National Aeronautics and Space Administration

Headquarters
Washington, DC 20546-0001

October 30, 2013

Reply to Attn of: Office of Communication
Headquarters, FOIA Office

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FOIA: 14-HQ-F-00034

Dear Mr. Greenewald:

Thank you for your Freedom of Information Act (FOIA) request dated and received October 24, 2013, at the NASA Headquarters FOIA Office. Your request was for:

> Title: (U) PROCEEDINGS OF MEETING ON PROBLEMS AND TECHNIQUES ASSOCIATED WITH THE DECONTAMINATION AND STERILIZATION OF SPACECRAFT JUNE 29, 1960, WASHINGTON, D. C
> View Full Text: (pdf) - 3 MB -
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> Personal Author(s): Posner, Jack
> Corporate Author: NATIONAL AERONAUTICS AND SPACE ADMINISTRATION WASHINGTON DC
> Report Date: Jan 1961
> Abstract: (U) A meeting was held of representatives of agencies concerned with the development of space vehicles and those investigating decontamination and sterilization procedures. Recommendations resulting from the deliberations include: (1) a body of related information be accumulated, (2) standard operating procedures be established, (3) acceptable limits of contamination be determined, (4) NASA policy be clarified, (5) new sterilizing agents be developed, (6) compatibility studies be pursued, (7) sterile manufacture of parts be investigated, and (8) a working level group should be formed to implement recommendations and procedures.
> Abstract Classification:Unclassified
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> Report Number: NASA-TN-D-771 (*NASATND771*) , XG - NASA (*XG*)

The NASA Headquarters program office(s) conducted a search for Agency records. Attached is the responsive document for your request. Fees for processing this request are less than $15.00 and are not being charged in accordance with 14 CFR § 1206.700(i)(2).

Please contact Lubna Shirazi at Lubna.M.Shirazi@nasa.gov or 202-358-2034 for further assistance.

Sincerely,

Josephine Sibley
Headquarters, FOIA Officer

TECHNICAL NOTE

D-771

PROCEEDINGS OF MEETING ON PROBLEMS AND TECHNIQUES ASSOCIATED WITH THE DECONTAMINATION AND STERILIZATION OF SPACECRAFT

JUNE 29, 1960, WASHINGTON, D.C.

Edited by Jack Posner
Office of Life Sciences Programs
NASA Headquarters
Washington, D.C.

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON
January 1961

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

TECHNICAL NOTE D-771

PROCEEDINGS OF MEETING ON PROBLEMS AND TECHNIQUES ASSOCIATED WITH THE DECONTAMINATION AND STERILIZATION OF SPACECRAFT*

June 29, 1960, Washington, D. C.

INTRODUCTION

The United States is about to embark on an ambitious program of Lunar and Space Exploration. This program will not only serve the needs of the national and international scientific community but will also enhance the prestige of the United States in the eyes of the peoples of the world.

During the next ten-year period the National Aeronautics and Space Administration is planning flights which include missions such as lunar orbiters (PIONEER), lunar hard landing (RANGER), lunar soft landing (SURVEYOR), and planetary and interplanetary missions (MARINER and VOYAGER).

There are many problems associated with such pioneering investigations, among which is included the effecting of adequate safeguards against biological contamination of celestial bodies with terrestrial microorganisms. In the not too distant future, the reverse problem of preventing contamination of our terrestrial body with extraterrestrial microorganisms must be considered.

In order to determine the current status of decontamination and sterilization procedures and to arrive at areas of research required in order to increase the knowledge in this field, the

*Edited by Jack Posner, Office of Life Sciences Programs, NASA Headquarters, Washington, D. C.

NASA sponsored a meeting at which time this problem was discussed. Invitations to attend this meeting were extended to agencies actively concerned with the development of spacecraft and launch vehicles as well as those groups involved in the investigation and development of decontamination and sterilization techniques. The meeting was held in Washington, D. C. on June 29, 1960. The recorded minutes of this meeting, which have been edited, are included in this paper, as well as a listing of recommendations resulting from the deliberations.

As a result of the meeting, a group was established at the working level under the leadership of Mr. George Hobby of the Jet Propulsion Laboratory. The responsibility of this group is to "insure that adequate decontamination and sterilization procedures are developed and effected . . . that adequate decontamination and sterilization procedures are applied to lunar and interplanetary probes from the very beginning of payload design, construction, assembly, and test through the launch and flight phase". The initial meeting of this group was held on September 28, 1960 and it is expected that the problems will be actively and forcefully pursued.

PROCEEDINGS

DR. CLARK T. RANDT: I am pleased to welcome you on behalf of the National Aeronautics and Space Administration.

Mr. Jack Posner, of the Office of Life Science Programs, will discuss the NASA space flight program including problems associated with decontamination and areas requiring additional study.

Dr. Charles Phillips will discuss the state of decontamination of large and delicate instruments, the requirements for sterility, and then demonstrate the sterilization chamber which he and his colleagues have developed.

Later in the morning there will be a period for general discussion. We will reconvene in the afternoon for further discussion of more specific details of sterilization problems.

I might briefly review the events that led up to consideration of this matter of sterilization of space probes that might impact extraterrestrial bodies.

A group led by Dr. Joshua Lederberg identified this problem area approximately a year and a half ago and made strong recommendations concerning the necessity for avoidance of extraterrestrial contamination. At the July 1959 Woods Hole Conference of the Armed Forces National Research Council Committee on Bioastronautics, Dr. Melvin Calvin and his panel reemphasized the indications for decontamination of space probes.

In October 1959, Dr. Glennan responded to a letter from Dr. Lloyd Berkner of the National Academy of Science, Space Science Board, saying that he agreed that a policy on sterilization should be established and that the NASA would participate. Late in October a contract was arranged with Dr. Phillips of the U. S. Army Chemical Corps Biological Laboratories at Fort Detrick, Maryland, for purposes of further investigating and advising NASA on this activity.

Mr. Jack Posner will present the NASA flight program and the decontamination problems incident thereto.

MR. JACK POSNER: I would like to discuss the NASA flight program and perhaps thereby indicate the time element with which we have to work. The table shown not only indicates the timing of the launch but also a breakdown of the mission of the particular payload.

Flight Schedule

Quarter	Calendar Year	Lunar Orbiter	Lunar Spacecraft	Lunar Impact	Lunar Soft Landing	Venus Probe	Planetary Spacecraft
3	1960	P-30 Atlas-Able					
4	1960	P-31 Atlas-Able					
3	1961		P-32 Atlas-Agena B				
4	1961		P-33 Atlas-Agena B				
	1962			P-34 Atlas-Agena B			
	1962			P-35 Atlas-Agena B			
	1962			P-36 Atlas-Agena B		P-37 Centaur	
	1962						P-38 Centaur
	1963				P-42, P-43, Centaur		P-39 Centaur

NOTES: -

P-32, P-33	Test Vehicles	P-42, P-43, proposed
P-34, P-35, P-36	High Resolution TV	
P-37, P-38, P-39	Test Vehicles	

During the third quarter of 1960, sometime within the next three months, Atlas-Able is scheduled to launch a lunar orbiter. During the last quarter of this year a second lunar orbiter is scheduled to be launched by an Atlas-Able.

The next shot that will approach the moon is scheduled for the third quarter of 1961, at which time an Atlas-Agena B, described as a lunar spacecraft, will be launched. This will be followed in the fourth quarter by a second Atlas-Agena B firing. They are called test vehicles, the test being, in a certain sense, to test the instrumentation that is on board and to test perhaps some of the guidance and control mechanism, but not to test whether it will hit the moon.

The next mission will be a lunar impact. This mission, of course, is something that we must be very strongly concerned with from the point of view of sterilization or decontamination. There are three such shots scheduled in 1962. These are hard landings, and a high resolution TV will be on board in all three cases. Finally, as far as lunar missions are concerned, two soft landings are proposed in 1963, using Centaur as a vehicle.

For planetary studies, the present schedule includes a probe directed towards Venus in 1962. Again we must be very strongly concerned with this from the point of view of sterilization. These shots are called test vehicles or probes in the same sense that were the lunar spacecraft.

I believe this indicates what time scale we have to work with, the urgency behind the problem, why it is that we are meeting here today, and why we hope that during this meeting ideas will be generated and submitted to resolve problem areas. We also hope that increased interest will be developed which will lead to additional activity in this area.

I have noted a few of the problems, and problem areas, with which we should concern ourselves. I emphasized the word "few" as this should by no means be considered complete or exhaustive. Nor is it intended to indicate a limitation on the kind of work that we want to do, or should do.

One of the problems that we are faced with is to what extent must we decontaminate? Must we have sterility or merely decontamination? What effective means are available for measuring the extent of decontamination that has been effected?

The physical scientist, of course, is concerned about what is going to happen to his payload, to his electronics, and to his structure when you subject the payload to a sterilization procedure, whatever the procedure might be. A major area for investigation is the compatibility of the component parts of a payload with the sterilization medium itself. Dr. Phillips has done some very fine preliminary work on this and I use the word "preliminary" because this is how he describes it.

We also face the problem of what to do at the launch site. To what extent should sterilization or decontamination procedures be effected and how do you do this on the pad or launch site? How do you sterilize or decontaminate in the laboratory during the construction phase or when you are conducting environmental tests on a payload package?

Associated with the launch site problem is the sterilization or decontamination just prior to launch and its maintenance during the initial stages of the flight. Provision must be made to sterilize and maintain sterility, or decontaminate, depending on what is desired, during these last stages on the ground and as the vehicle travels through the atmosphere, to prevent re-contamination.

One of the questions that we are faced with is what type of equipment is needed in order to effect and maintain a sterile condition. Is it expensive? Is it large? Is it effective? Where should it be located?

Another problem that must be faced is related to a lunar or planetary landing. Let us use as an example a lunar impact followed at a later date by a manned landing. It is essential that man know whether or not what he discovers was deposited by a previous landing or is indigenous to the particular body. This is an introduction to the thought of keeping track of those items that we have launched from earth to be placed on one of the celestial bodies. The question is to what extent should we keep track of our deposits? Should we merely keep a record of what is on the

payload or should we record chemical analyses of component parts? Should we establish a repository of duplicates of the parts that were actually landed on the extraterrestrial bodies? Assuming that we do have such a repository of information, where and how should this be established?

A problem that we are faced with is education. It is important in this relatively new field to educate both the bioscientists and the physical scientists with respect to the need for decontamination and sterilization procedures, the process for effecting these procedures, and to attempt to develop an appreciation of the requirement for sterilization.

Education is perhaps one of the most important matters for consideration because the requirement for effecting sterilization procedures is not accepted completely by some of the physical scientists. An associated consideration for implementing an educational program is the development in those who prepare the payload of an awareness of the need for including sterilization techniques in the environmental test program, and in the actual countdown at the firing pad.

There is a normal and natural resistance to accept a procedure that might delay very important work, particularly if there is not complete understanding and acceptance of the requirement for such a procedure.

Last, but not least, is the cost consideration in both time and money. We must establish the cost requirements for effecting sterilization techniques. We must be appreciative of the consequences of imposing a sterilization requirement and therefore must do so only when required and to an extent compatible with the mission.

I do want to mention one more problem for your consideration. That is, as Dr. Randt pointed out, at a later date we will be bringing samples back from extraterrestrial bodies. The problem of sterilization of those items we send to extraterrestrial bodies is simpler than the problem of sterilizing those that we return, for we will not have the convenience of an earth-bound base in which to effect our procedures. We should not forget that for a manned landing and return we must face the problem of decontaminating man himself.

DR. RANDT: Thank you very much. Dr. Phillips, I wonder if we might hear from you on the current status of decontamination procedures.

DR. CHARLES PHILLIPS: It is within current technical competence to produce a sterile man who does not have a single bacterium in him. We have done it with animals often enough to know we could do it with man.

The techniques have all been worked out at the LOBUND Institute at Notre Dame. The same methods used to obtain sterile guinea pigs, chickens, and rats, will produce a sterile man. All we will have to do is keep him in a germ-free cabinet for some twenty-five years following birth, meanwhile teaching him how to fly spacecraft.

However, what we can do, what we should do, what we want to do, or what is sensible to do, do not always match. A sterile spacecraft is a good sight easier to obtain than a sterile man. We can put a man in a sterile environment, and keep him there seeing that he is not exposed to possible biological contamination as he proceeds outwards into space. The first steps towards the maintenance of a sterile environment until the non-terrestrial biological forms which may exist in our solar system have been well studied and shown to be harmless, are the subject of this meeting.

(Dr. Phillips then proceeded to discuss the reasons why interplanetary probes should be sterilized, and the means by which this might be accomplished. These remarks were essentially similar to those in a paper prepared by Dr. Phillips for publication in Science Magazine. A reprint of this paper is included as an appendix to this report.)

Question by Dr. Davis: Do we know what the Russians did about sterilizing their lunar impact payload?

Answer by Dr. Phillips: Only that the Russians said they did something. However, regardless of what action was taken by the Russians, we must exercise caution. For example, even though some careless motorist is seen throwing away a lighted cigarette, we still do not take down our signs cautioning against doing this.

Answer by Dr. Seeley: Dr. Lloyd Berkner of the National Academy of Sciences recently asked the Russians if they sterilized that payload. They answered yes, then refused to give any further details or continue the discussion.

Following his talk, Dr. Phillips demonstrated a simple plastic chamber in which components could be placed, given an ethylene oxide treatment, and then removed and tested for functionability following treatment. He explained that engineers, being naturally concerned about the effect that the sterilizing treatment would have on their materials, should be equipped to perform such tests themselves, just as they, for example, perform standard "shake tests" to determine resistance to vibration. The need for a standard realistic test was emphasized, quoting the fact that contradictory reports had already come out of two laboratories. In fact, neither of the tests are considered to be valid since one could not tell just what ethylene oxide exposure was given. It was also pointed out that inquiries have been received from many laboratories concerning the ethylene oxide treatment and they probably will be performing tests of their own. The tests had best be standardized. The equipment shown was made in the Fort Detrick shops.

Dr. Randt asked a series of questions relating to the design, operation and cost of a chamber of the type demonstrated by Dr. Phillips.

Answers by Dr. Phillips: The device shown was made in the Fort Detrick shops. A sub-contract has been let with American Sterilizer Company to design a production model. Such equipment could and should be made available to all experimenters who will contribute a piece of equipment into the payload, or who design and test spacecraft. The cost, at this time, is estimated to be about two hundred dollars.

DR. RANDT: We would like to continue the discussion with a description of work being conducted in various laboratories and a description of the facilities available. The Jet Propulsion Laboratory has perhaps gone as far with this as anyone else. I would like to call on Mr. George Hobby to start this discussion.

MR. HOBBY: One of the difficulties in determining methods for sterilizing the Ranger series spacecraft has been the fact that it was not possible to start at the beginning of the conception of the vehicle and include in the design the requirements imposed by sterilization. Also, it is necessary to apply only the existing standard sterilization techniques, for developing new ones which are particularly applicable to the present problem probably cannot be done in time to meet existing space exploration schedules. Therefore, we are committed to use standard sterilization of the existing type spacecraft, and we are likewise committed to sterilize a spacecraft which is not completely adapted, engineeringwise, to the existing sterilizing techniques. Nevertheless, we expect that by insisting on a few minor changes in the spacecraft, and by the exercise of some ingenuity we can do the job.

In the sterilization of the spacecraft we are primarily interested in three areas: 1. Internal sterilization of materials and parts, 2. Surface sterilization, 3. The maintenance of sterility after final decontamination of the completed spacecraft.

Dr. Phillips' group at Fort Detrick has performed some preliminary tests to determine whether or not electronic components are contaminated with imbedded microorganisms. On the basis of these studies we must conclude that such contamination does exist, and proceed on the assumption that all materials and parts of the spacecraft contain internal bacterial contamination.

To achieve internal sterility, several methods are possible: Dry heating, irradiation with gamma radiation, sterilization of materials during production, and sterile assembly of the individual parts. The sterile fabrication of materials and parts would be a major undertaking, to say the least, for this would require the hundreds of vendors who produce spacecraft parts and components to initiate entirely new, expensive and time consuming concepts into their manufacturing philosophy. Therefore, we would prefer to treat these materials or parts by one of the standard sterilization methods in order to achieve internal sterilization. Only two such methods are currently available: Heat and high energy irradiation.

We are currently proposing that a time-heating schedule of 24 hours at 125°C under dry heat conditions will be sufficient to sterilize the inner volume of materials. However, this must be

subjected to a broad program of testing to provide complete confidence in this procedure.

Materials which are labile to the heat treatment will be irradiated with gamma radiation at dosage levels of 10^7 roentgens.

During the course of the assembly and testing of the spacecraft a considerable amount of biological surface contamination is expected because of contact with atmospheric dust and through human handling. Therefore, a final surface decontamination will be initiated after completion of assembly and testing. This will be accomplished in a manner which will achieve not only surface sterility, but will also maintain that sterility. To make this possible, the usual shroud or nose cone which protects the payload from aerodynamic forces during ascent, will be sealed from biological contamination and modified to permit introduction of ethylene oxide-freon gas mixtures for the decontamination process. The same shroud will protect the spacecraft from recontamination during the time the entire vehicle is raised to the top of the booster stages and during its ascent through the atmosphere. The shroud will eventually be jettisoned at a sufficiently high altitude to avoid risk of atmospheric recontamination. There will be no danger of contaminating the lunar surface by impact of the shroud, as the spacecraft will not have reached sufficient velocity at the time of release.

The current shroud design now being considered for the Ranger series of flights, consists essentially of a metal chamber having a suitable aerodynamic configuration, which will be mounted to the upper surface of the spacecraft adapter assembly and sealed at its base by means of an "O"- ring. This shroud will be modified for the purposes of the sterilization requirement by providing an inlet port near its base for introducing the sterilizing gas mixture, or a purging gas, and an outlet port near the top to permit expulsion of gases from the top during the filling procedure. As excessive pressure differences cannot be allowed to occur between the gas inside the shroud and the ambient atmosphere, a considerably larger port equipped with a bacteriological filter will be provided to permit expected gas exchange due to ambient thermal variations. This "breathing" port will be sealed during the filling operation.

In order to completely seal the shroud cavity a plastic diaphragm or bulkhead is being designed to close off the base of the

shroud from the lower stages. This diaphragm will contain the port for the bacteriological filter. In order to permit rapid evacuation of the shroud during ascent through the atmosphere, the plastic diaphragm will also contain a suitable automatic valve which will permit large rates of flow of the gases from the shroud, but will close before any back pressure can force air into the shroud through the valve from the outside. With this shroud device we hope to be able to perform the surface sterilization as well as maintain sterility during all subsequent operations and flight.

One of the areas in the overall sterilization procedure which presents an especially difficult problem is achieving or maintaining sterility during the assembly and test phases. Whenever substructures are bolted on to the spacecraft superstructure and surfaces are put into intimate contact, the probability arises that organisms will be trapped between these surfaces. The assembly and testing phases in preparation of the space vehicle are long and complicated and will consist of a great deal of disassembly and parts replacement. Under these conditions, such surfaces must be sterilized and resterilized as many times as they are put together. In order to find the easiest solution to this problem we would prefer to build a spacecraft which could be heated in entirety so that the whole device can be sterilized under the sealed shroud in a single operation after all assembly and testing have been completed. This is a big order, but it appears to be the simplest solution as far as sterilization is concerned. In this way internal and surface sterilization could be achieved in one relatively simple operation, and the sealed shroud would remain over the payload after treatment to protect it from being recontaminated. It appears at this time, that we should attempt to work towards building a spacecraft which will be completely thermo-stable under the heat sterilization environment. This is one way to simplify the whole procedure.

The possibility for at least partial heat treatment of the Ranger spacecraft appears fairly good at the moment. We estimate that approximately 85% of the electronic components on the spacecraft will survive the heat sterilization treatment of 125°C for twenty-four hours and preserve their tolerance rating. Perhaps 90% of the non-electronic equipment and materials will also survive this procedure. Sterilization of contacting surfaces, screw-holes, etc., during the assembly and testing phases is still a problem which must be solved. However, we feel fairly certain that at least

90% of the surface sterilization and 80 to 90% of the internal sterilization can be accomplished at this time with known and available techniques.

We feel, therefore, that we have made progress towards solving an extremely difficult problem.

Dr. Cole and Mr. Bates raised questions concerning the plastic diaphragm seal.

Messrs. Hobby and Mohl provided answers as follows:

1. Only the payload will be sealed off and sterilized - not the last stage engine.

2. Terminal sterilization using the gaseous procedure will be only of the surfaces. Internal sterilization must be effected by an appropriate technique prior to assembly.

3. The problem of making electrical connections through the membrane has not been solved as yet, although there appear to be several ways of doing this that are currently available.

4. The shroud material is plastic and metal.

Dr. Phillips, Mr. Brown and Mr. Bates asked several questions which indicate a concern of the possibility of the last stage as well as the payload impacting the moon.

Answer by Mr. Mohl: After burnout of the Agena and payload separation, the last stage is retarded and pushed out of the way to prevent it from following the payload.

DR. RANDT: Dr. Phillips, would you care to make any comment on the JPL program and the material that Dr. Hobby has presented?

DR. PHILLIPS: Yes, sir. I have some comments and I would also like to take advantage of the occasion to make one or more general statements. There are two things that I was delighted to hear about during previous conversations with the JPL people which I had not thought we would be able to do.

One, they believe that they will be able to treat the entire top assembly on the ground with the payload inside the shroud and then keep that assembly together, put it in place atop the gantry,all the while keeping peoples' hands off. This treatment which JPL plans as an assembly simplifies everything quite a bit. They have control of the shroud design, and are starting early enough that they can have the shroud itself serve as an exposure cabinet. Such a procedure would make it unnecessary to construct a plastic tent around the shroud and payload on top of the gantry.

Secondly, I was pleased to find out that they thought that by giving sufficient guidance to the manufacturers, they would get electronic components that would all be capable of being heat sterilized. Our survey work to date has certainly shown that we cannot count on these electronic components, with the possible exception of transistors, to be sterile internally.

If we are to achieve the ultimate goal we have been talking about and have a completely sterile payload, everything will be greatly simplified if it can be constructed of components all of which could withstand 125°C for an indefinite time.

Now, the general statements I wanted to comment upon concern the places I think where an advisory group, such as ours at Fort Detrick, can be of help to NASA in this program, and areas where work and information are needed.

First, we should, as rapidly as we can, build up bodies of readily available general information, basic principles and the like which are generally applicable to any effort to sterilize space vehicles. For example, what are transistors like as class? Are they sterile internally? Can we get transistors which are resistant to heat sterilization, or which can withstand sterilizing radiation, and similarly for other components and materials? The main problem is not that of developing new principles of sterilization, but that of finding out where our problems are, and investigating how they can be handled, usually with well known present day techniques. This information should be made available to all concerned. Every university or industrial firm which proposes to participate in a lunar or planetary experiment should have this information.

My second general point is that even after all the general information has been gathered and made readily available, there will still remain many specific problems which will have to be carefully worked out in applying these general principles to each particular launching. No two space probes will ever be identical, in all likelihood; therefore, you cannot expect to repeat exactly the treatments used in previous launchings. In other words, what the Army calls an SOP or Standard Operating Procedure will have to be worked out differently everytime a vehicle is launched. This will have to be worked out early, during the design stages. You must know how it is going to be sterilized in full detail before it is constructed, or else one could easily build into the probe something which will prevent complete sterilization. To see that this does not happen, people familiar with all the general sterilization principles must work closely with the design engineers and see that sterilization SOP's are worked out well in advance for each and every shot where sterilization is required. This is really nothing more than including sterilization in the environmental test and countdown procedures. Until the engineers are far more familiar with sterilization than they are at present, sterilization specialists can be of help to NASA in seeing that correct procedures are employed in each of the shots. Someone familiar with the art should make sure that sterilization principles are followed in each launching in which it is required; followed both in planning the shot, and designing the equipment as well as in the actual launching. People who might give advice should be called at the beginning, not two weeks before launching.

DR. RANDT: Thank you. We have representatives of four installations here who may be doing related work and have facilities that could help in this process. I would like to ask Dr. Stanley Levenson of the Walter Reed Army Institute of Research if he has any remarks to make.

DR. LEVENSON: Our work is concerned principally with what Dr. Phillips began and ended with, namely, the question of the sterile animal. I would just like to say in general that while Dr. Phillips' statement that animals can be kept germ free and so forth is true in a general sense, I am not sure we really know how true it is in a specific sense.

I think we can certainly say that animals can be kept free of bacteria; they can be kept free of fungi, and they can be kept free

of parasites. I don't think we have the objective information now to know whether they are truly free of virus and other types of infections. There is no evidence that viruses are actually present in the animals which we call germ free, but the evidence which has been sought has been very limited and I would think that one of the areas in which intensive work would have to be done is where it is considered that a live animal, hopefully germ free, would be included somewhere along the line in a payload. This would require a marked increase in effort now to actually define the animals which we now call germ free.

The reason why this has not been done is that it is much more difficult and much more expensive, labor, people, money, et cetera, to determine whether or not viruses are present in these animals than it is bacteria. By the same token, the determination of the state of, say, germ freeness of the animals is obviously contingent on the battery of tests you run. So I would think there would have to be a really marked increase in the testing for the possible presence of microorganisms. The basic information of how to go about it is at hand. It is just a question of applying the basic information to the precise situation.

In case some of you don't know, the species which have up to this point been raised germ free in the current sense of the terminology are rats, guinea pigs, mice, poultry, a few primates, a couple of sheep, some goats, no dogs, and a few rabbits. Theoretically I think you could hope to raise most any species in the germ free state.

Again it would be a question of applying present knowledge to the problem. We at Walter Reed are very anxious to get into the larger animals, particularly into the primates. There has been a limitation here because of special equipment which would be needed, money involved, et cetera, but again I think this is really just a question of application of effort.

DR. RANDT: Thank you, Dr. Levenson.

I wonder if you would speak to the point of the requirement for landing an animal on an extraterrestrial body. This is not clear to me. Why would you anticipate doing that?

DR. LEVENSON: One good reason to do it would be to detect microbial life on the planet if it exists there. You could use cultures, tubes, or similar procedures. The animal free of microorganisms is probably the best sort of culture media you can use. If there were just a few organisms on a planet and they got into the germ free animal, whose defense against ordinary bacteriological agents is very, very low, there would be a chance for the few microorganisms that might be present to multiply at an alarming rate. You would then have the animal with the organism in him which you could detect by biological means.

DR. RANDT: So you are making a point that an animal would be a better culture medium than could be produced otherwise?

DR. LEVENSON: That is right. I think that has been pretty well demonstrated in work with these animals. A decision would also have to be made as to which type of animal to use because interestingly enough there is really very marked species differential in sensitivity of so-called germ free animals. For example, a germ free guinea pig taken out of his isolator and put in an ordinary clean animal room is dead of an overwhelming infection within forty-eight hours. Infections which an ordinary guinea pig will handle without any trouble kill the germ free guinea pig. On the other hand, the germ free rat put in a clean animal room will just go along. We don't know what the primate would do. Perhaps the primate would be the ideal animal from this point of view if we are interested in investigating the things that might have application to man.

Then the other thing. Suppose you wanted to know whether animals of the higher levels could survive in a particular type of environment. We possibly would have to bring animals there to see whether or not they will survive or how they will adapt to this situation or things of that sort. Perhaps you might want to do this prior to actually sending man in terms of actually establishing residence there.

DR. RANDT: I think there is very little conjecture that man would not survive without protection from what we know of atmosphere and surfaces of extraterrestrial bodies. He would have to be provided with an artificial environment.

DR. PHILLIPS: Would it be possible to make one comment? Dr. Levenson has touched on a thing which we have discussed quite a bit with some of the biologists. That is the point about ignoring the viruses. With the germ free animals, while there has been no evidence of viral disease among them, no one is willing to stand up and say that they are virus free.

The same is obviously true with the sterilization techniques I have been discussing. They ignore viruses as a class. Our sterility testing does not determine whether viruses survive or not. We do know from general experience that any procedure which kills resistant bacterial spores by either chemical or heat will kill viruses. Radiation will also kill them, although they are a little more resistant to this than are the bacteria. Yet we are not testing for survival of viruses when we perform sterility tests. We have the philosophy that because viruses must have a host cell to propogate and because they are so highly specific to that host cell, if we are not carrying these viable host cells, we can just write off the probability that we could infect Mars or anywhere else with viruses. It would not only have to get there alive, but once there it would have to find a proper host. The probability of a proper host for an Earth virus existing on Mars, unless we carried it with us, is so slight that the only organisms we are worrying about are the husky, tough soil organisms which live on the very simplest kind of nutrient and media.

DR. LEVENSON: By the same token, you would not want to take a chance of introducing viruses on an extraterrestrial body which might somehow survive then introduce a terrestrial animal, contaminate that animal and then not know whether that contamination originated here or there.

DR. PHILLIPS: I agree.

DR. RANDT: The question has arisen as to whether or not bacterial and mycotic spores could not in themselves provide the cell on which a virus could survive.

DR. PHILLIPS: This is true. Even bacteria have virus diseases - bacteriophages, we call them. The safety director at Fort Detrick will not let us use, as a harmless simulant, any of the viruses that affect any animal in the whole animal kingdom even

though they have never been known to cause disease in man. He will let people breathe bacteriophage, however, believing them completely incapable of adapting to the higher animal forms. No virus disease of bacteria has been known to affect any higher organism. That may not be true in Mars or the other planets.

DR. VISHNIAC: I would like to make a comment concerning the use of animal as a culture tube. I can see its importance in testing for microorganisms that might be dangerous to man or animals, but I would like to take issue with the general statement that it is the best possible culture tube.

DR. LEVENSON: I would agree. If I implied it was the best possible, I retract that. I think it is an important part of the total battery of tests which one uses. That is the point I tried to make regarding the question of the definition of the germ free state. It is obviously limited to the amount of testing you do and the more extensive, the broader the testing, the better off you are. I think the germ free animals add a very important step in the testing program.

DR. RANDT: We have representatives of the National Institute of Allergy and Infectious Diseases of the National Institutes of Health. Dr. Davis, do you have any remarks to make about related work going on there?

DR. DAVIS: Our Institute, of course, is primarily concerned with diseases of man and diseases of other animals which are of importance to man. But in connection with that we do have a good deal of basic work going on in the biology of microorganisms. These include not only the bacteria about which we know so much, but also more recently the viruses and, also, the fungi.

Dr. Hasenclever is here from our Section on Biology and Dr. Cole is here from the Bacteriology Group. We are interested too in the basic problems and, as most laboratories are, we are working more and more in the general area of molecular biology. These are some of the basic things that one must do so that some years from now we will be better prepared to talk about the important practical things that have been brought up here. I think probably an organization such as ours can make our best contributions by continuing to work along these lines and make this information available through the usual channels.

I am interested in the comments on the viruses because really it is only by learning methods of testing for these that we learn much about them. Only a few years ago we more or less assumed that animals in whom we could not detect bacterial or mycotic infections with our usual methods of testing for viruses were, in fact, microbe free. We question this a little bit now as Dr. Levenson and Dr. Phillips have because we realize how inadequate our methods of testing are. The development of tissue culture methods opened up a whole new area of microbiology and virology. This is just an illustration which I don't mean to belabor.

Much of what we know comes about as a result of the methods that we use for testing. This will be increasingly true in some of these subjects which are being called molecular biology and related areas which are now beginning to demand a great deal of interest. We really don't know what the molecules do in our environment. What would they do in a different environment when exposed to different kinds of radiation, pressure, or gaseous environment? Our direct program at NIH is related to these basic things.

We also, as you know, have an extramural program. This is not a contract type program where we make contracts such as the military, NASA, or the Atomic Energy Commission do. The project actually originates with the investigator so we don't have the direct control over the projects. However, as you know, this does support a great deal of the basic biology that is going on in the country.

I am impressed with the remarks that have been made about being sure that certain space payloads are sterile and the methods used. In the operating room, we know the methods that result in a certain degree of sterility and yet we must realize that people make mistakes. If those mistakes are made, it is important to know it. The process of monitoring the procedures has always been important in biology because then you do know, at least, where you are. I think this is something which we all accept as being fundamental to science. We may have foolproof methods, but, if we get off the track, we will want to know it as soon as possible.

Maybe Dr. Cole or Dr. Hasenclever have some comments.

DR. COLE: I think the areas in which we are most concerned in the basic disciplines are those which might be called preventive

medicine. This is a little further in the future than the things we have been talking about so far this morning.

This question, which is of interest to me and to our area in general, is what is the return payload going to bring back. Aside from how we prevent some unknown organism from getting into the vehicle in the first place, if it does come back, what do we do when an organism with which we have had no previous experience gets into humans. As I say, it is something about which we know nothing at the present time and it is a little bit removed.

DR. RANDT: Dr. Hasenclever?

DR. HASENCLEVER: Since I am representative of the Mycology Section, I would like to bring up a point. I am sure that possibly all of you are aware that fungi, some of them at least, are a little different, in that they will utilize various substrates which most bacteria will not.

The fungi probably are not as resistant, under most circumstances, as bacterial spores, but they represent a pretty large portion of possible contaminants which might be taken along on vehicles into space. They would require organic matter for their growth, but this organic matter could be from many sources, not usable by bacteria. Thus the fungi are rather unusual under certain circumstances and cognizance must be taken of this fact.

DR. SEELEY: I think you would like to know, Dr. Davis, that Dr. Lederberg's committee has concerned itself and has had considerable discussions on the steps which might be required in quarantining or isolating material returned from other bodies before it might be declared safe to expose them to our environment.

DR. RANDT: We have representatives of the Army Ballistic Missile Agency from whom we would like to hear.

Dr. Young, do you have anything to say about these matters?

DR. YOUNG: The problem that the Missile Firing Lab of the Ballistic Missile Agency is concerned about, in particular, is what are they going to have to do at the launch site facility in maintaining sterility? This has already been discussed in some detail in

reference to the Ranger program. But the problem goes a little bit further than that. Probably if we think in terms of four or five years into the future when Saturn comes into being and we are faced with the prospect of landing boosters in the form of retrorockets or return vehicles, we will have to consider sterilizing the booster. The people at the launching sites have a great deal of interest in this problem and would like to get started because, as has been pointed out, a great deal of lead time is required to do an effective job in this area.

DR. RANDT: Mr. Brown from the NASA Langley Research Center. Do you wish to comment?

MR. BROWN: I don't think I have anything to add. I would like to ask one question:

Do I ascertain from the last remarks that there is some worry about the return of vehicles that will not come anywhere near these bodies to the earth's atmosphere?

DR. RANDT: No, the long range concern is in regard to the return of samples to earth.

AFTERNOON SESSION

DR. RANDT: The first matter on the afternoon agenda has to do with the interrelationship between the physical science and engineering parts of the decontamination problem and the biological ones.

Mr. Mohl of the Jet Propulsion Laboratory will go into some of the details of the engineering problems. I would hope that this will serve as a starting point for discussion in this area.

MR. MOHL: Basically, I work with the hardware. There are a few problems that do concern me about the question of sterilization. For example, you take modules of electronic components, batteries, and associated gear, add a mid-course motor and a high resolution TV, and assemble this onto a spacecraft. The assembly is designed so that each module is easily removed and replaced, but how easy is easy, if you have to sterilize it? For example, suppose we have a completely assembled spacecraft being tested and a module goes bad and we have to pull it out and put another one in. Now, how do you do it and maintain sterility and a schedule? Another problem is the development of a sterilant, perhaps liquid, that could be applied on connectors, one that could be put on magnesium or steel surfaces five or six months prior to flight and still be sure that there would be no damage from corrosion occurring in the next five to six months. The engineer must know from factual data and reports that he can take the liquid sterilant, apply it to a connector, put the connector together and still maintain the electrical characteristics that it would have if no sterilant was used.

There is an urgent need for special application devices to help on assembly. For example, little plastic boxes with handle ports that we could use to pull out a component or put a component in, introduce ethylene oxide, and then work through this box to get the component back down on its flanges and make the electrical connection.

Also needed would be a little chamber that would let us sterilize electrical connections. One in which a nonconnected connector could be placed, sterilized, and then physically connected inside the container before it is removed.

Plumbing will be a headache. On spacecraft there are tubes going to actuators for attitude control in space, tubes that conduct gas,

and just plain old piping. How do you connect up a piece of plumbing to an actuator? You have a sterile tube and plug on the end, but you have to take that plug off and connect it up with a wrench.

This is not easily adaptable to the tent device demonstrated by Dr. Phillips because all this piping is already physically attached to the basic structure. You would have to have something covering the entire structure. I would dislike getting involved in that but I don't know if we can avoid it. Maintaining a sterile assembly area would be extremely difficult.

DR. PHILLIPS: I could not agree with you more. It is a last resort. You don't do it if there is any other way. On the first spacecraft, the lunar orbiter, we are going through many of the technical procedures that we will have to utilize on the lunar impact shot. We will have an opportunity to learn of some of our problems and how to handle them without the ultimate responsibility of insuring a sterilized spacecraft for that particular round.

MR. MOHL: To point out the problems even a little more precisely: today I have a space airframe for the first lunar spacecraft in an oven being heated up to 125 degrees. This is a frame which holds electronic boxes and is made up of many, many bolts, castings, machine metal surfaces. The machine metal surfaces are laminar surfaced, used for reference planes, attitude control sensors, attitude control jets. Here we have these very fine surfaces and we want to know what will happen to them when we heat up this airframe. Will we lose our alignment? What will heat do to it? We had a very fine inspection made of the airframe and we will reinspect it after it comes out and see if that is a problem. If that would be a problem, this would mean as we assembled this airframe we would have to sterilize each and every bolt as we put it together. Then we are right back to the corrosion problem again. Or else we would have to learn how to build an airframe so that we could sterilize it by heat and then machine these surfaces afterwards. These are specific detailed problems.

Previously somebody asked about the shroud problem. We left one point unclear about the shroud. There is no basic disagreement in the laboratory on the concept of a sealed cavity for the spacecraft where all air coming in or going out must pass through a filter. By doing so, we have not eliminated the capability of getting at the

payload compartment on the gantry because our plumbing is such that it is right on the shroud. We have a valve in the shroud and if we would have to pull off the shroud on the gantry, we could pull it back down, introduce ethylene oxide through the valve, just wait the proper length of time and we would be back in business without ever taking the spacecraft from the vehicle.

MR. GALVIN: However, I have seen them put a man in a bucket at the end of a crane and raise him to the level of the payload so he could work through a port in the side of the vehicle and do an emergency repair job.

MR. MOHL: Another point to be made is the need for a well-documented concise statement that ethylene oxide is a safe gas and does not tend to promote explosions. What happens when you permit free oxide or free hydrogen to change the mixture? I think there is a need for studies and reports that spell out this problem very clearly. The application here is one where, with so many people working in a gantry, you cannot go by guess.

How sure do you have to be? It is the same as sterilization. What confidence level is desired to avoid a dangerous mixture of ethylene oxide gas if it leaks out of a filter? Being heavier than air, it could work its way down to the oxygen fuel mixture.

DR. PHILLIPS: If you are resterilizing, this can be done before you take on oxygen, and in any case the ethylene oxide in the amounts to be used is the equivalent of a cup full of aviation gas. The amount of gasoline could do little damage unless it were to set on fire a much greater amount of combustible material. Ethylene oxide is flammable to about the same extent as aviation gasoline. But this ethylene oxide will surely be released and away before it is mixed with liquid oxygen. In addition, I think it won't be too much trouble, to see that six hours of ethylene oxide treatment are carried out before oxygen is brought up and pumped in the rocket.

DR. RANDT: Before we leave this, Dr. Phillips, do you subscribe to the gathering of further data on the explosive mixtures?

DR. PHILLIPS: This could be done but I think we could almost say in advance that with oxygen rather than air it would go through an explosive range.

MR. HOBBY: We did have a test run on the ethylene oxide mixtures that we have in which we kept the pressure constant in the vessel and then added oxygen so that we were displacing the ethylene oxide mixture and raising the concentration of oxygen. Under these conditions, at thirty percent oxygen did explode.

DR. PHILLIPS: You still must have a detonator. You are dealing with material like highly volatile gasoline. This is handled all the time even though it does burn in air. With a cupful of such material, it is explosive only if confined. Otherwise it is just combustible.

MR. MOHL: But the need for referenced documentation definitely exists.

DR. PHILLIPS: I am quite sure the carboxide mixture will not take pure oxygen dilutent without passing through a flammable range. It will take air. That is well documented. The amount of dilutent needed, whether it be carbon dioxide or freon, so that no mixture with air is flammable is well documented.

DR. RANDT: Do you think it would be possible to document this other aspect?

DR. PHILLIPS: Yes. Except I can tell you right now we will find out it will burn if you mix it with oxygen.

DR. RANDT: You have been talking about the explosion hazard. How about the fire hazard?

DR. PHILLIPS: I don't think there is an explosion hazard; I think it is fire, really. You can't get an explosion unless the gases are tightly confined.

MR. MOHL: Could it create an explosion, if you drop a match?

DR. PHILLIPS: In the right place if it were to ignite your fuel, that is true. But you are dealing with, again, combustible material and there must be a certain amount of other combustible material about. It can be arranged by safety regulations that you don't use ethylene oxide mixtures at any time in the presence of lox. That could be a primary safety rule.

Of course, there are problems with all your other combustible materials. Take, for example, the hospital attitude. Wherever there is oxygen about, one takes all kinds of safety precautions, yet it is not possible to keep every type of combustible material away.

MR. MOHL: I don't see this is a real problem unless we reach the circumstances where we try to do emergency sterilization on the gantry.

DR. PHILLIPS: The problem could be solved by saying just don't use ethylene oxide mixtures when oxygen is present. Use other disinfectants. For example, for emergency treatment if you touch one part of the payload while it is on the gantry, you could use compounds which are completely noncombustible, propiolactone in water, for example. Have a squirtgun and put some on the spot touched. This will sterilize in a minute or so and will vaporize off very quickly. It would not damage anything you can let water touch. I think if the payload stands the high humidity they have at Cape Canaveral it must be able to take a little water.

MR. MOHL: We would have to decontaminate a big area.

DR. PHILLIPS: If somebody walks in and touches one spot, you can go back to that spot. If you take the whole thing apart, then you are going to redo the whole payload.

MR. MOHL: The only situation you get in is that you move the payload to the missile. After you got up there, before you started loxing in the case of the Atlas, you would have to break the shroud open.

DR. PHILLIPS: Once you finish sterilizing, you would not be carrying ethylene oxide up under that shroud. It would dissipate in minutes once the shroud were no longer sealed.

MR. MOHL: The only case I can visualize where it would be probable is as follows: suppose we got on a gantry and there was some minor defect that we felt we could correct by breaking the shroud open and getting in and closing up again, but to get inside we would have to expose the whole payload to surface contamination; we would be forced to go through a standard surface decontamination cycle with the length of time specified.

So you could have a choice, either to sterilize with ethylene oxide on the gantry, or pull the whole thing back to the assembly area. In either case a substantial time would be added to the launch countdown.

DR. PHILLIPS: This difficulty is not great. The amount of propiolactone vapor you can get in the air is not in a combustible range. It is not volatile enough for its vapor to be combustible. It is an organic liquid which will burn, like kerosene, but again whose vapors like kerosene cannot be ignited.

DR. RANDT: Would beta-propiolactone have an application in the emergency procedure?

DR. PHILLIPS: It could. Small amounts of beta-propiolactone could be used which would not be flammable in air. I would not guarantee that the vapor would not be flammable if it mixed with pure oxygen but that could be quickly tested.

We cannot have more than ten milligrams per liter of BPL vapor in air. With ethylene oxide, which is highly flammable, it takes three percent by weight of air to reach the bottom limit of flammability. With beta-propiolactone, we can't begin to get three percent vapor in air.

One very general comment, I want to say that I think my earlier point is being proved, namely that we need all the technical specialists sitting around a table with blueprints, mockups or models before them to plan such procedures in detail. The technical representatives should include people who are going to be in charge of the countdown procedure and people who are familiar with sterilization techniques as well as those familiar with the rockets and payloads. They should plan now how they will avoid having ethylene oxide and pure oxygen ever get together.

MR. MOHL: Another question I was going to ask, but I think some of that was discussed this morning, what tests will we have to insure sterility?

DR. PHILLIPS: This point was brought up. I have it in my notes and I did not answer it. The answer is simple. Absolutely none. No surgeon about to perform an operation picks up a scalpel

and says, "Nurse, will you run down to the laboratory and have them check this instrument to see if it is sterile? Meanwhile we will hold up the operation until we get the lab report back". Instead one sets up administrative control within the hospital. This control insures that the scalpel was given the standard sterilizing treatment, which was shown to have been effective on other scalpels. The scalpel being used, however, must be assumed to be sterile without having been tested itself. The same will have to be done here. Standard operating procedures, known to be effective will have to be set up, and administrative controls inaugurated to see that they are followed. In that way we can be reasonably assured that the tests conducted in all laboratories will be equivalent.

At this point Dr. Levenson remarked that, although he agrees in concept, a more appropriate analogy to use would be that of the germ free laboratory. The difference between the two analogies being that in the case of the hospital operating room, instruments remain sterile only until they are unwrapped and exposed to the atmosphere whereas in the germ free laboratory sterility can be maintained -- which is the objective in the problem under discussion.

MR. MOHL: I was thinking not so much of testing the flight article as I was wondering whether we can extrapolate the data used for determining the length of time, concentration, and pressure for sterilization of a small test unit to the actual case where you are inside the shroud, for example, the volume and area relations. How long do I have to run ethylene oxide into a shroud, how long should you bleed it? Should I keep strengthening it? For what period of time? Just what is the period of time I actually have to have to sterilize the hardware inside the shroud, not just the general case of a test unit?

DR. PHILLIPS: You have these shrouds. They always make two or three of them. Put some things in a shroud. Put test organisms on a piece of metal and have them around in the shroud and try it, show that it works. Do such things as put a space probe under the shroud and make sure you have the right concentration. I think it would be highly improper if the first time you ever put ethylene oxide under a shroud of your particular design was the time that it was being used prior to actual flight. This is the one time it, like the scalpel, should not be tested to see if the treatment worked. But you certainly have checked out the entire procedure beforehand.

MR. MOHL: The other point that is of some interest to me is the explosive squibs. I know they are supposed to be self-sterilizing, but are they?

DR. PHILLIPS: I would not say it is obvious. Organisms do live through explosions. This is another technical point that should be checked in the laboratory. We cannot solve them here. I believe that we at Fort Detrick are in a position to recommend a medium for effecting sterilization and procedures for its use. However, compatibility between the sterilizing agent and the hardware being sterilized can best be verified in a laboratory such as JPL since we at Fort Detrick are not equipped to conduct such tests of equipment.

MR. POSNER: We have spent some time here talking about a piece of hardware that is now in design. For future pieces of hardware on which engineering has not yet started, or is about to start, I believe in the engineers' capability to design these problems out. Given an understanding of the situation, if the sterilization problems are recognized and are included as an input into the original design, then the design can be varied such that these problems are minimized. Perhaps, for example, the squib can be placed elsewhere on the body so that it does not follow along with the payload. Many of the sterilization problems can be engineered out in the original design.

MR. MOHL: The other thing on my list was, is there a difference in definition between sterilization requirements to the moon and the planets?

DR. RANDT: Does anyone here have data on this subject?

DR. VISHNIAC: I think that even though there is a possibility that organic matter might exist on the moon, I think the idea is that there is no life on the moon.

So with the moon we are primarily concerned, I should think, with organic pollution by organic material which might confuse the chemical examination of the lunar surface.

We do not so much expect that introduction of a single microorganism would cause a biological explosion as introduction of enough organic material of biological or nonbiological origin may upset whatever chemicals land on the moon.

This is different from what we may expect on Mars. Assuming there is life on Mars, conceivably there are conditions under which life might exist, then the introduction of a single bacteria may set up a biological disaster and in that respect there is a difference. Sterility is a much more urgent problem in planetary exploration. Contamination with organic material is more of a lunar problem.

DR. YOUNG: On the other hand, it has been pointed out at other meetings that the moon should be a testing ground for techniques and if we are going to perfect these techniques for Mars, we had better learn how to do it now.

DR. RANDT: This brings us to the point of the criteria for sterilization. Does this mean no microorganisms or is there an allowable limit?

Would you like to speak to this?

DR. VISHNIAC: The large scale standardization is a difficult problem, of course. As one deals with apparatus we have to consider the probabilities and there are many different ways of computing such probability figures. I think it means that we have each component of the final vehicle sterilized with the degree of confidence that we place in laboratory equipment. It really means not a single viable organism.

DR. RANDT: Do you believe from your experience that this is a reasonable goal?

DR. VISHNIAC: I think so.

DR. RANDT: Even though you have just stated that with large complicated pieces of equipment it is a statistical probability?

DR. VISHNIAC: It is a statistical probability also because it is very hard to place an accurate evaluation on the means by which you determine whether or not you have achieved your objective.

DR. RANDT: This brings us to another problem that is related, concerning what probability can be accepted of impacting an extraterrestrial body that makes it necessary to accomplish the decontamination procedure. Is it one in twenty, one in a hundred, one in a hundred thousand? Does anyone wish to comment on that? I am talking about circumlunar and fly-bys of Mars and Venus.

DR. PHILLIPS: I asked for that figure six months ago and was given a number of about one percent probability that a lunar orbiter would impact. My understanding of the one part in a million organisms quotation that has been mentioned as an acceptable limit for spacecraft contamination is that there should not be as much as one chance in a million of putting one living microorganism on Mars. Such a value is really incapable of any mathematical treatment. No one has said how it should be calculated. It expresses more of a feeling and the amount of urgency put to that feeling.

DR. RANDT: To conclude our consideration of the problem of the inadvertent impact, are there any figures suggested for the Mars or the Venus fly-by?

DR. PHILLIPS: I believe that the intent of the biological members of the National Research Council Committee on Bioastronautics in making their recommendation can hardly be expressed mathematically. But I also believe that the intent of NASA in following these recommendations, and issuing statements such as "you will sterilize to the extent technically feasible", can be interpreted in several different ways. I would like to suggest that the intent of NASA in making such statements be expanded so that it cannot be misinterpreted. I was asked by JPL what I thought "the extent technically feasible" meant. My reply was that with the lunar shots, even those intended to impact, we would sterilize all areas which we could reach, and probably not call off the shot, if we had not as yet sterilized the interior of transistors, for example. I don't think any of the members of the Space Science Board would raise really violent objections if we inadvertently put a few organisms on the moon, provided we kept their number as low as we could without indefinitely delaying the shoot. However, by the time you get ready to launch a Mars probe, the same people might well recommend delaying the entire venture until such time as the sterilization problem has been solved.

DR. RANDT: This is a statistical probability. It is not a solution to the problem.

DR. PHILLIPS: There are cases where there is no statistical doubt whatsoever. It is not a statistical matter when the probe is contaminated. This is a known thing. It always gets to be a statistical probability, however, in whether it is indeed sterile.

DR. RANDT: We have two problems here and it seems to me that the one in a million has been established for a reasonable degree of sterility, but we have not yet established what missions might inadvertently impact and therefore should be recommended for the sterilization procedure.

Is there any expression of opinion on this?

DR. VISHNIAC: If we accept the numbers quoted, we will then say that the vehicles need to be sterilized only to a degree of certainty but this is clearly absurd. You can't sterilize anything a little bit. You either do it or do nothing.

DR. RANDT: The problem I am trying to get at is which ones must be decontaminated? Which missions will require this type of preparation? This bears upon the point of the inadvertent occasion of impact where it was not planned.

DR. VISHNIAC: The ideal thing would be to sterilize all those probes for which there is any chance whatsoever that they might impact.

DR. PHILLIPS: You are talking of the planets?

DR. VISHNIAC: Yes.

MR. MOHL: All probes with escape velocity?

DR. VISHNIAC: Ideally. Let us say that anything that comes fairly close, an orbit shot which may have a fair chance of crashing onto a planet.

DR. RANDT: Would you care to speak further on what you call a fair chance? This is really what we are trying to get at. A fair chance is not specific enough to make a recommendation as to whether all of this should be undertaken or whether it is not worthwhile.

DR. VISHNIAC: The question was raised whether anything that attains escape velocity; this is not what I meant.

DR. RANDT: The one percent chance was spoken of in regard to the circumlunar mission. We have not been able to get a recommendation of the critical probability for the planetary fly-by.

DR. PHILLIPS: Where you are deliberately aiming to be somewhat near one of the planets, I think you might say within a sizable fraction of both the time period and the location in space, then I think you should treat the probe as if you were planning to hit it.

DR. RANDT: Would you care to define what you consider to be sizable?

DR. PHILLIPS: I would say if you are going within five percent or something like that, that is if you plan to come as close to Mars as five percent of its distance from us. In other words, you are trying to get in its vicinity. This would indicate that anything planned as an orbit shot around Mars or Venus, should be treated as if it were a planned hit. In a planned orbit around the sun or a shot to some area where the planets had not been for nine months, or so, I suppose you could miscalculate so that a hit occurred, but I think this would be extremely unlikely.

MR. BATES: As an engineer, I would strongly suggest to anyone that all shots that are aimed in the vicinity of a planet be sterilized. Furthermore, very shortly after the fly-by shots are programmed shots that will either orbit or land on some of the planets.

On listening to this discussion this morning, it becomes evident we are laying out research programs involving structures and various aspects of the vehicles for the future. I find our engineers generally agree that sterilization has to be accomplished, but they have not completely considered the wherewithal of doing it.

It seems to me the thing that a group of biologists in general could spell out is that vehicles which are intended to approach an extraterrestrial body must be sterilized, then give the degree of sterilization and the technique of sterilization insofar as you know it. This will, to some extent, determine what the people can do research on or the techniques they will utilize in designing vehicles.

We are considering inflatable structures to be put on the moon's surface which may be made out of flexible materials. These materials might not be able to withstand your 125 degrees centigrade

here on earth. This is something the engineers have not fully considered in the process. They are more concerned about how to do it mechanically.

If you could lay this out as an accepted principle of another environmental facet that must be considered in the design of the vehicle and get it accepted and explain it to engineers and work closely with them as to the method by which you will accomplish this, then I think you will have made a step forward.

DR. RANDT: This is what we are implementing today. We intend to disseminate the edited record of these proceedings among those people of whom you speak. This is one of the reasons that I am asking these questions, in order that we present the problems for comments, suggestions, or alterations in a way that is reasonable and timely.

DR. SEELEY: Are you working on a better sterilizing agent which will more closely meet engineering requirements, and not incidental to that, would it not be of value to medicine if you can develop a better agent?

DR. PHILLIPS: I presume that question of Dr. Seeley was aimed at me. The answer is yes, but it would be extremely unfortunate if we were to have our plans depend entirely on this program.

The Army in 1943 started to look for new methods of sterilizing very odd and unusual objects. From the very beginning of the program, we decided that decontamination requirements might be such that we must consider objects that previously it had never been necessary to sterilize.

So we have a seventeen-year program which has been considering all kinds of odd ways to sterilize. Out of this program have arisen new accepted methods based on ethylene oxide and beta-propiolactone. Although the Army is continuing this program at but a modest rate, the space program is benefiting from its accomplishments. Only past accomplishments can aid those space programs now in the design stage, however further research may come up with things that will be helpful ten years from now.

DR. RANDT: There is the hope that the participants or readers of the transcript will provide us with assistance in approaching these problems.

DR. LEVENSON: Going back to your question to me this morning, Dr. Randt, of why germ free animals in space, I did not express that the basic decision was not whether the animal should be germ free at all, but whether the animal should be in such a vehicle.

Once you made the decision that an animal should be in the spacecraft, then it should be germ free for the obvious reason of the contamination, the scatter and what have you.

DR. YOUNG: With reference to the question of probabilities, there are two people responsible - with the biologist having the responsibility of saying there is zero probability of contamination. In other words, the biologist says it is a sterile vehicle. Then it is up to the engineer or firing people who have control of the vehicle to say there is zero possibility that a vehicle, if not sterile, will impact. So we have to ask the engineers what the probabilities are in answer to this question.

DR. RANDT: That is right. Is there any further discussion?

DR. VISHNIAC: If you are looking for ideas, I would like to mention one method of sterilization which has not come up this morning. Not so much a method of sterilization as the manufacture of some components. This is a practical procedure in the manufacture of surgical supplies where each material is made on a throwaway basis. It is manufactured under sterile conditions, used once and thrown away.

This includes plastics, it was mentioned this morning that plastics may contain bacteria in their midst which are trapped there by polymerization. It is possible to sterilize before the plastic is made, by distillation and then manufacture plastics which we are assured are at least sterile inside and only finally may need surface sterilization.

Electronic components I think can be made under such conditions. They already are made under dust-free conditions and with very few additional processes we could be sure that at least the interior of the components like capacitors would not contain microorganisms.

I am sure this could be expanded. As a matter of fact, squibs were mentioned, they could probably be made under antiseptic conditions.

DR. RANDT: This is a pertinent suggestion. I would hope that the more detailed consideration of the problems will point to the specific areas that require that kind of treatment from the start.

DR. PHILLIPS: This has been touched on, of course. It is a thing that the pharmaceutical industry has done more than anyone else. A certain amount of sterile assembly, can be done through glove ports in the cabinets we demonstrated this morning. Sterile handling and assembly tend to be tedious and expensive, however.

MR. MOHL: Apparently the biggest bugaboo is calibration. A lot of electronics could stand that temperature, but could not stand it without changing the calibration. On quite a few components the engineers writing the specs for particular components are insisting that they maintain their operating characteristics in 160 degrees Fahrenheit environment. The extrapolation between 160 and 257 or 260 degrees Fahrenheit, which is 125 degrees C, is not too far removed.

The ideal in my estimation of the final way to go is to get everything eventually capable of withstanding 125 C. This is not today or tomorrow at the moment. I guess it just brings back again the fact that the more stringent the sterilization requirement, the more the need for participating in the very early design concepts of payload packaging.

DR. RANDT: Is there any further discussion?

DR. QUIMBY: I think that Dr. Seeley made a very good point about the desirability of maintaining a research and development effort on trying to find more effective and more rapidly acting disinfectants.

It is true that the vehicles that are under design now will have to use ethylene oxide or some similar compound but I see no reason why we should rely totally on the modest program that the Army now has in this field.

DR. RANDT: Dr. Phillips, would you care to make some recommendations as to our future course in this area?

DR. PHILLIPS: I really have four things I would like to recommend, all of which have already been discussed. These are the four most obvious ones:

First, I would recommend that NASA policy on sterilization be made better known and expanded so that there is no possibility that the intent be misinterpreted. Some people have the impression that if sterilization proves to be difficult they may be excused from attempting it.

Second, a concerted effort should be made to overcome the communication problem. All the facts bearing on the problem now available, and those being currently under investigation at Fort Detrick, JPL, and elsewhere should be gathered, collated, put into manuals, or other readily available documents that can be sent to all the various people now interested in this area, or who may become involved in the future. This list of interested parties is a growing one, and not restricted just to NASA.

A third recommendation is that it should be a definite policy that with each new project started a detailed technical planning conference be held as often as necessary to see how the general sterilization principles referred to in the second recommendation will be applied in that particular project. These should be working conferences between biologists familiar with sterilization techniques, and those concerned with designing, constructing, and launching that particular space vehicle.

My fourth recommendation is that even though for the next year or two we are going to be concerned only with the best application of existing knowledge to the problem in hand, we should follow Dr. Seeley's suggestion and make every effort to see that we do more actual research in this field which will help us as the program moves on in future years.

DR. RANDT: Thank you very much, Dr. Phillips. I wish to thank all of the participants in this conference. It will serve as an impetus for further accomplishment in this area.

CONFERENCE RECOMMENDATIONS

The following are the recommendations that were made during the course of the discussions. These are presented here in the order in which they were mentioned, therefore there is no indication, implied or otherwise, of an order of importance or priority.

1) A body of information relating to sterilization techniques and procedures should be built up and made available to all interested parties currently working in this area or who may be involved in the future.

2) A standard operating procedure should be established for each scheduled launch at a stage early enough so that decontamination or sterilization are considered as another environmental factor for design considerations.

3) Studies be made relating to the sterilization of explosive squibs.

4) Work be done in the area of determining the probabilities of inadvertent impact coupled with the statistical limitations of implanting live microorganisms on celestial bodies.

5) NASA further clarify its policy and intent with respect to decontamination and sterilization.

6) An increased effort be put on the development of new and better sterilizing agents that will more closely meet engineering requirements.

7) Efforts be extended at the same time towards developing structures and component parts that are compatible with sterilizing agents.

8) The manufacturing process be studied to determine the feasibility of producing materials and components that are internally sterile.

9) A group at the working level be established to discuss details and problems of implementing sterilization techniques.

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APPENDIX

STERILIZATION OF INTERPLANETARY VEHICLES*

Charles R. Phillips and Robert K. Hoffman

For the past several years, biologists have been expressing their increasing concern that man, as he proceeds in his race toward outer space, may unwittingly be propagating biological contamination. An international committee, the Committee on Contamination by Extraterrestrial Exploration (CETEX), has formally recommended (1) that all efforts be made to prevent any such contamination of the moon or other celestial bodies. Lederberg and Cowie (2), Davies and Communtzis (3), Sagan (4), and Lederberg (5) have published on this subject in detail. The reasons proposed for this concern have not all been the same, and speculation has been varied as to what evidences of exobiology or nonterrestrial life might be expected on the moon or the planets and how contamination might affect such life, if any exists. There is complete agreement in these articles, however, that the spreading of biological contamination or pollution should be avoided most carefully until we have conducted careful biological studies on these extraterrestrial bodies. Now that the first physical contact has been made with the moon and since there is a probability that such contacts will become increasingly frequent, specific plans for implementing these recommendations are required, if this caution is to be observed.

In this country the National Aeronautics and Space Administration is actively investigating means of preventing extraterrestrial biological contamination. In this undertaking it has enlisted the cooperation of the U. S. Army Chemical Corps, through a government interagency agreement, because of the Chemical Corps' considerable success in developing techniques for the sterilization of unusual objects ranging from delicate laboratory equipment to rugged 6 by 6 Army trucks. The Russian Government, apparently, is similarly concerned. It was announced over Radio Moscow that the probe which

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the Russians landed on the moon just prior to Khrushchev's visit to this country had been sterilized.

Three questions should justifiably be asked concerning the biologists' contention that no living organisms should be transported to the moon or the planets, and that even nonliving organic matter so transferred be minimal. First, why should objects launched from earth which might hit the moon or one of the planets be free of terrestrial life forms? Second, granted that they should be, would not all life forms be automatically killed in passage because of the rigors of interplanetary space? Third, if the answers to the first and second questions indicate that all objects which may intercept extraterrestrial bodies should be sterile prior to launching, can this sterilization be accomplished without adding crippling restrictions to the space exploration program? The answers to these three questions require some discussion.

Why Contamination Must Be Avoided

The first question which concerns the desirability of avoiding accidental contamination of extraterrestrial bodies with terrestrial forms of life, is intimately related to an unsolved scientific question of fundamental importance, that of the origin of life itself. According to the old but now more or less discredited panspermia hypothesis of Arrhenius, spores of living organisms drifted through space and seeded suitable planets upon which they came to rest. Most present-day biologists believe, however, that life might arise independently on any world where suitable physical conditions have existed for a sufficiently long time. Resolution of these divergent views requires evidence of a sort that might well be provided by the moon and the planets, as long as their present biology remains unaltered until it can be investigated.

By many, for example, the moon has been thought to have existed for several billion years as an airless, barren body with no biology of its own, but capable of sweeping up the debris of outer space and preserving it in its nooks and crannies, whence it can be recovered and examined to see if any of this interstellar material shows evidence of organic origin. Sagan and Firsoff, on the other hand, contend that simple organic compounds or even life may have arisen on the moon (4, 6). Whatever may be the true situation, if the moon's surface becomes contaminated with living microorganisms

from earth, or even with considerable amounts of organic debris from earth, before the "moondust" (2) has been examined, a priceless opportunity to assess these possibilities may be lost. If all microorganisms or biological material carried to the moon were to remain within a limited area around the point of impact, this danger of contamination would be of less concern, for impact areas could then be easily identified and avoided in biological studies. Unfortunately, however, this would probably not be the case. Because the moon is almost totally lacking in atmosphere, particles ejected by a hard landing would encounter too little frictional resistance for their velocity to be appreciably diminished. Hence, even particles as small as bacteria might be expected to land anywhere on the moon's surface, even on the far side, depending upon their initial trajectory.

With Mars, and possibly with Venus, the concern over possible contamination is even greater. To the best of present knowledge, terrestrial microorganisms might not only survive on these planets but might find nutrient, thrive, and multiply. Indeed, bacteria did multiply when introduced into chambers that reproduced the conditions believed to prevail on Mars (7). Furthermore, spectroscopic evidence and seasonal color changes on Mars lead scientists to believe that some form of life, possibly similar to low forms of terrestrial vegetation, already exists there. If so, man may soon for the first time be able to examine in their native habitat life forms other than those which arose on earth. Extreme caution is needed, however, to ensure that Martian life, if it does exist, is not destroyed or irreversibly changed before it has been studied.

Just over 100 years ago Darwin pointed out the constant struggle for the survival of the fittest which goes on between all life forms occupying the same environment. The perilous knife-edge balance maintained by such competing forms is never more evident than when a new form is suddenly introduced and a new balance must be attained. The results of the transfer of rabbits from Europe to Australia and of Japanese beetles from their homeland to the eastern seaboard of the United States are well-known examples. Another example is the unrelenting effort of crab grass to invade and overwhelm the bluegrass of American lawns. It has been stated (3) that, to prevent the possibility of any such biological accident, no probe that allows as much as a one-in-a-million chance of landing a viable organism on the planetary surfaces should be launched toward either Mars or Venus. It has also been pointed out (5) that if it is possible

for earthly life to infect neighboring planets, the reverse is also true, and that here much more is at stake than the loss of an unparalleled opportunity for scientific investigation. In the not too distant future, interplanetary quarantine regulations may become even more necessary than present national and regional regulations.

The first of the three questions posed above has thus been simply answered. All earthly forms of life must be kept away from nearby celestial bodies to avoid jeopardizing, if not altogether losing, the unique chance to gather reliable data on possible extraterrestrial life. Nothing that will be done in the next decade or so, with the possible exception of creating from fuel exhausts a trace atmosphere on the moon, could permanently affect these bodies in any way except biologically. Even if, for example, good measurements of the gravitational field of the moon are not obtained this year or next, this force will remain unchanged a decade (or a century) hence, no matter how many probes have landed meanwhile on its surface. We have no such assurance concerning the biology of these bodies. Their biology, and indeed the biology of the earth as well, may be changed irreparably, and in a comparatively few years, unless unusual caution is exercised.

Resistance of Life Forms in Space

If one grants that it is desirable to prevent contamination, the second question then needs to be answered. Won't the sterility required be automatically achieved by passage of the vehicle through interplanetary space? This is by no means certain. The conditions believed to exist in outer space were recently discussed by Newell (8). In the 1958 Leeuwenhoek Lecture before the Royal Society, Keilin (9) carefully reviewed the resistance of various life forms to harsh environmental conditions when in a state of suspended animation, or kryptobiosis, to use the term he coined. A comparison of the information in these two documents can lead only to the conclusion, which has been presented before in considerably more detail (3), that spores or other earth life forms could indeed survive such a journey. Briefly, the penetrating radiations of outer space are not of sufficient intensity to assure sterility. The ultraviolet radiation is intense enough, but so easily shielded that only organisms uncovered on the surface of the space vehicle would be exposed. Cold, even down to a slight fraction of a degree above absolute zero, has no

lethal effect (8, 10). Heat is lethal, even if the resistance of organisms in an evacuated dehydrated state is greater than we have supposed (11), but the temperature within space vehicles is carefully controlled at more or less room temperature so that the instruments will perform satisfactorily. This temperature can be maintained with remarkable accuracy merely by having the surface of the vehicle contain a predetermined ratio of reflecting and absorbing areas.

Vacuum has no deleterious effect on microorganisms, at least as far as it has been measured, although admittedly experiments with extremely high vacuum, paralleling those of Becquerel (10) with extremely low temperatures, have not been performed. As for the hazards in landing on an extraterrestrial body, the momentary heat and pressure of a high-velocity landing on a hard surface should not exceed the levels achieved for brief fractions of a second in explosions, which bacteria have survived, nor would the atmosphere of Mars or Venus necessarily consume a space vehicle coming in at high speed, as micrometeorites are heated and consumed in the earth's atmosphere. In short, only by sterilizing space vehicles before they leave the earth can it be assured that living earth forms will not be transported to other celestial bodies.

Sterilization Techniques

The third question now requires an answer. Are relatively simple techniques available whereby space vehicles may be sterilized? The answer appears to be yes. Heat, radiation, and chemical sterilization techniques in various modifications are of proved efficiency. The choice of the method for space vehicles is governed by two considerations: (i) when and where must the treatment be applied, and (ii) will the treatment damage any part of the vehicle? It will be seen that although all of these sterilizing techniques in one form or another may be used on individual components, the final treatment given the fully assembled vehicle will, almost of necessity, be chemical, with the sterilizing agent in the gaseous state.

One of the first considerations which leads to this conclusion is the dual aspect of the sterilization problem. Not only must the vehicle be sterilized but it must be kept sterile until it has left the earth's atmosphere and started on its lonely journey through space. The second half of this problem could well be the more difficult technically. After the final treatment the vehicle must not be touched,

handled, or moved unless completely sterile technique is observed in these manipulations; otherwise it becomes recontaminated. Sterile handling techniques have of necessity been evolved, as in hospital surgery for example, but at best they are extremely tedious, and even with well-trained personnel, accidental breaks in sterile technique often occur. The difficulties involved in sterile handling can be largely avoided if the final sterilization treatment is given at the last possible moment before launching, after the vehicle has been placed into position and thoroughly tested. This implies a treatment which can, if necessary, be carried out in the cramped quarters atop a launching gantry. It would be difficult indeed to place the necessary amount of cobalt-60, for example, with its accompanying shielding material, in such a location that the assembled payload would receive a sterilizing dose of gamma radiation. Applying sufficient heat to sterilize the payload in such a position would also be almost prohibitively complicated. Moreover, if any single component of the space vehicle could not withstand the necessary amount of heat or radiation, it would be impossible to shield that component while treating the rest of the payload. Chemical sterilization with ethylene oxide gas, however, can be applied almost as conveniently at 120 feet in the air as at ground level. Fewer types of materials are damaged by this technique than by any other known sterilization method (12). If some component should prove to be sensitive to ethylene oxide, moreover, it could be sterilized prior to assembly, by another technique, and shielded from subsequent ethylene oxide exposure simply by building a gas-tight barrier around it. Anything incased in metal or certain plastics would be protected.

Although ethylene oxide sterilization is a relatively new development, there have been an increasing number of new applications of this technique within the past decade. The method is slow, requiring up to six hours' exposure time, but it is effective with many types of materials and objects that would be hopelessly damaged by other methods of sterilization. The gas diffuses readily through many types of porous materials but cannot, of course, penetrate hermetically sealed areas. When mixed in proper proportions with fluorinated hydrocarbons, the product is nonflammable (13), and can be packaged in convenient light-weight metal cans. Although elaborate automatic ethylene oxide sterilizing equipment is now available commercially, particularly for hospital use, the method can be adapted for use in extremely simple exposure chambers; for example, a simple polyethylene bag tightly closed at the neck serves adequately

as a device to contain the gas (14). Within such a plastic container, sterilization is achieved at ambient temperatures and relative humidities, and at essentially ambient pressures, since the bag can expand as the liquefied chemicals volatilize. The same technique can be used to sterilize objects of any size or shape by building about them a bag or tent of heat-sealed plastic sheeting and admitting the sterilizing mixture into this container. At concentrations of about 300 to 400 milligrams of ethylene oxide per liter of air and at room temperature, sterilization will occur in six hours or less (14).

The question of how and where to place the plastic covering could well be answered separately for each space vehicle, since no two are likely to be identical in design. How the cover is to be removed before launching and how the space vehicle will be kept sterile once the cover has been removed must also be considered. In many cases, sterilization of other items, not just of the space vehicle itself, must be considered. For example, if the design is such that the last rocket stage will follow the space vehicle rather than return to earth or be shunted off elsewhere into space, it too must be sterilized. Perhaps it might be best to consider one typical example and discuss in general terms how the procedure might work in this case.

A Hypothetical Case

Such a typical example might be a space vehicle atop a third-stage rocket, with both of them covered by a nose cone or fairing. The chief function of the fairing is to furnish environmental protection on the ground and, more particularly, during flight though the earth's lower atmosphere. The fairing in this assumed case would open and fall back to earth after the vehicle had reached a height (200,000 feet, for example) at which air pressure would be too low to damage the vehicle passing through it at high velocity. The third stage, we can assume, would separate from the space vehicle but would follow it throughout its flight and would be expected to impact any body upon which the vehicle itself might land. In such a hypothetical case, a sheet of plastic large enough to extend out beyond the fairing could be placed underneath the third-stage rocket during assembly. When the final check had been made on the assembly, a complete covering could be made by sealing other sheets of plastic to this base sheet, enveloping the third stage, vehicle, and fairing. Ethylene oxide gas would be admitted into this inclosure and, since

the fairing would not present a hermetically sealed barrier, the gas would sterilize the space vehicle and the third stage as well as the fairing itself. After six hours' exposure, the plastic sheet could be cut away and removed, and the ethylene oxide gas would be dissipated in a matter of minutes. Before launching, the outside of the fairing would almost certainly become recontaminated but it would act essentially as a petri dish cover does, preventing airborne organisms from entering underneath it and recontaminating the space vehicle or the third stage. To keep objects cool while the vehicle is still on the ground, conditioned air is sometimes blown under the fairing. This air can be kept sterile by the simple process of passing it through bacteria-tight filtering material, such as cotton, asbestos, or spun-glass fibers. Filters of the necessary efficiency are readily available. Upon launching, when the vehicle would be rising through the lower atmosphere, the fairing would protect the sensitive components of the payload against the heat and mechanical effects of atmospheric friction. With proper design, the fairing would continue to furnish biological protection in flight as well as on the ground, again acting like a petri dish cover. The sterile air under the fairing would diffuse outward as the atmosphere became less and less dense, and nonsterile outside air should not contact the probe. When the fairing was discharged, at the outer fringes of the atmosphere, the probe would be high enough in the air so that unshielded ultraviolet rays from the sun would prevent surface contamination from then on, if any microorganisms exist that high.

It was mentioned above that if the last-stage rocket or other equipment was also expected to impact on the lunar or planetary surfaces, these sections as well as the payload itself should be sterilized. Some preliminary calculations have indicated, however, that an extremely simple sterilization technique might be available for such material, which is required to function only during take-off and which is essentially inert thereafter. It was also stated above that temperature within the space vehicle itself could be carefully regulated by controlling the relative amounts of absorbing and reflecting areas on the surface. If the odd bits of metal that are not required to perform except during take-off had surfaces which were entirely adsorptive--that is, surfaces that were painted black--they might well, at rates dependent upon their particular geometry, slowly become hot enough in outer space so that they would be sterile before impact.

Once the decision has been made to perform the final sterilization with ethylene oxide at the last possible moment before

launching the probe, one can proceed backward and design a probe suited to the ethylene oxide treatment, just as one designs the probe to withstand the forces of acceleration or vibration to which it will be exposed during launching.

Design Considerations

Two design considerations are involved in constructing a probe to be sterilized with ethylene oxide. First, the design engineers should test all the materials, such as paint and adhesives, that will be used in construction, to satisfy themselves that the treatment will cause no damage. Secondly, the designer should see to it that the vehicle contains no hermetically sealed areas -- areas that cannot be reached by the gas -- unless the interior of such areas has been sterilized before sealing or can be sterilized by other techniques after sealing. Once any such component is sealed, the interior cannot be recontaminated, since bacteria cannot enter any space inaccessible to the gas. There is no concern about external recontamination, for this will be taken care of in the terminal sterilization process.

For example, welded aluminum tubing may be used as the basic framework for a space vehicle. Once this tubing has been welded together, no gas can penetrate the interior. The design engineer in this case would have two simple ways of making sure that microorganisms would not be transported inside this framework. He might bore a series of small holes in the framework, which would allow the sterilizing gas to enter, without reducing the strength of the framework. Or, more simply, he could easily heat the framework in an oven to sterilizing temperatures before he attached any heat-sensitive material to it.

This concern over possible contamination in hermetically sealed areas is based on the consideration that in a crash landing on a hard surface, the space vehicle might shatter completely. Any organisms that might be present in such inaccessible locations would be released. Bacteria might survive such a crash landing far better than metal or plastic objects. Thus, the requirement is not only that all accessible surfaces of the object should be sterile but that no viable organisms should be entrapped within the object which would be released if it were broken apart. Examples other than welded tubing of components in which organisms might be entrapped beyond

the reach of a sterilizing gas are individual electronic components, assemblies of electronic components that have been encased or potted in plastic, tight metal-to-metal surfaces (particularly those held together with a sealing mastic), and aluminum or plastic honeycomb sheets.

Exploratory Experiments

Certain practical experiments have been conducted recently at Fort Detrick to gain information on problems such as these. The theory behind such studies is that, although each space vehicle may well be distinctly different from all others, it will of necessity be constructed from a limited number of components and materials. These components can be studied separately, to see if they already carry within them living microorganisms as received from the manufacturer, or if assembly techniques will further entrap living microorganisms. The practical experiments along these lines have been, to date, largely exploratory. They are reported here merely to show how the problem of designing a space vehicle which will be sterile internally and externally is being attacked.

The experiments were performed in a sterile environment inside an airtight, transparent plastic chamber (Fig. 1) whose inner contents and surfaces could be sterilized with ethylene oxide gas and then flushed with filtered sterile air. The equipment is very similar to that recently developed for conducting germ-free animal experiments (15) or for use as bacteriological safety cabinets (16). The cabinet is typical of the sealed plastic covering that will be utilized in the terminal sterilization treatment of the assembled space vehicle.

In all cases, ethylene oxide-fluorinated hydrocarbon mixtures were used at ambient temperatures and admitted into areas not previously evacuated, displacing air so that the operation was carried out unpressurized. Six hours' exposure was adequate to insure sterilization in such a cabinet. It should be pointed out that cabinets of this type could also be used for sterile handling and assembly of certain components, should it prove more advantageous, or even necessary, to sterilize certain individual components, assemble them in a sterile atmosphere, and then seal them in a unit, rather than to sterilize after assembly.

The first tests were concerned with various electronic components -- primarily transistors, capacitors, resistors, transformers, diodes, and the like, all small sealed units. The question was whether these units were manufactured under conditions which permitted the entrapment of viable organisms in their inner areas, where ethylene oxide could not penetrate.

The test procedure involved placing inside the chamber the electronic components to be tested, together with sterile broth blanks, forceps, hammers, mortars and pestles, a metal hammering block, metal saws, pliers, and a can of ethylene oxide-fluorinated hydrocarbon mixture. The chamber was closed, and the ethylene oxide was released to sterilize the exterior of all the items and the atmosphere within the chamber. After a six-hour exposure of the chamber to ethylene oxide, air sterilized by filtration through a cotton filter was passed through the chamber for 16 hours to remove all ethylene oxide gas.

Two of each type of electronic component being investigated had been placed in the chamber. After the ethylene oxide treatment, one component from each pair was placed, whole, in a broth blank. These served as controls and indicated that the exterior surfaces had indeed been sterilized by the ethylene oxide treatment. The other component was then sawed, hammered, or otherwise broken open and ground up as much as possible, and the pieces were placed in another broth blank. These broth blanks were sealed, removed from the chamber, incubated for seven days, and then examined for cloudiness which might indicate bacterial growth. The bottles were then opened and aliquots of the broths were streaked on agar to check further for bacterial growth. The broth of each cloudy blank was also examined microscopically to check for bacterial cells. Anaerobic bacteria could grow in the broth but would not grow on the agar surface. Thus, only a microscopic examination would confirm the presence of these bacteria.

Following this, each broth blank was seeded with approximately 100 cells of Staphylococcus aureus, and after incubation an aliquot of this broth was streaked on agar. This last step was taken to assure that the blank was still capable of supporting microbial growth even though no growth had occurred in it when the electronic component was added. Positive growth in this step indicated that the material of which the component was made was not bacteriostatic or bactericidal.

Typical results obtained in these exploratory tests are given in the following table:

Electronic components with internal contamination

Type of component	No. contaminated: No. tested
Transistor	1/17
Capacitor	13/62
Resistor	6/41
Diode	0/2
Transformer	1/1

As is evident, transistors as a general class are more likely to be sterile internally than are capacitors, although at least one non-sterile example was found in each class. Considerably more investigations of this type will be necessary before definitive answers can be given concerning the biological status of all types of electronic components. Such data are now being routinely collected. Also, the ability of these components to perform satisfactorily after they have received various types of sterilization treatment is under investigation.

After various electronic components have been assembled into an electronic device, they are often potted or imbedded in plastic to give the object greater mechanical strength. When metal-to-metal contacts are made, plastics are also often used to strengthen the bond. A mastic may be used about screw threads, for example. Investigations have shown that if certain dry microorganisms of a hardy type are incorporated in the plastic monomers, they are quite capable of surviving the polymerization process. When the hardened plastics were treated with ethylene oxide, the surfaces were sterile, but living microorganisms were recovered when the plastics were sawed or cracked open. How long these bacterial spores would survive imbedded in plastic is yet to be determined, but again the resistance of certain life forms to harsh treatment is well exemplified. It appears that incorporation of a small amount of disinfectant, such as paraformaldehyde, in the plastic base may solve this problem. But this again emphasizes that, although the answer to the third question posed is in the affirmative -- space vehicles <u>can</u> be sterilized --

it is affirmative only if attention is given to the sterilization requirement in all stages of design and construction.

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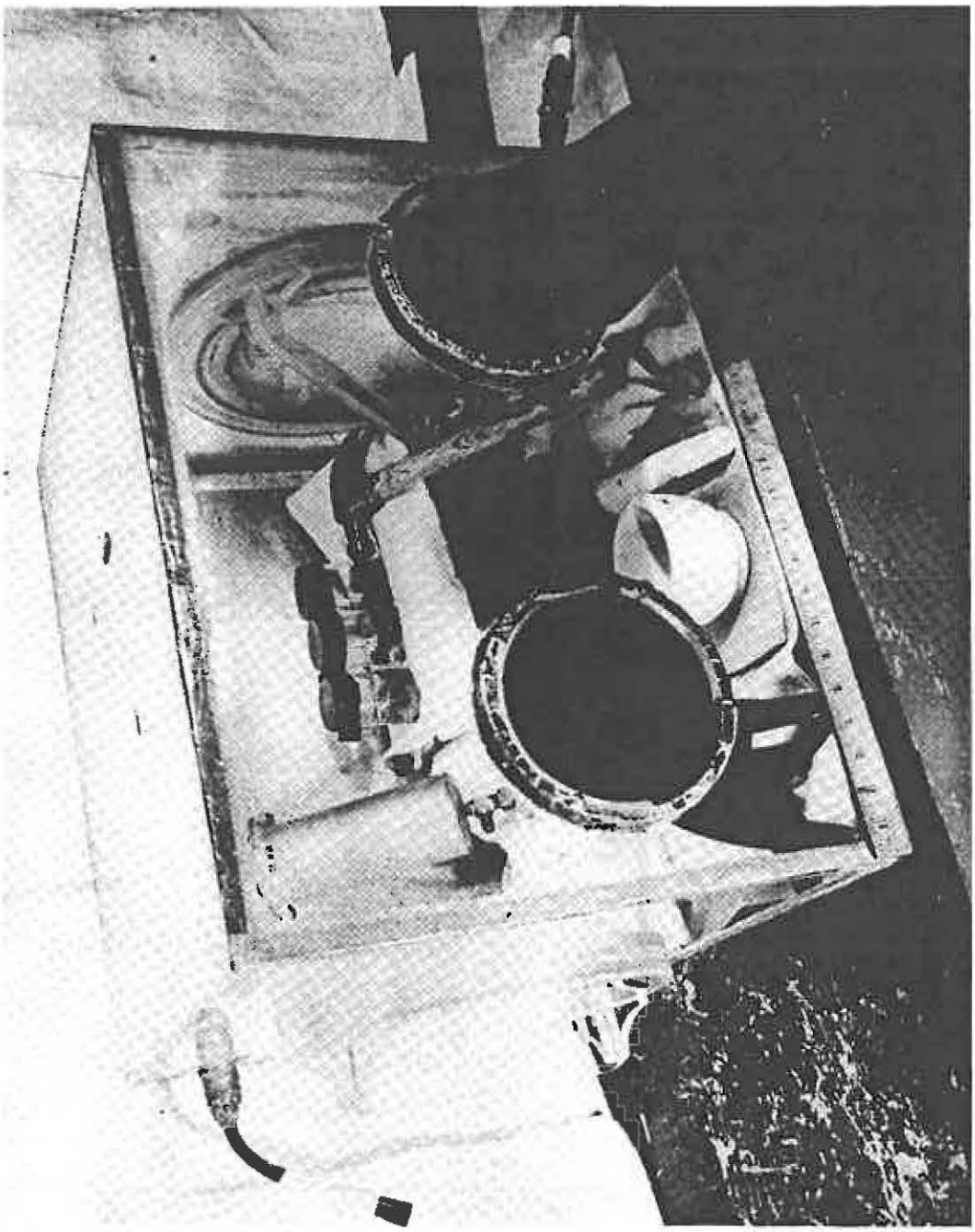

Figure 1.- Airtight plastic chamber. L-60-6977

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