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1. In the past, studies on dry heat resistance of bacterial spores have been conducted in a forced air oven (Precision Scientific model no. 625). Stainless steel strips (1/2" x 1/2") and unlaquered #1 paper clips were cleaned and sterilized separately, assembled aseptically, and inoculated with spore suspensions suspended in either water or 95% ethanol. Three strips were suspended from wire hangers in the oven for each individual heating interval. After the door was closed, the comeup time to 125 C for the strip-paper clip units was 2.5 minutes. This oven has been modified to allow insertion and removal of samples without opening the door. Six 1" x 5" ports were installed in the oven top. Hangers were constructed of bronze rod, fiberglass sheeting and #30 aluminum aligator clips, each capable of holding a maximum of six quintuplicate samples. Under this system the comeup time of a stainless steel strip to 125 C was 1.5 minutes in any portion of the oven as measured by thermocouples. The $D_{125C}$ values of the standard B. subtilis var. niger spore crops as determined in the modified oven was identical to the value obtained with the previous procedure (i.e., 15 min.).

   In the past, strip-paper clip units were inoculated with 0.05 ml of ethanol or water suspensions from a 1 ml pipette. Since this procedure was very time consuming pipette droppers (Scientific Products stock no. B-11861, 0.025 ml) were evaluated as a more rapid means of strip inoculation. Using the 0.025 ml dropper, the mean coefficient of variation between numbers of spores recovered from 20 strips was 9.5%, and consequently, this instrument will be used routinely in future studies.

2. As reported last quarter, addition of 0.2% yeast extract and 0.1% soluble starch to TSA improved recovery of both heated and unheated bacterial spores. During routine survival tests, several isolates showed survival curves with "shoulders" indicating a requirement for "heat activation". When the TSA with supplements was compared to standard TSA as a recovery medium, the supplemented medium appeared to satisfy a portion of the "heat activation requirement" for spores in the unheated controls (See Report #24, Figs. 1, 2 and 3). $D_{125C}$ values calculated from the logarithmic death portions of the survivor curves were equivalent when either TSA or supplemented TSA was used as the recovery medium.

   To investigate the possible relationship between heat activation and constituents in the plating medium upon recovery of unheated and heat-injured spores, two spore crops in 95% ethanol were used: 1) isolate G-2 sporulated at 35 C for 48 hours on AK Medium #2 (BBL) supplemented with 20 ppm magnesium sulfate and 80 ppm calcium chloride, and 2) isolate G-2 sporulated in the same manner on TAM Sporulation Agar (Difco) supplemented with 20 ppm magnesium sulfate and 80 ppm calcium chloride. Triplicate strips of each spore crop were suspended in a forced air oven for each of six time intervals, and processed as usual (Report #19). The suspension was plated in triplicate with both TSA and TSA supplemented with yeast extract and soluble starch. The suspensions were then heat shocked in an 80 C water bath for 15 minutes. Replicate portions were again plated in triplicate with both media. Survivor curves are shown in Figs. 1 and 2. Supplemented TSA was superior to regular TSA as a recovery medium in every instance. When plated with regular TSA without conventional 80 C moist "heat shock", both spore crops exhibited increased viable counts (shoulders) compared to the unheated controls during the first 30 minutes of exposure to 125 C dry heat. Recovery during this period was appreciably improved by 80 C "heat shock" prior to plating. Heat shocking

at 80 C failed to appreciably increase recovery of G-2 (TAM) spores after 30 minutes of exposure at 125 C. In addition, recovery of the G-2 (AK) spores decreased rapidly after 45 minutes at 125 C followed by heat shock. The most dramatic increases in recovery were noted when both crops were plated with supplemented TSA. Heat shocking the suspension prior to plating with this medium yielded very little, if any, improvement in recovery. Table 1 illustrates the effects of 80 C heat shock and supplemented TSA upon recovery of spores which were not exposed to dry heat. The greatest increases with both crops were noted when supplemented TSA was compared to regular TSA.

Although complete explanation of the above results is unclear at the present time, several observations can be made. Classically, heat shock or "heat activation" is thought to involve loss of spore constituents (for example, calcium dipicolinic acid) into a liquid medium or certain enzymatic rearrangements or a combination of both. The great majority of studies concerning spore activation has dealt only with moist heat. As can be seen by the survivor curves presented here, dry heating of dessicated spores apparently satisfys a portion of the heat activation requirement. The mechanisms involved may or may not be similar to those of moist heat but certainly could provide a tool for more exact definition of dry heat activation. Whether or not the increased recoveries noted upon addition of yeast extract and soluble starch to the TSA recovery medium involve physical or nutritional mechanisms is also open to speculation. It is evident, however, from the "shoulders" in Figs. 1 and 2 that some factor(s) necessary for maximum recovery of the viable unheated spores is still required even after application of a heat shock treatment and use of the supplemented medium.

3. As mentioned last quarter, the Planetary Quarantine Officer, NASA, has requested production of a standard spore crop of B. subtilis var. niger. The crops was prepared employing the liquid medium described by Lazzarini and Santangelo (J. Bacteriol. 94:125-130):

SSM-10 (Synthetic Sporulation Medium-10), per liter

| | | |
|---|---|---|
| $K_2HPO_4 \cdot 3H_2O$ | 7.3 | g |
| $KH_2PO_4$ | 2.4 | g |
| Sodium Citrate | 1.0 | g |
| Tris (hydroxymethyl) Aminomethane (free base) | 3.6 | g |
| Glutamic acid | 4.4 | g |
| Glucose | 1.5 | g |
| Tryptophan | 25.0 | g |
| Methionine | 25.0 | g |
| $CaCl_2$ | 111.0 | mg |

| | | |
|---|---|---|
| $MgSO_4 \cdot 7H_2O$ | 246.5 | mg |
| $ZnCl_2$ | 6.81 | mg |
| $MnCl_2 \cdot 4H_2O$ | 9.89 | mg |
| $FeCl_3$ | 2.703 | mg |

The above materials with the exception of the metal salts were dissolved in 900 ml of distilled deionized water. The metal salt solution was made up in ten-fold concentration and 100 ml were added aseptically after sterilization for 15 min at 121 C. Three hundred milliliter portions of the complete medium in 1 L baffled flasks were inoculated with 5 ml of a 24 hr B. subtilis var. niger culture (obtained from A. Irons, Jet Propulsion Laboratory) in the SSM-10 liquid medium. Flasks were then incubated at 136 RPM and 32 C for 5 days (ca. 90% spores) in a Psychro-Therm Incubator Shaker (New Brunswick Scientific Co.). After incubation, growth in 800 ml of medium was harvested with a refrigerated continuous flow centrifuge at 936 x g. Pellets were pooled and exposed to ultrasonic energy to disrupt clumps and cell debris. The bulk of vegetative cell material was removed by repeated centrifugation. Three quarters of the cleaned spores were suspended in distilled water ($5.6 \times 10^9$/ml). Preliminary $D_{125C}$ values were 40 min for the ethanol suspension and 36 min for the water suspension.

4. In conjunction with the study to obtain a frequency distribution of $D_{125C}$ values of spores associated with pertinent spacecraft, one-hundred and forty-five spore isolates from the Mariner '69 spacecraft were received from Cape Kennedy. As cultures were received, they were streaked on TSA, and 12 impure cultures or cultures other than gram positive rods were discarded. Pure cultures were streaked on quadrant plates of TAM Sporulation Agar supplemented with 20 ppm magnesium sulfate and 80 ppm calcium chloride. Plates were incubated at 32 C for a maximum of 96 hr and growth was stained to observe spore production. Eighteen cultures of gram positive rods not forming spores were discarded. Growth showing any degree of sporulation was harvested in 3 ml of sterile buffered distilled water, exposed to ultrasonic energy, washed twice and suspended in 2 ml of 95% ethanol and stored at 4 C for subsequent titering. A total of 115 spore crops were prepared. The frequency distribution of $D_{125C}$ values will be obtained using the modified dry heat oven described above in part 1 and a rapid assay system.

5. As discussed in Quarterly Report #23, variations in death rate of a particular spore isolate can be attributed to cultural conditions as well as the method and/or location in which a dry heat assay is performed. Table 2 shows a summary of $D_{125C}$ values obtained at this laboratory from several different suspensions of B. subtilis var. niger spores. This summary serves to emphasize the care which must be taken in interpretations of data employing such a "standard reference organism" with regard to heat resistance. Argument has been made in the past (Reports 20-24) against using a subcultured spore preparation, particularly B. subtilis var. niger spores, as an absolute dry heat resistance index of naturally occurring spores.

Studies on the dry heat resistance of naturally occurring spore populations from spacecraft assembly and testing environments is underway and results will be reported later.

6. The Virginia Polytechnic Institute (VPI) technique for recovering anaerobic bacteria was evaluated at Cape Kennedy using samples of microbial contamination associated with spacecraft. This technique was compared with the one specified in the "NASA Standard Procedures for the Microbiological Examination of Space Hardware" in three ways. (1) Naturally contaminated components were insonated using the VPI procedure (i.e., use of pre-reduced rinse fluid and containers flushed with nitrogen gas prior to insonation) and the rinse fluid was split and assayed by each recovery technique (pre-reduced agar and roll tube vs TSA pour plate. (2) Additional components from the same batch were both insonated and assayed using the NASA technique (TSA pour plates). (3) A series of swabs taken from adjacent areas on the surfaces of spacecraft were assayed using both the VPI and NASA techniques.

   Table 3 shows the mean values of anaerobic microorganisms in 90 split samples from components and the mean values of anaerobic spores in 74 split samples. The results of 110 additional component assays using the NASA technique are also presented. The VPI and NASA techniques produced comparable mean values for total anaerobic microorganisms in the split samples. Components insonated using the NASA technique, however, gave lower values than the VPI method suggesting that insonation in presence of oxygen may have had a detrimental effect on the recovery of certain anaerobes. The NASA anaerobic spore assay technique used on the split samples gave markedly higher results than the VPI method and even the mean value obtained using the NASA insonation technique was three times that of the VPI value.

   Table 4 presents the results of assays for total anaerobes on matched swabs taken on three different days. The results obtained by both techniques was comparable on the first and third days. On the second day the VPI technique indicated a much higher level of anaerobes than the NASA technique. No apparent explanation for this inconsistency was evident. The results of anaerobic spore assays on swabs taken on these three days are presented in Table 5. Although the levels were very low, both techniques appeared to give comparable overall results. In contrast to the results from the component assays the NASA technique for recovering anaerobic spores from swabs did not result in markedly higher levels than the VPI method.

   On the basis of these data it is obvious that there was no consistent superiority of either technique in the recovery of anaerobic microorganisms associated with spacecraft. Considering the relative simplicity of the NASA technique it would appear to remain the assay method of choice.

7. The Apollo 9 (CSM-104) spacecraft was studied during its residency at the Manned Spacecraft Operations Building (MSOB), Vehicle Assembly Building (VAB) and at Launch Complex 39A. The levels of microbial contamination present on the Command Module (C/M), Instrument Unit (I.U.) and the Saturn S-4B engine are presented in Table 6. Contamination levels for the C/M remained basically the same throughout the study period except for the sample taken 2/11/69 when a marked increase was observed. Samples taken from five sampling sites, (a total of fifteen sites were sampled), revealed relatively high levels of microbial contamination, which was reflected in the high mean obtained for that sampling period. The I.U. and S-4B were sampled only once due to scheduling difficulties. The levels of aerobic mesophilic microorganisms were lower than those observed for the I.U. and S-4B sampling areas

with hypergolic blankets plus the forcing of high volumes of air beneath the blankets could have caused the reduction by means of dessication and physical removal.

Tables 7 and 8 present all of the quantitative data collected on the interior and exterior surfaces of the ascent and descent stages of the Lunar Module 3 (LM-3). The levels of microbial contamination in the interior of the LM-3 (Table 7) ranged from $1.8 \times 10^4$ to $1.2 \times 10^5$ per square foot during the sampling period. The exterior surfaces of the LM-3, ascent and descent stages, showed some variation in the contamination levels at different sampling period (Table 8). A one to two log reduction in contamination levels was noted when the LM-3 was enclosed in the SLA and located at Launch Complex 39A. The exterior surfaces of the LM-3 were continually being purged with large volumes of filtered air while at Complex 39A and this may have contributed to the reduction in contamination levels.

The percentage of aerobic spores and molds detected on the interior surfaces of the Apollo 9 Command Module (CSM-104), the I.U. and the S-4B are shown in Table 9. The levels for both the aerobic spores and molds were relatively low for the Command Module. Although the I.U. revealed lower levels of aerobic mesophilic microorganisms (Table 6) than was reported for the I.U. of Apollo 8 (Report #24), the percentage of aerobic spores and molds was found to be twice as high for the aerobic spores and four times as high for molds. Similar results were obtained with the S-4B and the interior surfaces of the LM-3 (Table 10). The percentage of spores and molds on the exterior surfaces of the LM-3 (Table 11) was greater than those observed on the interior surfaces of the Command and Lunar Modules. An increase in percentage of spores and molds detected on the LM-3 while it was located at Launch Complex 39A correlates with the decrease in the aerobic mesophilic levels (Table 8) observed during the same period. The constant flushing with filtered air might have selectively reduced the microbial vegetative population, resulting in a relatively high population of sporeformers and molds.

Studies were initiated on the Apollo 10 and Apollo 11 spacecraft. This includes the Command Module (CSM-106) of the Apollo 10 and the Lunar Module 5 (LM-5) of the Apollo 11. Results of the preliminary sampling are presented in Table 12. The initial data appears to be similar to those obtained with previous Apollo Command and Lunar Modules.

The types of aerobic mesophilic microorganisms isolated from the Command Modules of Apollo 8 (CSM-103) and Apollo 9 (CSM-104) are shown in Table 13. A total of 1561 isolates were identified from the interior of CSM-103. Most of the contaminants isolated from Apollo 8 Command Module (CSM-103) were Staphylococcus spp. and Micrococcus spp. (ca. 98%). Aerobic sporeformers (Bacillus spp.) accounted for less than 2% of the population. The Apollo 9 Command Module (CSM-104) revealed a higher percentage (ca. 21%) of gram positive non-sporeforming rods (Corynebacterium-Brevibacterium group) than were found on CSM-103. Whether this increase is due to seasonal variation is yet to be determined. The percentage of molds was low in both command modules. The types of aerobic mesophilic microorganisms detected on LM-3 and LM-4 are shown in Tables 14 and 15. In both lunar modules the predominant types of microorganisms were Staphylococcus spp. and Micrococcus spp. A total of 2561 microorganisms was isolated from the Apollo 8 spacecraft and

4262 microorganisms from the Apollo 9 spacecraft. For both spacecraft, 6823 microorganisms were isolated, and to date 4698 have been identified. The remaining 2124 isolates are in the process of identification and will be reported in the next quarter.

Figure 3 shows the percentage of microorganisms considered to be indigenous to humans (Staphylococcus spp. and Micrococcus spp.) compared to those associated with soil and dust in the environment (Bacillus spp., molds and actinomycetes). A decrease in the types of microorganisms indigenous to the environment was noted in the two month period that the CSM-103 was in the Manned Spacecraft Operations Building (MSOB). A slight increase was observed after moving the spacecraft from the VAB to Launch Complex 39A. The levels remained stable while located at Launch Complex 39A.

8. At the request of Mr. George Mallison, Chief, Biophysics Section, Epidemiology Program, National Communicable Disease Center, air samples, using sterile millipore field monitors, were taken in three different environments at Cape Kennedy, Florida, and in four different environments at Phoenix. Continuous seven hour samples were taken in each of the following environments: 1) outside the Spacecraft Bioassay Laboratory, 2) inside the MSOB high bay area, 3) inside the Spacesuit Clean Room (Class 100,000), 4) outside the Phoenix Laboratories, 5) inside the Phoenix bioclean room (Class 100), 6) inside a microbiological laboratory in the Phoenix Laboratories, and 7) in the hallway serving the administrative offices in the Phoenix Laboratories. The purpose of this study is to investigate the resistance of naturally occurring microbial contaminants recovered on membrane filters to ethylene oxide.

9. The Protocol for the Identification Schemes for Microorganisms Isolated from Apollo Spacecraft was completed. A total of 2,000 isolates from each Apollo spacecraft during the next several missions will be identified to species by these procedures.

10. In accordance with a request from the Planetary Quarantine Officer, a study for detecting virus on space hardware was initiated. Swab samples were taken from the interior surfaces of the CSM-106 and Lunar Module 5 and sent to the EIP Phoenix Laboratories for virus assay. Specific methods and preliminary results will be described later.

TABLE 1. EFFECTS OF MEDIUM AND MOIST HEAT SHOCK UPON RECOVERY OF BACTERIAL SPORES.

| Recovery Medium and Moist Heat Treatment[3] | Ratio of Colony Counts[1] | |
|---|---|---|
| | Isolate G-2 (TAM)[4] | Isolate G-2 (AK #2)[5] |
| Supplemented TSA[2]:TSA | 17.6:1 | 20.7:1 |
| Supplemented TSA + Heat Shock:TSA + Heat Shock | 8.9:1 | 2.5:1 |
| Supplemented TSA + Heat Shock:Supplemented TSA | 1.6:1 | 1.7:1 |
| TSA + Heat Shock:TSA | 3.1:1 | 14.7:1 |

[1]Counts obtained from unheated controls; see Figs. 1 and 2.

[2]TSA supplemented with 0.2% yeast extract and 0.1% soluble starch.

[3]80 C, 15 minutes.

[4]Sporulated on TAM Sporulation Agar (Difco) supplemented with 20 ppm magnesium sulfate and 80 ppm calcium chloride.

[5]Sporulated on AK Medium #2 (BBL) supplemented with 20 ppm magnesium sulfate and 80 ppm calcium chloride.

TABLE 2. $D_{125C}$ VALUES OF VARIOUS B. SUBTILIS VAR. NIGER SPORE PREPARATIONS[1].

| Suspension Designation | Source | Sporulation Medium | Suspending Fluid | $D_{125C}$ Value (min) |
|---|---|---|---|---|
| BgHA | Phoenix Laboratories | TAM + minerals[2] | 95% ethanol | 15 |
| BgAK+ | Phoenix Laboratories | AK #2 + minerals[3] | 95% ethanol | 15 |
| BgTAM-S | Phoenix Laboratories | TAM + soil extract[4] | 95% ethanol | 18 |
| BgC | Phoenix Laboratories | Charcoal Agar[5] | 95% ethanol | 13 |
| BgLRL | Lunar Receiving Laboratory, Houston | not known[6] | distilled water | 15 |
| BgSSM10A | Phoenix Laboratories | SSM-10[7] | 95% ethanol | 40 |
| BgSSM10W | Phoenix Laboratories | SSM-10 | distilled water | 36 |
| BgJPL | Jet Propulsion Laboratory, Pasadena | SSM-10 | distilled water | 21 |
| BgS | Sandia Laboratories, Albuquerque | TAM | distilled water | 36 |
| BgT | Taft Sanitary Engineering Center (R. Angelotti) | ---[8] | distilled water | 15 |

[1]D-values obtained at Phoenix Laboratories by standard procedure (Report #19).

[2]TAM Sporulation Agar (Difco) supplemented with 20 ppm magnesium sulfate and 80 ppm calcium chloride.

[3]AK Medium #2 (BBL) supplemented with 20 ppm magnesium sulfate and 80 ppm calcium chloride.

[4]Prepared with a boiling water-soil extract:clean filtrate of 100 g soil/100 ml distilled water to make 100 ml medium.

[5]Difco

[6]Suspension made at Phoenix Laboratories from B. subtilis var. niger spore powder originally cultured and prepared by the U.S. Army Biological Laboratories.

[7]Synthetic Sporulation Medium-10 (liquid), see this report for composition and procedure.

[8]Seitz filtered glucose, 0.25%; casamino acids (tech.), 0.25%; yeast extract 0.5%; $MnSO_4 \cdot H_2O$, 0.001%; $FeSO_4 \cdot 7H_2O$, 0.0014%.

TABLE 3. COMPARISON OF VPI AND NASA TECHNIQUES FOR RECOVERY OF ANAEROBIC MICROORGANISMS FROM COMPONENTS.

| | Split Samples | | | Separate Samples | |
|---|---|---|---|---|---|
| | No. Assayed | Mean No. Microorganisms Recovered | | No. Assayed | Mean No. Microorganisms Recovered |
| | | VPI | NASA | | NASA |
| Total Anaerobes | 90 | 23 | 17 | 110 | 7.6 |
| Anaerobic Spores | 74 | 2.2 | 13 | 110 | 6.7 |

TABLE 4. COMPARISON OF VPI AND NASA TECHNIQUES FOR RECOVERY OF TOTAL ANAEROBIC MICROORGANISMS FROM SWABS.

| Day | No. Samples Assayed by Each Technique | Mean No. Anaerobic Microorganisms Recovered | |
|---|---|---|---|
| | | VPI Technique | NASA Technique |
| 1 | 40 | 98 | 105 |
| 2 | 40 | 109 | 17 |
| 3 | 40 | 50 | 66 |

TABLE 5. COMPARISON OF VPI AND NASA TECHNIQUES FOR RECOVERY OF ANAEROBIC SPORES FROM SWABS.

| Day | No. Samples Assayed by Each Technique | Mean No. Anaerobic Microorganisms Recovered | |
|---|---|---|---|
| | | VPI Technique | NASA Technique |
| 1 | 40 | 2.5 | 4.7 |
| 2 | 40 | 2.3 | 0.8 |
| 3 | 40 | 0 | 0.1 |

TABLE 6. MICROBIAL CONTAMINATION DETECTED ON THE APOLLO 9 COMMAND MODULE (CSM-104), INSTRUMENT UNIT AND THE SATURN S-4B.

| Source | Date Sampled | Area Sampled[1] (sq.in.) | Microorganisms per square foot | | | |
|---|---|---|---|---|---|---|
| | | | Aerobes | Anaerobes | Aerobic spores | Anaerobic spores |
| Command Module (CSM-104) | 10-29-68[2] | 108 | 12,053 | 3,845 | 158 | 43 |
| | 11-14-68[2] | 104 | 18,086 | 5,484 | 27 | 14 |
| | 11-26-68[2] | 120 | 11,865 | 7,747 | 24 | 30 |
| | 12-19-68[3] | 56 | 52,949 | 19,872 | 115 | 0 |
| | 1-17-69[4] | 56 | 13,051 | 3,021 | 91 | 13 |
| | 2-11-69[4] | 60 | 140,904 | 8,266 | 46 | 48 |
| | 2-21-69[4] | 60 | 11,549 | 2,606 | 84 | 36 |
| Instrument Unit | 2-12-69[4] | 60 | 6,019 | 1,123 | 763 | 84 |
| S-4B | 2-12-69[4] | 40 | 18,331 | 3,931 | 1,022 | 163 |

[1]Swab-rinse technique.

[2]Samples taken from the interior of Command Module while located in the Manned Spacecraft Operations Building.

[3]Samples taken from the interior of Command Module while located in the Vehicle Assembly Building.

[4]Samples taken from the interior of the Command Module, Instrument Unit and S-4B while located at Launch Complex 39A.

TABLE 7. MICROBIAL CONTAMINATION DETECTED ON THE INTERIOR SURFACES OF THE LUNAR MODULE 3 OF THE APOLLO 9 SPACECRAFT

| Source | Date Sampled | Area Sampled[1] (sq.in.) | Microorganisms per square foot | | | |
|---|---|---|---|---|---|---|
| | | | Aerobes | Anaerobes | Aerobic spores | Anaerobic spores |
| Lunar Module 3 | 7-2-68[2] | 116 | 28,101 | 16,647 | 87 | 37 |
| | 7-18-68[2] | 84 | 17,803 | 7,526 | 231 | 43 |
| | 7-31-68[2] | 112 | 47,131 | 9,144 | 1,080 | 919 |
| | 8-19-68[2] | 96 | 100,901 | 35,467 | 143 | 45 |
| | 8-28-68[2] | 108 | 65,707 | 29,030 | 274 | 46 |
| | 9-13-68[2] | 92 | 82,987 | 38,909 | 157 | 16 |
| | 9-24-68[2] | 92 | 54,187 | 19,915 | 94 | 32 |
| | 10-10-68[2] | 100 | 57,744 | 20,275 | 151 | 43 |
| | 11-29-68[3] | 112 | 17,654 | 8,986 | 86 | 19 |
| | 12-20-68[4] | 116 | 61,603 | 40,032 | 173 | 49 |
| | 1-16-69[5] | 56 | 67,507 | 28,426 | 141 | 52 |
| | 2-11-69[5] | 40 | 21,139 | 4,090 | 72 | 19 |
| | 2-20-69[5] | 60 | 115,200 | 43,920 | 533 | 25 |

[1]Swab-rinse technique.

[2]Samples taken while Lunar Module was located in the Manned Spacecraft Operations Building.

[3]Samples taken 5 days after LM-3 was placed in the Spacecraft Lunar Module Adapter.

[4]Samples taken while LM-3 was located in the Vehicle Assembly Building.

[5]Samples taken while LM-3 was located at Launch Complex 39A.

TABLE 8. LEVEL OF MICROBIAL CONTAMINATION DETECTED ON THE EXTERIOR SURFACES OF THE LUNAR MODULE 3 ASCENT AND DESCENT STAGES.

| Source | Date Sampled | Area Sampled[1] (sq.in.) | Microorganisms per square foot | | | |
|---|---|---|---|---|---|---|
| | | | Aerobes | Anaerobes | Aerobic spores | Anaerobic spores |
| Lunar Module 3 Ascent Stage | 7-03-68[2] | 116 | 19,744 | 3,997 | 248 | 155 |
| | 7-17-68[2] | 120 | 12,816 | 2,232 | 132 | 30 |
| | 8-01-68[2] | 120 | 1,843 | 346 | 43 | 0 |
| | 8-14-68[2] | 120 | 6,408 | 2,333 | 101 | 43 |
| | 8-28-68[3] | 120 | 53,698 | 20,333 | 259 | 86 |
| | 9-13-68[2] | 120 | 15,293 | 4,162 | 379 | 91 |
| | 9-26-68[2] | 116 | 13,579 | 3,010 | 266 | 75 |
| | 10-09-68[2] | 120 | 11,722 | 4,003 | 72 | 12 |
| | 11-29-68[2] | 120 | 21,096 | 2,232 | 475 | 138 |
| | 12-19-68[4] | 120 | 10,570 | 1,195 | 30 | 12 |
| | 1-16-69[5] | 60 | 2,534 | 432 | 0 | 29 |
| | 2-11-69[5] | 60 | 907 | 518 | 108 | 36 |
| | 2-20-69[5] | 60 | 5,731 | 1,642 | 72 | 12 |
| Lunar Module 3 Descent Stage | 7-03-68[2] | 116 | 7,703 | 2,886 | 75 | 25 |
| | 7-17-68[2] | 88 | 15,357 | 2,790 | 49 | 33 |
| | 8-01-68[2] | 112 | 10,411 | 2,002 | 65 | 26 |
| | 8-14-68[2] | 120 | 8,770 | 2,434 | 30 | 42 |
| | 8-28-68[3] | 92 | 48,571 | 12,686 | 212 | 32 |
| | 9-13-68[2] | 104 | 15,350 | 4,334 | 187 | 27 |
| | 9-26-68[2] | 104 | 47,246 | 5,040 | 346 | 125 |
| | 10-09-68[2] | 120 | 8,323 | 2,333 | 158 | 12 |
| | 11-29-68[2] | 48 | 33,120 | 4,867 | 965 | 230 |
| | 12-19-68[4] | 48 | 19,598 | 4,306 | 60 | 0 |
| | 1-16-69[5] | 60 | 997 | 120 | 36 | 29 |
| | 2-11-69[5] | 60 | 7,920 | 3,226 | 274 | 12 |
| | 2-20-69[5] | 60 | 2,995 | 648 | 158 | 97 |

[1]Swab-rinse technique.

[2]Samples taken while Lunar Module 3 was located in the Polarity Fixture Work Stand in the Manned Spacecraft Operations Building.

[3]Samples taken while Lunar Module 3 was located in the Altitude Test Chamber in the Manned Spacecraft Operations Building.

[4]Samples taken while Lunar Module 3 was located in the Vehicle Assembly Building.

[5]Samples taken while Lunar Module 3 was located at Launch Complex 39A.

TABLE 9. COMPARISON OF AEROBIC BACTERIAL SPORES AND MOLDS DETECTED ON THE APOLLO 9 COMMAND MODULE (CSM-104), INSTRUMENT UNIT AND THE SATURN S-4B.

| Source | Sample Number | Date Sampled | Area Sampled[1] (sq.in.) | Percent[2] | |
|---|---|---|---|---|---|
| | | | | Aerobic Bacterial Spores | Molds |
| Apollo 9[3] (CSM-104) | 1 | 10-29-69 | 108 | 1.30 | 0.17 |
| | 2 | 11-14-68 | 104 | 0.15 | 0.04 |
| | 3 | 11-26-68 | 120 | 0.20 | 0.20 |
| | 4 | 12-19-68 | 56 | 0.20 | 0.05 |
| | 5 | 1-17-69 | 56 | 0.70 | 0.10 |
| | 6 | 2-11-69 | 60 | 0.03 | 0.02 |
| | 7 | 2-21-69 | 60 | 0.73 | 0.00 |
| Instrument Unit[3] | 1 | 2-12-69 | 60 | 12.68 | 14.66 |
| S-4B[3] | 1 | 2-12-69 | 40 | 5.58 | 2.85 |

[1]Swab-rinse technique.

[2]Percentage of total aerobic mesophilic microorganisms.

[3]Interior surfaces.

TABLE 10. AEROBIC BACTERIAL SPORES AND MOLDS DETECTED ON INTERIOR SURFACES OF THE LUNAR MODULE 3 OF THE APOLLO 9 SPACECRAFT.

| Source | Sample Number | Date Sampled | Area Sampled[1] (sq.in.) | Percent[2] | |
|---|---|---|---|---|---|
| | | | | Aerobic Bacterial Spores | Molds |
| Lunar Module 3 | 1 | 7-02-68 | 116 | 0.30 | 0.09 |
| | 2 | 7-18-68 | 84 | 1.30 | 0.24 |
| | 3 | 7-31-68 | 112 | 2.30 | 0.00 |
| | 4 | 8-19-68 | 96 | 0.10 | 0.01 |
| | 5 | 8-28-68 | 108 | 0.40 | 0.01 |
| | 6 | 9-13-68 | 92 | 0.20 | 0.00 |
| | 7 | 9-24-68 | 92 | 0.20 | 0.06 |
| | 8 | 10-10-68 | 100 | 0.30 | 0.10 |
| | 9 | 11-29-68 | 112 | 0.49 | 0.15 |
| | 10 | 12-20-68 | 116 | 0.28 | 0.06 |
| | 11 | 1-16-69 | 56 | 0.21 | 0.13 |
| | 12 | 2-11-69 | 40 | 0.34 | 0.09 |
| | 13 | 2-20-69 | 60 | 0.50 | 0.00 |

[1]Swab-rinse technique.

[2]Percentage of total aerobic mesophilic microorganisms.

TABLE 11. AEROBIC BACTERIAL SPORES AND MOLDS DETECTED ON THE EXTERIOR SURFACES OF THE LUNAR MODULE 3 ASCENT AND DESCENT STAGES.

| Source | Sample Number | Date Sampled | Area Sampled[1] (sq.in.) | Percent[2] | |
|---|---|---|---|---|---|
| | | | | Aerobic Bacterial Spores | Molds |
| Lunar Module 3 Ascent Stage | 1 | 7-03-68 | 116 | 1.30 | 0.19 |
| | 2 | 7-17-68 | 120 | 1.00 | 3.04 |
| | 3 | 8-01-68 | 120 | 2.30 | 0.31 |
| | 4 | 8-14-68 | 120 | 1.60 | 0.66 |
| | 5 | 8-28-68 | 120 | 0.50 | 0.50 |
| | 6 | 9-13-68 | 120 | 2.50 | 15.58 |
| | 7 | 9-26-68 | 116 | 2.00 | 0.18 |
| | 8 | 10-09-68 | 120 | 0.60 | 0.47 |
| | 9 | 11-29-68 | 120 | 2.30 | 2.10 |
| | 10 | 12-19-68 | 120 | 0.30 | 0.20 |
| | 11 | 1-16-69 | 60 | 0.00 | 1.42 |
| | 12 | 2-11-69 | 60 | 11.90 | 10.70 |
| | 13 | 2-20-69 | 60 | 1.30 | 1.70 |
| Lunar Module 3 Descent Stage | 1 | 7-03-68 | 116 | 1.00 | 0.80 |
| | 2 | 7-17-68 | 88 | 0.30 | 0.70 |
| | 3 | 8-01-68 | 112 | 0.60 | 0.87 |
| | 4 | 8-14-68 | 120 | 0.30 | 0.70 |
| | 5 | 8-28-68 | 92 | 0.40 | 0.39 |
| | 6 | 9-13-68 | 104 | 1.20 | 1.90 |
| | 7 | 9-26-68 | 104 | 0.70 | 0.35 |
| | 8 | 10-09-68 | 120 | 1.90 | 0.57 |
| | 9 | 11-29-68 | 48 | 2.90 | 1.20 |
| | 10 | 12-19-68 | 48 | 0.30 | 1.00 |
| | 11 | 1-16-69 | 60 | 3.60 | 3.61 |
| | 12 | 2-11-69 | 60 | 3.50 | 1.60 |
| | 13 | 2-20-69 | 60 | 5.30 | 6.24 |

[1]Swab-rinse technique.

[2]Percentage of total mesophilic microorganisms.

TABLE 12. MICROBIAL CONTAMINATION DETECTED ON THE SURFACES OF APOLLO 10 COMMAND MODULE (CSM-106) AND LUNAR MODULE 5 ASCENT STAGE OF THE APOLLO 11 SPACECRAFT

| Source | Date Sampled | Area Sampled[1] (sq.in.) | Microorganisms per square foot | | | |
|---|---|---|---|---|---|---|
| | | | Aerobes | Anaerobes | Aerobic spores | Anaerobic spores |
| Command Module 106 (Interior) | 12-18-68[2] | 60 | 22,565 | 8,870 | 97 | 84 |
| Lunar Module 5 (Ascent Interior) | 2-27-69[2] | 60 | 47,866 | 12,643 | 48 | 25 |
| Lunar Module 5 (Ascent Exterior) | 2-27-69[2] | 60 | 4,637 | 1,152 | 48 | 12 |

[1]Swab-rinse technique.

[2]Samples taken while modules located in the Manned Spacecraft Operations Building.

TABLE 13. TYPES OF AEROBIC MESOPHILIC MICROORGANISMS ISOLATED FROM THE APOLLO COMMAND MODULE 8 (CSM-108) AND COMMAND MODULE 9 (CSM-104)

| Microorgamisms | CSM-103 (Interior) | | CSM-104 (Interior) | |
|---|---|---|---|---|
| | No. | Percent | No. | Percent |
| *Staphylococcus epidermidis* | 204 | 13.1 | 75 | 18.8 |
| *Staphylococcus aureus* | 3 | 0.2 | 0 | 0.0 |
| *Micrococcus* spp. | 1,087 | 69.6 | 220 | 55.2 |
| *Corynebacterium-Brevibacterium* Group | 139 | 9.0 | 81 | 20.4 |
| Miscellaneous gram negative rods | 5 | 0.3 | 1 | 0.2 |
| *Bacillus* spp. | 17 | 1.1 | 8 | 2.0 |
| Molds | 10 | 0.6 | 1 | 0.2 |
| *Sarcina* spp. | 10 | 0.6 | 0 | 0.0 |
| *Gaffkya* spp. | 47 | 3.0 | 11 | 2.8 |
| *Streptococcus* spp. | 38 | 2.4 | 1 | 0.2 |
| Yeasts | 0 | 0.0 | 1 | 0.2 |
| Actinomycetes | 1 | 0.1 | 0 | 0.0 |
| TOTAL | 1,561 | 100.0 | 399 | 100.0 |

TABLE 14. TYPES OF AEROBIC MESOPHILIC MICROORGANISMS ISOLATED FROM THE APOLLO LUNAR MODULE 3 OF APOLLO 9 SPACECRAFT

| Microorganisms | Ascent Stage Interior | | Ascent Stage Exterior | | Descent Stage Exterior | | Total | |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % |
| *Staphylococcus epidermidis* | 278 | 31.5 | 151 | 16.2 | 179 | 19.4 | 608 | 22.2 |
| *Staphylococcus aureus* | 0 | 0.0 | 2 | 0.2 | 1 | 0.1 | 3 | 0.1 |
| *Micrococcus* spp. | 527 | 59.6 | 590 | 63.2 | 563 | 61.1 | 1680 | 61.4 |
| *Corynebacterium-Brevibacterium* Group | 18 | 2.0 | 71 | 7.7 | 59 | 6.5 | 148 | 5.4 |
| Miscellaneous gram negative rods | 7 | 0.8 | 5 | 0.5 | 28 | 3.0 | 40 | 1.5 |
| *Bacillus* spp. | 7 | 0.8 | 31 | 3.3 | 36 | 3.9 | 74 | 2.7 |
| Molds | 19 | 2.1 | 31 | 3.3 | 22 | 2.4 | 72 | 2.6 |
| *Sarcina* spp. | 0 | 0.0 | 2 | 0.2 | 3 | 0.3 | 5 | 0.2 |
| *Gaffkya* spp. | 23 | 2.6 | 31 | 3.3 | 17 | 1.9 | 71 | 2.6 |
| *Streptococcus* spp. | 4 | 0.5 | 3 | 0.3 | 7 | 0.8 | 14 | 0.5 |
| Yeasts | 1 | 0.1 | 12 | 1.4 | 4 | 0.4 | 17 | 0.6 |
| Actinomycetes | 0 | 0.0 | 4 | 0.4 | 2 | 0.2 | 6 | 0.2 |
| TOTAL | 884 | 100.0 | 933 | 100.0 | 921 | 100.0 | 2738 | 100.0 |

TABLE 15. TYPES OF AEROBIC MESOPHILIC MICROORGANISMS ISOLATED FROM THE LUNAR MODULE 4 OF THE APOLLO 10 SPACECRAFT

| Microorganisms | Ascent Stage Interior | | Ascent Stage Exterior | | Descent Stage Exterior | | Total | |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % |
| *Staphylococcus epidermidis* | 47 | 23.5 | 40 | 20.5 | 23 | 11.6 | 110 | 18.5 |
| *Staphylococcus aureus* | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| *Micrococcus* spp. | 112 | 56.0 | 106 | 54.4 | 135 | 67.8 | 353 | 59.4 |
| *Corynebacterium-Brevibacterium* Group | 24 | 12.0 | 23 | 11.8 | 21 | 10.6 | 68 | 11.5 |
| Miscellaneous gram negative rods | 0 | 0.0 | 0 | 0.0 | 3 | 1.5 | 3 | 0.5 |
| *Bacillus* spp. | 1 | 0.5 | 1 | 0.5 | 3 | 1.5 | 5 | 0.8 |
| Molds | 0 | 0.0 | 5 | 2.6 | 3 | 1.5 | 8 | 1.4 |
| *Sarcina* spp. | 2 | 1.0 | 0 | 0.0 | 1 | 0.5 | 3 | 0.5 |
| *Gaffkya* spp. | 11 | 5.5 | 19 | 9.7 | 6 | 3.0 | 36 | 6.1 |
| *Streptococcus* spp. | 1 | 0.5 | 0 | 0.0 | 2 | 1.0 | 3 | 0.5 |
| Yeasts | 0 | 0.0 | 1 | 0.5 | 1 | 0.5 | 2 | 0.3 |
| Actinomycetes | 2 | 1.0 | 0 | 0.0 | 1 | 0.5 | 3 | 0.5 |
| TOTAL | 200 | 100.0 | 195 | 100.0 | 199 | 100.0 | 594 | 100.0 |

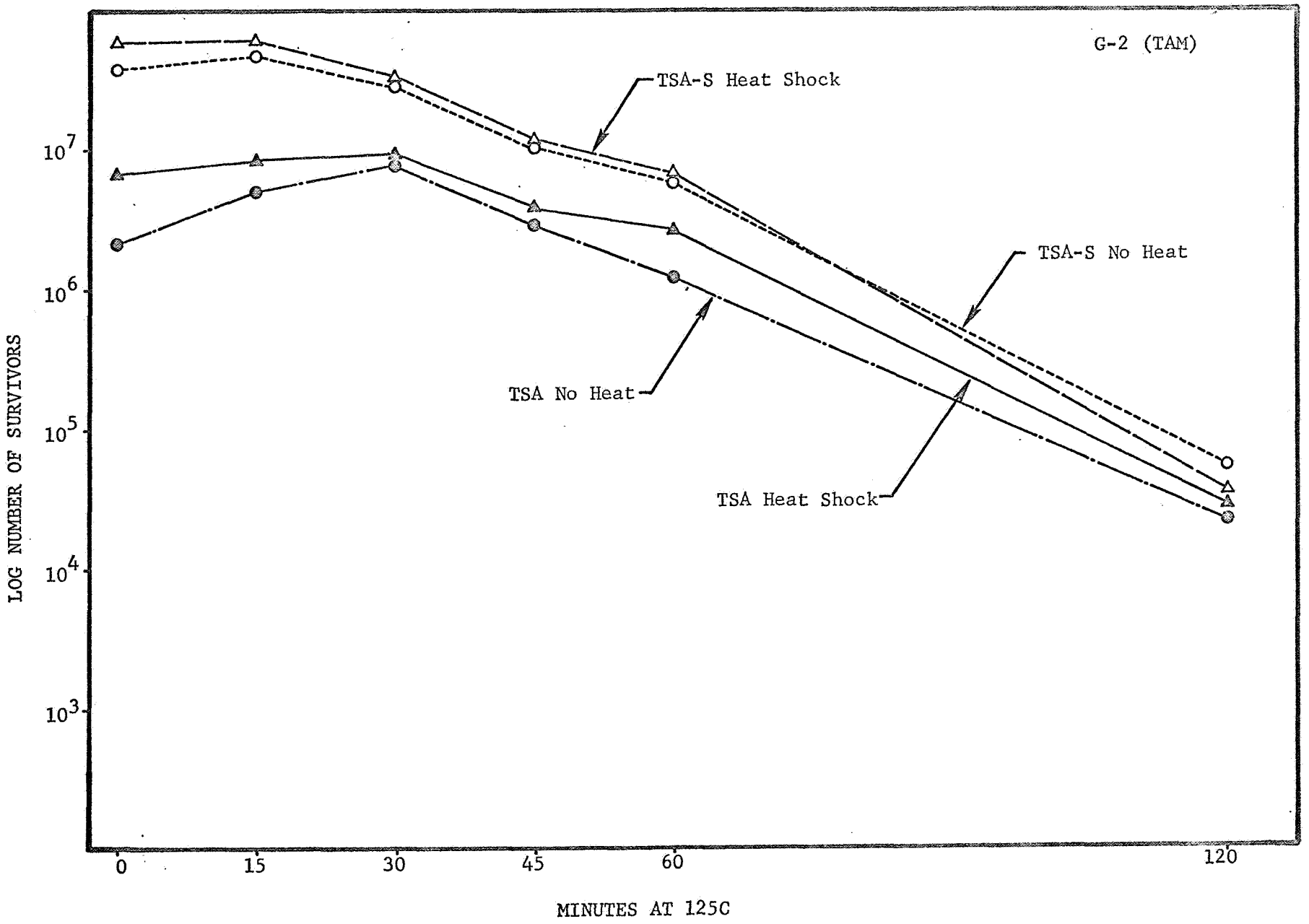


FIG. 1. COMPARATIVE DRY HEAT SURVIVOR CURVES AT 125 C OF ISOLATE G-2 SPORULATED ON TAM AND RECOVERED WITH TSA AND SUPPLEMENTED TSA:HEAT SHOCK, NO HEAT SHOCK.

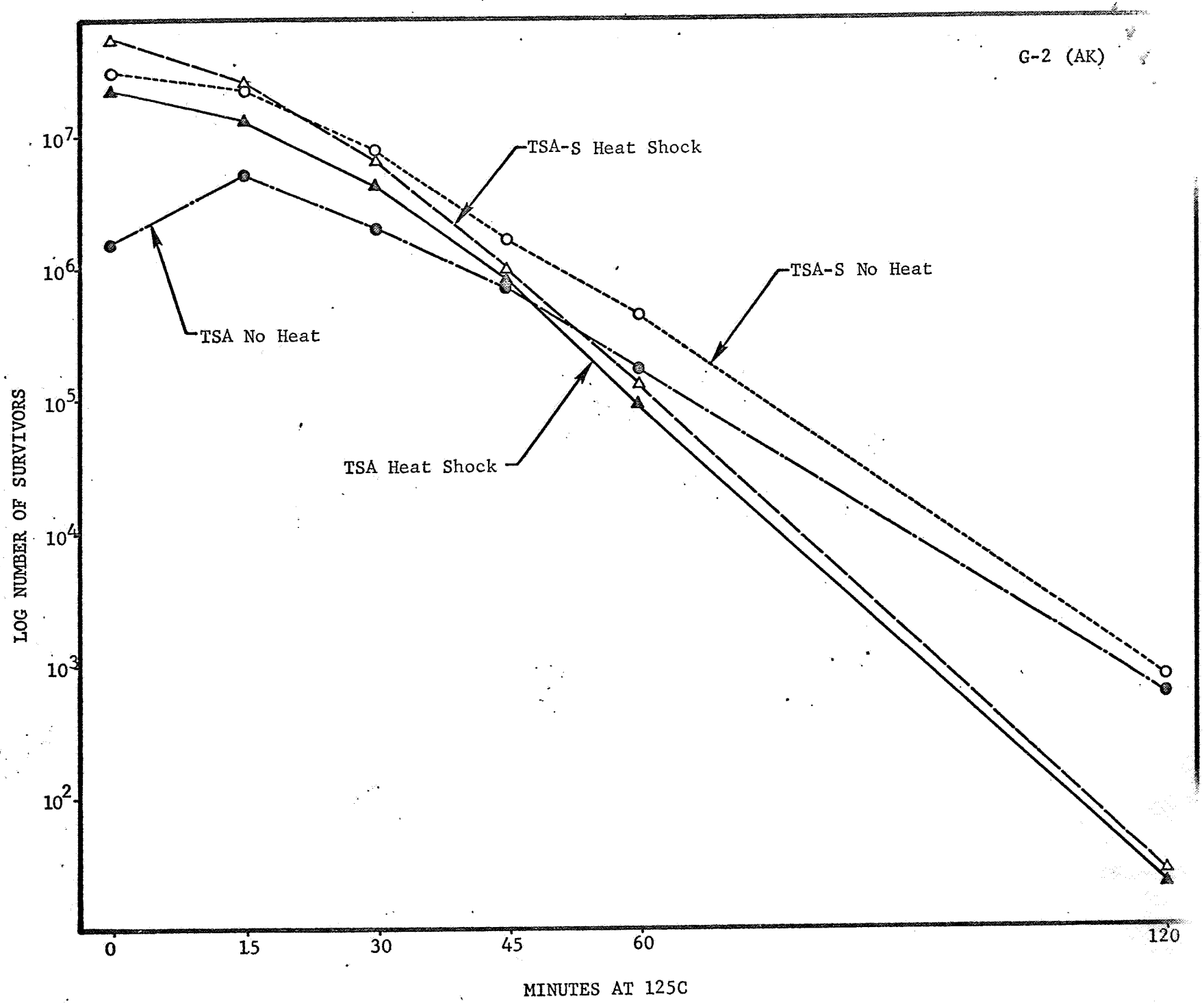


FIG. 2. COMPARATIVE DRY HEAT SURVIVOR CURVES AT 125 C OF ISOLATE G-2 SPORULATED ON AK #2 AND RECOVERED WITH TSA AND SUPPLEMENTED TSA:HEAT SHOCK, NO HEAT SHOCK.

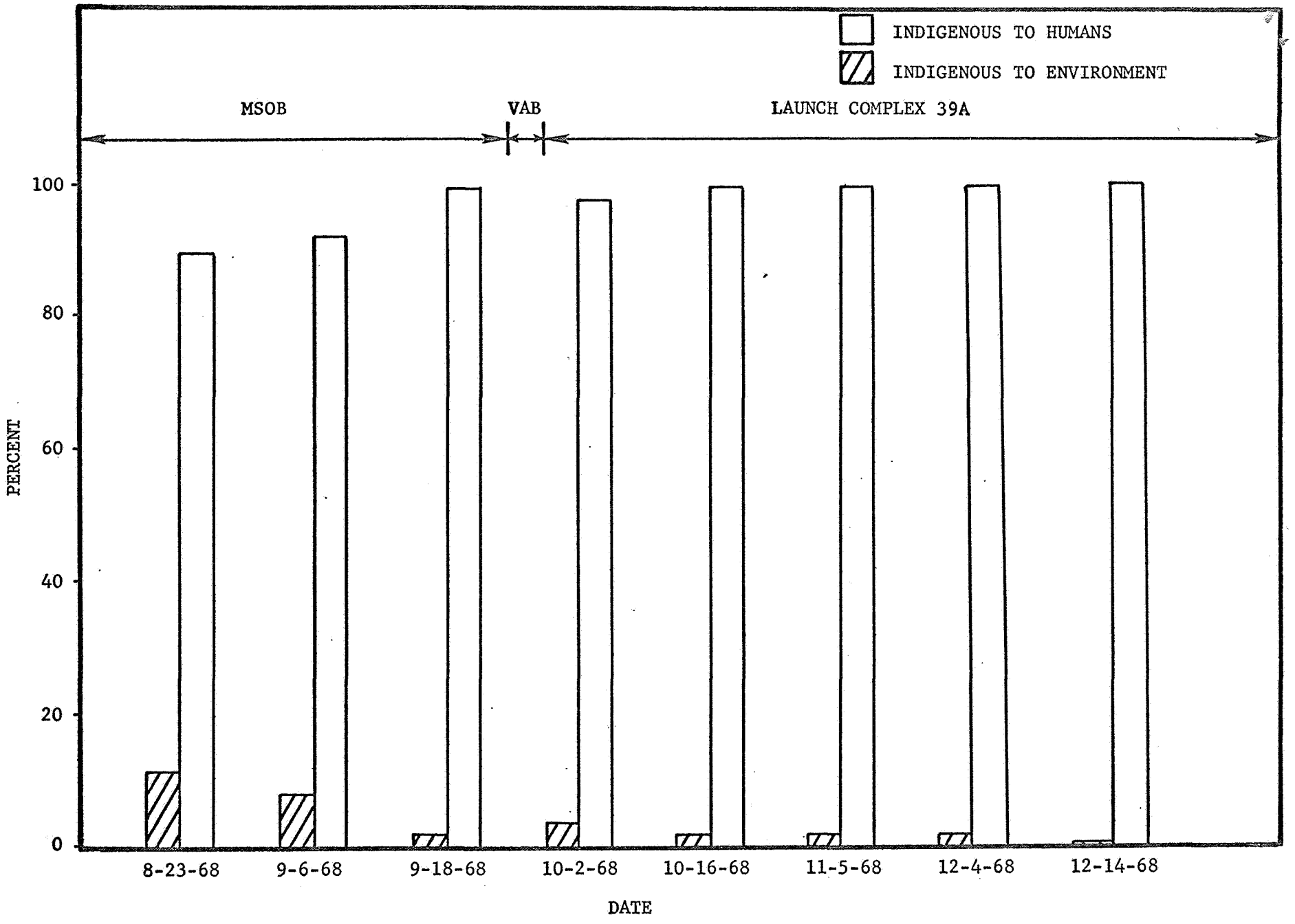


FIGURE 3. GENERAL TYPES OF MICROORGANISMS ON THE APOLLO SPACECRAFT DURING A FOUR MONTH PERIOD.