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SUMMARY OF THE PROCEEDINGS OF THE MARS SURFACE SAMPLE RETURN SYMPOSIUM

AMES RESEARCH CENTER
Moffett Field, California
October 24-25, 1973

Symposium organized by
Dr. Richard S. Young, Chief,
Planetary Biology Office
NASA Headquarters
Washington, D. C.

Summary Prepared by
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CONCLUSIONS

1. The scientific value of a sample returned from Mars would be very high. The most desirable kind of sample would be one that is kept intact and not altered by heat, radiation or chemical treatment before return.
2. Some scientists strongly argue against now actively considering the return of an unsterilized sample. The bioscience community is widely divided on the back contamination issue and the expressed intent to return an unsterile sample would very likely precipitate a widespread and public debate on the issue. The attitude of the regulatory agencies is conservative and they are not likely to regard lightly the back contamination hazard.[1] The international implications have not been thoroughly explored, although clearly, there is international interest.
3. It is possible to plan the return of a sterile sample from Mars to the Earth. In order to accomplish this, considerable research needs to be done. For example, what is an acceptable sterilization mode for the sample? The sterilization procedure must be evaluated as to its ability to kill terrestrial organisms while doing the least damage to the scientific content of the sample. These two objectives are to some extent divergent; however, a compromise solution seems attainable. This is an area where considerable work must be done. Although dry heat treatment was the method of choice for spacecraft sterilization, other methods (wet heat, heat and radiation, radiation, chemical treatment, etc.) may be more suitable for this purpose. Two research programs will be needed if we proceed. 1) The effectiveness

of various sterilization methods for killing organisms in soil, and 2) The effect of these techniques on the inorganic, organic, and biological information in that soil.

4. The least expensive and simplest mission mode for returning a Mars sample to Earth is the direct entry mode. In this case the sample is lifted from Mars to Mars orbit, transferred to the orbiting return vehicle and returned directly to the Earth where it could be put into orbit or brought directly to the surface. The sample container is contaminated externally and in the process of transfer, the return spacecraft is contaminated internally in the sample holding area. Present design studies have not yet resolved the problem of sterilization of the return spacecraft and container or of achieving a sterile transfer of the sample container. This problem must be solved before return to Earth or Earth orbit can be considered.

5. The Viking mission to Mars must be considered to be the <u>beginning</u> of Martian exploration. While it has as one of its objectives the search for life, it should by no means be considered capable of determining unequivocally the presence or absence of life. If life is present on Mars Viking has a reasonable chance of detecting it - it cannot possibly prove the absence of life. The life detecting ability of Viking is the best that can be designed on the basis of our present limited knowledge of Mars. Additional spacecraft, of the Viking class, can add appreciably to our store of knowledge about Mars and its potential for life, so that in time, an unsterile sample return might be feasible. In the event that life is found on Mars, additional Viking class spacecraft can begin the task of

characterizing that life and assessing to some degree the hazard such life might pose for the Earth.

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1. Although it was not discussed at the meeting, it has been suggested that the regulatory agencies would require containment of the sample behind major quarantine barriers since sterility of a returned sample, even after extensive sterilization treatment, could not be demonstrated. In other words, containment procedures would be the same for unsterile or sterilized samples.

Short Summary

Regulatory agencies involved with the importation of biological material will probably require the construction of major quarantine barriers if the absence of a biohazard in a returned Martian sample cannot be demonstrated.

Preliminary mission analysis indicates that the probability of uncontrolled impact upon return of a sample to earth is high. Analysis of the mode of a sterilized sample return is incomplete in both the biological and hardware aspects.

The effect of a dry heat sterilization procedure on inorganic materials would be minimal if temperatures were kept below 250°C. Potential organic geochemistry data would suffer, probably in an assessable manner, if sterilization were effected at temperatures above 150°C. Morphological biological analysis would be strongly degraded in most heating regimes. However, the use of fixative agents which can also act as sterilants, may offer a hope of salvaging some biological data from sterile samples.

Support for the return of an unsterile sample was expressed with

the consideration that the chances of interaction between a Martian and terrestrial biology are so remote as to be highly improbable. This view was opposed by one which suggests that the probability, while low, must be measured against the values at stake; hence stringent precautions must be observed.

A consensus seemed to be arrived at suggesting that if sterility of the sample could be assured with maximum retention of inorganic and organic geochemical information, the mission would be of high value and of great scientific importance, yet with minimum risk to terrestrial values. If biological information could be retained, the value of the mission would be markedly increased.

The major questions to be solved appear to be based upon the biological problem of sterility, a total assessment of the effect of heating an organic and inorganic geochemistry, and a thorough examination of techniques for sterilizing the Earth-entry package and for recovery of the return package on Earth.

INTRODUCTION

A symposium was convened on October 24-25, 1973 at the Ames Research Center to discuss various technical and scientific aspects of a Mars Surface Sample Return (MSSR) mission. Particular attention was focused on the question of back-contamination. The purpose of the meeting was to sample the opinions of the scientific community on MSSR, to define some of the problems inherent in the back contamination issue and return sample missions, and to highlight areas where additional research was needed, in order to be able to react to a decision on pursuing an MSSR mission. It was assumed that such a mission is scientifically desireable and feasible in concept.

The agenda for the symposium is shown in Attachment I. The invited participants (Attachment II) were carefully selected to represent all aspects of thought on a MSSR mission: engineers and scientists, NASA personnel and University scientists, NASA grantees and independents, geoscientists and bioscientists, and arguments for both sides of the question of the risk of back-contamination. The following is a brief discussion of the two-day symposium.

Acknowledgement: We would like to express our appreciation to Professor Thomas Jukes, who had the foresight to record the presentations.

PLANETARY QUARANTINE REQUIREMENTS

The government agencies responsible for quarantine policy are the Center for Disease Control, Department of Agriculture, and the Department of the Interior. In addition, the World Health Organization and COSPAR have an impact on such decisions. The position of the governmental organizations with regard to the introduction of any biological material into the United States is, in general, the following: If the absence of a biohazard cannot be demonstrated, all precautions must be taken. The return of a Martian sample falls under these constraints and therefore presents the problem of back-contamination. Prevention of back-contamination is accomplished through quarantine techniques, essentially the construction of partial or complete barriers.

Part of the quarantine program must be an assessment of the risks of contamination during all phases of the process of sample return including the development of analysis techniques for automated sample package insertion, containment and sealing, and methods of fail-safe container sterilization.

MISSION OPTIONS

MSSR mission opportunities exist in 1981, 1983/84, and 1986 which would utilize existing launch vehicles or shuttle combinations depending upon the mission. At Mars, options exist for direct descent, or descent from orbit, and for direct ascent to a parking orbit around Mars or to a Mars orbital rendezvous. Return to Earth can be accomplished by direct entry or orbital capture. All of these missions have in common an approximate 1000 day timeline, dictated by a 400 day stay on the Martian surface or in orbit in order to ensure a low energy return trajectory. Such a timeline allows sufficient opportunity for science testing and/or sterilization activities during the mission, should such inclusions be desired.

The results of the joint LaRC/JPL Mars Sample Return Study were summarized. This study was described as a "pre-phase A" effort to determine the engineering requirements for a minimum mission to return a nominal 200 gm sample. The cost of such a mission was estimated at $600 M, and this figure excluded any science on the Martian surface or in transit, sterilization of the sample, retrieval from Earth orbit, storage in an orbiting lab, Earth-based containment facilities, or procedures for returning an unsterilized sample in an uncontaminated vehicle.

Each of the possible mission modes at Mars or in the vicinity of Earth has its own set of manuvers which can result in contamination of the Earth return capsule and associated vehicles.

Current engineering activity involves a conceptual study to analyze the feasibility of rendezvous and docking in Mars orbit, and the sterile transfer of samples from the lander to return spacecraft.

RISK

In the event that direct Earth entry of an unsterilized sample (in a capsule whose exterior is uncontaminated) is chosen as a mission design, a reliability analysis was described which analyzed risks associated with failures in return guidance maneuvers, separation, and deflection of bus and capsule, and capsule failure upon re-entry. In summary, this analysis showed that the probability for back-contamination, defined as unhindered access of unsterilized surfaces to the terrestrial environment was high, 1 chance in 100 if the bus-deflection option was chosen (a less complex design) and 1 chance in 10,000 for the capsule-deflection mode (a more complex design). This risk can be greatly reduced by designing a spacecraft sterilization technique, sterile sample transfer technique or by redesign of the spacecraft to include features providing greater engineering reliability in the system. All of these would, of course, increase the cost.

EFFECTS OF STERILIZATION

(1) On inorganic data

Data were reported on the effect of dry heat sterilization using common rock models, such as are found on Earth or in lunar samples. The data indicate that serious degradation of information would occur if the sample were heated above 300°C, but that heating at lower temperatures would preserve considerable data. In general, the lower the temperatures employed for sterilization, the more information preserved. Among the types of events which occur, to varying extents, during heating are volatilization of water and of some elements such as sulfur, possible phase changes; loss of gases, and contamination by the containment vessel. At low sterilization temperatures (below 300°C) the scientific value of the sample is nearly unaltered, and the inclusion of differential thermal analysis capabilities and a trap for volatiles would assure those which could be obtained from the sample during and after sterilization..

(2) On organic data

Three physico-chemical processes of organic materials, volatilization, chemical reaction and racemization would be influenced by sterilization regimes. These processes would have different effects depending upon whether the organics were absorbed on the sample as small molecules, were present as polymers, or alternatively were implanted in the sample. Except for implanted species, heating to 400°C would cause severe degradation of sample information. At 250°C for 1 hour, about 10% of the polymeric material would be degraded and volatilized and extensive chemical reactions would occur. Adsorbed molecules would be nearly completely volatilized or lost by chemical reactions. Racemization of amino acids, if present, would be complete. At 200°C, about 70%, of the

small adsorbed organics would be volatilized, but only a small degree of chemical reaction would occur. Polymeric material would be little affected by the treatment but racemization could be extensive. The above analysis was done at 10^{-6} Torr. At standard pressure, but in a nitrogen atmosphere, an examination was made of the effect of heating at 196° for 24 hr on the racemization of amino acids. A comparison was made between the effects on free amino acids and those bound in a matrix of a meteorite. The results suggest that racemization is restrained by inclusion of the amino acid in a matrix and that considerable variation in rate occurs dependent upon the structure of the amino acid.

It was stressed that the amount of data available from a sample after heating could be increased significantly by the use of a trap for volatiles during the heating process. Suggested traps included coldtraps and chemical sequestering methods.

(3) On biology

Preliminary experiments have been carried out in order to assess the biological value of a sterilized sample. Various sterilization regimes were applied to terrestrial samples and the degree of data loss was found to be highly dependent upon the method used. Electron microscopy was used as the detection device. When samples are heated to 200°C for 24 hours in air there are essentially no surviving biological structures that are recognizable. Heating at 160°C for 3 hr does not sterilize the sample, but some cells, membranes, and congealed cytoplasmic material remained. Standard autoclaving (121°C 120 min., two cycles) does sterilize the sample and structural survivability is about as above. Since electron microscopy was used for sample observation, two

established methods of sample fixation were examined as sterilizing agents. Glutaraldehyde treatment combined with heating to about 90°C and treatment with osmium tetroxide were both very effective sterilants and, as expected, resulted in significant preservation preservation of biological structure.

Two disadvantages of optical techniques for biological characterization are the requirement for large numbers of cells in the sample and the difficulty associated with identifying biological entities in a background of soil debris. However, preservation by chemical fixation combined with characterization by electron microscopy can lessen these disadvantages.

ARGUMENTS FOR RETURN OF AN UNSTERILIZED SAMPLE

One suggested mode of sample return is direct, unsterilized introduction of the material to the Earth. An argument supporting this type of return is based upon two lines of evidence; 1. the functioning of the evolutionary process observed on Earth, and 2. the fact that evolution of many host-parasite relationships have developed in concert. Considering the enormous number of variations of RNA and DNA sequences possible as a result of evolution, it is highly improbable that by pure coincidence a Martian infectious agent similar to a virus could invade and multiply in a terrestrial host, in which the requirement for specific proteins an nucleic acid sequences could be met. Host-specific bacterial or protozoological infections could be similarly ruled out. Non-specific terrestrial infectious agents could be easily contained by routine quarantine techniques.

Another argument offered in support of the position that the hazard to terrestrial life of returned viable Martian organisms would be negligible was based on the large differences between Earth and Mars of three biologically important environmental parameters: partial pressure of oxygen, average temperature, and water activity.

A probability equation was advanced which proposed that the chances of a "Mars plague" occurring on Earth following unsterilized sample return was equal to the probability that organisms exist on Mars, times the probability that these organisms could survive on Earth, times the probability that these organisms would be infective to terrestrial life forms. The probability of organisms existing on Mars was assumed to be 1, based on the presumption that a sample return mission would not be attempted without prior evidence that life exists on Mars. The probability that such organisms would be infective on Earth, if

they survived, was estimated to be a conservative 10^{-3}. The probability that Martian organisms would survive on Earth was proposed to be 10^{-6} (which also happens to be the probability that terrestrial organisms would grow on Mars) based on the wide differences in environmental conditions between Earth and Mars. For example, the partial pressure of oxygen in the Earths atmosphere is 40,000 fold higher than in Mars'. On Earth there has been strong evolutionary pressure for oxygen tolerance, and partial pressures of oxygen only 50 times higher than ambient are lethal for terrestrial organisms . Similarly, the average temperature on Mars is some 80°K cooler than Earth. One can speculate as to the fate of life on Earth if the temperature was suddenly increased by 80°K. Finally, the activity of water on Mars is estimated to be about 3 orders of magnitude lower than the lowest water activity at which terrestrial microorganisms grow; and terrestrial experience shows that high water activities are lethal for microorganisms adapted to live at low activities.

The product of these probabilities, therefore, is 10^{-9}; and these probabilities were proposed as being on the conservative side.

ARGUMENTS AGAINST RETURN OF AN UNSTERILIZED SAMPLE

As an argument against direct unsterilized sample return it was pointed out that Martian organisms would probably have evolved quite different proteins and cell wall materials than terrestrial organisms; and that, if such were the case, potential parasites would not be recognized either by the specific immune systems which Earth organisms have evolved, or by the general defense systems, such as lysozyme. Many parasitological situations observed on Earth are not the result of long term co-evolving parasitism, but rather result from the accidental, or adventitious, invasion of a host. Several examples of such adventitious parasitism were mentioned, including tropical sprue, possibly caused by an alga, aspergilosis, fungal infections involving toxin release as well as the toxins released by anaerobic bacteria during infection.

In additional support of the view that an unsterile sample should not be returned directly to Earth were the following points: It is difficult, and may be impossible, to detect pathogens, either because we cannot recognize them because of non-commonality, or because they take too long to develop. Even though Martian and terrestrial genomes may be totally different there is a chance that the genomes would interact, and furthermore, the more similar the genomes, the more likely the presence of enzymes able to attack components of terrestrial cells. Assuming again similarity of genomes, it was suggested that Martian selection pressures would be quite different from those on Earth and that the possibility exists of altered selection pressures which could result in long-term pathogenicity as evolutionary adaptation occurred. Finally, it was stated that it is impossible on the basis of present

knowledge to evaluate the probability of pathogenicity and that in such a situation it would be best not to return an unsterile sample. Numerical and statistical methods were used to demonstrate the dangers of a catastrophic event which could result from a mistake in judgment.

SUGGESTED EXPERIMENTS

During the course of the two day symposium, it became apparent that there are a number of areas where laboratory research is needed in order to clarify some of the issues raised and to generate a data base from which important decisions could be made. They are listed below and in no particular priority. This listing is incomplete in the sense that it does not include the engineering assessments and related studies, or quarantine risk analyses. Emphasis here is on experiments required to elucidate some of the open scientific questions.

1. Efficiency of current biological barrier systems.
2. The effect of heat, chemical or radiation treatment:
    - A. a. on biological information preservation
        - b. on inorganic and organic information preservation
        - c. on biological killing
    - B. The time - treatment reciprocity on killing and the preservation of scientific information
    - C. The synergistic relationships of:
        - a. chemical combinations
        - b. chemical and heat combinations
        - c. humidity and heat relationships to preservation of science content
        - d. humidity and heat relationships to killing
3. Use of dry heat:
    - A. Minimum temperature for all science
    - B. Effect on biological structure
    - C. Effect of gas pressure and composition on all science and on killing

4. Techniques for possible use during sterilization:

   A. Inclusion of differential thermal analysis (DTA)

   B. Use of a water trap

   C. Use of a trap for organics and prevention of movement of organisms into traps

ATTACHMENT 1

AGENDA
MARS SAMPLE RETURN SYMPOSIUM

I.	Introduction	
	The Problems	R.S. Young
II.	Quarantine	
	From Mars with Safety	L. Hall
III.	Effects of Sterilization	
	a. On Inorganic Data	E. King
	b. On Organic Data	J. Hayes
		K. Kvenvolden
	c. On Biology	E. Casida
IV.	Back Contamination	
	Arguments for Unsterilized Sample Return	T. Jukes
		N. Horowitz
	Arguments Against Unsterilized Sample Return	J. Danielli
		J. Lederberg
V.	Mission Options	
	a. Options	J. Moore
	b. Reliability	B. Swenson

ATTACHMENT I

FINAL AGENDA

MARS SAMPLE RETURN PROBLEMS

24 October 9:00A.M. - Ames Research Center
Building #239 - Basement Conference Room
Tel. 415-965-5000

I. Introduction

a. "From Mars With Love" - Problems - Young - 15 min.
b. Why are we all here? - Young - 15 min.

II. Quarantine

a. From Mars With Safety - Hall - 15 min.

III. Mission options (how to get from there to here)

a. Directly home - Moore
b. Meeting with shuttle sortie lab. - Moore } 15 min
c. Reliability - Swenson - 5 min.

IV. Effects of sterilization on sample science

a. Inorganic - King - 20 min.
b. Organic - Hayes - 20 min.
c. Biology - Morphology, Biochemistry - Casida - 20 min.

V. Back contamination

a. Why we should worry about back contamination - Lederberg - 15 min.
   a. Pathogenicity - Danielli - 15 min.
b. Why we should not worry about back contamination - Horowitz - 15 min.
   a. Evolution of Pathogenicity - Jukes - 15 min.

VI. Questions and answers - Young

a. Is sterilization of samples necessary?
   a. Why?
   b. Why not?
b. What is the best method?
   If it is heat - what temperature?
c. What is an acceptable mode for non-sterile return?
   a. What guarantee do we need that:
      1. spacecraft won't crash?
      2. container won't split?
      3. barrier system won't leak?

} as required