SURVIVAL OF MICROORGANISMS IN A SIMULATED MARTIAN ENVIRONMENT

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Abstract. Studies were conducted to determine the effects of a simulated Martian environment on the survival of terrestrial microorganisms. Mariner IV data were utilized to establish the Martian model. Day/night cycling, temperature, humidity, pressure, atmospheric constituents and solar irradiation were controlled in a 3600 ft^3 simulation chamber at the Boeing Kent Space Simulation Laboratory.

Microorganisms used in this study included suspensions of: *Bacillus subtilis* var. *niger* spores, a psychrophilic sporeformer, and the organisms contained in a soil emulsion prepared from several soils. Aliquots of each suspension were placed on separate sterile stainless steel planchets, air dried, and positioned in 4 layers in sterile limonite. One-half of the samples placed on the surface received the total solar spectrum (0.44 earth solar constants); the remainder of the surface samples received the above treatment minus the ultraviolet (UV) portion of the spectrum. Subsurface samples were placed in limonite at depths of 0.5, 1.5 and 3 in.

Survival data were obtained for chamber exposure periods of 2, 4, and 8 days. These data indicate that: (1) organisms exposed to the total solar spectrum did not survive, (2) a time dependent, 1 to 3 log, reduction in numbers occurred in the surface samples that received the solar spectrum minus UV, and (3) subsurface survival varied with depth and type or organism.

1. Introduction

Terrestrial microorganisms may reach the surface of Mars through dislodgement from unsterilized orbiting or fly-by vehicles; by accidental impact of these unsterilized vehicles on the planet's surface; or by inadequate sterilization of Martian lander vehicles. This investigation was initiated to determine the effect of a Martian environment on the survival of these organisms. An estimate of the survival probability is required in order to properly evaluate this factor within the total planetary quarantine probability allocation set forth by the Committee on Space Research (COSPAR, 1964) and the National Aeronautics and Space Administration (Hall, 1968).

Numerous investigations have been undertaken to ascertain the effect of individual Martian environmental parameters on terrestrial organisms, Microorganisms have been exposed to simulated Martian temperature cycles (Hawrylewicz, *et al.*, 1965; Young *et al.*, 1968), atmospheric pressures (Roberts, 1963; Hagen and Jones, 1963; Silverman *et al.*, 1967); solar irradiation (Silverman *et al.*, 1964, 1967); and moisture levels (Hagen *et al.*, 1967) to determine what effect each parameter has on growth and survival. While studies of the effects of individual parameters are important, it is necessary to combine these parameters into a single test in order to estimate the probability of microbial survival for use in planetary quarantine calculations. Zhukova

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and Kondratyev (1964), and many others have pointed out that organisms may respond differently to individual climatic parameters acting successively than they will to combined environmental conditions. Therefore, this study was initiated in an attempt to obtain improved estimates of microbial survival in a Martian environment by combining as many individual parameters as possible into a 'total' simulation program. Simulation tests were conducted for 2, 4 and 8 day exposure periods.

2. Methods

A. ENVIRONMENTAL PARAMETERS

Mariner IV data were utilized to establish the environmental test parameters. These data indicated that the Martian atmosphere was primarily composed of carbon dioxide with lesser concentrations of nitrogen and an inert gas. The atmospheric pressure was calculated to range between 4 and 8 mm Hg with only traces of bound water estimated to be present (Kliore *et al.*, 1965).

A surface temperature profile was selected for that calculated at 45 °S lat. during the Martian summer. This temperature range appears to be the most conducive to microbial growth and, therefore, a conservative condition upon which to base probability estimates for use in planetary quarantine calculations. The model of the Martian environmental parameters simulated in this program are summarized in Table I.

TABLE I

Model of martian environmental parameters[a]

Day-Night Cycle	16 h light and 8 h dark
Temperature	Surface temperature max, 25 °C min, −60 °C Subsurface temperature: soil at 6 in., −40 °C
Humidity	Trace (calculated at less than 1 %)
Atmospheric Composition	Carbon dioxide (CO_2)–70 % Nitrogen (N_2)–25 % Argon (A)–5 %
Pressure	6 mm Hg
Solar Simulation	0.44 Earth solar constants (45 °S lat. at midsummer)
Spectral Range	2000 Å to 25,000 Å
Ultraviolet (UV)	Total UV between 2000 Å and 3000 Å = 7000 erg/cm²/sec
Soil	Limonite

[a] The following parameters were monitored and recorded continuously throughout the tests. (1) Temperature: Copper-constantan thermocouples tied to a Brown 24-channel multipoint strip chart recorder. (2) Atmospheric Composition: Varian residual gas analyzer with an ion pump. (3) Atmospheric Pressure: Wallace & Tiernan Bourdon Tube-Type Pressure Gage (0–20 Torr range). (4) Soil Pressure: Pirani Gage. (5) Solar Intensity: TRW Model DR-2 Total Radiometer.

B. TEST FACILITIES

The tests were conducted in Chamber 'B', (Figure 1) at the Boeing Kent Space Simulation Laboratory. The chamber has a net working space that is 8 ft in diameter and 8 ft high. The total volume is approximately 2000 ft^3. The chamber is equipped with a Spectrolab Model A-1200 Solar Simulator which, through a series of mirrors

Fig. 1. Space simulation chamber 'B'.

and lenses, projects a collimated 42 in. diameter beam onto the test samples at the bottom of the chamber. The solar simulation system contains nineteen 2.5 kW zenon arc lamps. Each of these lamps illuminates the full beam diameter, thus the light intensity received by the samples may be varied incrementally by utilizing various numbers of lamps. In addition, the power of each lamp may be varied approximately 25% to control the intensity.

To determine the effect of ultraviolet (UV) light on the survival of organisms under the test environment, the experiment was designed so that one-half of the surface of the cell was exposed to the total solar spectrum and the other half to the total spectrum minus wave lengths of 3200 Å and less. The UV light was filtered from one-half of the projected beam by placing a piece of plate glass above the test specimens. Quartz plates that allowed passage of the total solar spectrum covered the other half of the beam to balance the energy level and assure a uniform temperature across all surface samples.

A temperature gradient through the simulated Martian soil was produced by placing the sample box containing 6 in. of soil on a fluid controlled cold plate maintained at $-40\,^{\circ}C$. A cylindrical black shroud measuring 28 in. in diameter and 6 ft high was positioned above the samples and was maintained between -78 and $-84\,^{\circ}C$. The cold plate and shroud in combination with the heat supplied by the solar simulator produced the desired temperature gradients and cycling during the tests.

The Martian atmosphere was simulated by maintaining a composition of 70% CO_2, 25% N_2, and 5% A at a total pressure of 6 mm Hg. To achieve this atmosphere, the chamber was evacuated to 4 mm Hg and then backfilled to 40 mm Hg with CO_2.The chamber was again evacuated to 4 mm Hg. At this time the Bemco heat exchanger, which cooled the cold plate, was activated. The chamber was backfilled to 40 mm Hg with a mixture of CO_2, N_2 and A in approximately the above proportions. The chamber was evacuated again to the operating pressure of 6 mm Hg and the heat exchanger controlling the shroud was put into operation. Discrete additions of the individual gasses were made, as necessary, during the duration of each test and monitored on a mass spectrograph. The gasses were supplied from cylinders to a common manifold and then into the chamber through a sterile filter and shutoff valve

C. TEST MICROORGANISMS

A broad spectrum of microorganisms was selected for use in this program. The microorganisms were chosen to meet one of the following criteria: (1) availability to contaminate spacecraft, and (2) physiological characteristics that may permit survival in the Martian environment. The 3 test inocula used throughout the tests included the following:

(1) Mixed Population containing:
   (a) Heterogeneous population obtained from a composite soil.
   (b) Spores of the fungus *Cephalosporium acremonium.*

* Source: Douglas King, Western Regional Laboratories, Albany, California.

(c) Spores of the fungus *Byssochlamys fulva* NNRL A 13, 158* heat resistant, will grow in small quantities of oxygen (less than 1% O_2).

(2) *Bacillus subtilis* var. *niger* spores – ATCC No. 9372 resistant to dry heat.

(3) *Bacillus psychrosaccharolyticus* sp. Strain T15A* psychrophile, facultative anaerobe.

D. SAMPLE PREPARATION

The 1 in. stainless steel planchets were thoroughly cleaned prior to use. The planchets were boiled in a Haemo-Sol (commercial detergent) solution, then rinsed successively in tap water, distilled water, iso-propyl alcohol, and ether. They were then air dried, placed in petri dishes, and sterilized in a dry heat oven at 204°C for 4 h.

The mixed population of microorganisms was prepared from a composite of soils. The soils were mixed together, air dried, and ground with a mortar and pestle. Spores of the fungi *Cephalosporium acremonium* and *Byssochlamys fulva* were added to the soil mixture. 1 g of this mixture was diluted in 100 ml of sterile distilled water and then filtered through sterile gauze. The resulting suspension was used as the mixed population inoculum.

The *Bacillus subtilis* var. *niger* spores were suspended in sterile distilled water. The inoculum of *Bacillus psychrosaccharolyticus* sp. was prepared by growing the organism on agar plates. The growth was then scraped from the agar surface and suspended in sterile distilled water.

Each of the inocula was placed in sterile Erlenmeyer flasks containing magnetic stirring bars. The suspensions were stirred continuously during the inoculation process. All of the planchets for one test were inoculated at the same time using 0.1 ml aliquots of the suspensions. Controlled numbers of the 3 groups of microorganisms were spread evenly on each planchet and the planchets were allowed to air dry, overnight, in the air flow of a Class 100 clean bench.

E. SAMPLE ARRANGEMENT

The planchets were arranged in oven sterilized limonite in 4 layers within a 2-ft diameter circle. 1 layer of planchets was placed on the surface of the limonite and the other 3 layers were placed at 0.5, 1.5, and 3.0 in. depths beneath the surface. An aluminum sample box was used to contain the limonite and planchets.

The surface layer was divided into halves. 48 planchets (16 replicates of each of the 3 test inocula groups) were randomly arranged, within a previously determined grid, in each half. One-half was exposed to the total solar spectrum and the other half was exposed to the solar spectrum minus UV as previously described. The subsurface layers contained the same number of randomly distributed replicates of the 3 groups of microorganisms as each of the surface halves. The planchets in the 3 subsurface layers were offset so that they were not directly below a planchet in the layer above. This was done to facilitate heat transfer and maintain even heating gradients. In

* Source: John Larkin and J. L. Stokes, Department of Bacteriology and Public Health, Washington State University, Pullman, Washington.

addition, 8 sterile planchets were included in each subsurface layer. 8 sterile planchets were also placed in the UV and non-UV surface areas. These sterile planchets were used as controls to monitor for possible background microbial contamination.

Included on the surface layer were inoculated planchets which were turned upside down, i.e., the inoculated area was not exposed to the solar radiation. 8 planchets containing the mixed population and 8 planchets containing *B. subtilis* spores were used for this facet of the study.

To simulate the possible surface contamination by spall ejected from a spacecraft as the result of meteoroid impact (Olson *et al.*, 1967) solar panels were ground into small particles, heat sterilized, and inoculated with *B. subtilis* spores. After drying, the spall was mixed thoroughly and 0.1 g aliquots were placed in planchets. 16 planchets were placed on the surface layer in the sample box. 8 of these were positioned to receive the total solar radiation and the other 8 were positioned to receive the same treatment minus the UV.

F. SAMPLE EXPOSURE

After the sample box was loaded, the lid was closed to prevent contamination. The motorized, sliding lid was kept closed at all times except during the actual test in the simulation chamber. Extreme care was taken to avoid getting limonite into any of the surface layer planchets.

The loaded sample box was placed in the test chamber on the fluid-cooled plate attached to the bottom lid of the chamber. The sides of the box and the bottom of the cold plate were insulated with layers of aluminum foil. Cables were installed for monitoring the environment. The chamber was closed and the atmosphere was established by the previously described technique. At this time, the remotely operated motorized lid of the sample box was opened exposing the samples and starting the test. The test cycle was always started at the beginning of the 8 h Martian 'night'.

After the required number of 'night' and 'day' cycles, the test was concluded by closing the motorized box lid, warming the cold plate to 7 °C and backfilling the chamber to 100 mm Hg with gaseous nitrogen. The chamber was then evacuated to 6 mm Hg and again backfilled with gaseous nitrogen to atmospheric pressure. During this time, the sample box was allowed to warm gradually. This temperature stabilization period ranged from 6 to 8 h after test completion to removal of the sample box from the chamber.

The sample box was taken to the Microbiology Laboratory and placed in a Class 100 clean bench before the lid was opened. Each of the surface planchets, containing the test microorganisms, was removed with sterile forceps and placed in a bottle containing 60 ml of 0.1% peptone water. The subsurface planchets were retrieved by scooping out the excess limonite to a point $\frac{1}{2}$ in. above the layer being extracted.

A template was then placed on the limonite and by gently probing each hole in the template, all of the planchets were recovered. One complete layer of planchets was analyzed before removing the next layer from the sample box.

Each planchet was analyzed separately to obtain the number of surviving micro-

organisms per planchet. A schematic diagram outlining the procedural steps of the microbial analyses for the mixed population of microorganisms is presented in Figure 2. The other test cultures were analyzed by plating 5 replicate 10 ml aliquots in Trypticase Soy Agar.

The sterile planchets from the surface and subsurface layers were analyzed by placing each one in a petri dish. The direct surface agar pour (DSAP) technique was used to determine if contamination had occurred throughout the test cycle.

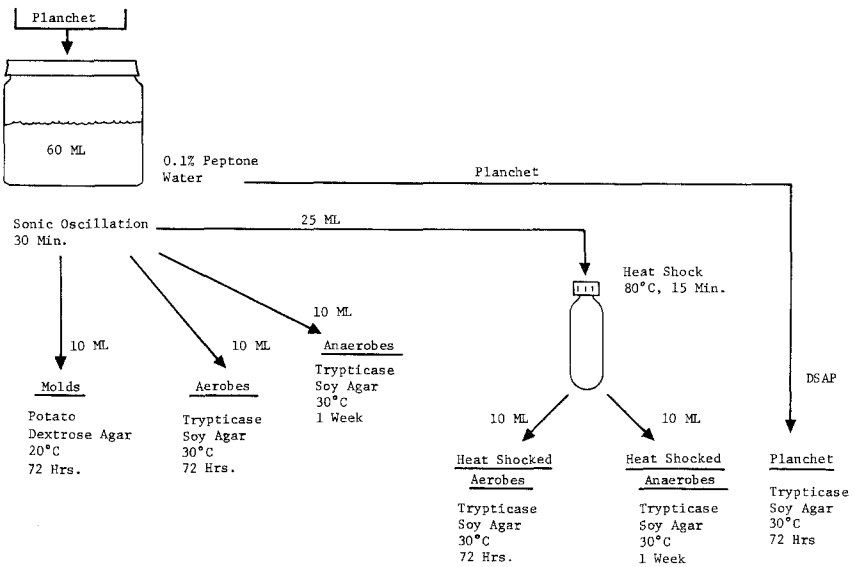

Fig. 2. Analysis of mixed population.

G. TEST CONTROLS

A set of planchets inoculated with the test microorganisms was maintained in an ambient environment. One-half of these planchets were exposed to a surface environment and the other half were exposed to a subsurface environment in limonite. All of the planchets were enclosed in a metal can which was maintained in the Microbiology Laboratory for the duration of each test cycle. The analyses of these planchets followed the same procedure as outlined above.

Sterile planchets were also processed through each of the outlined procedural steps. Procedural controls were performed prior to and after processing the test planchets to obtain data on possible contamination occurring during handling.

3. Results

The effects of the simulated Martian environment on the survival of microorganisms was investigated for 2, 4 and 8 days of exposure. However, for purposes of limiting the volume of data, the environmental data from one representative day (day 5 of 8-day row) was selected for inclusion in this paper.

A. ENVIRONMENTAL PARAMETERS

Copper-constant thermocouples were used to measure the planchet temperatures at each layer. Figure 3 shows the typical planchet temperatures recorded on day 5 of the 8-day test. The atmospheric composition and total pressure were measured con-

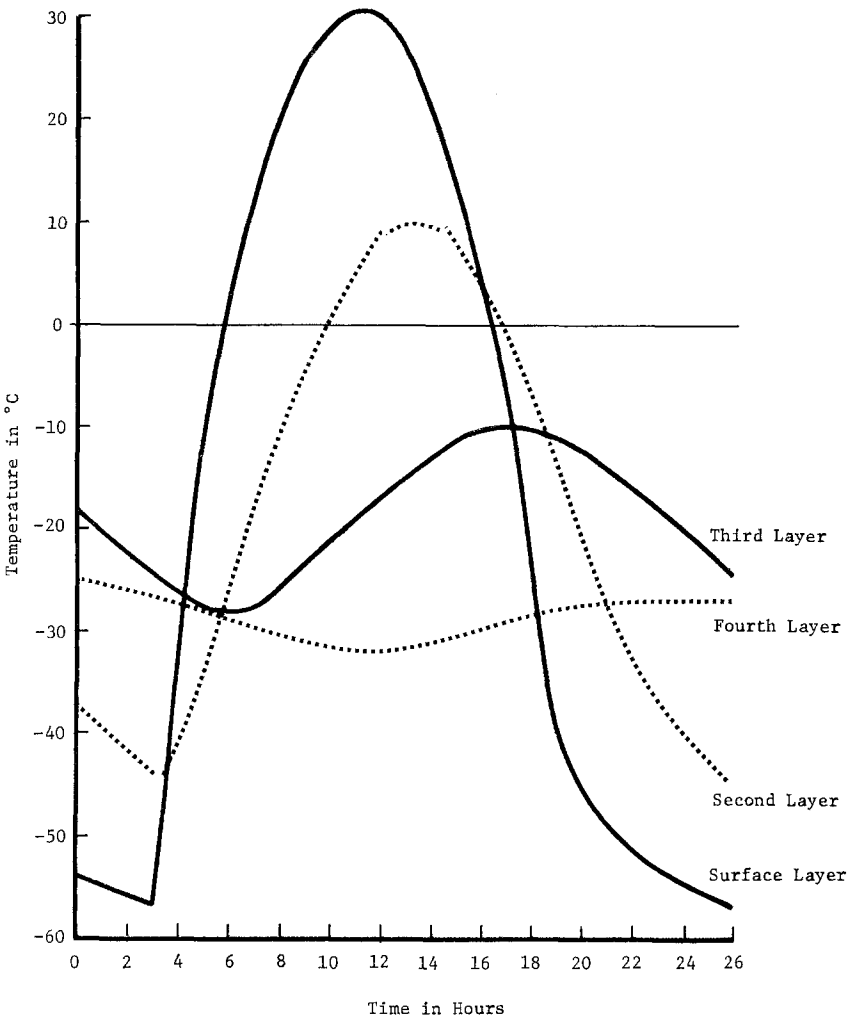

Fig. 3. Typical planchet temperature profile.

tinuously throughout the tests. The means of these measurements taken during day 5 of the 8-day test are 70.18% CO_2, 24.02% N_2, 5.78% A, and 5.97 torr total pressure.

The solar radiation incident on the surface test planchets was measured with a TR W Model DR-2 Total Radiometer. Figure 4 gives the radiation measured for day 5 of the 8-day test.

B. BIOLOGICAL ANALYSIS

A summary of the survival data is presented in Tables II, III, IV and V. An analysis of variance technique was used to evaluate the survival data obtained from the environmental chamber tests. Comparisons were made between surface samples and

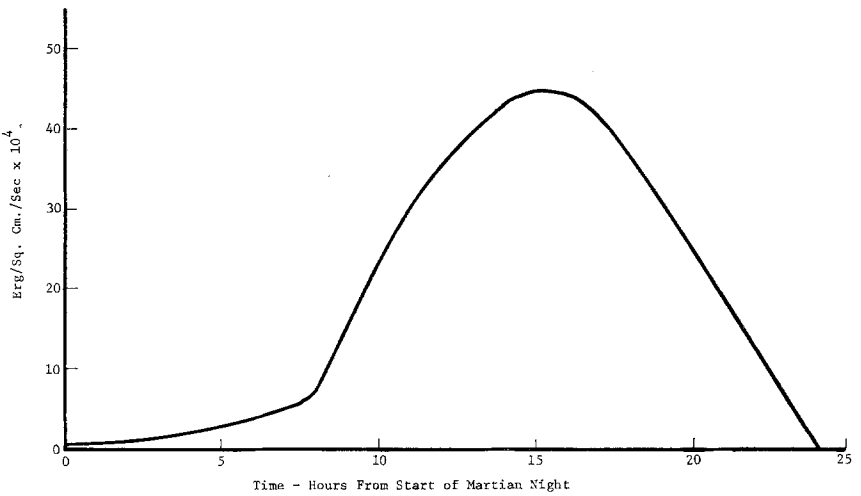

Fig. 4. Surface solar radiation (5th day of 8 day test).

TABLE II

Surface comparison – 2 day simulation test
Means for total number of microorganisms recovered per planchet
(Based on 16 replicates)

Microorganisms	Surface ambient	Martian environment		
		Surface[2b] upside down	Surface no UV.	Surface UV.
Soil aerobes	26500	22600	479	87
	a[a]	b	c	d
Soil anaerobes	2430	1080	112	6
	a	b	c	d
Heat shocked	9540	6130	271	19
Soil aerobes	a	b	c	d
Heat shocked	2850	2500	47	4
Soil anaerobes	a	b	c	d
Soil molds	100	79	20	2
	a	b	c	d
Bacillus subtilis	621	526	5	0.0
spores	a	a	b	c
Spall[b]	526	N0 test	266	5
Bacillus subtilis	a		b	c

[a] Means in each row followed by the same letter are not significantly different at the 0.05 level of probability, based on the Duncan Multiple Range Test.
[b] Means based on 8 replicates.

TABLE III

Subsurface comparison – 2 day simulation test
Means for total number of microorganisms recovered per planchet
(Based on 16 replicates)

Microorganisms	Subsurface ambient	Martian environment		
		Second layer ($\frac{1}{2}''$)	Third layer ($1\frac{1}{2}''$)	Fourth layer ($3''$)
Soil aerobes	18800	37100	36800	22900
	a[a]	b	b	a
Soil anaerobes	3590	No data	No data	No data
Heat shocked	7670	11400	11200	9140
Soil aerobes	a	b	b	b
				a
Heat shocked	3530	2560	1740	807
Soil anaerobes	a	b	c	d
Soil molds	58	86	101	44
	a	b	b	a
Bacillus subtilis	613	628	667	579
spores	a	a	b	c

[a] Means in each row followed by the same letter are not significantly different at the 0.05 level of probability, based on the Duncan Multiple Range Test.

TABLE IV

Surface comparison – 8 day simulation test
(Based on 16 replicates)

Microorganisms	Surface ambient	Martian environment		
		Surface[b] upside down	Surface no UV.	Surface UV.
Soil aerobes	533000	395000	6896	6.8
	a[a]	b	c	d
Soil anaerobes	68000	29000	301	2.6
	a	b	c	d
Heat shocked	513000	400000	5774	8.0
Soil aerobes	a	b	c	d
Heat shocked	148000	185000	1728	0.4
Soil anaerobes	a	a	b	c
Soil molds	177	248	25	1.9
	a	b	c	d
Bacillus subtilis	670	505	0.6	0.0
spores	a	b	c	c
Bacillus	19200	No test	0.8	0.0
psychrosaccharolyticus	a		b	b
Spall[b]	514	No test	145	4
B. subtilis	a		b	c

[a] Means in each row followed by the same letter are not significantly different at the 0.05 level of probability, based on the Duncan Multiple Range Test.
[b] Means based on 8 replicates.

TABLE V

Subsurface comparison – 8 day simulation test
Means for total number of microorganisms recovered per planchet
(Based on 16 replicates)

Microorganisms	Subsurface ambient	Martian environment		
		Second layer ($\frac{1}{2}''$)	Third layer ($1\frac{1}{2}''$)	Fourth layer ($3''$)
Soil aerobes	644000	575000	609000	638000
	a[a]	a	a	a
Soil anaerobes	70000	57000	78000	73000
	a		a	a
	b	b		b
Heat shocked	544000	523000	477000	527000
Soil aerobes	a	a		a
		b	b	b
Heat shocked	146000	134000	153000	141000
Soil anaerobes	a	a	a	a
Soil molds	177	178	194	199
	a	a	a	a
Bacillus subtilis	641	676	650	636
spores	a	a	a	a
Bacillus	18900	13900	17300	19100
psychrosaccharolyticus	a	a	a	a

[a] Means in each row followed by the same letter are not significantly different at the 0.05 level of probability, based on the Duncan Multiple Range Test.

between subsurface samples to determine if the various environmental conditions significantly affected the survival of the microorganisms. The survival data obtained from the samples maintained at Earth ambient conditions have been included in the analysis. The survival data from the 4-day simulation test are not included because the initial inoculum on the samples was too small to give statistically valid results. However, the trends were comparable with those obtained for the 2 and 8 day tests.

The sterile planchets placed on the surface and in the subsurface layers to detect microbial contamination were analyzed. All of the sterile control results indicated that no contamination occurred during the tests.

4. Discussion

The environmental conditions that were simulated in this series of tests were as follows: (1) direct surface solar environment, (2) the surface solar spectrum minus UV, (3) the surface environment minus solar radiation (upside down planchets), and (4) the Martian subsurface environment.

The data indicate that the probability of microorganisms surviving exposure to the

total Martian surface environment is very low. As an example, the soil aerobes were reduced by 5 logs within 8 days. This general trend is exhibited in all cases and substantiates the conclusion. Microbial death is probably caused by a combination of factors including temperature cycling and dehydration; however, the primary cause of death in this case is solar radiation.

A significant reduction was observed when microorganisms were exposed to the surface environment minus the UV portion of the spectrum (Tables II and IV). This reduction, which was exhibited in all of the tests, may be attributable to a combination of factors including: sensitivity to the white light portion of the spectrum; or temperature variations in combination with the reduced pressure which would affect dehydration of the organisms. The water which would be released from the samples would be trapped on the cold wall and, in essence, develop a molecular flux away from the microorganisms. Various investigations including Murrell and Scott (1966), Angelotti (1968), and Fox and Pflug (1968) have reported that water activity has an influence on resistance of microorganisms to dry heat and that dehydration may be the mechanism primarily responsible for lethality of dry heat. While water activity is undoubtedly an important factor in survival under these conditions, white light appears to have a significant influence. This conclusion is reached when the results from these samples (minus UV) are compared to the surface upside down and subsurface data. When microorganisms were placed in surface planchets turned upside down (surface environment, no solar exposure) less than one log reduction was demonstrated in all the samples tested. The organisms on these planchets were not subjected to the solar radiation. The dehydrating environment was modified in this case since it is probable that the position of the planchets over the microorganisms afforded some protection to dehydration. The planchet temperatures were approximately the same in the exposed planchets as those that were upside down.

No log numerical reductions were demonstrated in the subsurface samples in the 8-day test. These samples were not exposed to extreme temperature variations or solar radiation as were the surface samples. They were exposed to the same pressure which was confirmed by the pressure measurements taken during the tests. The subsurface environment probably had some negating effect on dehydration. However, the fact that log reductions were not observed in the subsurface environment, provides substance to the conclusion that solar radiation was the primary cause of death in this study.

5. Conclusions

These data support the conclusion that the Martian surface environment is a very hostile environment in which survival probabilities for exposed microorganisms would be very small. However, if microorganisms are protected from the solar irradiation or on spall which might result from meteoroid impacts, the probability for survival increases. Microorganisms protected in subsurface environments would be expected to survive for extended periods.

References

Angelotti, R.: 1968 'Protective Mechanisms Affecting Dry Heat Sterilization', *COSPAR Technique Manual Series* **4**, 59–74.

COSPAR Information Bulletin No. 20, November 1964, 25–26.

Fox, K. and Pflug, I. J.: 1968, 'Effect of Temperature and Gas Velocity on the Dry Heat Destruction Rate of Bacterial Spores', *Appl. Microbiol.* **16**, 343–348.

Hagen, C. A. and Jones, R.: 1963, 'Life in Extraterrestrial Environments', Quarterly Status Report, Sept-Nov. NASr 22: IITRI Proj. C194.

Hagen, C. A., Hawrylewicz, E. J., and Ehrlich, R.: 1967, 'Survival of Microorganisms in a Simulated Martian Environment, II. Moisture and Oxygen Requirements for Germination of *Bacillus cereus* and *Bacillus subtilis* var. *niger* Spores, *Appl. Microbiol.* **15**, 285–291.

Hall, L. B.: 1968, 'The Importance of Sterilization Techniques in Space Exploration', *COSPAR Technique Manual Series* **4**, 3–18.

Hawrylewicz, E. J., Hagen, C. A., and Ehrlich, R.: 1965, 'Response of Microorganisms to a Simulated Martian Environment', *Life Sci. Space Res.* **III**, 64–73.

Kliore, A., Cain, D. L., Levy, G. S., Eshleman, V. R., Fjeldbo, G., and Drake, F. D.: 1965, 'Occulation Experiment: Results of the First Direct Measurement of Mar's Atmosphere and Ionosphere', *Science* **149**, 124–3–1248.

Murrell, W. G. and Scott, W. J.: 1966, 'The Heat Resistance of Bacterial Spores at Various Water Activities', *J. Gen. Microbiol.* **43**, 411–425.

Olson, R. L., Green, R. H., Gustan, E. A., and Pilgrim, A. J.: 1967, 'Microbial Survival After Simulated Meteoroid Impact', *Develop. Ind. Microbiol.* **8**, 235–245.

Roberts, T. L., 1963, 'Studies with a Simulated Martian Environment', *J. Astronaut. Sci.* **X**, 65–74.

Silverman, G. J., Davis, N. S., and Keller, W. H.: 1964, 'Exposure of Microorganisms to Simulated Extraterrestrial Space Ecology', *Life Sci. Space Res.* **II**, 372–384.

Silverman, G. J., Davis, N. S., and Beecher, N.: 1967, 'Resistivity of Spores to Ultraviolet and Gamma Radiation while Exposed to Ultrahigh Vacuum or at Atmospheric Pressure', *Appl. Microbiol.* **15**, 510–515.

Young, R. S., Deal, R. H., and Whitfield, O.: 1968, 'The Response of Spore Forming vs. Non Spore Forming Bacteria to Diurnal Freezing and Thawing', *Space Life Sci.* **1**, 113–117.

Zhukova, A. I. and Kondratyev,: 1964, 'On Artificial Martian Conditions Reproduced for Microbiological Research', *Life Sci. Space Res.* **III**, 120–127.