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STUDIES WITH A SIMULATED MARTIAN ENVIRONMENT

Bacterial Survival and Soil Moisture Content

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USAF School of Aerospace Medicine
Aerospace Medical Division (AFSC)
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FOREWORD

This report was prepared by the following personnel in the Astrobiology Branch:

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The authors express appreciation to Richard C. McNee of the Biometrics Department for the statistical evaluation of the data.

ABSTRACT

In a simulated Martian environment based on the latest available data, colony counts of a sporeforming bacterium increased. There were no changes in soil moisture. Multiple entry into Mars jars did not affect counts or soil moisture.

This technical documentary report has been reviewed and is approved.

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1. INTRODUCTION

Ecologic screening of the solar system indicates that Mars presents the best planet for consideration of xenobiologic study (1-6). After a study of such factors as soil, moisture, atmosphere, and pressure within a simulated Martian environment, it was reported by Davis and Fulton (7, 8) that certain soil bacteria survived and that at least one of three species studied multiplied. However, information concerning conditions on Mars has been revised recently (9). Within a simulated environment based on these latest available data concerning Mars, the present study was undertaken to attempt confirmation of the findings cited above. The effect of multiple entry into the simulated environment was also evaluated.

2. MATERIAL AND METHODS

Conditions used to simulate the Martian environment are listed in table I. The crushed red sandstone employed for soil (7) was dried and sterilized in a vacuum oven at 175° C. and 20 cm. Hg for 72 to 80 hours. Bacteriologic tests showed no viable organisms; moisture content was 0.2 percent. The test bacterium was the gram-positive sporeforming rod ("42-58") used by Davis and Fulton (7). This organism was tentatively identified by morphologic and biochemical means as a strain of *Bacillus cereus*. Approximately 4×10^{12} vegetative cells and spores from a 24-hour nutrient broth culture were mixed with 80 gm. of the treated soil. Aliquots of about 0.15 gm. of this mixture were dispensed to open glass tubes of 2.0 ml. capacity. Thirty of the tubes were placed in each of 16 "Mars jars" (7). The jars were evacuated to approximately 4 mm. Hg, flushed several times with moisture-free nitrogen, and filled with the gas mixture (table I) to 65 mm. Hg STP. All jars were sealed and held at room temperature (about 22° to 25° C.) for 3 hours, then temperature cycled (16 hours at −25° C. followed by 8 hours at room temperature) each day for 60 days.

TABLE I

Conditions used to simulate the Martian environment

Factor	Condition	
Atmospheric composition	Nitrogen	93.54%
	Argon	4.24%
	Carbon dioxide	2.21%
	Oxygen	0.01%
Atmospheric pressure	65 mm. Hg (STP)	
Moisture	About 0.5% per gram soil	
Temperature	22° to 25° C. diurnal −25° C. nocturnal	
Soil type	Red sandstone	

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Colony counts were performed in nutrient agar, with triplicate platings from each of the 3 tubes used for each data point. For determination of moisture content, the treated soil was placed in moisture-free 7 ml. screw-cap glass vials, and the vial and contents weighed. Caps were removed, and the vials and caps were heated in a hot-air oven at 105° C. for 48 hours, an then reweighed. Finally, the vials were emptied and weighed again.

To evaluate the effect of re-entry on colony counts and moisture content of soil, 4 series of 4 jars each were employed, from which triplicate samples were extracted for the

TABLE II

Effect of re-entry on colony counts and soil moisture in a simulated Martian environment

Jar series	Day entered or re-entered									
	1		8		15		30		60	
	Count*	Percent moisture	Count	Percent moisture	Count	Percent moisture	Count	Percent moisture	Count	Percent moisture
A	4.0	0.48	4.7	0.46	6.2	0.43	7.0	0.52	9.2	0.40
	3.9	.49	4.5	.46	6.2	.45	6.9	.53	9.2	.44
	3.9	.48	4.7	.45	6.3	.49	6.8	.50	9.4	.43
	3.9	.49	4.5	.44	6.4	.47	6.6	—	9.0	.47
B			5.3	0.20	6.9	0.48	6.6	0.51	9.5	0.42
			5.3	.42	7.1	.42	6.7	.53	9.1	.41
			5.2	.42	6.8	.49	6.4	.51	9.0	.45
			5.2	.42	6.8	.46	6.8	.48	9.0	.44
C					6.9	0.47	7.3	0.53	9.2	0.44
					6.9	.45	7.3	.49	9.2	.43
					6.6	.45	7.5	.49	9.1	.41
					6.7	.47	7.3	.52	9.2	.45
D							7.3	0.47	9.4	0.45
							7.4	.48	9.2	.45
							7.4	.47	9.5	.44
							7.2	.49	9.5	.42

Time zero: 3.4×10^7 colonies per gram soil; 0.48 percent moisture.

*Times 10^7 colonies per gram soil.

determinations at times shown in table II. Data for zero time were obtained from 15 tubes.

3. RESULTS AND DISCUSSION

In confirmation of the findings of Davis and Fulton (7), colony counts increased in all jars with time. There was no change in soil moisture during the experimental period employed. Re-entry did not cause statistically significant variation in either counts or moisture content. Statistical evaluation indicated appreciable variation in both counts and moisture between samples within jars and among jars; nevertheless, all series showed similar trends. Statistically, the percent moisture in every jar was significantly higher ($P < .05$) at 30 days than at other times. It is believed that this isolated difference was due to uncontrollable fluctuations in relative humidity during analysis.

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2. Extraterrestrial life

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