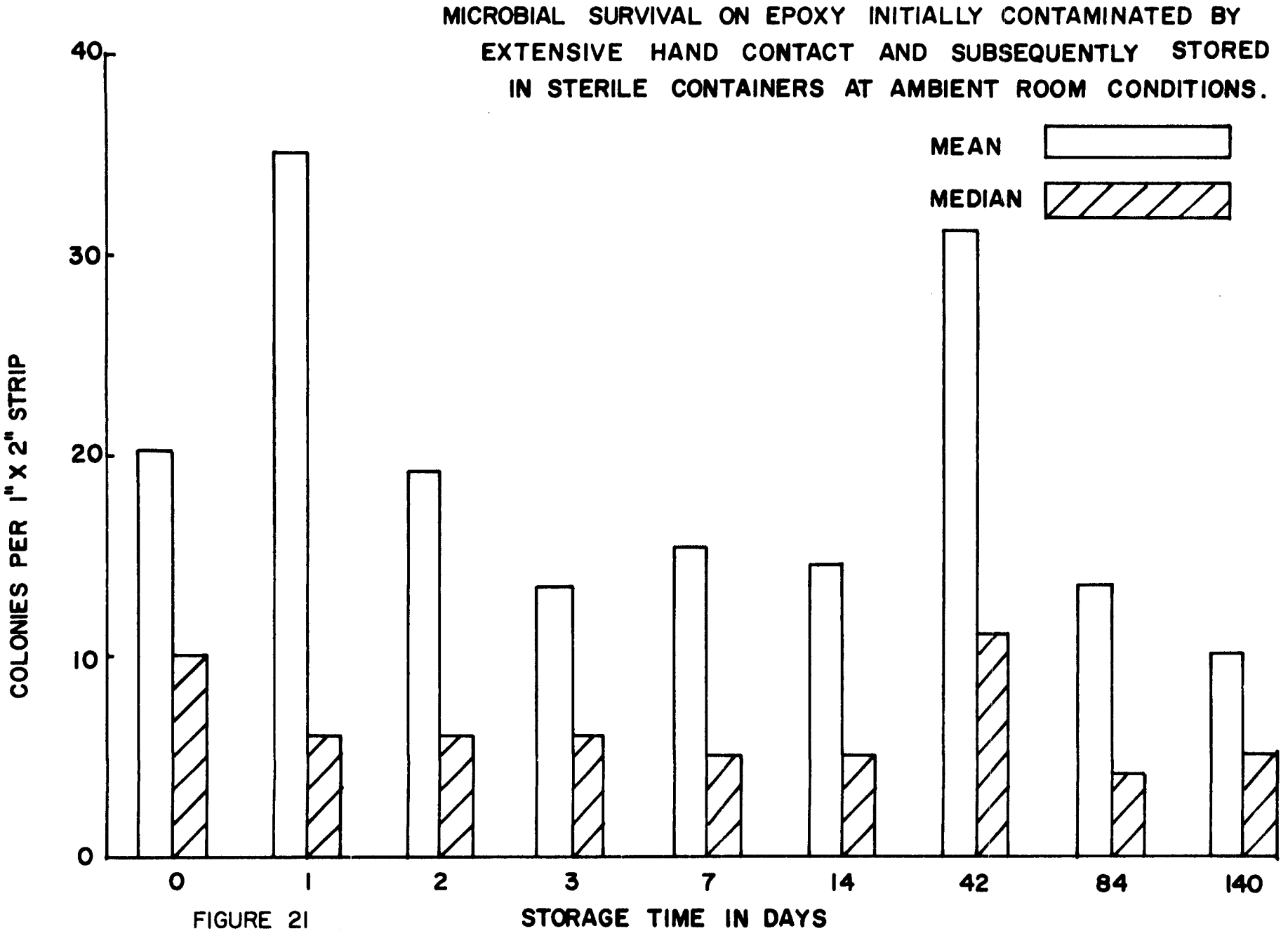

MICROBIAL SURVIVAL ON EPOXY INITIALLY CONTAMINATED BY EXTENSIVE HAND CONTACT AND SUBSEQUENTLY STORED IN STERILE CONTAINERS AT AMBIENT ROOM CONDITIONS.
MEAN
MEDIAN
COLONIES PER 1" X 2" STRIP
40
30
20
10
0
0
1
2
3
7
14
42
84
140
STORAGE TIME IN DAYS

FIGURE 21

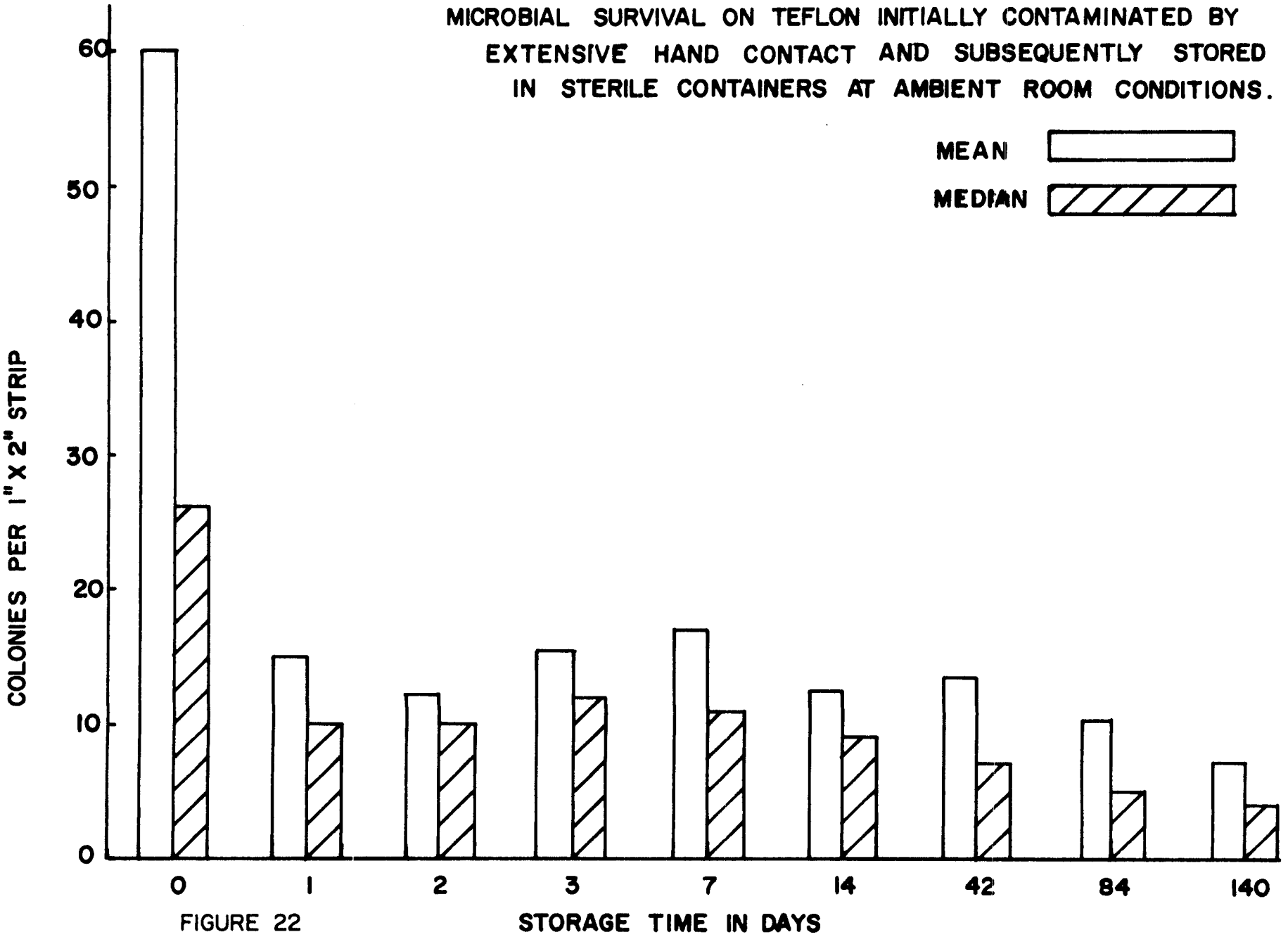

MICROBIAL SURVIVAL ON TEFLON INITIALLY CONTAMINATED BY EXTENSIVE HAND CONTACT AND SUBSEQUENTLY STORED IN STERILE CONTAINERS AT AMBIENT ROOM CONDITIONS.
MEAN
MEDIAN
COLONIES PER 1" X 2" STRIP
0
10
20
30
40
50
60
0
1
2
3
7
14
42
84
140
STORAGE TIME IN DAYS

FIGURE 22

MICROBIAL SURVIVAL ON LUCITE INITIALLY CONTAMINATED BY EXTENSIVE HAND CONTACT AND SUBSEQUENTLY STORED IN STERILE CONTAINERS AT AMBIENT ROOM CONDITIONS.

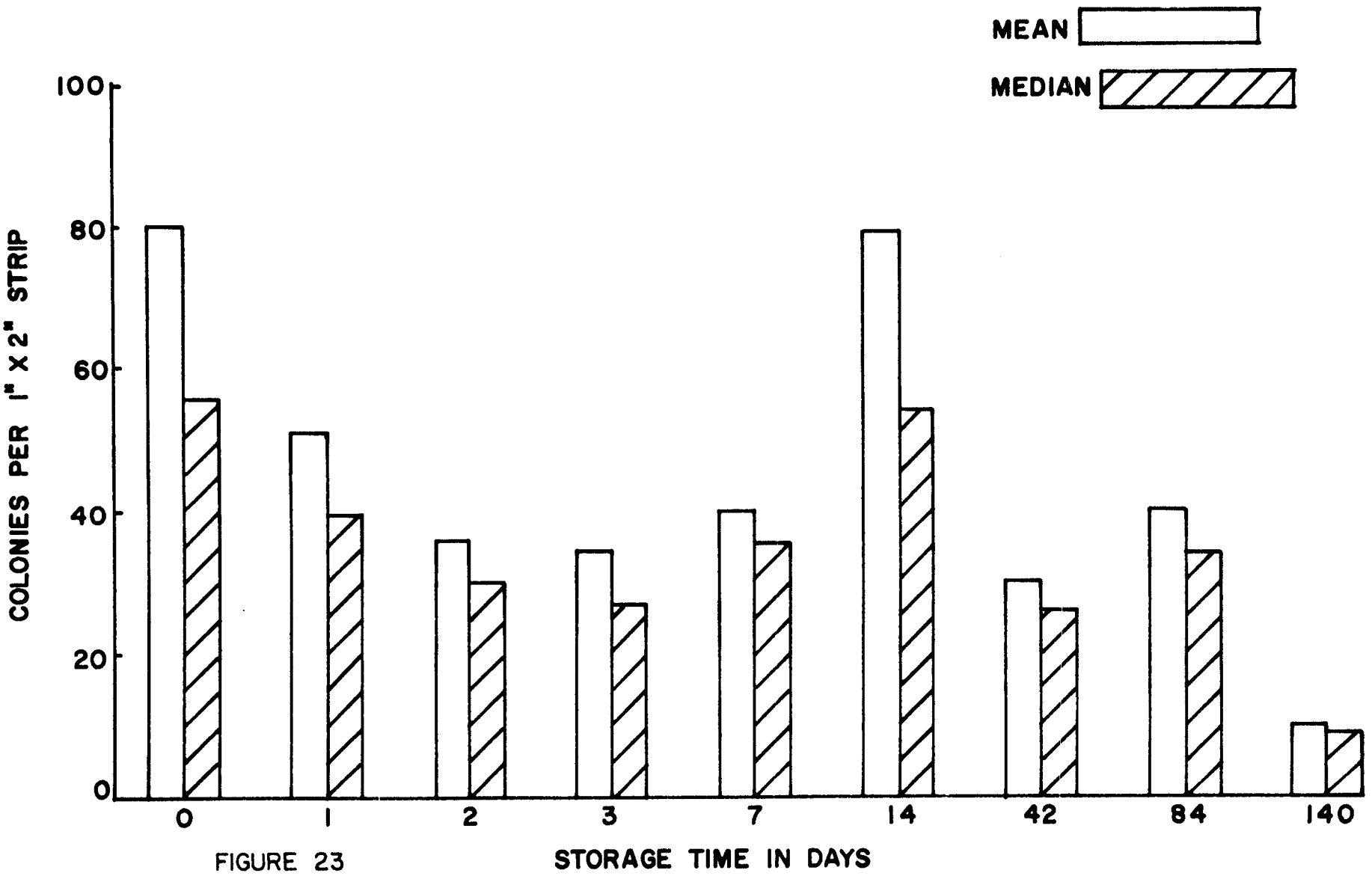


FIGURE 23

mean counts were observed on these materials, even as late as 14 to 42 days after storage. At the moment, these apparent anomolous fluctuations are difficult to interpret. The composition of materials, changes in room air mass, humidity and other factors of storage may have some effects on this phenomenon.

A compilation of actual mean count values for this study of microbial survival on materials is presented in Table IX. From these data, it is evident that highest die-off rates were observed on stainless steel, where mean counts dropped from 239 viable particles per strip to less than one, over a period of 20 weeks storage. Thus, it appears that simple storage effected a 99 per cent reduction in the mean microbial plate count for the steel strips. The aluminum strips and solder plated strips yielded mean concentrations of less than two organisms per strip after 20 weeks. This value represents a 90 per cent reduction from the initial contamination levels deposited on these materials.

Lowest rates of die-off were observed on copper strips and on the non-metallic materials (epoxy laminate, lucite and teflon). Although yielding lowered mean counts following storage, these strips still retained mean contamination levels of 7 to 29 viable particles per strip at the end of 20 weeks storage. Highest mean numbers of 20 weeks survivors were found on the copper.

In conjunction with the studies of microbial die-off under storage, a parallel series of analyses provided data on the characteristic types of organisms surviving the storage periods between 12 and 20 weeks. A breakdown of microbial types among 1,513 survivors isolated from stored strips is shown in the data of Table X.

By far the predominant group of organisms isolated from these materials

TABLE IX

SURVIVAL AFTER STORAGE OF MICROORGANISMS ON AEROSPACE COMPONENT MATERIALS CONTAMINATED THROUGH HANDLING.

STORAGE TIME (in days)	MEAN MICROBIAL PLATE COUNTS* PER 1" X 2" STRIP						
	stainless steel	copper	aluminum	soldered copper	epoxy laminate	Teflon	Lucite
0	239.9	101.4	17.7	25.6	20.1	59.8	55.5
1	253.2	86.5	11.9	6.3	34.9	14.9	39.5
2	140.6	39.6	3.4	7.6	19.2	12.2	30.0
3	28.8	98.2	4.9	8.0	13.6	15.4	27.0
7	36.4	63.7	3.0	26.5	15.4	17.1	35.5
14	5.6	38.4	10.9	4.4	14.5	12.4	54.0
42	1.1	71.6	2.6	3.6	30.8	13.4	26.0
84	1.0	26.9	1.1	2.2	13.6	10.2	40.2
140	0.9	29.1	1.1	1.9	10.1	7.4	9.6

* Represents mean count of 50 strips for each time interval through one week and 20-25 strips for subsequent time intervals.

TABLE X

MORPHOLOGICAL TYPES OF MICROORGANISMS ISOLATED FROM STRIPS INITIALLY HANDLED BY PERSONNEL PRIOR TO STORAGE IN STERILE CONTAINERS FOR 12 TO 20 WEEKS AT AMBIENT ROOM CONDITIONS*

STRIP MATERIAL	NUMBER OF ISOLATES & MICROSCOPIC CHARACTERIZATION				
	TOTAL ISOLATES EXAMINED	GM.+ BACILLI (SPORES) per cent	GM.+ BACILLI (NO SPORES) per cent	GM.+ COCCI per cent	OTHER FORMS per cent
STAINLESS STEEL	21	0.0	90.4	4.8	4.8
COPPER	314	82.2	17.2	0.6	0.0
ALUMINUM	50	66.0	28.0	6.0	0.0
SOLDER	219	0.5	9.1	82.6	7.8
LUCITE	466	56.8	39.7	3.2	0.03
EPOXY	249	39.8	60.2	0.0	0.0
TEFLON	194	81.4	17.5	1.1	0.0

*TEMPERATURE RANGE: 68-76°F AND 50-65% R.H.

were the Gram positive bacilli. In almost every case, this group comprised over 90 per cent of the isolates from colonies on strips plated after 12 to 20 weeks. Only in the analyses of the solder strips were the Gram positive cocci found in greatest abundance. However, these data appear anomolous when compared to those for other materials and, since some problems of media contamination occurred during analyses of solder, the validity of these data remains questionable. Other forms of microorganisms, such as yeasts, molds, Gram negative bacteria, etc. were either not detected or made up less than 10 per cent of the total isolates. Of great potential importance was the fact that these determinations revealed a very high proportion of potential spore forming species among the microbial types surviving after 12 and 20 weeks on these materials. It is precisely this fraction of the microbial spectrum which is of greatest concern to persons involved in spacecraft sterilization programs.

Because sporeformers were detected among contaminants on handled strips, the question concerning the ease of dry heat sterilization arose. For this reason, the heat susceptibility of the heterogeneous microflora deposited on stainless steel by hand contact was investigated in a single experimental series. The 250 strips analyzed had been contaminated by handling as described previously. An initial mean level of 76.5 viable particles per strip was the detectable contamination achieved. This load was subjected to the heat treatment process.

The results of this experiment are presented in Table XI. Of the total 200 strips exposed to 135° C., only two demonstrated growth in media tubes after heat treatment. These were from groups of strips exposed for thirty minutes or less. More significant is the fact that no growth was observed from any cultured strips which had been treated at 135° C. for one hour or

TABLE XI

EFFECT OF DRY HEAT TREATMENT ON HETEROGENEOUS CONTAMINATION NATURALLY DEPOSITED ON STAINLESS STEEL STRIPS BY HANDLING

number of contaminated strips* tested	holding time at 135° C	number of strips showing growth after heating
50	15 min.	1
50	30 min.	1
50	60 min.	0
50	24 hrs.	0

* Analyses of 50 additional strips indicated a mean contamination level of 76.5 colonies per strip and a range of 7 to 461 colonies per strip.

longer. Thus, these preliminary data suggest that dry heat exposure times of greater than one hour at 135° C. may be adequate to sterilize a mean contamination load of 76.5 viable particles per strip, if the contaminants are similar to the heterogeneous mixtures ordinarily deposited from handling.

Studies concerned with the effectiveness of different control measures for reducing microbial contact contamination were also of a preliminary nature. Nevertheless, the data from experiments with precautionary hand care and special barrier techniques demonstrated that certain procedures were effective for reducing the transfer of microorganisms to component materials. The various experimental assembly trials attempted and the results of each are listed in Table XII.

Mean contamination levels reached 122.6 viable particles per single assembly unit when no precautionary measures were used. Washing with ordinary soap yielded erratic results, including in one instance a four-fold increase over the observed counts for controls (no treatment) from the nut and screw assemblies. Hexachlorophene soap scrubs appeared to be moderately effective in reducing transfer of contamination from hands to assembly units.

The greatest contamination control was obtained with a combination of Phisohex scrub plus the use of sterile, disposable gloves. Use of this procedure reduced mean levels of contamination to less than one organism per assembled unit. These data indicate that even modest precautions in hand care combined with the use of effective barriers, such as sterile gloves, can reduce microbial contamination considerably below the levels attained under conditions ordinarily prevalent in uncontrolled circumstances.

TABLE XII

LEVELS OF MICROBIAL CONTAMINATION DETECTED DURING SIMULATED COMPONENT ASSEMBLY* TRIALS UNDER DIFFERENT CONTROL METHODS.

Microbial Colony Counts – TSA medium

method	per five assemblies			per single assemblies
	mean	median	range	mean
Open Laboratory				
No hand care	611.3	432.0	20-1904	122.6
Two min. Ivory soap wash	66.5	31.5	11-280	13.3
Two min. Phisohex wash	6.6	2.0	0-37	1.3
Specialaire Hoods				
No hand care	77.1	57.0	12-288	15.4
Two min. Ivory soap wash	299.3	46.5	0-3044	59.9
Two min. Phisohex wash	20.8	15.5	5-51	4.2
Two min. Phisohex wash plus sterile gloves	0.9	0.0	0-10	0.2

*nut-washer-machine screw assemblies.

CHAPTER IV

EVALUATION OF LAMINAR FLOW ROOMS

The concept of the vertical laminar flow clean room indicated a possible dramatic reduction in microbial loading on a spacecraft assembled in such a facility. The data accumulated on the environmental microbiology of conventional clean rooms, and confirmed by several research laboratories, had indicated that a minimum of approximately 10^3 total colonies per square foot could be expected to accumulate on space hardware from aerial fall-out during the duration of its stay in the best of conventional facilities. This figure is independent of time due to the plateau effect discussed in Chapter II. Thus, a basis for comparison was available and N.A.S.A. was anxious to learn whether a significant improvement could be affected by the use of laminar flow.

The facility constructed for the University of Minnesota is a pre-fabricated model, 8 feet by 10 feet in area with a 7 foot 6 inch ceiling and utilizing a 4 foot by 4 foot anteroom. The wall surfaces are anodized aluminum and the flooring is aluminum grillwork set over media prefilters. It was designed and built by Envirco, Inc. of Albuquerque, New Mexico. Admittedly, the facility employs a minimum of pre-entrance precautions and thus provides an excellent test of the capability of the vertical laminar flow principle itself. The facility is diagrammed in Figure 24.

Following its construction and check-out, the room was initially cleaned by repeated scrubbings with an iodophor solution.[1] The design called for a constant temperature of 72° F. $\pm$ 2° and relative humidity of 40-45

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[1] Mikroklene, Economics Laboratory, St. Paul, Minnesota

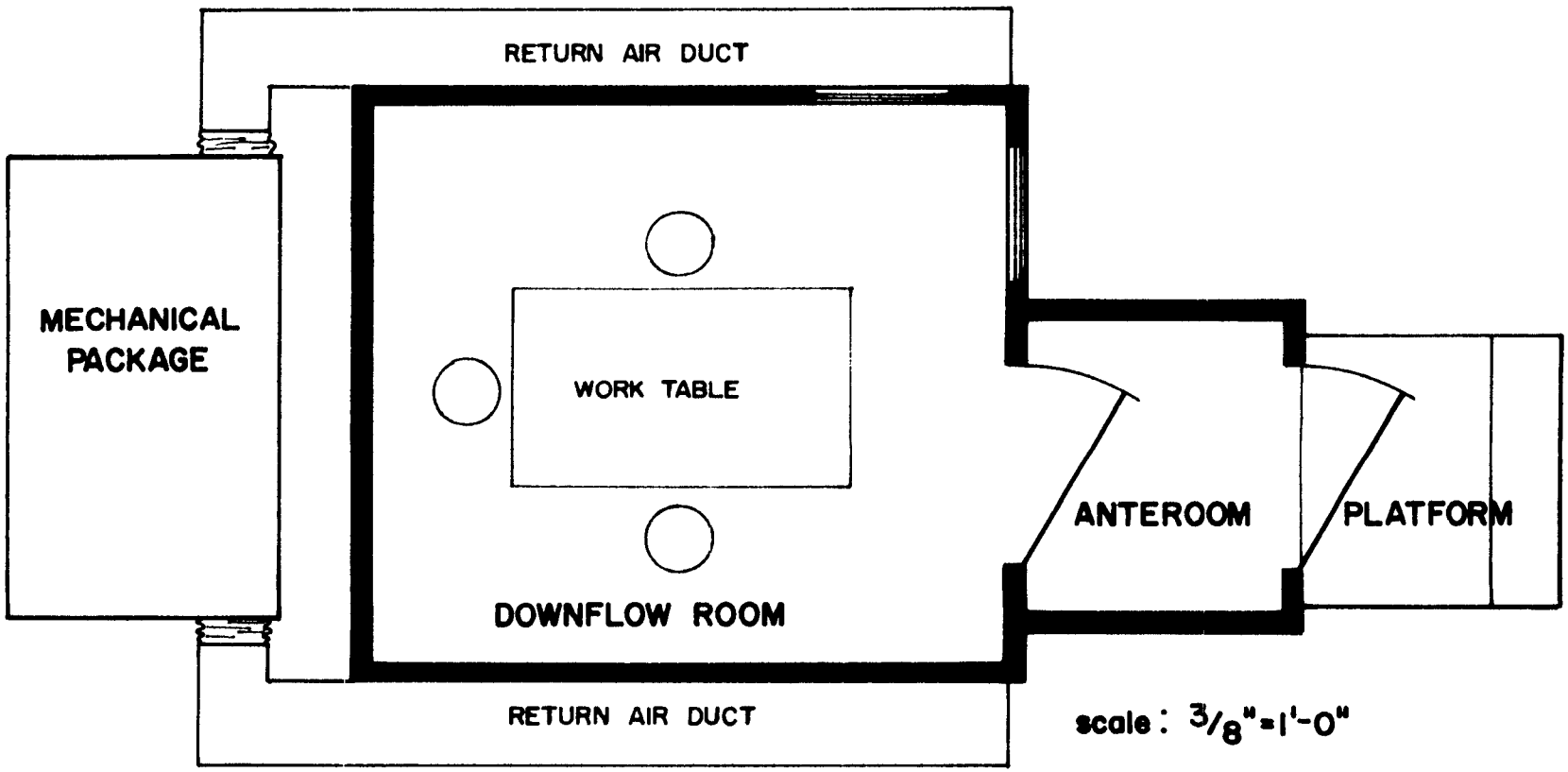

FIGURE 24

FLOOR PLAN, LAMINAR DOWNFLOW ROOM FACILITY
SCHOOL OF PUBLIC HEALTH, UNIVERSITY OF MINNESOTA

per cent.

Furnishings consisted of only three chairs and two 4 foot by 2 foot 6 inch home-made tables with perforated aluminum tops for minimal impedance of laminar flow. Later, these tables were replaced by a conventional 5 foot by 3 foot clean room table with pedestal legs, and conventional clean room chairs were added.

Activity in the room during monitoring periods consisted of three persons performing simulated space hardware assembly experiments. These experiments were designed primarily to provide information on human contact contamination and the efficacy of various barriers on prevention of contamination.

The parameters used to measure the microbial characteristics of the laminar flow room were very similar to those used in conventional clean rooms. They were:

1. Volumetric air sampling using Reynier slit samplers.
2. Fall-out onto open Rodac plates at bench top and floor levels.
3. Rodac plate sampling of room surfaces.
4. Fall-out onto 1 inch by 2 inch stainless steel and epoxy laminate strips during a 20 week period.

METHODS

The sampling methodology was very similar to that used in the conventional clean room work and described in detail in Chapter II. However, there were several major modifications.

The slit samplers were replaced by new models with an improved o-ring closing mechanism and electric clock motors. During these evaluations 6 and 2 hour clocks were used in place of the one hour clocks used previously. They have the obvious advantage of longer sampling intervals with less manipulation

needed by sampling personnel. However, the six hour sampling period seemed to result in a lower sampling efficiency and it was decided to use the two hour clocks exclusively during the later stages of the evaluations. Two samplers were placed on the work tables in close proximity to the actual area of assembly. Samplers were started just prior to the day's activity and run for 2 to 6 hours, including the duration of the entire assembly operation. Samples were collected on Trypticase Soy Agar and incubated for 72 hours at 32° C.

Additional information on microbial fall-out in the assembly area was gathered by placing a series of ten open Rodac plates containing Trypticase Soy Agar on the bench top in the vicinity of the activity during each assembly period (about 2 hours). An additional ten such plates were simultaneously placed on the floor near the workers' feet. The plates were left uncovered during the entire assembly period then covered and incubated at 32° C. for 72 hours.

The Rodac surface samples were confined to wall surfaces in the downflow room and to wall and floor samples in the anteroom. It was not practical to sample floors or bench tops due to the perforations. All sampling sites were randomly selected and samples were collected approximately every two weeks with no cleaning whatsoever in between for the purposes of this experiment.

The final parameter of interest in comparing the laminar flow and conventional clean rooms was microbial fall-out onto 1 inch by 2 inch stainless steel and epoxy laminate strips placed on bench tops in the assembly area for a period of 20 weeks. In this experiment 60 stainless steel and 60 epoxy strips were placed on the benches in open, empty Rodac dishes at the start of the period. The strips had been prepared and sterilized as

described in Chapter II. At two week intervals six strips of each type were randomly selected for analysis.

For total count after aerobic incubation, two strips of each type were placed into 25 ml of sterile phosphate buffer and mechanically shaken for five minutes. The 25 ml eluate was then poured into a 150 mm plate and molten T.S.A. was added in sufficient quantity to yield a solid agar plate after cooling. The strip itself was then placed directly into a standard Petri plate and covered with T.S.A. Plates were incubated aerobically at 32° C. for 72 hours and counted. The total count for the strip was taken as the sum of the counts from the pour plate and the direct plating of the strip.

The four additional strips of each type were placed into dry, sterile, four ounce bottles, and dry heat treated at 80° C. for 15 minutes. Following this treatment, 25 ml of sterile phosphate buffer was added to the bottles and they were shaken mechanically for five minutes. The eluates were poured into 150 mm dishes as before with the molten T.S.A. Two plates from each type of strip were incubated aerobically and the remaining two anaerobically. Again the strips themselves were plated directly in T.S.A. and two of each incubated aerobically while the remaining two were incubated anaerobically. Aerobic incubation was at 32° C. for 72 hours while anaerobic incubation was at 32° C. for 1 week.

During the twenty week period the room was in continuous operation for the first eleven weeks. It was then shut down for repairs during the entire twelfth week, although assembly activity continued without the laminar air flow or environmental control. The air flow was turned on again the day that the twelve week series of strips was picked up for analysis.

RESULTS

Table XIII presents data from volumetric air sampling and aerial fall-out

TABLE XIII

THE EFFECT OF PERSONNEL DRESS PROCEDURES ON MICROBIAL AIR-BORNE CONTAMINATION AND FALLOUT IN A LAMINAR DOWNFLOW ROOM DURING ASSEMBLY PERIODS.

SAMPLING METHOD AND LOCATION	———	FULL DRESS PROCEDURE	LAB COATS ONLY
REYNIERS VOLUMETRIC AIR SAMPLES AT BENCH TOP LEVELS	TOTAL CU. FT. SAMPLED	6200	10,080
	MEAN COL./CU. FT.	1.4×10^{-3}	2.3×10^{-2}
FALLOUT ONTO OPEN RODAC PLATES AT BENCH TOP LEVEL	NUMBER OF SAMPLES	170	190
	MEAN COL./RODAC PLATE	0.11	0.25
	MEAN COL./SQ. FT.	3.96	9.00
FALLOUT ONTO OPEN RODAC PLATES AT FLOOR LEVEL	NUMBER OF SAMPLES	165	186
	MEAN COL./RODAC PLATE	0.34	0.26
	MEAN COL./SQ. FT.	12.24	9.36

onto agar surfaces during assembly periods in the laminar downflow room. The data are broken down to show a comparison between a complete clean room dress procedure and a limited dress procedure.

The full dress consisted of a dacron clean room uniform of coverall style with tight ankles and wrists, a full hood leaving only the face exposed, a surgical mask, and clean room booties. All parts of the uniform were sterilized prior to use. Participants dressed in the anteroom. Hands were covered with sterile gloves about half the time and subjected to five minute Phisohex scrubs on remaining occasions, depending on the particular assembly experiment in progress.

The partial dress procedure consisted of clean, cotton laboratory coats, paper caps and plastic boots. Hand barriers were again divided between the sterile gloves and the Phisohex scrub.

The data indicate a level of approximately one colony per 1,000 cu. ft. of air during the full dress procedure, and two colonies per 100 cu. ft. for the lab coats only. It should be pointed out, however, that except for one very high sample the mean count for lab coats only would have been about 5 colonies per 1,000 cu. ft. Nevertheless, this does indicate the potential difficulty which can be encountered, even if only rarely by relaxation of stringent dress procedures, even with laminar flow. By comparison, in conventional clean rooms a minimum of approximately 1 colony in 4 cu. ft. was the best that could be achieved (see Chapter II). It is also interesting to note that fall-out onto Rodac plates at floor level was very similar regardless of dress procedure. However, on the work bench there was a two-fold increase in these counts when the less stringent controls were exercised. In all cases, the fall-out was less than 13 colonies per square foot and is very low indeed for a two hour period with activity.

Table XIV demonstrates the "build-up" of contamination on room surfaces during a nine week period of occupancy following disinfection, with no further cleaning measures employed. It is obvious from these data that a minimum of routine housekeeping will be necessary in such a facility. Even the anteroom, without laminar flow, but with positive air pressure from the laminar flow room to the outside, exhibited very little contamination accumulation. The levels on the downflow room walls, including two occasions with no colonies at all in 80 samples is, of course, extremely low.

Table XV summarizes the results of the 20 week accumulation of contaminants on the stainless steel and epoxy laminate strips. It can be seen that not one single colony was recovered from these strips during the first ten weeks of the experiment, while the room was in operation. After a one week shutdown, while activity continued, total counts between 5 and 10 thousand per square foot were quickly recorded. Then after several weeks of renewed operation, counts quickly subsided to several hundred colonies per square foot. It is interesting to note that the numbers on heat treated strips at this time were approximately equal to those on the unheated strips, indicating that only the most resistant microorganisms remained at that time. It is also interesting to note that this situation prevailed at approximately the same level for the remainder of the twenty week period.

While it is not practical to claim that the zero levels encountered on stainless steel strips during the first ten weeks can be depended upon as reliable consistent estimates of aerial fall-out in a laminar downflow room, they are certainly indicative of the potential of this facility to effect a significant reduction in microbial loading on a spacecraft compared to a conventional clean room facility.

TABLE XIV

BUILD-UP OF MICROBIAL CONTAMINATION ON SURFACES IN A LAMINAR DOWNFLOW FACILITY FOLLOWING CLEANING AND DISINFECTION

ELAPSED TIME IN DAYS	COLONIES/RODAC PLATE		
	ANTEROOM FLOOR (10 samples per period)	ANTEROOM WALLS (20 samples per period)	DOWNFLOW ROOM WALLS (80 samples per period)
0	3.50	0.40	0.06
3	1.80	0.85	0.34
22	9.20	2.00	0.00
36	6.50	0.25	0.03
50	9.30	1.00	0.05
67	16.40	1.10	0.00

TABLE XV

ACCUMULATION OF MICROBIAL CONTAMINANTS ON 1" X 2" STAINLESS STEEL AND EPOXY STRIPS DURING 20 WEEKS OF LAMINAR FLOW ROOM OPERATION

ELAPSED TIME IN WEEKS	REMARKS	COLONY COUNTS/SQ. FT. (MEAN OF 2 STRIPS/CONDITION/TIME PERIOD)					
		AEROBIC INCUBATION		*DRY HEAT TREATED-AEROBIC		*DRY HEAT TREATED-ANAEROBIC	
		STAINLESS	EPOXY	STAINLESS	EPOXY	STAINLESS	EPOXY
2	ROOM IN OPERATION	0	0	0	0	0	0
4	"	0	0	0	0	0	0
6	"	0	0	0	0	0	0
8	"	0	0	0	0	0	0
10	"	0	0	0	0	0	0
12	ROOM SHUT DOWN FOR 7 DAYS	5148	9792	1512	2196	612	1080
14	ROOM IN OPERATION	216	288	360	144	0	0
16	"	108	252	108	144	36	0
18	"	36	72	144	144	0	0
20	"	144	36	252	108	0	0

* 80°C FOR 15 MINUTES

CHAPTER V

SUMMARY AND CONCLUSIONS

The immediate mission of the Planetary Quarantine Office is to assure sterility of space hardware inpacting on the planet Mars. To accomplish this task, technical and administrative procedures must be decided upon and implimented on a tight schedule, well in advance of the actual mission. Decisions have thus already been made on the basis of available knowledge, often without the advantage of having that knowledge confirmed by repetition and careful analysis. In this regard many of the data reported herein have been disseminated far in advance of this document. Indeed, the chapter on evaluation of a laminar flow room resulted from a decision based, in part, on the preliminary data from the comparison of conventional clean room facilities. As such, this document provides no new or startling information. Instead it is hoped that it will provide some measure of comfort to the Planetary Quarantine Office in that the data seem to confirm the early indications and empirical judgments and, thus, vindicate the decisions which have been made thus far on subjects related to this research. Some of the more important conclusions can be summarized as follows:

1. Microbial evaluations of conventional clean rooms revealed contamination levels in the best of these facilities about one order of magnitude below previously determined "minimum" levels in critical areas of hospitals.

2. Predictably, the contaminants were approximately 75% species associated with shedding from human sources and no more than 10% appeared to be spore forming varieties.

3. Spores isolated from clean room environments are almost entirely common species and pure isolates of these varieties are readily killed by

dry heat cycles less severe than those contemplated for sterilization of space hardware.

4. The "plateau phenomenon" indicating no build-up of contaminants over an extended time period is borne out by all experiments performed and seems to hold true for plastic materials as well as stainless steel, and to some extent for spores as well as non-sporeforming species.

5. Contamination transfer to various materials from human contact proved to be predictably variable among individuals and groups of individuals, but of smaller magnitude than had been expected. Plastic based materials generally are more readily contaminated by human contact than metallics and retain contaminants for longer time periods.

6. The laminar downflow room can achieve a further reduction in contamination levels of several orders of magnitude below the best conventional clean rooms. However, every precaution should be taken to avoid overreliance on such rooms which might result in relaxation of aseptic technique. The room will never be able to compensate for careless techniques by workers involved.

Thus, there is every indication that a combination of rigid adherence to the techniques of sterile assembly and the use of a vertical laminar flow facility for final assembly can greatly reduce the microbial loading on the spacecraft prior to terminal sterilization. Every viable microbe which can be killed or diverted from the spacecraft before it enters the terminal sterilization chamber is one less which can possibly survive the cycle, thus improving the odds for successful sterilization.

A great deal has already been accomplished by the sterilization program. The determination to succeed is great and it is hoped that this report will contribute some small measure to that eventual success. At the same time it

is the belief of the authors that the benefits from this program have implications far beyond the immediate goals. The spur to microbiologists to probe more deeply into the techniques and measurement of ultra clean assembly are already bearing fruits for other applications in medicine and industry.

Techniques for aseptic care of low resistance patients and for manufacture of pharmaceuticals are examples of application already under development.

Indeed, man's newly acquired ability to "reach for the stars" will undoubtedly open up currently unthought of benefits for improvement of his own plight right here on Earth and further justify the expense and effort which such ambition requires.

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