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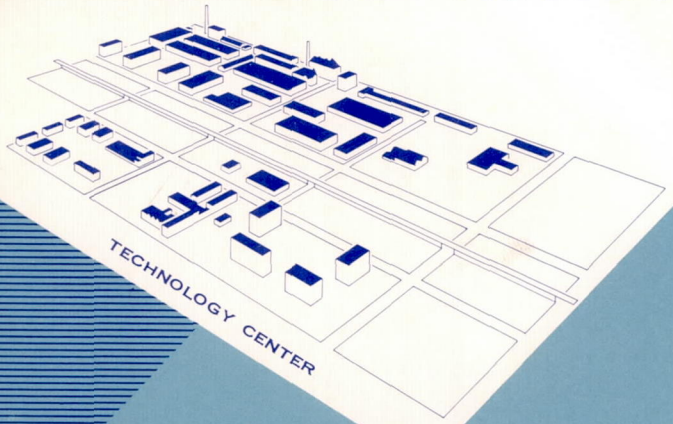

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Report No. ARF 3194-9
(Quarterly Status Report)

LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

National Aeronautics
and Space Administration
Washington, D.C.

Report No. ARF 3194-9
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LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

ARF Project C 194
Contract No. NASr-22

February 15 to May 15, 1963

I. INTRODUCTION

The objective of this phase of the program is to study the effect of a simulated Martian environment on the survival of terrestrial microorganisms. During the period covered by this report the survival of a coccus isolated from a California desert soil was studied.* This microorganism had a high survival rate after the inoculation and flushing procedures and also after 28 days in the Martian environment. After inoculation and flushing with Earth atmosphere 44% of the cells were recovered. From the tubes flushed with Martian atmosphere 30% of the cells remained viable. After 28 days in the Martian environment little change in the viable cell count occurred: 3×10^7 cells/g present initially, 3.5×10^7 cells/g present after 28 days. This microorganism, tentatively identified as a Micrococcus, had a wide temperature range of growth: from 5°C (lowest temperature tested) to 37°C. It displayed a heat resistance capable of surviving 60°C for 10 min.

*One of 5 desert soil samples supplied by Dr. R. E. Cameron of Jet Propulsion Laboratory, California Institute of Technology.

When sterilized soil of the type that this organism was isolated from was used instead of Martian soil, the percent survival after inoculation and flushing procedures was increased. Flushing with Earth and Martian atmospheres resulted in a survival of 90 and 54% of the organisms, respectively.

Flushing with the Martian atmosphere decreased the percent viable cells recovered. After 1 freeze and thaw cycle in the Martian environment the viable cell count was higher than the initial count. There was little difference in the survival rate of the Earth control and Martian experimental groups. Thus, it appears that after the initial shock of the Martian environment upon the cells the diurnal freeze-thaw cycle had no significant effect on the cell's survival.

Biochemical tests were also performed with this organism. Glucose, sucrose, and lactose were not utilized; indole and acetylmethlycarbinol were not produced; nitrate was not reduced; and the methyl red reaction was negative. Characteristic of some Micrococci, this organism utilized inorganic nitrogen (ammonium phosphate) for growth.

The moisture, organic, and inorganic composition of desert soil samples and of the Martian soil (uninoculated and inoculated with microorganisms) was determined. Not unusual was the fact that the desert soil listed as clay had the highest moisture (3.5%) and organic material (2.6%) of the five desert soil samples. The percent organic material present in the simulated Martian soil was 3.8%.

To confirm previous observations that the Martian soil with dehydrated bacteriological material absorbed more water in a closed system then Martian soil without the bacteriological medium, studies were performed in desiccators containing various types of soil. The moisture in the Martian soil increased 334% while the moisture in the Martian soil containing 10% dehydrated bacteriological medium increased 2038% during the same time period.

II. EXPERIMENTAL

The simulated Martian atmosphere previously described in Report ARF 3194-5 was used. The methods of growing, harvesting, inoculating, and sampling Mars tubes and the types of media used are described in detail in Reports ARF 3194-2, -3, -4, and -7.

The procedure for gravimetric determination of moisture, organic, and inorganic composition of soil was as follows:

1. Moisture was determined by weight loss at 105°C
2. Organic matter was determined by weight loss at 500°C
3. Inorganic matter was determined by weight loss at 800°C.

The biochemical tests performed with the desert soil isolate were in accordance with methods set forth in Manual of Microbiological Methods, A Guide to the Identification of the Genera of Bacteria and Bergey's Manual of Determinative Bacteriology.[1,2,3]

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[1] Conn, H. J., "Manual of Microbiological Methods," Williams and and Wilkins Co., Baltimore 2, Maryland, 1957.

[2] Skerman, V. B. D., "A Guide to the Identification of the Genera of Bacteria," Williams and Wilkins Co., Baltimore 2, Maryland, 1959.

[3] Breed, R. S., Murray, E. G. D., and Smith, N. R., "Bergey's Manual of Determinative Bacteriology," Williams and Wilkins, Baltimore, 1957.

III. RESULTS AND DISCUSSION

The moisture, organic, and inorganic composition of Martian soil and of 5 desert soils is given in Table 1. The Martian soil does not resemble any of the desert soils in moisture, organic, and inorganic content. As would be expected, the desert soil described as clay (Sample c, No. 68) had the highest moisture and organic content. Desert soil b (sandy loam, No. 62) was the soil from which the Micrococcus was isolated. This soil was used in place of the Martian soil in an experiment designed to test the survival of the isolated Micrococcus in the Martian atmosphere and temperature cycle.

The moisture, organic, and inorganic composition of the Martian soil and the desert soil b, 7 days after inoculation with the Micrococcus, is shown in Table 2. The only difference noted was a decrease in the moisture content of the desert soil. This moisture loss was the result of the flushing procedure. The addition of culture to the soils did not increase the organic or inorganic content of the soils.

Table 3 shows the effect of inoculation and flushing procedures on the survival of the Micrococcus in Martian soil and desert soil b (the soil from which it was isolated). The total count decreased more in the Martian soil after inoculation and after flushing with either Earth or Martian atmospheres than in the desert soil. Since both soil types have relatively low moisture and organic content and

Table 1

MOISTURE, ORGANIC, AND INORGANIC COMPOSITION OF MARTIAN SOIL AND OF FIVE DESERT SOILS

	% Moisture	% Organic	% Inorganic
Martian soil	0.23	3.82	95.4
Desert soils*			
a - 51 sand	0.18	0.37	98.4
b - 62 sandy loam	1.08	1.24	94.1
c - 68 clay	3.47	2.57	89.2
d - 70 sand	0.21	0.67	98.8
e - 76 sand	0.73	1.58	97.4

*Samples supplied by Dr. R. E. Cameron of Jet Propulsion Laboratory, California Institute of Technology.

Table 2

MOISTURE, ORGANIC, AND INORGANIC COMPOSITION OF MARTIAN SOIL AND DESERT SOIL NO. 62 AFTER INOCULATION WITH MICROCOCCI

	% Moisture	% Organic	% Inorganic
Martian soil			
Earth control	0.18	3.50	95.3
Martian experimental	0.18	3.60	95.0
Desert soil No. 62			
Earth control	0.53	0.90	95.0
Martian experimental	0.51	0.80	94.9

Table 3

THE EFFECT OF INOCULATION AND FLUSHING PROCEDURES ON THE TOTAL VIABLE CELL COUNT OF A MICROCOCCI IN MARTIAN SOIL AND IN ITS NATURAL DESERT SOIL

	Martian Soil		Desert Soil No. 62	
	Total Count/g	% Survival	Total Count/g	% Survival
Inoculum	101×10^6	100	167×10^6	100
After inoculation	65×10^6	64	135×10^6	81
After flushing with Earth atmosphere	44×10^5	44	148×10^6	89
After flushing with Martian atmosphere	30×10^5	30	90×10^6	54

similarly high inorganic content the difference in survival rates is of interest. It is to be noted that the moisture content of the desert soil after flushing was 3 times the moisture content of the Martian soil which may have influenced the survival rates, Table 2.

Table 4 shows the morphological and biochemical characteristics of the Micrococcus used in these experiments. It is recognized that some of the Micrococcaceae form spores; but spores have not been demonstrated in this Micrococcus.

The ability for this organism to grow at low temperatures, to survive at high levels afterthe diurnal freeze-thaw cycle certainly makes it a likely candidate for surviving on Mars. Ultraviolet experiments are beirg initiated with this organism to determine its resistance to ultraviolet irradiation. Initial experiments with this organism in an atmosphere of 90% nitrogen and 10% carbon dioxide have demonstrated an inability to grow.

Figure 1 shows the survival of the Micrococcus in Martian soil in the Martian environment. Essentially the same growth pattern occurred with this microorganism that occurred with Bacillus subtilis var globigii of previous experiments. There was an increase, or peaking, in the total count at 1 day in the Martian experimental group. This increase in total count at 1 day was followed by a decrease at 7 and 14 days with an increase at 28 days. The Earth control group showed little change in total count at 1, 7, and 14 days. At 28 days a slight increase in total count had also occurred in this group.

Table 4

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF A MICROCOCCUS ISOLATED FROM DESERT SOIL

Spheres: 1.0 to 1.5 microns; occurring singly, in pairs, and in irregular clumps. Non-motile, gram positive.

Agar Colonies: Circular, smooth, orange, glistening, entire.

Agar Slant: Abundant, opaque, orange, smooth, moist.

Broth: Slight turbidity, no pellicle, orange slimy sediment.

Litmus Milk: No change.

Nitrites: Not produced from nitrates.

Indole: Not formed.

Nitrogen Source: Utilizes $NH_4H_2PO_4$ as a source of nitrogen. No acid from glucose, lactose or sucrose.

Catalase: Positive.

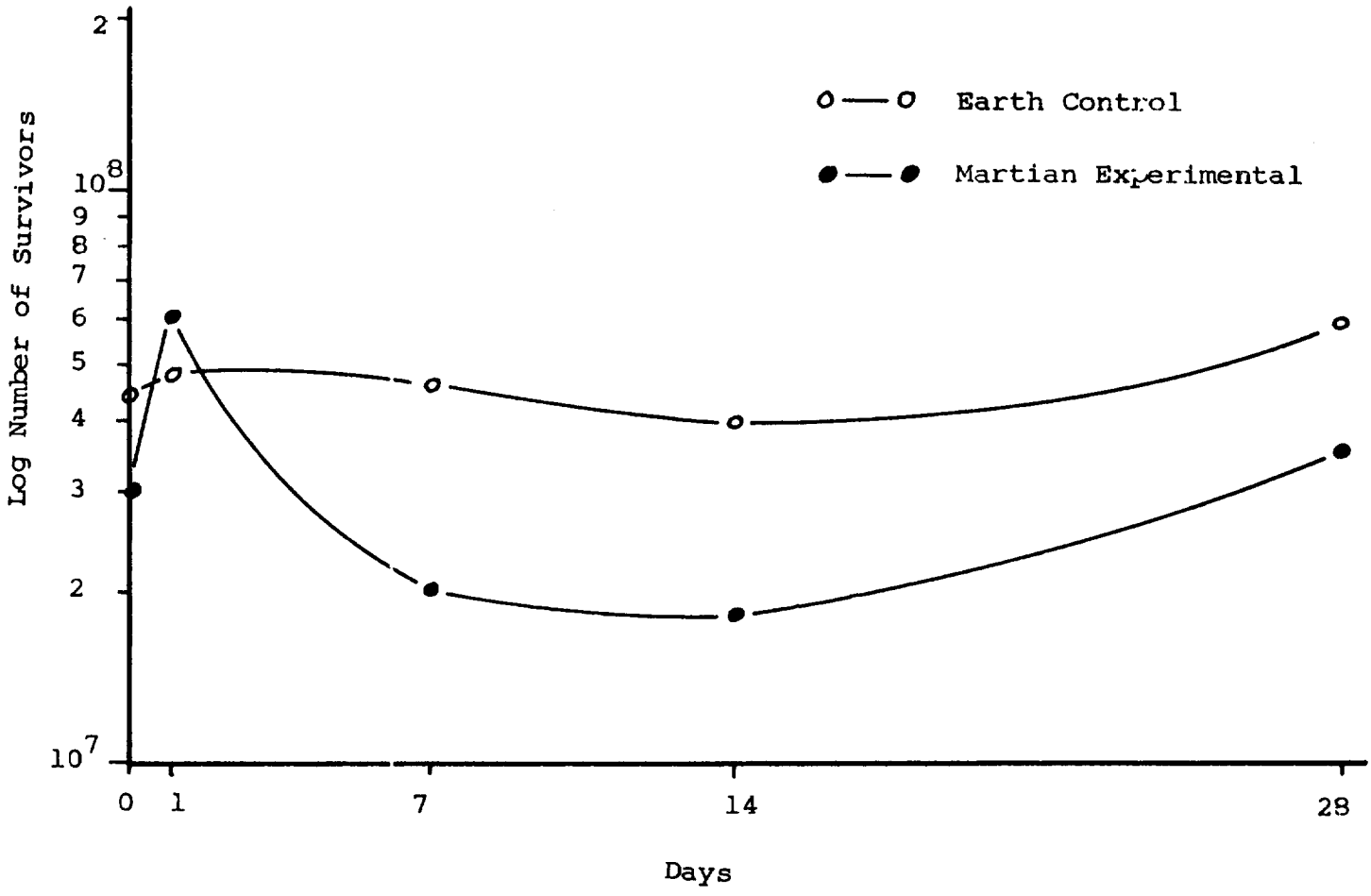
Oxidase: Negative.

Growth: At 5°, 27°, 35°, and 37°C.

Stability: Withstands 60°C for 10 min but not 80°C for 10 min. Obligate aerobe.

Figure 1

SURVIVAL IN MARTIAN ENVIRONMENT
OF A MICROCOCCUS IN MARTIAN SOIL



When this Micrococcus was inoculated into sterile desert soil and placed in the Martian environment a survival pattern similar to that described above occurred, Figure 2. The desert soil used was the same type soil the microorganism was isolated from. The Martian experimental group displayed an increase in total count at 1 day followed by a decrease at 7 and 14 days with an increase at 28 days. The Earth control group showed little change in total count at 1, 7, and 14 days. At 28 days a slight increase in total count had also occurred in this group.

Table 5 shows the moisture uptake of the Martian soil and the Martian soil with 1 and 10% dehydrated bacteriological medium (AC medium, Difco). The experiments were conducted with desiccators, containing test tubes with soil or soil-medium, to which 2 ml of water was added (average desiccator volume was 2744 ml). Martian atmosphere at 85 mm Hg pressure was established prior to addition of water.

The soil with 10% medium absorbed more water and at a faster rate than either the soil or soil with 1% medium. The increase in moisture relative to the zero day was 334, 544, and 2038% for soil, soil with 1% medium, and soil with 10% medium, respectively.

IV. CONCLUSIONS

A Micrococcus, non-spore former, isolated from a California desert soil, survived the Martian environment with little change in total viable count for at least 28 days. Survival in the Martian environment of this microorganism was enhanced when the Martian soil was replaced with sterile desert soil from which it

Figure 2

SURVIVAL IN MARTIAN ENVIRONMENT
OF A MICROCOCCUS IN DESERT SOIL

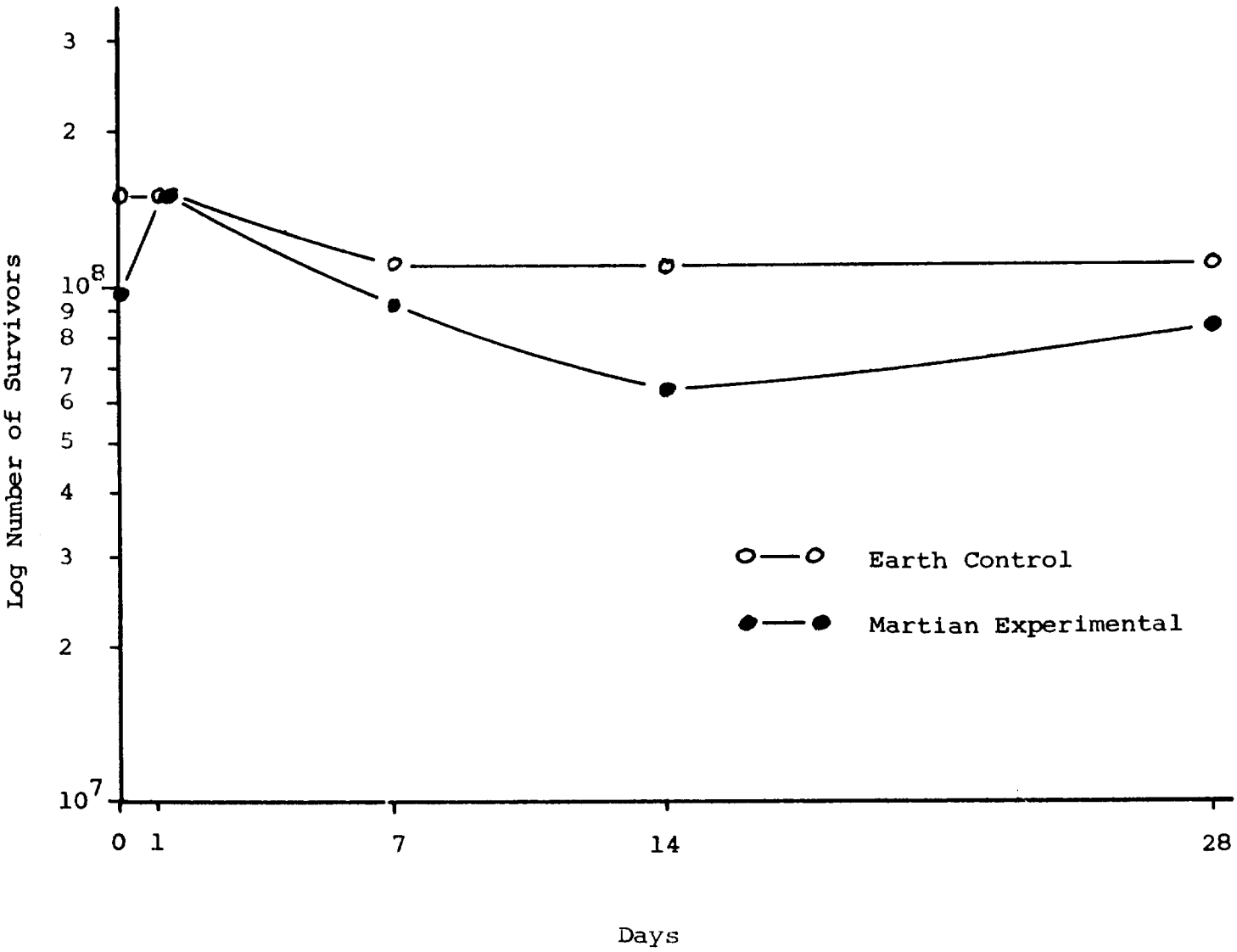


Table 5

MOISTURE UPTAKE OF MARTIAN SOIL AND OF MARTIAN SOIL WITH 1 AND 10% ORGANIC MATERIAL ADDED

Days	Soil Moisture		Moisture Soil with 1% Medium		Moisture Soil with 10% Medium	
	g	%	g	%	g	%
0	.0009	.09	.0009	.08	.0016	0.24
1	.0038	.35	.0050	.45	.0154	2.13
3	.0039	.38	.0058	.55	.0342	3.90

was isolated. The decrease in total viable count as a result of the inoculation procedure and flushing with either Earth or Martian atmosphere was partially overcome by replacing the Martian soil with the desert soil.

A partial listing of this microorganism's morphological and biochemical characteristics are given.

The moisture, organic, and inorganic composition of the Martian soil and of 5 desert soils are reported.

The moisture uptake of Martian soil and of Martian soil with 1 and 10% dehydrated bacteriological medium are indicated.

V. RECORDS AND PERSONNEL

The experimental data are recorded in ARF Logbook C 13248. Technical assistance was given by J. Rush.

Respectfully submitted,

ARMOUR RESEARCH FOUNDATION
of Illinois Institute of Technology

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Associate Bacteriologist
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Approved by:

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CAH/cg

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