

PHYSIOLOGIC CHANGES OBSERVED IN CLOSTRIDIUM SPOROGENES

**IRVING DAVIS, Major, USAF, MSC
THOMAS L. ROBERTS, M. S.
SYLVESTER LASSITER, Airman Second Class, USAF**

Microbiology-Cellular Biology Branch

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**SCHOOL OF AEROSPACE MEDICINE
USAF AEROSPACE MEDICAL CENTER (AFSC)
BROOKS AIR FORCE BASE, TEXAS**

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The biologic effects of space radiations require investigations at the macrobiologic and microbiologic levels. Microorganisms provide a unique tool for such studies because of their rapid growth and multiplication, and their biochemical, physiologic, and genetic characteristics which, in certain cases, are analogous and applicable to other forms of life, including man. Space radiation research establishes certain requisites of the biologic entity. First, the cell must be able to withstand the temperature extremes, prolonged storage, and rigors of

handling encountered during space experimentation. Second, the cell must provide a sensitive indicator that will be altered in a quantitative manner when exposed to radiation. The *Clostridia* spore-labilization system, reported previously (1), appears to fulfill these requirements. The rationale for this system is diagrammed in figure 1. This paper reports results obtained utilizing the *Clostridia* spore-labilization system on biologic specimens recovered from the Discoverer XVIII space flight.

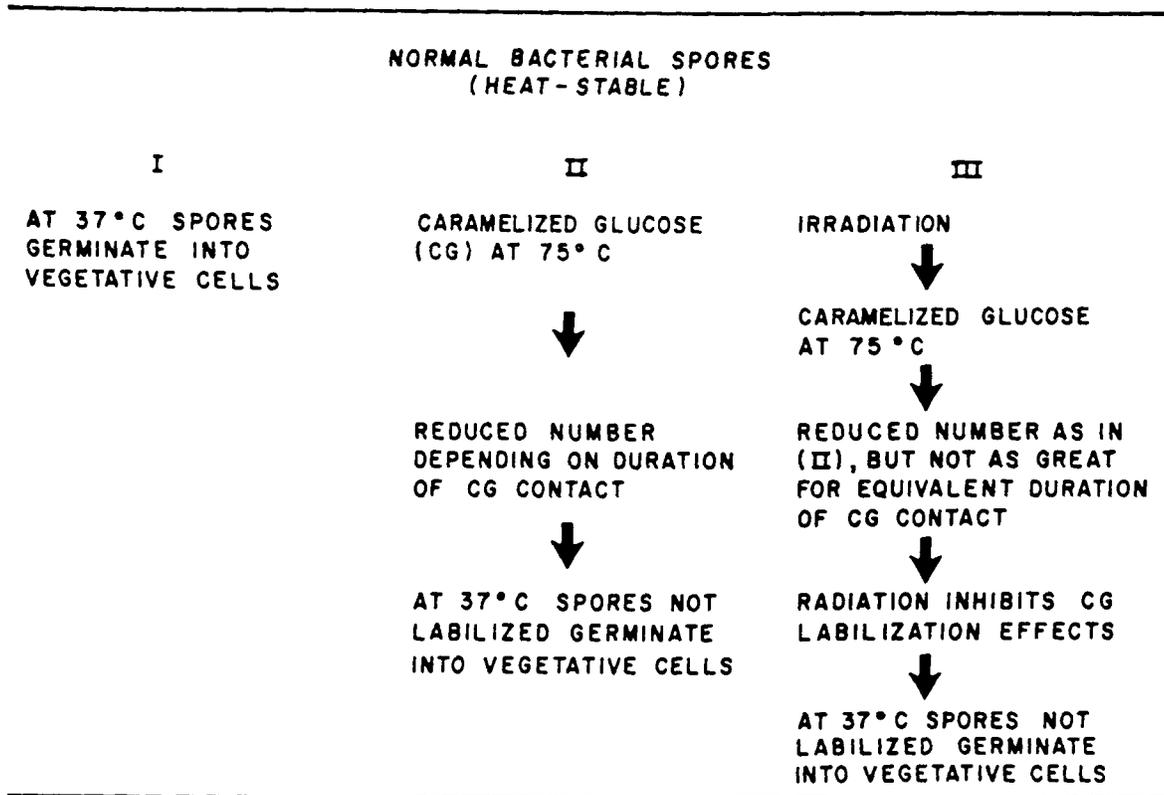


FIGURE 1

Rationale of the *Clostridium* spore labilization system to quantitate radiation.

MATERIALS AND METHODS

Clostridium sporogenes, ATCC No. 7955 (NCA, "Putrefactive Anaerobe" 3679) was used. Preflight and postflight experimental procedures were followed as described in an earlier study (2). The physical description of the Discoverer XVIII space flight and the SAM recoverable biopack is provided by Crawford in Report 62-39 of this series (3).

The recoverable biopack aboard Discoverer XVIII contained twelve ampuls of *Clostridia* spores. Four ground-control ampuls accompanied the flight ampuls to Vandenberg Air Force Base, Calif., and were returned with them to the laboratory after the flight. Flight and ground-control ampuls were maintained at approximately 5° C. during preflight and postflight times until they were subjected to postflight caramelized glucose treatment in the laboratory. During the flight period, ground-control ampuls were kept at ambient room temperature (approximately 25° C.). The four laboratory control ampuls remained at approximately 5° C. until analyzed along with the flight and ground-control ampuls. All ampuls were selected randomly from a homogeneous group.

RESULTS

Each figure in table I is the average count of the number of spores that were capable of germinating and forming visible colonies in the germination count medium. Each average value represents triplicate aliquots from each ampul and duplicate germination counts for each aliquot, i.e., the average of six colony counts. The values shown in parentheses in this table are the percentage of labile spores for each indicated time period. These spores were affected by the caramelized glucose treatment at 75° C. and failed to germinate when subsequently placed in appropriate nutrient substrate at 37° C. A statistical evaluation was carried out with these data. Statistical results were significant at the 5 percent level.

Preflight handling operations and environment encountered had no apparent effect on the spore system. This is observed by comparing

results in table I for ground-control and laboratory-control ampuls at zero time and each of the posttreatment times. The percentage of labilization variability between ampuls in both of these groups was not statistically significant. Therefore, the percentage labilization results from table I for the two control groups were each pooled and graphed as indicated in figure 2.

The flight data for Discoverer XVIII were processed similarly to those previously described for Discoverer XVII (2), i.e., individually for each ampul and collectively as a group. Survival of the flight group was not statistically significant when compared to the combined control groups. When the same comparison was made with regard to the average postflight caramelized glucose treatment time, however, statistical significance was observed ($P < .05$). On this basis the percentage of labilization data for each ampul at each treatment time was evaluated against similar data for the combined controls. The analysis indicated that 4 of the 12 flight ampuls (Nos. 2 and 7 ($P < .05$); 3 and 12 ($P < .01$)) showed statistically significant results after a 90-minute caramelized glucose treatment. The curves in figure 2 show this separation of the flight ampul group into two divisions. Although small, the inhibition of the percentage labilization shown by these 4 flight ampuls at 90 minutes is apparent.

An attempt was made to relate the number of recovered spores in each flight ampul after flight and after return to the laboratory, but before the caramelized glucose treatment with its physical positioning within the recoverable biopack. The type of relationship referred to in the results of Discoverer XVII (2), in which a "lethal" gradient appeared to be related to the physical positioning of the ampuls within the biopack, was not apparent in the results of Discoverer XVIII.

DISCUSSION

Heat-stable *Clostridia* spores incubated at 75° C., in the presence of caramelized glucose, become heat labile and are killed quantitatively

TABLE I

Number of residual *Clostridium sporogenes* spores (PA 3679) following caramelized glucose treatment at 75° C. for indicated time periods

Ampuls	Time (minutes)						
	0	30		45		90	
	Control	Control	Treatment	Control	Treatment	Control	Treatment
Discoverer XVIII flight numbers							
1	105*	103	84 (18%)†	102	46 (55%)	102	1 (99%)
2	105	103	82 (20%)	104	52 (50%)	100	14 (86%)
3	106	106	86 (19%)	102	48 (53%)	102	22 (78%)
4	107	104	91 (13%)	105	47 (55%)	101	10 (90%)
5	105	102	85 (17%)	102	46 (55%)	100	13 (87%)
6	103	104	87 (16%)	102	47 (54%)	100	13 (87%)
7	110	107	87 (19%)	103	48 (53%)	104	15 (86%)
8	105	107	86 (20%)	104	53 (49%)	101	13 (87%)
9	107	104	86 (17%)	105	52 (50%)	103	12 (88%)
10	109	106	82 (23%)	107	48 (55%)	104	5 (95%)
11	107	108	87 (19%)	106	47 (56%)	104	9 (91%)
12	104	107	86 (20%)	105	49 (53%)	105	19 (82%)
Ground control numbers							
1	104*	106	84 (21%)†	99	50 (50%)	101	2 (98%)
2	106	105	87 (17%)	104	50 (52%)	101	7 (93%)
3	106	108	85 (21%)	103	47 (54%)	103	8 (92%)
4	110	104	85 (18%)	109	46 (58%)	105	4 (96%)

TABLE I (Contd)

Ampuls	Time (minutes)						
	0	30		45		90	
	Control	Control	Treatment	Control	Treatment	Control	Treatment
Laboratory control numbers							
1	105	104	84 (19%)	102	46 (55%)	99	2 (98%)
2	111	108	92 (15%)	105	54 (49%)	104	13 (88%)
3	114	108	87 (19%)	111	50 (55%)	106	12 (89%)
4	110	107	87 (19%)	106	48 (54%)	106	6 (94%)

*Each figure represents triplicate aliquots per ampul, duplicate counts per aliquot, i.e., each figure is the average of six colony counts.

†Figures in parenthesis are the percent of labile spores at the indicated time period, i.e., $\frac{\text{control} - \text{treatment}}{\text{control}} \times 100$.

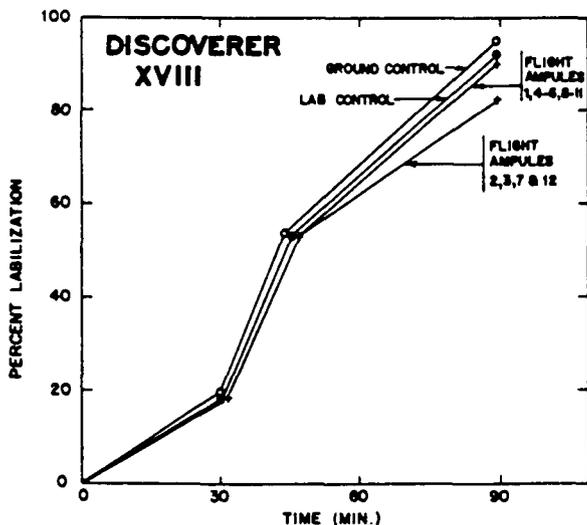


FIGURE 2

Effect of caramelized glucose treatment at 75° C. for indicated time periods on postflight *Clostridium sporogenes* spores (PA3679). Ground control data represent average of 6 ampuls. Laboratory control data represent average of 2 ampuls. Flight data are the average of the ampuls indicated.

as a function of the contact time with the caramelized glucose. When these spores are exposed to laboratory-controlled radiations and subsequently treated with caramelized glucose, the usual labilization effect is quantitatively

change as a result of the radiation dose (1). The radiation dose received by the flight ampuls in the Discoverer XVIII flight may be approximated from such a laboratory-controlled radiation curve.

As discussed in the previous report (2), another convenient standard measurement in these studies is the "percent inhibition of labilization." The spores in the 4-flight ampuls that revealed significant data after a 90-minute caramelized glucose treatment showed a 12 percent inhibition of labilization.

The response of the bacterial spores to the test system reflects a quantitative difference between the two Discoverer flights. The spores in 3 of 6 flight ampuls recovered from Discoverer XVII showed an average inhibition of 71 percent.

Since neither of these two radiation indexes—physical dosimetry or biologic dosimetry—has been worked out completely for space radiations, cautious reliance on either system is suggested. It seems appropriate and imperative, however, that a baseline for comparison be established for both of these radiation-detection and measurement systems. Accomplishment of this objective is being pursued by using 730 Mev protons.

SUMMARY

Spores of *Clostridium sporogenes* were part of the payload in the SAM recoverable biopack that was aboard the successful space flight of Discoverer XVIII. The *Clostridia* spore-labilization system was employed to detect and quantitate the effects of the space radiations on the cells.

Four of the 12-flight ampuls containing spores showed statistically significant results after a 90-minute caramelized glucose treatment, after flight. A comparison is made of

the biologic results from Discoverer XVII and Discoverer XVIII.

Criteria established for biologic entities for space experimentation are fulfilled by *Clostridia* spores. Preflight conditions had no apparent effect on the spore system. A "lethal" gradient within the several ampuls of spores related to their physical positioning within the biopack was not noted in this space flight.

The authors appreciate the statistical evaluation of the experimental data that was completed by Dr. Phelps P. Crump and his colleagues of the Biometrics Branch.

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