Report No. IITRI-L6023-3
(Quarterly Status Report)

LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

Contract No. NASr-22

National Aeronautics and
Space Administration

IIT RESEARCH INSTITUTE

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August 15 to November 15, 1965

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I. INTRODUCTION

Bacillus cereus spores exposed to either 100 or 130°C for as long as 30 min were not remarkably sensitive to a modified Martian environment with diurnal freezing and thawing cycles. Although vegetative cell growth and sporulation were delayed, population densities after 28 days of exposure compared favorably with previous results from nonheated spores.

Exposure of *B. cereus* and *B. subtilis* spores to a simulated Martian environment with barometric pressures over the 5 to 12 mm Hg range indicated that the growth response was unusually delayed or inhibited when compared to the growth response of these organisms in a similar environment but with a pressure of 75 mm Hg.

Manometric studies were initiated to determine the extent of adaptation and/or damage that an organism's respiratory system undergoes as a result of prolonged contact with a simulated extraterrestrial environment. Determination of suitable systems were investigated and included; phosphate buffer and glucose concentrations, the effect of l-alanine and manganese sulfate as germinating agents, and different densities of B. cereus and B. subtilis spore suspensions.

II. EXPERIMENTAL PROCEDURES

The Bacillus species used were the IITRI strain of B. subtilis and a B. cereus strain isolated from California desert soil. The spore suspensions, prepared in the manner previously described, were heat-shocked for 10 min at 80°C immediately before use.

Experiments investigating the effect of heat upon the growth response of B. cereus and the effects of barometric pressure upon the growth response of B. cereus and B. subtilis were conducted. The environmental conditions for both studies were: a daily freeze cycle of 8 hr, a felsite/limonite soil containing 1% organic medium, and 7 to 9% moisture.

For the heat studies the tubes containing the soil were inoculated with 0.01 ml of spore suspension and were placed in a hot air oven preset at the desired temperature. When the tubes equilibrated to the preset temperature (in 5 to 6 min), they remained at this temperature for 15 or 30 min and were then removed and rapidly cooled in an ice water bath. The 75-mm Hg pressure was established after the addition of water to give the required 8 to 9% moisture.

The pressure studies utilized 5-, 9-, 12-, and 75-mm Hg barometric pressures. Since water is in the vapor state at 25°C under barometric pressures of 5, 9, and 12 mm Hg, the moisture in these tubes was frozen in a dry ice-acetone bath prior to the establishment of pressure. This freezing was conducted to maintain the moisture at a constant level although it was realized that when the frozen moisture in the tubes thawed, the water vapor contributed to the pressure of the system in accordance with Dalton's law of partial pressures.

Total and spore counts were performed in both studies immediately and at 1, 2, 7, and 28 days unless otherwise stated.

In all experiments the recovery medium was trypticase soy agar (BBL). B. cereus was incubated for 24 hr at 35°C, and B. subtilis was incubated for 48 hr at 35°C.

Manometric studies with B. cereus and B. subtilis spore suspensions were conducted according to the techniques set forth by Umbreit, Burris, and Stauffer.[1] The harvesting and washing of the spore suspensions were described in previous reports.

III. RESULTS AND DISCUSSION

The growth responses of B. cereus spores after exposure to dry heat at 100 and 130°C for 15 and 30 min are shown in Figures 1 and 2. In all experiments, less than 50% of the spores survived the initial heat treatment. There was a

[1] Umbreit, Burris, and Stauffer, "Manometric Techniques", 4th Ed., Burgess Publishing Co., Minneapolis, 1964.

Figure 1

EFFECT OF DIURNAL FREEZE-THAW CYCLES ON BACILLUS CEREUS SPORES INITIALLY TREATED WITH DRY HEAT AT 100°C FOR 15 AND 30 MINUTES

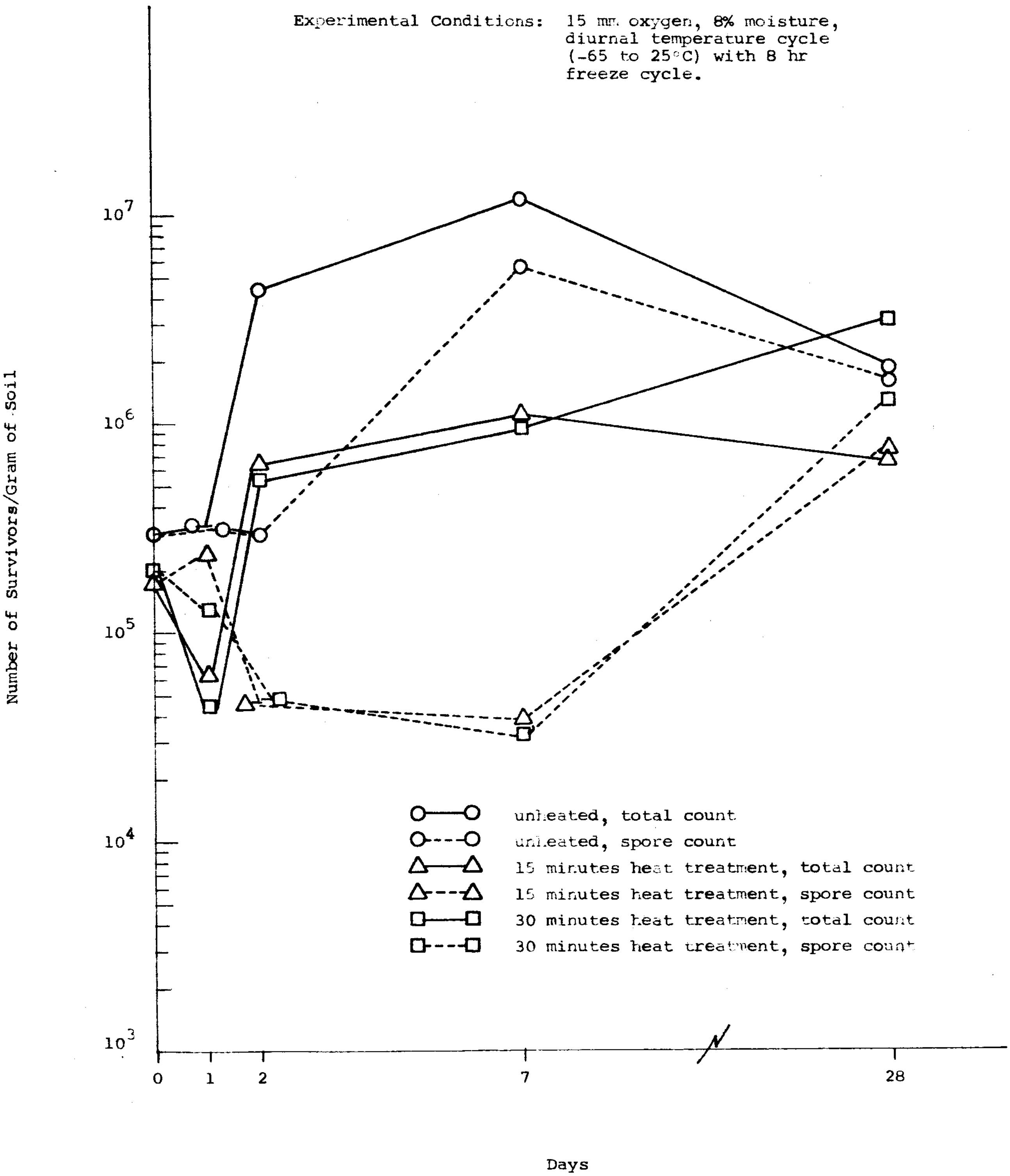
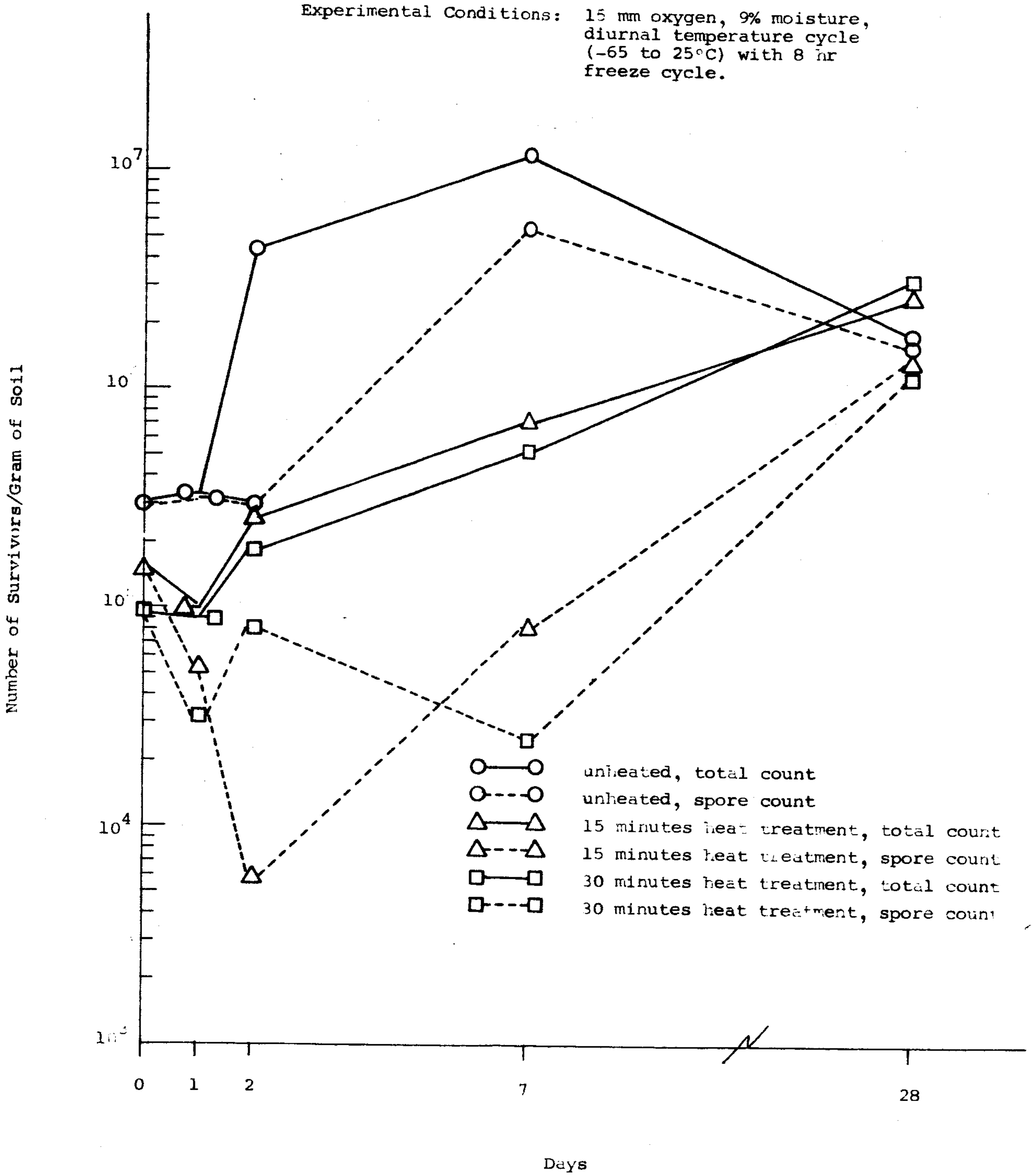


Figure 2

EFFECT OF DIURNAL FREEZE-THAW CYCLES ON BACILLUS CEREUS SPORES INITIALLY TREATED WITH DRY HEAT AT 130°C FOR 15 AND 30 MINUTES



delayed growth response noticed for both vegetative cell growth and sporulation of spores heated at 100 and 130°C, compared to the unheated control culture. Maximum vegetative cell growth of the unheated spores occurred between the second and third day in the simulated environment (not shown in Figures 1 and 2), while the vegetative cell growth of the spores heated at 100 and 130°C did not reach maximum vegetative cell growth after 28 days of exposure to the environment.

Sporulation was similarly delayed in the heated spores. The unheated spores reached maximum sporulation between 3 and 7 days, and the spores heated at both 100 and 130°C appeared to reach maximum sporulation after 28 days of exposure.

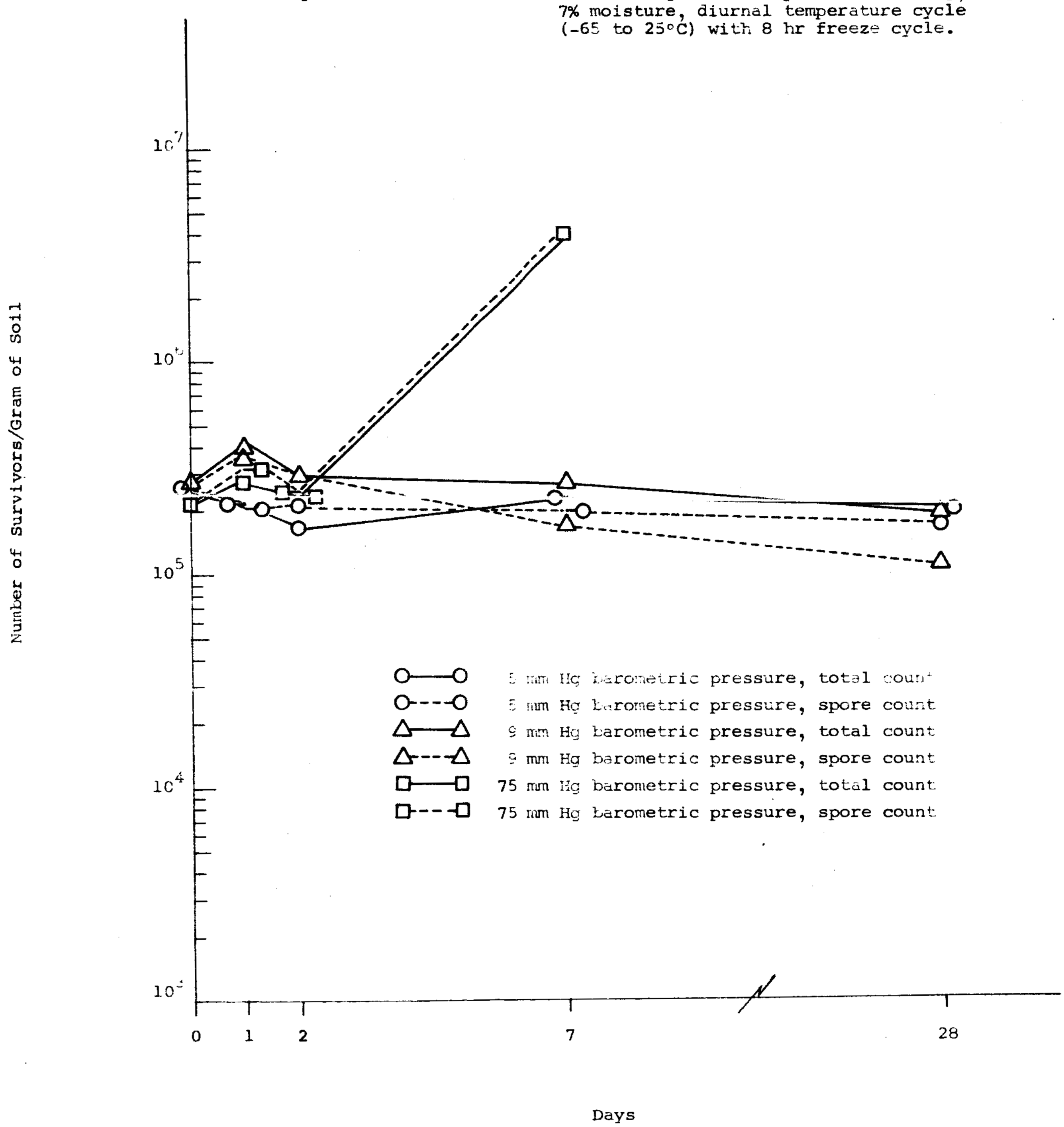
Extension of these heat studies to include 16- and 20-hr freeze cycles would be of interest since extending the freeze cycle length delays or depresses the growth response of the bacterial organisms.

The results of reduced barometric pressures upon the growth response of B. cereus are presented in Figure 3. With other conditions being similar, a lowering of the barometric pressure to 9 or 5 mm Hg resulted in an unusually delayed growth response of B. cereus. This growth inhibition may be significant because B. cereus spores germinate with subsequent vegetative growth in the absence of oxygen. The significant factor appears to be the availability of water since water is in the vapor state above 2 and 14°C at the reduced barometric pressures of 5 and 9 mm Hg. If water in the vapor phase were

Figure 3

EFFECT OF BAROMETRIC PRESSURE ON THE GROWTH RESPONSE OF BACILLUS CEREUS IN A SIMULATED MARTIAN ENVIRONMENT

Experimental Conditions: No flushing of tubes prior to sealing, 7% moisture, diurnal temperature cycle (-65 to 25°C) with 8 hr freeze cycle.



not available to the organism, the growth cycle of that organism would be confined to the region below the temperature maxima of 2 or 14°C, depending upon the barometric pressure.

Figure 4 presents similar results of the growth response of B. subtilis in a simulated Martian environment with barometric pressure reduced to 12 mm Hg. The growth response of this organism was negative with a slight loss in viability over the 28-day exposure period. The results with B. subtilis are not as significant as those with B. cereus since it has been demonstrated previously that B. subtilis spores require at least 10-mm Hg partial pressure of oxygen for spore germination and vegetative cell growth.

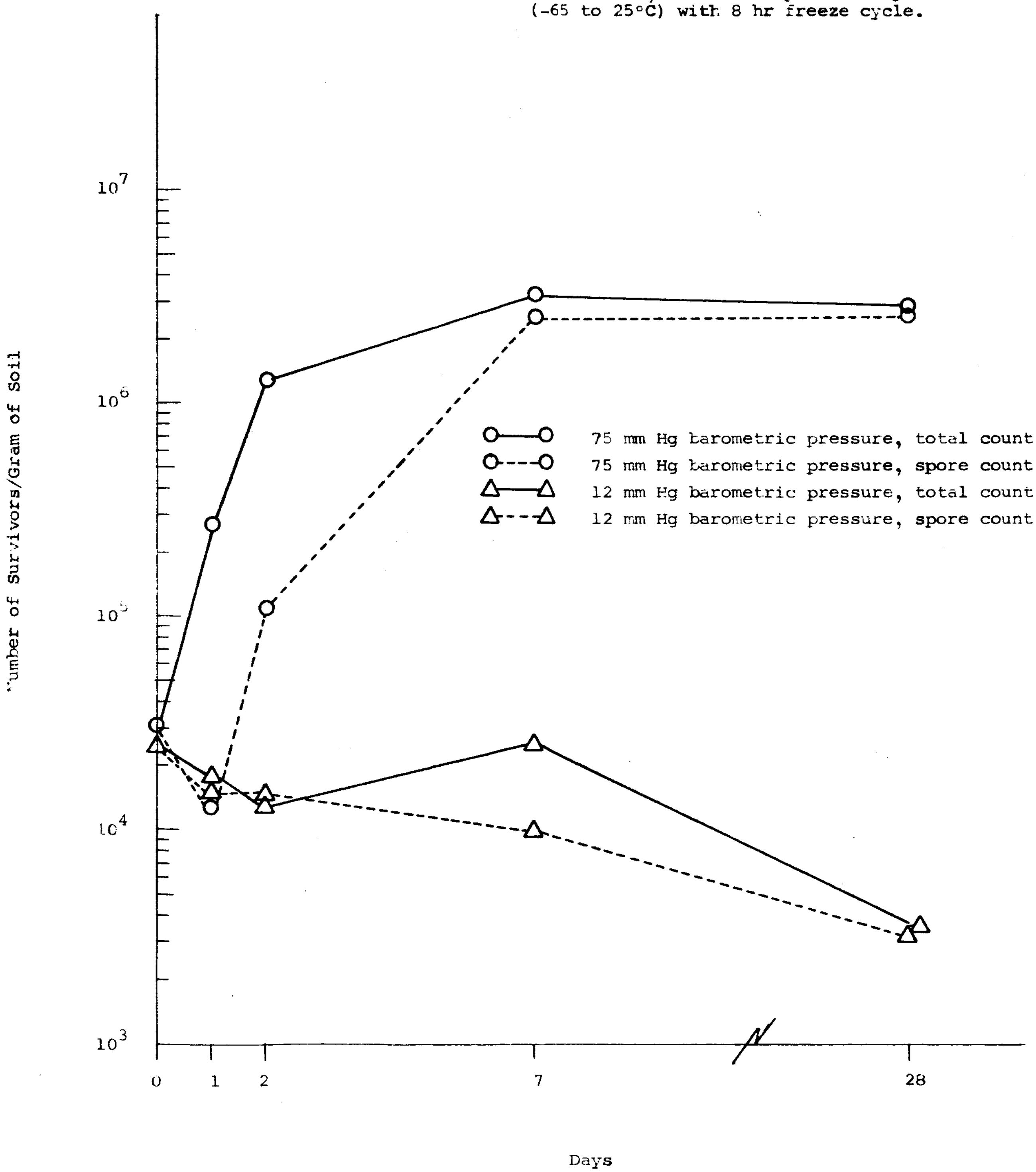
If the above results from reduced barometric pressures are confirmed, they will focus the problem of the prevention of contamination on Mars upon two large groups of microorganisms, wild or mutant types: (a) psychrophilic organisms and (b) halophiles, or, more generally, those organisms having a low water activity (A_w) requirement for growth.

Another ramification of these results would be that if growth were delayed rather than inhibited, the massive or explosive type of bacterial contamination on a planet like Mars could not occur as otherwise was indicated by studies performed at higher barometric pressures.

Figure 4

EFFECT OF BAROMETRIC PRESSURE ON THE GROWTH RESPONSE
OF BACILLUS SUBTILIS IN A SIMULATED MARTIAN ENVIRONMENT

Experimental Conditions: No flushing of tubes prior to sealing, 9% moisture, diurnal temperature cycle (-65 to 25°C) with 8 hr freeze cycle.



Superimposed upon the above problem areas is the complete lack of knowledge regarding the Martian soil's moisture content and moisture retention at different depths, factors which have significant bearing on studies of this nature.

By utilizing the techniques of Levinson and Hyatt,[2] spore germination, emergence, and cell division of spores produced in severe environments can be studied. Similar techniques can also be used to study nonsporulating bacteria in order to determine whether a cell's respiratory system is adapted or is damaged as a result of prolonged contact with a severe environment.

Manometric studies with B. cereus investigated the influence of 0.05 and 0.01 M phosphate buffers at pH 7.0 with 5 and 0.5 mg/ml of glucose as substrate. Heat shocked B. cereus spore suspensions of 3×10^6 and 3×10^8 spores/ml did not respond favorably to these systems even with the addition of 0.01 M l-alanine and trace amounts of manganese sulfate. Respiration rate (μliters $O_2/10^6$ cells/min) were not greater than the respiration rates of the endogenous control flasks. Respiration rates of B. subtilis spores at concentrations of 3×10^6 and 3×10^8 spores/ml were measurable over a 3 hr-period when a complex medium was used as the substrate.

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[2] Levinson and Hyatt, Ann. N. Y. Acad. Sci., Vol. 102, Art. 3, pp. 773-788, 1963.

IV. SUMMARY

B. cereus spores exposed to 100 and 130°C dry heat exhibited a delayed response in vegetative cell growth and sporulation in a simulated Martian environment compared to nonheat treated spores.

A reduction of the barometric pressure to 5 or 12 mm Hg delayed, or inhibited, spore germination and subsequent vegetative cell growth of *B. cereus*. Reduction of the barometric pressure to 9 mm Hg produced similar results with *B. subtilis* spores. We believe that it was the lowering of the barometric pressure that caused the decrease of available water, especially in the case of *B. cereus*.

Manometric studies initiated with *B. cereus* and *B. subtilis* spores investigated suitable substrates for future experiments, which will study adaptation or damage to a cell's respiratory system as a result of prolonged contact with a severe environment.

V. PERSONNEL AND RECORDS

Experiments were planned with the counsel of Dr. E. J. Hawrylewicz and with the technical assistance of Miss Marjorie Ewing and Miss Vivian Tolkacz.

Experimental data are recorded in Logbooks C16249 and C16394.

Respectfully submitted,

IIT RESEARCH INSTITUTE

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