NASA SP-108

SPACECRAFT STERILIZATION TECHNOLOGY

BECKMAN AUDITORIUM

Pasadena, California

November 16-18, 1965

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

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Foreword

UPON THE RECOMMENDATION OF THE SCIENTIFIC COMMUNITY, the National Aeronautics and Space Administration has established as a major goal the search for extraterrestrial life. So that the data obtained in this search will not be compromised, NASA has embarked on a program designed to prevent external contamination of the planets.

This National Conference on Spacecraft Sterilization Technology was convened to bring together for exchange of knowledge those scientists, engineers, and administrators who play a role in spacecraft sterilization technology. Recognizing the rapid accumulation of knowledge in this area of scientific endeavor, it was deemed essential to broadcast this information as rapidly as possible, to establish a firm base upon which newer techniques could be built, and to provide a forum in which differences could be aired and guidelines revised or established. The Conference was hosted by the Jet Propulsion Laboratory, California Institute of Technology, and was coordinated by the American Institute of Biological Sciences. Special acknowledgment should be given James R. Miles for his contribution to the success of the Conference.

Conference participants were invited to discuss the progress of work undertaken in their respective areas of interest. These areas include the NASA sterilization requirements; microbiological control and monitoring, and sterilization techniques applicable to spacecraft components, payloads, and facilities. More than 30 papers were presented during the 3-day conference which represented the combined efforts of the academic institutions, the aerospace industry, NASA, and its Centers. Thus, this volume represents the comprehensive technology information, research, policies, and goals available relating to this subject through the fall of 1965. The compilation of this volume will serve as a medium of information dissemination to the more than 700 persons who were in attendance at Pasadena and to the remaining thousands of persons directly or indirectly associated with the spacecraft sterilization program.

Lawrence B. Hall

Planetary Quarantine Officer, Bioscience Programs
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Sterilization Requirements

CHAIRMAN: Homer E. Newell

Associate Administrator for Space Science and Application, NASA

KIMBALL C. ATWOOD
University of Illinois

Basis for the Sterility Requirement

The necessity for sterilization of spacecraft, space probes, or any other objects that are intended to or may by accident land at an extraterrestrial location will now be discussed. There are two facets to this subject: (1) the scientific gains that may not be realized if some significant effect of contamination of the extraterrestrial sites should occur, and (2) the probability that any significant effect will occur. The current view is that the scientific gains that might be lost are of very great value. We cannot put an actual value on them, but if we could and could multiply it by the probability of a significant effect occurring, which is a very small value, we would obtain a result that justifies the action that we are taking for spacecraft sterilization — a responsible action that cannot be discontinued until we have complete confidence that no harm would be done.

First let me discuss the second facet of the subject, that of the probability that any significant effect will occur, in the context of Mars. First of all, we can distinguish significant contamination from slight contamination. That is to say, the surface area of Mars is very large, and if we dropped some organisms there that merely distributed themselves and remained but did not multiply, the chance that we would later pick them up, that they would confuse experiments intended to detect indigenous life, or that they would change the natural environment is, of course, infinitesimally small and unimportant. We therefore can conclude that it is necessary for organisms to multiply if contamination is to be significant.

It could be argued that, in a place where the environment is very hostile and to which terrestrial organisms are not well adapted, if they grow at all they would grow very slowly. Indeed, in the case of Mars, this sounds reasonable, since it is very cold and, even at best, it does not have the microenvironment where one might expect to find the conditions to be those to which contaminants from Earth are initially well adapted. However, if an organism had a generation time of 1 month, which we would rate as rather slow, it would take only 8 years to cover the entire surface with 1 g/sq cm of organisms. Of course, this is a preposterous idea, because most of the surface shows considerable variation and most of it is obviously

unsuitable for multiplication of organisms, but it does show that slow multiplication is not exactly a safeguard.

Now, after the organisms are landed on Mars, assuming that they are, they would not only have to multiply in order to make a significant contamination but they would also have to spread. If they multiplied in one spot and never spread to any other, future investigation of the planet would reveal that other locations had whatever the indigenous condition was, and the spot contaminated would probably not be revisited by chance. If it were revisited on purpose, we could draw the correct conclusion relative to how organisms of terrestrial type happened to be found there.

One approach to the question of whether organisms could spread is the simulated-environment experiment. The trouble with the simulated-environment experiment is that we do not know precisely the range of environments that it is necessary to simulate; we know only the average environment. The simulated average environment, it turns out, does not allow the multiplication of organisms that have been experimentally tested in it, and therefore we can make the assumption that, although some terrestrial microorganisms will survive, none of them can multiply. On this assumption, the establishment of a population would require the introduction of the organisms into a suitable microenvironment, and the presence of suitable microenvironments is a perfectly open question.

This, of course, makes the significant contamination somewhat less probable because the micro-organisms have to land in just the right place. Multiplication would be more or less delayed, depending on the circumstances and the accessibility to the special environments. No matter how direct the initial introduction, multiplication will be a selective process; that is to say, in the case where the spacecraft contains several species of contaminants, all the different ones certainly would not have an equal chance to survive. The subsequent spread one could imagine taking place would be by direct extension, in case the local environment goes out as a network of some sort, by wind, or on indigenous organisms. The direct extension is limited by geographical barriers; I think the Mariner IV pictures show quite clearly that in that varied terrain there would be no chance whatever for direct extension of micro-organisms from an inoculant point.

The spread by wind is a more likely basis for planetwide distribution of contaminants, but there are several difficulties with this. The first one is the rate at which organisms become windborne. If in order to grow they must be at one of the rare places where some water exists on Mars, then it would be less likely that

they would become windborne than if they were in a place devoid of water. We know that duststorms occur on Mars. The dust rises from the light areas and is deposited on the dark areas where, strangely enough, it seems to disappear within a short time. We therefore should not expect the areas that are most likely to be subject to transport by wind to be the same areas at which contaminants might be expected to be concentrated. In addition, windborne organisms or any organisms that are on the surface are exposed to the solar flux which is essentially unshielded and ultraviolet, and this would definitely hinder the spread by wind. Of course, one could say that the organisms can be attached to opaque particles or that dust clouds could be effective shields against the ultraviolet rays. This is a valid uncertainty, inasmuch as the dust may consist of iron oxides, which are extremely opaque to ultraviolet; however, only a fraction of the organisms could be surrounded by opaque material and hence be able to survive indefinitely. On the whole, though, the obstacles to the spread of micro-organisms on Mars seem to be very severe indeed.

Another point is what would happen if Earth contaminants were picked up in an assay of the Martian matter. When sterilization of spacecraft was first suggested several years ago, I believe the question of mistaken identity of contaminants for indigenous organisms during a search for life there was the thing that was uppermost in people's minds. Biology has changed a good deal since that time and now we have an even more fundamental reason for wanting to investigate the point but perhaps a less urgent reason for worrying about whether there are terrestrial contaminants present. Let us examine the differences between the situation if all the organisms present are contaminants and if they are indigenous ones.

It is obvious, first of all, that the number of species introduced will be small because the sources of contamination during assembly of the vehicle are nonrandom with respect to species. An unsuccessful attempt at vehicle sterilization has a selective effect and further attenuates the number of species that would be transported. On the other hand, one suspects that if the biota are native to the landing place they will comprise very many types. One also suspects that organisms would evolve in any places that are appropriate to the variety of ecological opportunities. For example, an absence of autotrophs would be very suspicious indeed. The terrestrial autotrophs, I think, would be quite unlikely to be contaminants, and yet the indigenous flora, if you want to call it that, should, simply on thermodynamic grounds, contain a biomass

of autotrophs larger than that of heterotrophs, so that it has a steady-state cycle with solar energy.

The question of the identification of the possible exobiota should be considered. The identification of the exobiota as members of an Earth taxon would prove that they were adventitious and also that they were recently introduced, recently, that is, in the scale of planetary evolution. This statement applies to a question on the main value of investigating extraterrestrial sites which will be discussed later in this paper. Most of the evolution of any Martian organism may have occurred, at least in the early stages, from matters of necessity rather than chance. We are most anxious to discover which stages during life's origination are questions of physical necessity and which are matters of chance. Even if the early stages are necessity rather than chance, no organism could fit into a lower taxonomic category now present on Earth that had evolved for most of its time elsewhere. We know this simply because sufficient barriers exist here to test it with terrestrial organisms and any taxonomist can tell the difference between organisms that have spent most of their evolutionary history under quite different conditions. Moreover, if there are native indigenous organisms on Mars they should be present under the surface in the permafrost, if there is any, and generally distributed, in contrast with a very superficial distribution of the recent contaminants.

Finally, we could consider what would happen under contamination to the surface composition of the planet which had gone through the chemogenic stage in which the conditions were right for abiogenesis, or origination of life, but where, for some reason, this had not occurred. We now have a deposit of the "soup," or the Oparin sea, organic compounds on the planet, and no life there. One could imagine that contamination under those conditions would greatly alter such a surface. In the case of Mars, of course, the surface composition would show little alteration, since a great deal, or even most, of it will still be preserved in dried, frozen, or buried regions. The trouble with this analysis is whether it is feasible to apply such criteria from the distance that we find ourselves from that site. It would be quite easy in an Earth-based laboratory to make such decisions. If you are asked to find out whether you contaminated the Gobi Desert, I am sure you could easily do so, but the criteria are extremely difficult with life detection devices and devices capable of doing biological experiments at the level of bit return that we may expect from Mars. There are two ways of looking at the results of contamination of Mars: What harm would it do at first and what in the long run? It is obvious that a long

period of confusion will result before these results are determined if the contamination is significant.

I have made a case that the probability that contamination would be significant is quite low for Mars. Now, let me make the case that if contamination were significant, it would do a great deal of harm to the scientific gains that we expect to make in biology. As mentioned previously, the biological interest that Mars holds for us does not depend on what conditions are there at the present time; it merely depends on the early history of the planet. That is, did the planet pass through a stage when the surface conditions were such as to make possible the origination of life? Some argument about that has been seen recently because of the Mariner IV results. The question may be answered by the age of the surface. If the people who think that it is 2 billion years old are right, then probably the conditions did not ever exist for abiogenesis. On the other hand, if the people who think it is between 300 and 800 million years old are right, then, in all probability, the conditions did exist since this would mean that there has been an extensive erosion of craters. This erosion implies processes, such as more water on the surface than there is now, that would have favored abiogenesis. If we do not find that some form of life actually originated, then we might find the remnants of the organic syntheses that occurred in a prelife era, and these would be extremely interesting indeed. The point I wish to make is why it seems so important to biologists to obtain and to be able to examine extraterrestrial organisms, if any exist.

To sum this up, the reason for the importance of extraterrestrial organisms to biologists is that, beyond a certain point, we have no examples of the intermediate stages in the evolution of terrestrial organisms. It has become apparent in the last 2 or 3 years that every terrestrial organism, without known exceptions so far, shares the use of what we can call a genetic translation system. This is a very complicated system in which the genetic code, which is in nucleitide sequence, is caused to specify an amino acid sequence. It does not matter that viruses do not have a translation system because they use the translation system of their host cell; the point is that there are no organisms which have a defective system or one which represents an intermediate stage in the evolution of this system. This system is so complicated that it clearly must have required a very long time to evolve, and I will describe it briefly.

The system has 64 soluble ribonucleic acid (RNA) adaptor molecules, each one of which is for one of the 64 possible codons. (There may be only 60, it is probable that there are 64.) For each

one of these there is a specific enzyme which recognizes some site — the adaptor molecule which recognizes the amino acid that is the right one to go on that particular soluble RNA molecule. There is a mechanical tape-reading device, the ribosome, which itself is rather complicated and contains ribonucleic acid of two molecular axes, and an unknown, although probably small, number of kinds of protein. The ribosome, the adaptor or ribonucleic acid, the series of transfer enzymes which put the right amino acid on, and then, finally, one or more enzymes involved in the polymerization of the amino acid make up the complete system which needs all its parts to function correctly. Obviously, if it needs all its parts to function correctly and there are so many parts that they could not have assembled themselves spontaneously, the question arises as to how such a system could have evolved. This is an example at a biochemical level of problems that were faced in the 19th century on a morphological level. For example, Darwin was presented with the problem of how the electric organ of the torpedo ray could possibly exist. It could not always have existed because it is a very complicated thing and must have required a long time to evolve by slow stages. On the other hand, before it was essentially fully evolved, it would not work, and if it did not work then it would not have been of any use to the animal so it would not have been subject to the natural selection processes to produce it. The same thing is true with the genetic translation system; if it did not work, then there would be no force selecting it, and yet it has to have all its parts in order to work. It turned out that there was a solution to Darwin's problem, and, obviously, there must also be a solution for the genetic translation system.

For example, it is obvious that biopolymers were made nonenzymatically at some early time. We do not know how this was accomplished because we have been unable to make them nonenzymatically in any significant way or to make them specify each other nonenzymatically in the laboratory, but it may be that we just have not found the right conditions. It is obvious that the translation system went through a stage of not being as perfect as it is now. Probably it made a lot of mistakes, and amino acids were put in as members of a group, rather than as individual ones distinct for each code triplate. During this process, one can imagine that some of the events that happened were determined by stereochemical considerations and others were determined by chance. We have no idea at present of how to unscramble this problem. The chance to examine a form of life that we know went from its earliest times, or that we have very strong reason indeed to believe has grown out from its earliest times, in an extraterres-

trial location, and therefore does not share a common ancestor with any form of life now known, would be one of the most exciting things that any biologist could hope to have happen. It would give them a very important clue as to the scope of the forms of life and as to which formative events depended on chance factors and which depended on stereochemical factors.

Finally, I would like to comment on what criteria we could imagine as being important enough to permit relaxation of the requirement to sterilize spacecraft. Of course, a lot may be learned about the Martian surface before a landing that actually may make a difference. The Mariner pictures were of very little help to most biologists that I have talked to; all they showed was that there is a varied terrain. There is no evidence that there are not places that are interesting biologically, but we have to look with better resolution and with certain other things in mind. For example, are there local warm spots, deep crevices, or sheltered places? If one could be satisfied that no favorable microenvironments for terrestrial organisms existed or whether there are indigenous ones, one might consider relaxing the sterilization requirement, especially if, at the same time, we had already looked at the indigenous organisms. If it could be proved that the surface does not contain any interesting collection of organic compounds, or if evidence could be obtained that would make us confident that the history of the planet did not include any such early period for abiogenesis, it might be concluded that the chemogenic era did not proceed as it supposedly did and we might consider relaxing the requirement for sterility. However, all these criteria are really quite difficult to attain.

I want to suggest the somewhat different approach: that we should try to get evidence that no organisms that are like terrestrial organisms are present. This is a fairly lengthy argument, and I want to go through it in some detail. In the first place, one of the main points that has been previously considered in discussing life-detection instruments is that it is very difficult to know whether the instrument proposed would detect an exotic form of life. It may be simple to identify terrestrial forms of life, but on Mars one would always be worried about the correctness of the medium, the proper gestation temperature, or the possibility of assaying for the wrong biochemical reaction. Consideration of these problems led to the idea that it might be better to put some sort of enclosure over the ground, simply add a little water, presumably what is most lacking on Mars, and see whether anything happens than to try to grow organisms in an Earth-proved medium, since there is no way of guessing what the most

beneficial conditions for a Martian organism may be. In other words, it may be best to treat the environment gently and not do things that are the product of terrestrial experience.

I think that there is a case to be made for doing something a little different from now on. First, obviously, if there is significant contamination it will be picked up with instruments that are good for detecting terrestrial organisms because the significant contamination is, in fact, with terrestrial organisms. (We do know how to make instruments that are perfectly good at detecting terrestrial organisms.) If these instruments show persistently negative results after landings in various places, one could deduce that if there are any autoecious indigenous forms they will at least not grow under the same conditions as the terrestrial ones because they have not been picked up in the life-detection instruments. This may suggest further that there are not any Martian environments which are enough like the terrestrial ones to permit the terrestrial organisms to gain a foothold. This might give us some degree of confidence in deciding to relax the sterility standards where it is necessary to do so for some reason.

Of course, this leaves open the problem of how to proceed with biological exploration on a biochemical basis if we know that we cannot detect any Martian biota with instruments capable of detecting terrestrial life. Of course, we may discover a means; it may be that when we have good pictures of high resolution, something will turn up. It is an interesting problem, and unless it is solved we will be returned to having to guess what life is like in a place where we really have no idea what to look for.

In conclusion, we can regard the necessity for sterilization as the product of a very big and very little number. One is the scientific importance of extraterrestrial life, if it exists, or of the conditions for its formation, if they occurred, and the other is the probability that contamination of the place will interefere with our quest. A stratagem which may possibly be useful in arriving at some means of relieving the terribly hard requirements for sterilization would be the extensive use of life-detection instruments that are not especially designed to detect extraterrestrial biota but will detect terrestrial-like organisms in an extraterrestrial environment.

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The Role and Responsibility of NASA in Relation to Spacecraft Sterilization

The program of the National Aeronautics and Space Administration is broad, reflecting the magnitude and diversity of its mission and the many motivations which led to its creation — a Federal agency to establish and maintain an adequate place for the United States in the exploration of space. The National Aeronautics and Space Act of 1958 establishing the agency declared that U.S. activities in space "should be devoted to peaceful purposes for the benefit of all mankind." The act specifies certain objectives which clearly present the agency's "peaceful purposes," and they present a challenge to the mind and to the hand of man not before equaled in history.

In addition to, but in no degree less important than, the scientific objectives of the exploration of space is the fact that the exploration of space in itself — regardless of what we seek, or how we seek it, or even what we eventually discover — is an opportunity for man's most glorious adventure.

Three years ago, in a paper presented at the NASA-University Conference on the Science and Technology of Space Exploration and entitled "What We Have Learned and Hope to Learn From Space Exploration," I said:

> It does not appear likely that there are living forms on the surface of the Moon, because of the lack of an atmosphere, the lack of any observable water, and the extreme temperature ranges to which the lunar surface is subjected. Some believe, however, that there might be living forms existing at some distance below the hostile lunar surface. But even if there are no living forms on the Moon, other biologists point out that the Moon is still of interest in that it may carry the residue of previously living forms or possibly material that is in the nature of precursors to life. Controversy rages on this issue, with some scientists categorizing this reasoning on the part of the biologists as absolute nonsense. But the biologists can counter with the observation that if they should be right, walking all over the Moon with dirty feet, or plastering it with dirty material, can destroy a once-in-forever opportunity to make exbiological studies that may have great bearing on our understanding of terrestrial life.

At any rate, it seems clear that we must be careful about what we do in the case of Mars. A suggestion was made at the recent Space Science Summer Study conducted at the State University of Iowa by the National Academy of Sciences under NASA sponsorship, that Mars be made an ecological preserve, where steps are taken to protect the planet from undesirable contamination. According to the suggestion, Mars would be investigated in such a manner as to protect the interests and needs of the biologists who wish to search for and study any living forms or traces of life that might exist there. This proposal also included the suggestion that, although Venus and the Moon not be considered as ecological preserves, care be taken to minimize their contamination.

One might mention in passing, that those who are concerned about possible contamination of our neighbors in space through the introduction of terrestrial organisms, also point to the possible danger of back contamination of the Earth by the introduction of extraterrestrial organisms. Careful thought must be given to this problem, and in due time appropriate steps taken to remove any risks that are judged unacceptable.

NASA was developed and designed to meet the objectives set up by the National Aeronautics and Space Act of 1958. The major parts of its organization are shown in figure 1. The Administrator and his Deputy are assisted by a number of staff offices, including those for Legislative Affairs, General Counsel, Policy Planning, Technology Utilization, Public Affairs, and International Affairs. Line authority, however, flows to the Associate Administrator who has responsibility for the three major operating offices: the Office of Manned Space Flight, the Office of Advanced Research and Technology, and the Office of Space Science and Applications. The latter, the Office of Space Science and Applications, is directly concerned with the sterilization of unmanned outbound spacecraft,

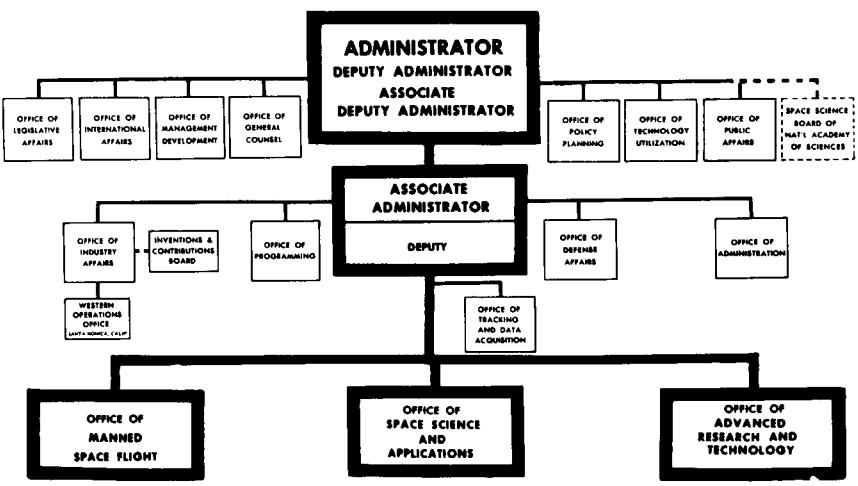

FIGURE 1.—NASA organization.

with the Associate Administrator of the OSSA specifically charged by management directive with responsibility for this matter.

The direction of the scientific policies and guidance of NASA's programs are not always fully understood by the space industry and the public. The agency does not base decisions on these matters solely on the knowledge and capabilities within the organization but has taken steps to obtain consultation and guidance from the foremost scientists available. The agency sought, from the first days of its existence, the valuable assistance of the National Academy of Sciences — National Research Council. Established within the Academy is the Space Science Board, chaired by Harry Hess of Princeton University. Its 15 members are leading scientists of the country. NASA provides funding for the Space Science Board, supplying full information on NASA planning and budgeting and enjoying the advantage of advice on scientific problems and investigations from the Board. In order to make this exchange effective, several members of NASA's top management meet monthly with members of the Space Science Board to discuss current problems. Members of the Board also serve on other NASA advisory committees, achieving a crossflow of information.

Although the Space Science Board does not take part in the actual decision making for NASA, its competence and scientific authority quite definitely influence NASA decisions. NASA's programs in space science match closely the recommendations of the Space Science Board.

Within NASA, the Associate Administrator for Space Science and Applications is responsible for the NASA Space Science Program, which includes a broad range of ground-based and flight experiments. Specifically, it is his responsibility to —

1. Define the scientific goals
2. Recommend to the Associate Administrator for approval the necessary supporting research and technology, as well as ground-based and flight programs to accomplish these goals
3. Delegate to appropriate offices responsibility for the planning and execution of approved programs and the public dissemination of acquired knowledge
4. Delegate to appropriate offices responsibility for the planning and direction of the overall scientific aspects of the OSSA program

To discharge these responsibilities, the Office of Space Science and Applications is organized into a number of Program Offices, as shown in figure 2. These programs include Bioscience, Communi-

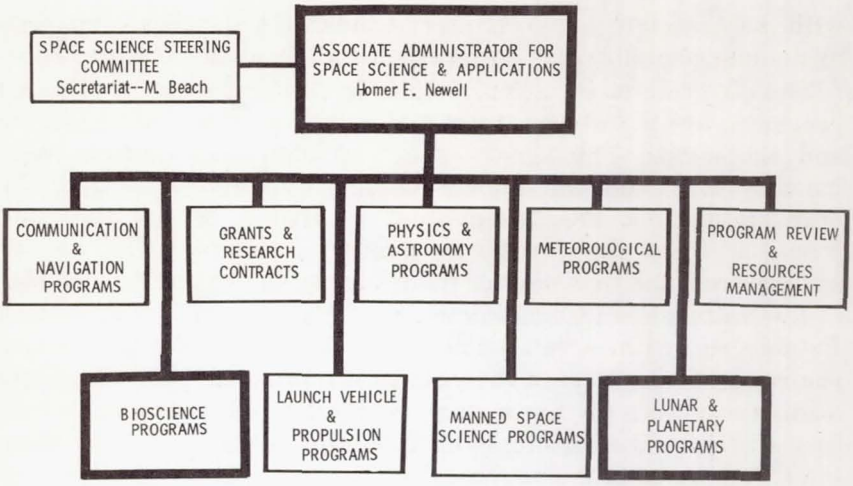

FIGURE 2.—Organization of the Office of Space Science and Applications.

cation and Navigation, Lunar and Planetary, Meteorological, Physics and Astronomy, and others.

Each program director, with his staff, is responsible for activities in clearly specified areas of space science. In carrying out its functions, the Program Office —

1. Establishes the scientific objectives in its area and determines the resources required to meet these objectives
2. Formulates and recommends the necessary short and long-range science or applications program
3. Recommends necessary flight missions and corresponding investigations and investigators
4. Supports and coordinates the research and development work of the grantees, contractors, and NASA field centers in executing approved programs and missions
5. Insures dissemination of scientific results in accordance with policy

The great mass of scientific detail is much too large to be surveyed, except in broadest terms, by the Space Science Board. To provide adequate consideration of the questions that arise and to insure that members of the scientific community outside NASA participate in its programs, the Space Science Steering Committee and its advisory subcommittees have been established. This structure has half its members from outside of NASA and yet, in part, functions along lines parallel to the established NASA administrative organization. The Director of Sciences, Office of Space

Science and Applications, is the Chairman of the Space Science Steering Committee, and he reports to the Associate Administrator for Space Science and Applications. The subcommittee chairmen, all of whom are staff members of the Office of Space Science and Applications, report directly to the Chairman of the Space Science Steering Committee.

Advisory subcommittees exist for Astronomy, Biosciences, Ionospheres and Radio Physics, Particles and Fields, Planetary Atmospheres, Planetology, and Solar Physics. Chairmen of the subcommittees are selected from the OSSA Division Offices, but the membership is composed of scientists from universities, from NASA and other Government agencies, and nonprofit organizations. These individuals are selected on the basis of professional competence in specific areas of science. They incorporate their experience, knowledge, and ability into recommendations related to NASA's space science activities. Further, because of the strong technical ties, the impact of the opinions and attitudes of the subcommittees quickly reaches the operating programs and is clearly reflected in their program direction.

The Space Science Board in the last few years has repeatedly and publicly pointed out the importance of the search for extraterrestrial life. The report of a study by the Space Science Board in 1964 states that —

> the biological exploration of Mars is a scientific undertaking of the greatest validity and significance. Its realization will be a milestone in the history of human achievement. Its importance and the consequences for biology justify the highest priority among all objectives in space science—indeed in the space program as a whole.

NASA and its Office of Space Science and Applications concurs in this position, recognizing that additions to the basic knowledge of life may have far reaching, though as yet undreamed of, benefits to mankind. Failure to place adequate emphasis on biological aspects of the planetary exploration program could result in failure to gain priceless knowledge awaiting only those who seek that knowledge with wisdom enough to insure against destruction of the opportunity.

Such destruction may well occur if, before biological explorations can be completed, the biota of the planets is intermixed. In certain minor respects this is the situation that now exists on the Moon. Early in the space program the scientific community urged that the Moon be kept free of contamination by terrestrial life, and U.S. lunar missions were to be sterilized. Unfortunately, the technology of sterilization had not then sufficiently progressed to insure that

our own vehicles (and possibly those of other nations) did not contaminate the lunar surface.

In the light of this situation, the scientific community reexamined the problem based on our newly acquired knowledge of the Moon and agreed that biologically clean — if not sterile — spacecraft could safely be landed on the lunar surface. This decision was based on the assumption that no life exists on the lunar surface, that terrestrial life cannot reproduce in that harsh environment, and that such terrestrial contamination as may have reached the Moon and may have, perhaps, penetrated below the surface will remain localized.

Despite this reasoning, terrestrial contamination of the Moon may yet prove to be a problem. When the first samples of lunar material are returned to Earth by manned flight, any evidence of life detected will pose serious questions on the character of the microbial life found. Identification of that life as being of terrestrial or extraterrestrial origin will require time, delaying release of the lunar samples for detailed examination of their physical characteristics. The greater the terrestrial contamination of the Moon, the greater the probability of confusion.

To minimize this potential problem and to meet the requirements of the scientific community, it is NASA's policy to launch lunar landing spacecraft with as small a microbial burden on board as can be attained without serious prejudice to the success of the flight. The Surveyor, Lunar Orbiter, and IMP spacecraft are being cleaned during assembly by the most efficient known techniques which remove approximately 90 percent of the existing contamination. Much of the remainder of the viable life will "die away" during the last 20 days before launch while housed in Class 100, downward laminar flow, clean facilities. Launch operations and transit through the Earth's atmosphere will be designed to prevent any serious degree of recontamination.

Fortunately, there is time available to develop sterilization technology for planetary exploration. Inevitably, however, if traffic between the planets increases with the years, as now seems probable, there will be a transfer from one planet to another and reproduction of the alien life will occur if the environment permits. The time when the planets are infected by Earth life can probably be delayed until at least the early, instrumented, and unmanned biological detection payloads have been landed. Indeed, the landings of the early biological experiments must have upon them no terrestrial life or those experiments will be valueless. Such a terrestrially contaminated experiment would detect the Earth life it carried with it. Thus, since we at NASA are serious about the

biological exploration of the planets, the flights of exploration must present a minimum hazard to the opportunity that will exist only once in all the history of space exploration. To this end, it has been, and is, NASA's policy to use as goals the constraints recommended by the Space Science Board and by authoritative international bodies as they now exist and as they may be revised in the light of new knowledge gained from space exploration or from more exact analysis of the problem.

At the present time these goals require that the probability of a single viable organism being aboard a spacecraft intended for Mars landing, or penetration of the Martian atmosphere, be less than 1×10^{-4}. Similarly, the probability of an accidental impact or atmosphere entry by an unsterilized flyby will be kept to 3×10^{-5}, or less. These goals are being used, and will be used, as the basis for the development of specific operational procedures. Unfortunately, although the goals are easily established, it is another matter to prove directly either that the numbers defining the goals have genuine validity or that the goals have been attained. Standard procedures to achieve the required goals have been established for the sterilization *per se*, but the proof of attainment of sterility must be by inference based on knowledge of the efficiency of the sterilization environment to which the capsule has been subjected.

An even more difficult situation faces us in the matter of insuring that nonsterile spacecraft do not impact the planet. Goals, in terms of probability, have been established and every effort is made to comply with these goals; but it will never be possible to prove that they have been attained. Further, standard procedures cannot be used because every mission and trajectory vary from all others. NASA will, nevertheless, strive diligently and conscientiously to meet the established goals by observing the following guidelines:

1. Every possible available means will be utilized to insure that all factors which might contribute to the total Mars contamination probability are considered
2. Probabilities which are assigned to each individual factor will be determined by experiment, testing, or experience whenever possible and practical
3. If probabilities for certain factors cannot be determined by experiment, testing, or experience, conservative methods will be used for statistical calculations of these probabilities
4. The mathematical model used for final combination of probabilities for individual factors will be based on standard statistical procedures
5. NASA adopts the estimate that the preliminary biological

exploration of Mars will have been accomplished by the year 2021. Missions need not be sterile if they are designed to be flown in Martian orbits that will not decay and will not, either in whole or in part, enter the planet's atmosphere before 2021.

The problems presented by these necessary policies are legion, and many are difficult to solve. In dealing with these problems, NASA management takes the position that successful biological exploration of the planets is a goal that supersedes in importance any program, flight, or schedule that would endanger the eventual acquisition of knowledge of extraterrestrial life. We ask that all individuals and groups concerned with the problems bend their efforts not only to the success of the interplanetary flights, but also to the success of the scientific objectives of these flights.

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NASA Program Scope and Definition

The program of the National Aeronautics and Space Administration for Planetary Quarantine is defined in NASA Management Manual Instruction 4–4–1 of September 9, 1963. The document relates only to unmanned spacecraft designed to fly past, orbit, impact upon, or enter the atmosphere of the Moon or the planets. NASA regulations pertaining to the Moon, as implemented by this document, are based on the very low probability of terrestrial life reproducing on the surface of the Moon and on the presumption that subsurface contamination will remain localized. Although it is NASA policy to protect the Moon from widespread or excessive contamination, the intention is not to require sterile landings, but to try to keep contamination within reasonable bounds.

In the case of the planets, NASA policy acknowledges the possibility of extraterrestrial life and recognizes that terrestrial contamination would make investigation of extraterrestrial life difficult, if not impossible. NASA regulations, therefore, require that unmanned flights to Mars be sterile.

The basic responsibility for this program lies with the Associate Administrator for Space Science and Applications; in the discharge of this responsibility, he provides overall direction and certifies the sterility of missions to the Administrator of NASA. Within the Space Science and Applications Office, the responsibility has been delegated to two units: the Lunar and Planetary Programs Office and the Bioscience Programs Office.

The organization of the Bioscience Programs Office is shown in figure 1. Note that Planetary Quarantine is placed at a staff level, above the operating programs, in order to give it some degree of autonomy and authority over the individual program offices that might be dissuaded by other motivations from observing the sterility requirements. The responsibilities of the Bioscience Programs Office include defense of the scientific basis for sterilization;

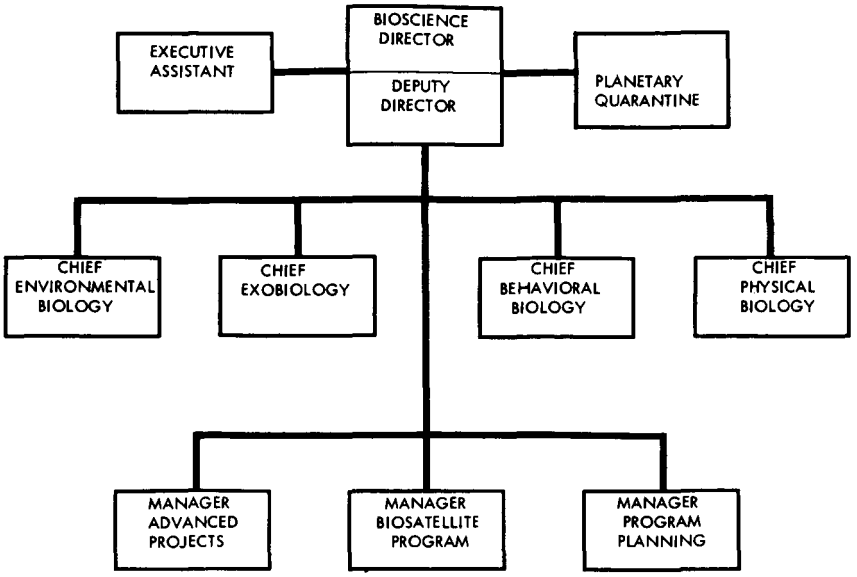

FIGURE 1.—Organization of Bioscience Programs Office.

development of techniques for sterilization; definition of the reliability of these techniques; conduct of bioassays; monitoring of all phases of manufacture, assembly, and launch; approval of procedures proposed by the Lunar and Planetary Programs and certification of sterility to the Associate Administrator for OSSA.

The organization of the Lunar and Planetary Programs Office is shown in figure 2. Responsibility for the research of this group is centered in Advanced Projects and Technology. Most of this work is directed to the evaluation or development of sterilizable flight hardware. Decontamination operations are the responsibility of the mission programs, Surveyor, Lunar Orbiter, and Mariner; sterilization operations are the responsibility of the Voyager program. The overall responsibilities of the Lunar and Planetary Programs under this management arrangement are to develop sterilizable spacecraft; insure that nonsterile spacecraft do not impact the planets; propose facilities, techniques, and procedures for approval by the Bioscience Programs Office; develop, build, and operate sterilization equipment; and perform all sterilization operations.

The present work of the Bioscience Programs Office on sterilization includes a large effort on Supporting Research and Technology. Support of this effort is shared between Planetary Quarantine and Exobiology. The program currently includes definition of the heat cycles, improved methods of bioassay, reduction of the

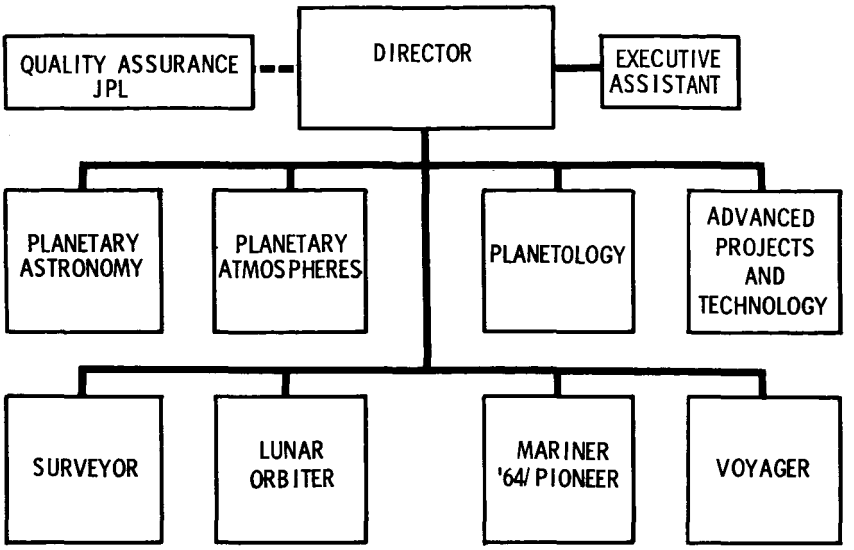

FIGURE 2.—Organization of Lunar and Planetary Programs Office.

spacecraft biological burden, survival of terrestrial life in Martian atmospheres, the identification of highly resistant organisms, and the development of sterile insertion and repair techniques.

The monitoring program of Planetary Quarantine is now being established. For the Lunar missions, Headquarters Planetary Quarantine personnel and laboratories will provide the necessary monitoring services. For planetary missions, such as Voyager, a much more thorough monitoring program will be required, with primary reliance placed upon the fabricator of the capsule during manufacture and assembly phases. Laboratories and personnel, certified by NASA, will be needed for this function. To achieve maximum assurance that the monitoring is accurate, the fabricator's monitoring activities will be inspected by personnel of the cognizant Center and the work of both will be reviewed by a Headquarters Sterility Control Group. The Sterility Control Laboratory, operated for NASA by the U.S. Public Health Service in Phoenix, Ariz., will serve as the final reference authority on microbiological aspects of the monitoring program. The capsule will be given continuous surveillance and monitoring by Center and Headquarters personnel from the beginning of the sterilization cycle through launch.

An increasingly important factor in the Planetary Quarantine Program is training; for there are, at the present time, too few persons trained or experienced in environmental microbiology to staff this and other essential programs. This effort is taking the

form of a fellowship program that will support four engineers or physical scientists for 1 year of graduate study and a series of 1-week short courses designed to give engineers and members of allied disciplines a general acquaintance with environmental microbiology. To supplement these and other programs, training aids are being developed for on-the-job training, particularly in control of the biological burden.

The Planetary Quarantine organization needed to carry out these functions is shown in figure 3. Lawrence Hall occupies the

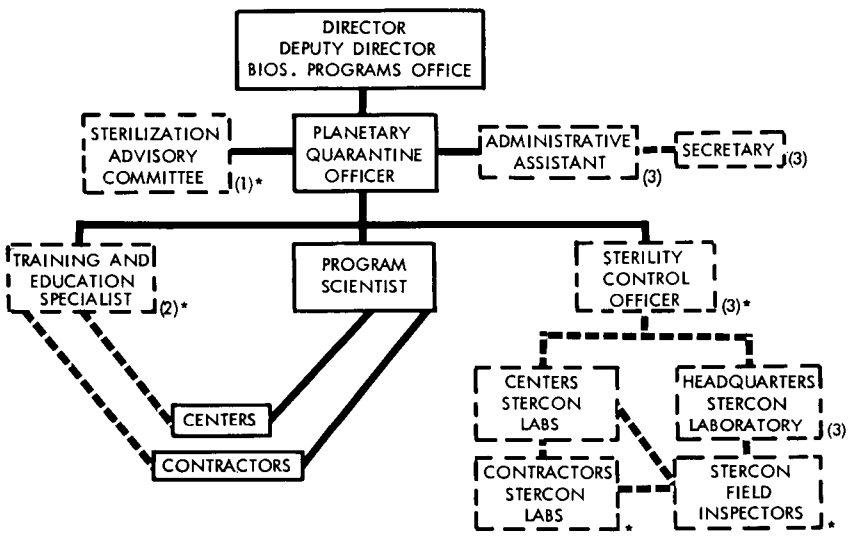

FIGURE 3.—Organization of Planetary Quarantine Office. (1) indicates NASA contract with American Institute of Biological Sciences; (2) indicates NASA contract with George Washington University; (3) indicates NASA contract with U.S. Public Health Service. An asterisk indicates that contract is under negotiation.

position of Planetary Quarantine Officer. Advising him on scientific matters pertaining to sterilization is a Sterilization Advisory Committee, currently being established under the chairmanship of Richard Bond, School for Public Health, University of Minnesota. The position of Program Scientist, occupied by Carl Bruch, is responsible for the conduct of the Supporting Research and Technology effort. The position of Sterility Control Officer is occupied by Sanitary Engineer Director Jack H. Fooks, on detail to NASA from the Public Health Service. The Training and Education Specialist position is filled under contract with George Washington University by Donald Wright. The program is carried out

through support of NASA centers, grants to universities, and contracts with industry.

The operations of the Planetary Quarantine Program are not, of course, confined to the Bioscience Programs Office alone. The lines of contact with the other OSSA Offices, Programs, and Centers are diagramed in figure 4. Working contacts are direct and informal,

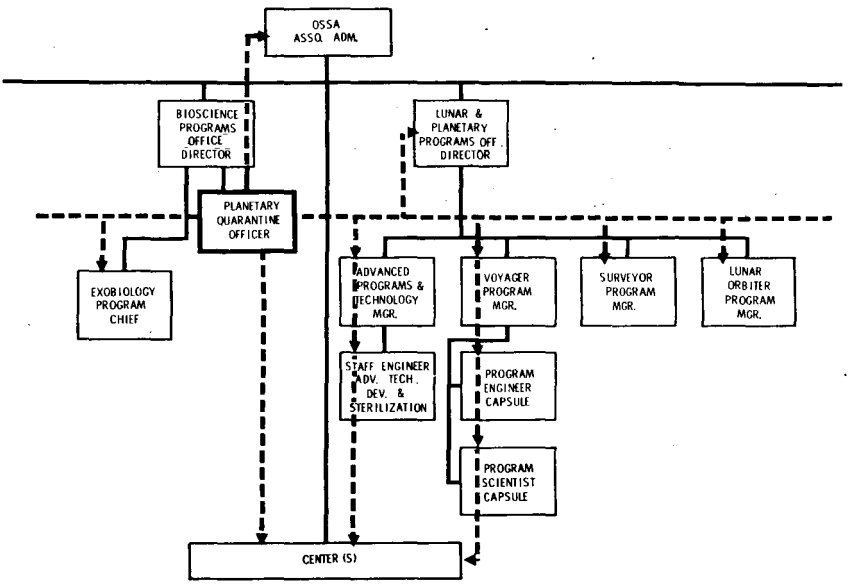

FIGURE 4.—Organization of the Office of Space Science and Applications with respect to Planetary Quarantine.

with only major agreements and policies being formalized in writing and passed through channels for approval.

The important work of the Lunar and Planetary Programs Office in Supporting Research and Technology includes development of sterilizable batteries, tape recorders, vidicon tubes, and many other components. These will be reported in detail in Sessions IV and V of these Proceedings. Up to the present time, this work has been conducted under the Supporting Research and Technology label; but the Voyager project takes over responsibility on July 1, 1966, for all tasks of direct interest to the Voyager mission. The Advanced Projects and Technology section of the Lunar and Planetary Programs Office will continue to study alternate approaches and longer range tasks in this program.

The NASA Centers are assigned specific tasks, most of them being assigned to the Jet Propulsion Laboratory, but also to the Marshall Space Flight Center, the Ames Research Center, the

Langley Research Center, and the Goddard Space Flight Center. These Centers either do the work in their own laboratories or contract the tasks to industries, universities, or nonprofit institutions. In general, Headquarters handles directly most grants to universities and to nonprofit groups, while the cognizant Centers handle contracts with industry, including those on direct project-oriented tasks as well as supporting research in advanced development on nonproject tasks.

This discussion of the NASA program and methods of operation may be useful in evaluating the technical discussions in this area.

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NASA Requirements for the Sterilization of Spacecraft

Before entering into a discussion of sterilization requirements or guidelines, it is desirable that we agree on terminology. A few of the more critical terms can be defined as follows:

Planetary quarantine, in NASA usage, means prevention of the transfer of life between planets and between planets and the Earth's Moon. For the immediate future, at least, it is restricted to problems associated with unmanned spacecraft, outbound from the Earth to the planets. The methods used to prevent transfer of life may include both the killing of all life on board the landing capsule as well as measures to insure that contaminated spacecraft do not impact on the planets or enter into their atmospheres.

Sterilization, on the other hand, includes only those measures that kill *all* life on the surfaces and inside the solids of the landing vehicle. Its companion word, "sterility," is an absolute term. It either is or it is not. There can be no such thing as "almost sterile" or "nearly sterile." Due to specific shortcomings of our usually adequate English language, there appears to be no single, convenient word that is a counterpart to "sterilization," which defines those measures insuring avoidance of impact of a nonsterile object. Frequently, in the recent literature, "sterilization" has been pressed into use, improperly, to include this latter meaning. In reading the literature on this subject, one must be alert to avoid confusion.

The word *decontamination* is also occasionally misused and more often misunderstood. It means a reduction of the quantity of living material to a lower level, but not to zero, which would be sterility. Decontamination is frequently a prelude to sterilization, but the meanings of the two words are quite different.

To avoid confusion, let us understand that for the Voyager program the word *capsule* refers to the package that lands on the planet, whereas *canister* is used to designate the sealed barrier that protects the capsule from recontamination during launch and passage through the Earth's biosphere. *Spacecraft* is used to

designate the nonsterile vehicle and bus that do not enter the atmosphere of the planet. However, in describing other missions such as Ranger and Surveyor, in which there are no flybys and no separate landing capsules, the more all-inclusive word "spacecraft" is used to designate the landing hardware.

With these definitions in mind, let us examine the basic requirements for planetary quarantine and sterilization. The foundation for these requirements by the National Aeronautics and Space Administration rests upon the policies that have been adopted by NASA to meet the requirements of the national and international scientific communities. Thus, NASA has accepted as goals, subject to further study and revision if justified, the

> Sterilization level such that the probability of a single viable organism aboard any spacecraft [capsule] intended for planetary landing or atmospheric penetration would be less than 1×10^{-5}, and a probability limit for accidental planetary impact by unsterilized flyby or orbiting spacecraft of 3×10^{-5} or less.

These probabilities of contamination, then, are the bases for the present guidelines — procedures needed to achieve these goals. It must be emphasized, however, that for NASA these goals and guidelines are not final and immutable. The scientific basis of the goals is under continuing study. Should justification be found for relaxation of the goals and national and international scientific community agreement be attained, the goals will be revised. Similarly, the procedures required to meet these goals are under study. The present guidelines are based, wherever possible, on known quantities but where the values cannot be determined with accuracy, the *"worst case"* situation must be assumed. As systems analysis studies are completed and as research in microbiology and reliability of space hardware provides further answers, the "worst case" situations will be replaced by known and, we hope, more favorable values. There is, therefore, reason to expect that the guidelines for sterilization may be eased in the future; but until those developments take place, plans must be based on the sterilization guidelines as they now stand.

Before detailing the requirements for sterilization, let us review the requirements for the Moon. The precautions against the contamination of the Moon, once strict, have now been relaxed in view of our developing knowledge of the inhospitable environment for terrestrial life that exists on the lunar surface and the belief that landed contamination, if it survives, will remain localized. For these reasons, lunar landing spacecraft may have on board a low level of microbial life — they must be decontaminated, but not sterile.

Therefore, the requirements, the least rigorous that will meet the objectives, are as follows:

Primary reliance for decontamination shall be placed on the die-away of micro-organisms over a period of time and shall be accomplished and supplemented by one of the three methods that follow:

Method A:

1. Initial assembly of spacecraft shall be in clean rooms designed, constructed, and operated at the level of cleanliness required by reliability considerations.
2. Final assembly, test, checkout, insertion of pyrotechnics, and launch shall be accomplished in spaces conforming to the NASA Laminar Flow Clean Room Specification. The total period of enclosure of the spacecraft in these clean facilities before launch shall be not less than 20 days (T − 20).
3. During any transport between clean facilities, the spacecraft shall be protected from contamination by wrapping and handling in a manner that shall prevent the accumulation of dirt, dust, and micro-organisms.
4. During the period required for item 2, every effort shall be made to avoid contact between the spacecraft surfaces and human skin or respiratory excretions.
5. Available surfaces of the spacecraft shall be wiped with sterile cloths moistened with sporicides approved by the Bioscience Programs Division, but in no case shall such treatment be used if it endangers the success of the mission.

Method B:

1. Initial assembly of spacecraft shall be in clean rooms designed, constructed, and operated at the level of cleanliness required by reliability considerations.
2. Air filter banks in the air-handling systems in the facilities in which the spacecraft will be housed in preparation for launch shall meet the requirements of Federal Specification 209 for high efficiency particulate air (HEPA) filters. They shall be tested in place with dioctyl phthalate (DOP) smoke detected by a Sinclair-Phoenix Photometer.
3. The spacecraft shall be covered whenever active work is not underway.
4. HEPA filters, tested in place by the procedure outlined in item 2, shall be used as final filters in the air supply to the shroud.
5. The spacecraft, before enclosure in the shroud, will be

cleaned as thoroughly as practical by the use of vacuum cleaners and by rubbing accessible surfaces with a cleaning solvent where such treatment will not endanger the reliability or thermal properties of the spacecraft.

6. After closure, capsule air temperature shall be kept at 85° F or above and relative humidity at not less than 45 percent for not less than 5 days, or more if consistent with flight constraints.

Method C:

Decontamination by exposure of the spacecraft to ethylene oxide in a concentration of 300 milligrams per liter of space for 4 hours, preceded by exposure for 72 hours to more than 35 percent relative humidity, may be used as complete or partial substitution for the procedures of method A or B.

To insure the biological effectiveness of these procedures, it will be necessary to monitor the spacecraft visually or photographically during its preparation for launch. Bioassays will also be required at frequent intervals during this period. Assay procedures will be in accord with standard methods of spacecraft bioassay currently being developed (paper in press entitled "Standard Methods for Bioassay of Spacecraft").

Requirements for the sterilization of planetary landing capsules are, of course, much more rigorous than those for decontamination. The general plan includes four major phases:

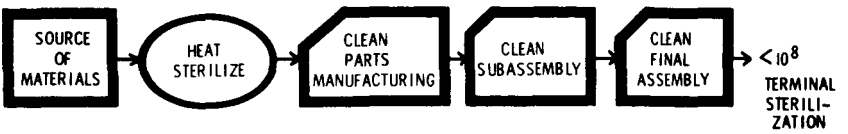
1. Development of sterilizable capsule hardware
2. Limitation of the quantity of viable biological loading
3. Application of terminal sterilization
4. Protection of the sterile capsule from recontamination through launch

Phase 1 is the subject of sessions IV and V of this conference and is discussed fully in those sessions.

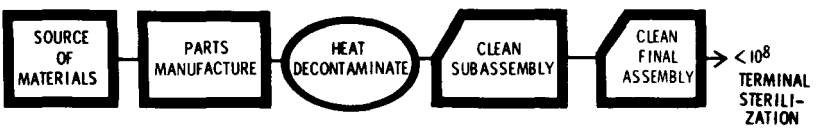
Phase 2, however, has been the subject of much research and concern. The viable burden on a capsule has been estimated to be as high as 10^9 organisms or more. The maximum that planning has permitted for the terminal sterilization cycles considered is 10^8 organisms with the expectation that it will be possible to assemble a spacecraft with fewer contaminants.

There are several alternative methods of limiting the viable burden on a capsule. Selection of the final method will probably be based on reliability factors. Because of reliability considerations, it has in the past been accepted that the capsule could be heated once and once only. To achieve an assembled device under this constraint would mean heat sterilization of the raw materials to kill

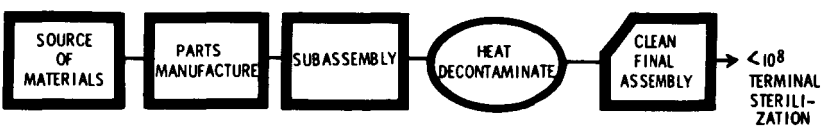
any internal life, followed by clean-room or clean-bench manufacture, subassembly, and final assembly to keep the final microbial burden to a reasonably low level before terminal sterilization. This sequence is diagramed in figure 1(a). It is quite clear that this procedure would pose an enormous and expensive problem to maintain the necessary levels of biological cleanliness in the multitude of plants, laboratories, and assembly areas that supply the thousands of parts making up the final capsule.



(a) Clean handling throughout.



(b) Heat decontamination of parts, clean subassembly.



(c) Heat decontamination before clean final assembly.

FIGURE 1.—Limitation of biological loading.

A second alternative to the difficult and perhaps impossible task just described would relax the restriction against double heating to use low-level heat decontamination in lieu of clean room parts manufacture. In this plan, diagramed in figure 1(b), parts would be manufactured under normal industrial conditions or under the clean conditions required for quality assurance. They would then be heat treated at low levels to kill the internal contamination and would be sealed in sterile packaging. These decontaminated parts would be used to make up subassemblies followed by final assembly, both in clean facilities.

This plan would markedly reduce the complexity of the problem and the number of clean facilities needed. Because representative samples of the parts, relatively small in size, could be assayed for contamination, strong control could be exercised; and there could

be assurance of the quantity of the biological burden on and in the capsule at the time of terminal sterilization.

A third plan is also under consideration that would permit parts manufacture and subassembly to be conducted under normal space hardware conditions. Because the subassemblies would be too large and complex to assay with any degree of accuracy for biological burden, low-level heat cycles cannot be depended upon to decontaminate to a satisfactory level. However, the need for bioassay of subassemblies can be avoided if heat can be applied to the subassemblies sufficient to assure a reduction of viable organisms from the "worst case" situation to a total level that can be handled by the terminal sterilization cycle. In actual practice it may be decided for purposes of quality assurance that a flight acceptance heat cycle, equal to or in excess of the terminal sterilization cycle, may be applied to the subassemblies. Whichever high-level heat cycle is used, the decontaminated subassemblies would be assembled in a clean room. This plan, diagramed in figure 1(c), would greatly simplify the task of producing a spacecraft with minimum viable contamination at the time of terminal sterilization. In fact, if very careful final assembly measures are used under this method, it may be possible to produce sufficient assurance of a small enough microbial population to justify an appreciable reduction in the terminal sterilization cycle.

From the foregoing it is evident that the sterilization guidelines are undergoing constant re-evaluation and adjustment in order to meet satisfactorily the goals of sterility at the lowest program cost. at the present time, however, and subject to revision as additional knowledge is gained and applied, the NASA sterilization guidelines for planetary landing hardware are broadly as follows:

Guideline 1:

(A) Clean rooms used for control of biological contamination on NASA spacecraft or capsules shall be of the laminar flow type, air movement from ceiling to floor, and shall conform to Federal Specification 209, Class 100. Construction guidelines will be supplied.

(B) Personnel access to the clean room will be restricted to those with proven need. Positive means of control of personnel traffic shall be exercised.

(C) All persons in the clean room shall wear deflection or other types of masks and barrier clothing of a type approved by the Planetary Quarantine Office.

(D) No person, regardless of need except to save life in an emergency, shall enter the bioclean area if he has upon his body any open sores, has readily noticeable dandruff, has a cold, a fever, or is suffering from diarrhea.

(E) Portable laminar flow clean rooms may be used, with the approval of the Planetary Quarantine Office, where circumstances require.

(F) Laminar airflow workbenches may be used. Such workbenches shall conform to the requirements of Federal Specification 209.

(G) Sterile surgical rubber gloves or sterile disposable plastic gloves shall be used to handle decontaminated spacecraft parts.

(H) Only decontaminated tools shall be used in connection with decontaminated parts.

Guideline 2:

Biological loading in and on a spacecraft shall not exceed 10^8 viable organisms at the time it is brought to terminal sterilization if the heat cycles specified are to be applicable. If there is evidence that the viable contamination is any greater than 10^8, additional time of heating or higher temperatures shall be required.

Guideline 3:

Heat may be used for internal decontamination in place of ultraclean manufacture and assembly under certain conditions.

(A) *Assayable Items*

Any of the low-level heat cycles given in table I may be used in lieu of ultraclean room assembly for hardware items whose dimensions and physical characteristics are such that they can be satisfactorily bioassayed.

TABLE I. — *Decontamination Heat Cycles*

Temperature, °C	*D*-value,[a] hr	Minimum decontamination time, hr
100	30	120
110	12	48
120	4.5	18
130	1.7	7
135	1.0	4
145	.38	1.5
155	.14	.6
160	.09	.4

[a] Based on the thermal death-time curve (with extrapolations) for spores of *Bacillus subtilis* var. *niger* embedded in plastics.

Items which, at present, are assayable are:

(a) Small piece parts (such as resistors, capacitors, diodes, circuit boards, relays, connectors, small vacuum tubes, squibs, transistors, thermocouples, and microcircuits).

(b) Wires, cables, and most varieties of homogeneous bulk materials (such as lubricants, conformal coatings, pottings, adhesives, bulk liquids, stored gases, and solid propellants) which can be bioassayed on a sampling basis provided samples can be selected that are truly representative.

(c) Simple subassemblies, which can be disassembled into a very few parts, each of which is assayable. Examples of this are relays, accelerometers, and so forth.

The authorization to use low-level heat as the decontamination procedure for assayable parts and materials is subject to the following:

(a) Bioassays are to be performed, both before and after the decontamination procedure, on representative samples.

(b) Where the predecontamination assay indicates the initial internal load to be greater than 1×10^4 organisms, one *D*-value of heating time shall be added to the decontamination cycle for each log or fraction of a log of initial contamination beyond 1×10^4 organisms.

(c) If decontamination is performed at a location other than at the point of final assembly of the capsule, shipment shall be made in approved biological barriers.

B. *Nonassayable Items* (or assayable with low limits of confidence)

Major effort is now underway to improve the technology of bioassay. However, at present, complex subassemblies and some bulk materials cannot be satisfactorily assayed. Heat may be used to decontaminate such items, provided that *D*-values of heating time are applied to cover the possibility that any such item may have a high internal microbial loading. The minimum times required for the "worst case" situation currently anticipated are given in table II.

TABLE II. — *Minimum Decontamination Heat Cycles for Nonassayable Parts*

Temperature, °C	*D*-value,[a] hr	Decontamination time, hr
160	0.21	2.1
155	.31	3.1
150	.46	4.6
145	.73	7.3
140	1.1	11
135	1.8	18
130	2.8	28
125	4.4	44
120	7.0	70
115	11.0	110
110	17.5	175
105	28.0	280

[a] Based on spores in soil.

Items which now fall in this category are built-up items such as batteries, transformers, motors, clocks, actuators, heat shields, rocket motors, science instruments, vidicons, photomultiplier tubes, tape recorders, electronic subsystems, mechanical subsystems, and so forth.

C. *Decontamination During Manufacture and Testing*

It is probable that in the course of manufacture or testing, some parts, bulk material, and subassemblies will be subjected to a heat cycle that is equivalent to or greater than those specified for assayable parts or for nonassayable parts. In such cases, it will be considered that the requirements for decontamination have been met (provided the item is thereafter properly handled to maintain the decontamination achieved).

D. *Limitations*

The procedures described for assayable parts and for nonassayable sub-

assemblies do not apply to the complete capsule which must be assembled and tested under ultraclean room conditions, installed in its barrier (canister), and terminally sterilized by heat.

Guideline 4:

Surface decontamination may be achieved by the use of ethylene oxide. When spacecraft surfaces only have been contaminated (as in test and checkout procedures before terminal sterilization), the surfaces can be decontaminated by the use of ethylene oxide. Surfaces shall be exposed to not less than 300 milligrams of ethylene oxide per liter of space in the enclosure for 4 hours or more at not less than 70° F. Prior to ethylene oxide treatment, the surfaces shall be exposed to more than 35 percent relative humidity for more than 72 hours. Proof that decontamination has been achieved shall be obtained in each case by pre- and post-treatment culturing of representative parts from the group treated.

It should be noted that ethylene oxide *cannot* be used for internal decontamination.

Guideline 5:

Radiation sterilization of Mars capsules is not approved for general use. Approval may be granted for special use upon application to the Planetary Quarantine Office and the Director, Lunar and Planetary Programs. Applications must include proof of need for radiation sterilization, proof that it can be applied without compromising the sterility of the entire system, and proof that the reliability of the spacecraft will not be reduced below acceptable limits.

Guideline 6:

The use of ethylene oxide for sterilization of planetary spacecraft is *not* approved at the present time. There is hope, however, that at least limited use may be authorized in time to be of value.

Guideline 7:

Sterile insertion and repairs will *not* be permitted unless it can be proven that the specific proposed procedure (in exact detail to that to be employed) has a probability of contaminating the spacecraft equal to or less than the probability of the spacecraft being contaminated; that is, 10^{-4}.

Responsibility for proof of the reliability of the technique rests upon the proposer. Such proof must be submitted in advance of the need and in sufficient time for evaluation.

Guideline 8:

Terminal sterilization, based on the original concept of one heat cycle, is designed to sterilize 10^{5} viable organisms. The *D*-values and times required are given in table III.

Proof of biological loading appreciably less than 10^{5} at the time of terminal sterilization may permit the applications of fewer *D*-values than shown.

The monitoring of all decontamination and sterilization operations will be as follows:

Manufacturing

1. Spot visual monitoring of clean operations.

2. Spot visual monitoring of heat decontamination procedures and check of calibration of temperature — time indicators and recorders.

TABLE III. — *Minimum Spacecraft Sterilization Time, Temperatures, and D-Values (Based on the Assumed "Worst Case" of 10^8 Viable Organisms)*

Temperature, °C	*D*-value,[a] hr	Sterilization time, hr
160	0.21	3
155	.31	4
150	.46	6
145	.73	9
140	1.1	14
135	1.8	22
130	2.8	34
125	4.4	53
120	7.0	84
115	11.0	132
110	17.5	210
105	28.0	336

[a] Based on thermal death-time curve of heterogeneous mesophilic bacterial spores in soil.

3. Review of temperature — time records of heat decontamination procedures.

4. Microbioassays as required by contractor, Center, or Headquarters monitors.

Subassembly

1. Spot visual monitoring of clean operations.

2. Spot visual monitoring of heat decontamination procedures and check of calibration of temperature — time indicators and recorders.

3. Review of temperature — time records of heat decontamination procedures.

4. Microbioassays as required by contractor, Center, or Headquarters monitors.

5. Microbioassay of microbial contamination in air in all clean rooms and clean work benches. Not less than three samples shall be taken after the final filters have been changed or altered in any way and before assembly work is begun in the room. Not less than three samples shall be taken once each month that work is in progress.

6. Microbioassay of viable particle fallout on surfaces in all clean rooms and clean work benches. Not less than two strip samples shall be removed for analysis twice each week. Exposure of the strips shall be not less than 1 week.

7. Microbioassay of viable particles on and in components. Approximately 10 percent of the basic parts (such as capacitors, resistors, diodes, fuses, thermistors, transistors, crystals, relays, microcircuits, inductors, tapes, insulators, films, sheets, inks, lubricants, and greases) used in the subassemblies shall be made available for microbioassay and shall be so assayed. These shall be brought to analysis in the same packaging as they are delivered to the

subassembly operation. Tests may be destructive and parts tested will not be available for subassembly use.

8. Visual assay of protective packaging and transfer to other assembly operations.

Assembly, Test, and Checkout

1. Visual monitoring at all times during which work is being performed on the spacecraft.

2. Photographic monitoring shall be continuous during all periods that the assembly area is occupied by any personnel or during which the doors are unlocked and open to access by personnel.

3. The total number of physical particles, 0.3 micron or larger, in the clean room air shall be continuously monitored and recorded during the time that flight hardware is in the clean room by means of a light scattering electro-optical counter.

4. Microbial contamination of the clean room air shall be determined after installation of the final filters and before flight hardware is moved into the clean room. The number of samples taken shall be at the discretion of the NASA Headquarters or Center representative certifying the clean room for use. Following introduction of flight hardware into the room, three samples shall be taken of the room air at least twice each week.

5. Viable particle fallout on surfaces in the clean room shall be determined as part of the certification process. After flight hardware is introduced into the clean room, viable particle fallout on surfaces will be evaluated by exposure of strips for not less than 1 week. At least six strips from at least three locations in the room shall be removed for analysis twice weekly.

6. The biological contamination on clean room clothing, spacecraft covers, and similar flexible sheet materials in the clean room will be assayed as follows:

CLOTHING: Samples from at least three garments as available for wearing not less than once each week.

LARGE SHEET MATERIALS: Samples from at least six locations on each sheet before it is brought into the clean room. If sheeting is sterilized by steam autoclaving or dry heat, it may be used immediately thereafter without waiting for analysis results; but analyses shall still be made.

7. Microbioassay of viable particles on and in components: Approximately 10 percent of the basic parts (bolts, screws, nuts, washers, tubing, tapes, cord, wire, insulation, and cables — not subassemblies) used in assembly, test, and checkout shall be made available for microbioassay and shall be so assayed. These shall be brought to analysis in the same packaging as they are delivered to the assembly operation. Tests may be destructive, and parts will not be available for assembly use. Parts of the same type, from the same manufacturer and lot as assayed in subassembly, need not be so examined.

8. Microbioassay of contamination on tools: Samples shall be taken from tools by impression plate, swab, or total immersion techniques with the frequency and in the manner directed by the sterility control monitoring personnel.

9. Microbioassay of personnel: Assay shall be made not less than once a week of the quantity of biological contamination on the skin of each person

whose duties take him routinely into the clean room, in which flight hardware is present. Samples shall be taken from the cheek, chest, back, forearm, and palm and any other locations and at any other times required by the sterility control personnel or by medical officers.

10. Visual, photographic, and such other monitoring as is deemed necessary by the monitoring personnel shall be applied during 100 percent of the time from the beginning of terminal sterilization to launch.

We have thus reviewed the basic needs for decontamination and sterilization. Even more complex requirements must be imposed on the planetary quarantine portions of the problem. However, the procedures used to prevent contamination of a planet by avoiding the landing of nonsterile hardware are different for every mission. Many of the problems will be dealt with in the papers which follow.

By conscientious compliance with the rather simple, though detailed, requirements we can produce and successfully fly missions that will carry out one of the greatest opportunities of mankind — the study of life on the planets.

Reference

1. ANON.: Information Bull. 20, COSPAR, Nov. 1964.

NOTE

In the period between the Conference and the time this volume was printed, data resulting from continuing research have become available and have resulted in changes in the requirements listed in the above paper. These changes include:

1. On the basis of data on the death of spores in plastic (Lucite) and recommendations by the Spacecraft Sterilization Advisory Committee, the value for D_{125} in solids will be reduced from 4.4 to 3.5 hours. Values at other temperatures are being determined.

2. The assembly plan will require that ethylene oxide and heat cycles, equal to or greater than the levels applied for terminal sterilization, be applied to all subassemblies. Although these cycles are required for reliability assurance, they will also serve to decontaminate the interiors of the subassemblies. The decontamination heat cycles given in tables I and II will not apply.

3. Following the initial ethylene oxide and heat cycles, the sub assemblies will be kept in bioclean facilities. Contamination reaching the capsule during this period will rest on open and mating surfaces.

4. The design of the terminal sterilization cycle will be based on the number of heat resistant organisms known to be on and in the capsule. The loading has been estimated to be $<1\times10^7$ and may be as low as 1×10^4. The sterilization effect of heat-up and cool-down times and resistance due to location—open surface, mating surface, or interior—will be used in designing the heat cycles.

5. The effectiveness of the heat cycle will be judged on the basis of physical rather than biological indicators.

Session I—Discussion

Hedén, *Karolinska Institute (Stockholm)*: On behalf of the COSPAR Consulting Group on the Potentially Harmful Effects of Space Experiments, I would like to express appreciation for the concentrated efforts made by NASA to comply with international recommendations in regard to spacecraft sterilization. The difficulties involved are staggering and I find the NASA policy to disseminate information in this field most gratifying. The Consultative Group keeps the so-called standards under constant consideration for revisions which might be called for in regard to the nonsterile flybys. We are very worried about the complexities of computing the circular probability, particularly considering the frustrating pace at which the establishment of international cooperation develops. I feel that some cooperation and reconsiderations are in order.

I think that an attempt to seal off an uncontaminated area of the planet should be part of the very first mission. The hardware for such a probe, which would be devoid of telemetering devices and other complex features, might be suitable for international discussion. There should be possibilities for getting samples from the permafrost layer. One might also consider the possibility of making a manmade fossil with some epoxy so that there would be something to section for later electronmicroscopy in the event some missions fail in regard to sterility levels.

Lorsch, *General Electric* Co.: I am quite stunned by what I've heard. In the last 2 years we have spent half a million dollars of NASA money, and a considerable amount of G.E. money; now we can start over again. If I understood correctly, now everything that can't be properly assayed (which is probably something like 90 percent of the spacecraft) has to be load reduced for 10 cycles and then sterilized for 12. My question is, why not make it dirty, stick it in the oven, and load-reduce for 22 cycles — for 22 logs. You probably won't have more than 10^{18} organisms on the dirty spacecraft at the start. Why this duality now of two steps rather than just one?

Hall: This probably is something of a shock to many. It has also been a shock to me in the last few months but, frankly, a very happy one. We have proceeded for almost 2 years in the belief that the reliability requirements would impose absolute obstacles against heating the spacecraft more than once. However, it is the consensus of the reliability people that the spacecraft must have a

flight-acceptance test equal to the terminal sterilization test, so this means two cycles. It has made us happy because it reduces the sterilization problem many orders of magnitude. We can depend upon the flight-acceptance test to do the job that would otherwise have depended upon ultraclean work throughout the early phases of the spacecraft manufacture and assembly.

Slote, *Grumman Aircraft Corp.*: At what altitude does NASA consider the Martian atmosphere to begin insofar as sterilization is concerned? This is important to basic design, but nowhere is this figure spelled out. The second question relates to the figure of 10^8 organisms. Are we talking about total organisms, discrete organisms, colonial populations of 10^8, or discrete populations? All these are affected differently by heat treatment, but nowhere is this defined. Therefore, the figure of 10^8 organisms is subject to question in regard to the heat treatment that has been proposed unless we *know* what figures we are talking about.

Atwood: I have a brochure concerning the occultation experiment which shows the profile of the atmosphere, but I do not know what would be decided upon as its actual beginning.

Reynolds: We have a great deal of information about the Martian atmosphere as a result of work done during the last opposition, including the occultation data. These data are in the process of being considered, and debates between people with differing observations are continuing. At the moment I do not believe we have arrived at a standard for the Martian atmosphere. I think from the information and data now at hand, that an answer will be soon forthcoming. Preliminary examination of the data reveals the atmosphere to be considerably shallower than we had thought. I think that the selection of a standard number is something that will have to wait a few months.

Hall: Questions about the total number of organisms, the number of discrete organisms, and the number of colonial organisms we cannot answer completely. We cannot always distinguish nor certainly quantitate the number of discrete organisms. We do not have techniques for it that are really satisfactory. So, for the purposes of discussion at the present time, we must leave it on the basis that it is the number of colonial organisms as developed by the standard techniques used for both the evaluation of heat cycles and for the assays.

Shull, *General Electric Co.*: On the figure concerning the accepted cycles for decontamination and sterilization, three different scales of D-values were listed. Can you give some idea of the philosophy used in generating or applying those D-values to the respective tasks?

Hall: Actually, there are only two *D*-values: those for decontamination at the low level of heat, and those at the high level of heat for sterilization. The former are based on the knowledge available concerning the survival of organisms inside solids (dental plastics). These *D*-values are comparatively low, but we feel that they are satisfactory for decontamination if this procedure is to be used. We tend to forget that decontamination means only reduction; we do not have to get very close to zero. If a component has 10^6 organisms and four *D*-values are applied to get a four-decade reduction, the result is only a hundred organisms in that particular part. We can stand a lot of hundred-organism components in a spacecraft and still stay under 10^8. On the basis of the higher *D*-values, these are the worst-case situations for the survival of the resistant organisms in soil. We realize that we are not going to send soil on our spacecraft, but we are unable to define accurately the survival of these organisms inside the solids that are used within spacecraft. Therefore, we must accept the worst-case situation. Actually, there probably are worse situations than those used, but there has been a judgment applied by the microbiologists to the effect that it is impossible to find a more difficult situation. Instead, we take a situation that is 10 to 15 percent less than this worst value according to another curve that we have established through use of different culture media.

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Session II

Microbiological Contamination Control, Microbiological Monitoring, and Visual Monitoring

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Sterilization and Quarantine Parameters for Consideration During the Design of Planetary Vehicles

In exploring the planets, consideration must be given to preservation of unique evidence of any life forms that may have independently evolved. Otherwise, carefully designed experiments to determine the nature and form of any extraterrestrial life might be rendered meaningless by contamination from Earth. A national policy of planetary quarantine has been established by the National Aeronautics and Space Administration to preserve the ecology of the planets during the time these experiments are being carried out. To insure against contamination, each planetary vehicle for impact or landing during the next 50 years must be sterile so that our confidence in the probability of releasing even one live organism on the planet's surface is less than 1 in 10 000 (refs. 1 to 7).

Unmanned planetary explorations are to be carried out within the Voyager Program. Immediate explorations are aimed at the planet Mars. Careful observations indicate that it is the planet most likely to harbor life. It is of sufficient size and close enough to the Sun to have a thin atmosphere mildly warmed near the surface during summer exposure. The atmosphere is strikingly revealed by photographs of high thin clouds and large duststorms. The seasonal changes in surface colorations suggest some form of rapidly changing vegetation. In addition, the well-defined polar caps change in size and shape with the seasons indicating the possibility of ice and water (ref. 8). Although we presently do not have direct evidence of life on Mars, there is a strong possibility that some of the more primitive forms have evolved. Explorations and studies to find and elucidate the nature of such forms might lead to epochal changes in or knowledge of life. Thus a rigid policy of quarantine is of great importance during exploration of the planets.

Mission Constraints

For Voyager the following policy has been tentatively adopted as a constraint on all launches for Mars:

1. All aspects of the proposed launch, including the complex interactions of the spacecraft with the interplanetary environments, will be examined in order to isolate every conceivable source of contamination.
2. Each separate source of contamination will be investigated to assure an adequate understanding of the processes through which it occurs and, wherever possible, mathematical models will be formulated to characterize adequately the probability of contamination. These mathematical models will be based upon standard techniques, and the limitations and assumptions inherent in their formulation will be explicitly described in the explanation of their validity.
3. The effects of all possible sources of contamination will be estimated. These estimates will include those sources of contamination that can be adequately described by a mathematical model and, for these, formulas will be propounded to calculate the probability of possible contamination. In addition, conservative assumptions will be employed whenever uncertainties are present in the derivation of these formulas. Wherever an adequate mathematical model is impossible (for example, when the necessary assumptions are not meaningful), every effort will still be exerted to describe suitable ranges or bounds for the chances of planetary contamination.
4. The total probability of contamination resulting from a single vehicle will be constrained to be less than, or equal to, 10^{-4}, and allocated within the following areas:
   a. The capsule designed for planetary landing will be sterilized so that, given that the capsule reaches the planet, and given that no recontamination occurs during the interplanetary transfer, the probability of contamination from this capsule will be less than 10^{-4}.
   b. The probability of contamination of the planet due to recontamination of the previously sterile capsule, given that the capsule reaches the planet, will be less than 10^{-4}.
   c. The probability of contamination due to accidental impact of the launch vehicle or parts of the launch vehicle will be less than 10^{-4}.
   d. The probability of contamination due to accidental im-

pact of the unsterilized spacecraft and/or decay from planetary orbit of the unsterilized spacecraft will be less than 10^{-4}.

e. The total probability of contamination due to accidental impacts of various standard and nonstandard spacecraft ejecta will be less than 10^{-4}.

Whenever possible, numerical estimates of the probabilities of contamination from the above will be calculated; the spacecraft will be designed and constructed and the launch operations so formulated that these estimates conform to the numerical constraints. In addition, an explanation and evaluation of each possible source of contamination will be documented to demonstrate compliance with the above policy.

Sources of Contamination

Each of the modes of contamination with their respective numerical constraints can be affected by various separate mechanisms or sources of contamination. The following summary of currently known sources is presented for a more careful definition of the nature of the sources constituting each mode and to illustrate the vast number of problem areas that must be investigated to comply fully with the mission constraints:

1. Sterilization procedures for the capsule—
   a. One or more viable organisms left on the capsule in an unencapsulated condition
   b. One or more viable organisms, though encapsulated in the interior of the capsule, released on the planet's surface by disintegration of the capsule upon impact or by erosion of encapsulating surface areas
2. Recontamination of the previously sterile capsule—
   a. Capsule recontaminated by handling prior to launch or contaminated during launch, for example, by breach of the biological barrier
   b. Viable organisms from the unsterilized spacecraft penetrate the biological barrier during interplanetary transfer
   c. Recontamination at time of barrier release
   d. Recontamination caused by unsterile debris after the biological barrier release
   e. Recontamination at time of capsule ejection due to electrostatic attraction of contamination associated with the spacecraft
3. Accidental impact of the launch vehicle or its parts—

a. Failure of the launch vehicle retrograde rocket maneuver
b. Launch vehicle maneuver of insufficient magnitude
c. Debris associated with the launch vehicle (clamps, rings or bolts) placed on an impact trajectory
d. Launch vehicle detonates, scattering debris that is placed on an impact trajectory

4. Accidental impact of the unsterilized spacecraft or early decay of its planetary orbit—
   a. The spacecraft placed on an impact trajectory at interplanetary injection by the launch vehicle and then fails
   b. The spacecraft placed on an impact trajectory during the first maneuver and then fails
   c. The spacecraft placed on an impact trajectory during a later maneuver and then fails
   d. Large orbit determination errors near the planet causing impact
   e. Orbit insertion errors resulting in early orbit decay and impact
   f. Early planetary orbit decay due to miscalculation of the planet's atmosphere
5. Accidental impact of various standard and nonstandard spacecraft ejecta—
   a. Ejecta released during heliocentric cruise due to attitude control gas, outgassing, spalling during micrometeoroid impact, retrograde rocket propulsion exhaust gases
   b. Ejected biological barrier impacts
   c. Debris released at time of capsule separation impacts
   d. Debris in case of detonation during one of the cruise or orbit maneuvers scattered and placed on an impact trajectory
   e. Ejecta released during planetary orbit phase might decay into the planet's atmosphere

These sources of contamination are schematically depicted in figure 1.

Capsule Sterilization

The capsule to be landed on Mars must be sterilized before launch since it is evident that reliance cannot be placed on the harsh conditions of deep space to bring about sterility (ref. 9). Sterilization procedures, based on chemical decontamination and exposure to dry heat, have been selected as optimum. Accordingly, steps have been taken to insure the development of sterilizable

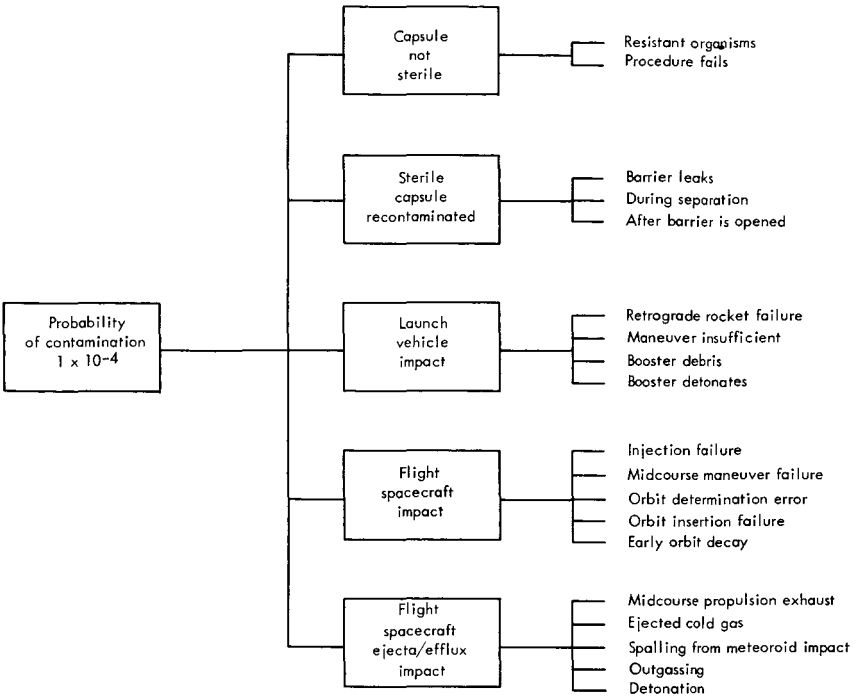

FIGURE 1.—Sources of contamination.

materials and components, including equipment and procedures, that will be required for a sterilizable capsule. Heating to 135° C for 24 hours has proved adequate for sterilization and has been used as a guide in establishing the NASA parts qualification program. Recent data indicate that other heating cycles are also adequate for sterilization. It is possible that a lower temperature for a longer time may be selected for the flight program.

Capsule sterilization is aimed at the inactivation of all micro-organisms. Although such microbial contamination could be expanded to include bacterial, fungal, algal, viral, and protozoan organisms, major concern is for two groups of micro-organisms; these are the fungi and bacteria. The latter group is considered as an index of microbial contamination for space hardware. Little information is available on viruses as contaminants, and viruses as a group do not fall within the scope of the quarantine policy since a virus can reproduce only in a specific Earth-type cell.

One family of bacteria, the *Bacillaceae*, contains two genera, *Bacillus*, aerobic micro-organisms, and *Clostridium*, anaerobic micro-organisms, that have the genetic capability of forming

spores. Bacterial spore formation is largely confined to this family. The spore is a refractile body formed within the bacterial cell and, as such, the spore is very resistant to environmental extremes, including heat. Due to this resistance, the spore is the major problem in the control of microbial contamination. Sterilization procedures must be directed toward the destruction of bacterial spores.

The fungi, yeasts, and molds also produce spores. However, fungal spores are not nearly so resistant to heat as are bacterial spores. These forms, including bacterial vegetable cells, are readily killed or inactivated by procedures necessary for bacterial spores.

When populations of micro-organisms are exposed to heat, the number of viable organisms decrease exponentially as a function of exposure time (ref. 10). The rate of death resembles that of a first-order reaction. Variations and exceptions to this logarithmic death rate do occur, but in general the deviations are not marked. The exponential concept is accepted as valid for establishing guidelines to sterilization procedures.

For a given sterilization procedure, plotting the number of surviving organisms on a logarithmic scale against time on a linear scale results in a good fit to a straight line. The resulting curve, termed a survivor curve, is described by the following equation:

$$K = \frac{1}{t}(\log_{10} N_0 - \log_{10} N)$$

derived from $N = N_0 \times 10^{-kt}$ where:

k = experimentally determined constant
t = time of exposure, in minutes
N_0 = number of organisms viable at the beginning of time t
N = number of organisms viable at time t

There are several factors which affect the value of k. These factors include dose rate or heat flux; the species or strain of micro-organisms (whether the organisms occur as spores on vegetative cells); and the environment history of the cells. If the microbial population being sterilized is a mixed population of various species and strains, the resistance of the population would probably exhibit considerable variability so that the death rate would be complicated beyond the simple straight-line relationship.

Much of the work on kill rates for resistant bacterial spores has been expressed in D-values, or the time required to reduce a population by 90 percent, assuming logarithmic rates of destruction. Since the logarithmic order of death implies that the same percentage of organisms die every D-value, then it is possible to theorize that true sterility is never attained. For example, if one

were to take a particular sample of 10^6 organisms having a D-value of 1 minute, the first minute of heating would reduce the number of organisms to 10^5 (1-log reduction). The second minute of heating would reduce the number to 10^4 (another log reduction). Thus, after 7 minutes of heating, the probability of one organism remaining would be 10^{-1}.

This example points out the necessity of longer heating periods in order to sterilize when higher numbers of organisms are present. If the total load or organisms on a capsule can be held to a low level, then the time of heating for sterilization can be reduced. Thus, control of the microbial load is of importance in building a sterilizable capsule. This can be accomplished by clean-room assembly, with provisions for regulated microbiological sampling to define the contamination load (McDade, J. J., et al., Am. J. Pub. Health, in press).

For a sterilizable capsule, it is expected that all subsystems, including the various science payloads, will be subjected to one chemical decontamination cycle (ethylene oxide), and one dry heat sterilization cycle as a part of the flight acceptance test before the start of final assembly. This treatment will greatly reduce the contamination load at an early stage so that concern for contamination can be limited to that accumulation which is caused by handling and test procedures during final assembly and checkout. Assembly and test can be accomplished within clean assembly facilities controlled to limit the microbial load. Frequent microbiological assays can be made to obtain contamination load data for use in calculations of final loading. This knowledge will insure selection of a heating time sufficient to achieve capsule sterility. The assembled capsule can be shipped and stored under bond to protect it from improper handling and contamination. After functional tests, it can be placed in a biological barrier, subjected to an ethylene oxide decontamination cycle, and sterilized by exposure to dry heat. Capsule assembly and sterilization events are shown in figure 2.

The capsule must be enclosed in a biological barrier to prevent recontamination. The barrier should be installed after final assembly, sealed at the completion of the sterilization procedure, and kept in place until after launch through the Earth's atmosphere. It may be advantageous to retain the barrier throughout the interplanetary cruise phase to help control temperature levels within the capsule and to serve as a micrometeoroid bumper.

Although the use of a sealed biological barrier is considered necessary to prevent recontamination, its use imposes several constraints. It will prevent direct access to capsule subsystems

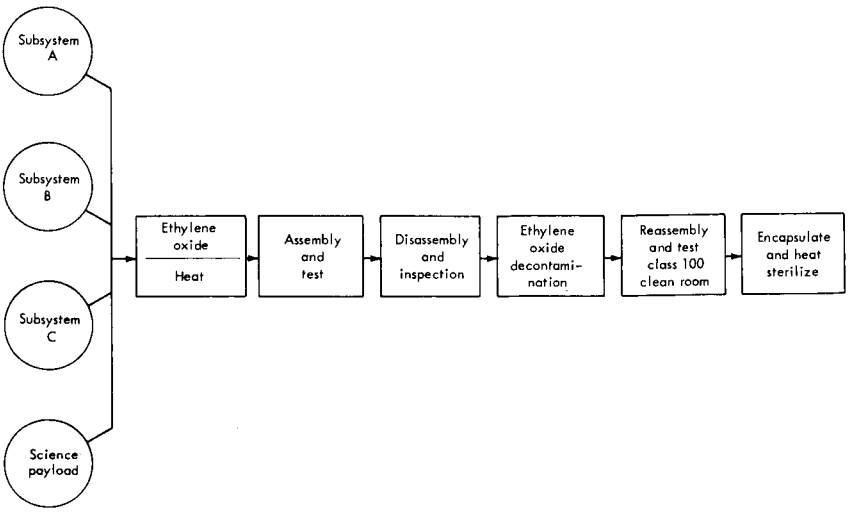

FIGURE 2.—Capsule assembly and sterilization procedures.

during final checkout and launch countdown. Positive pressure must be maintained within the barrier to prevent contamination through possible leaks. Provisions must be made to vent excess gas through biological filters to prevent barrier rupture during heating and launch.

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Environmental Microbiology and the Control of Microbial Contamination

Levels of microbial contamination have been presented for the intramural environments of 17 different facilities located in 3 geographical areas of the United States. The data obtained indicate that a well run clean room can and does reduce the level of intramural microbial contamination. Such studies also have shown that the primary source of microbial contamination in a clean room is the operating personnel. A large percentage of the microbial species present as contaminants in clean rooms are those commonly associated with humans. Soil-type species, such as bacterial and mold spores, did not appear prevalent in the total population of micro-organisms recovered within the clean rooms sampled in this survey. Elimination of microbial contaminants such as spores is of critical importance in obtaining sterility by dry heat, or at least in increasing the reliability that a given sterilization procedure successfully will meet the NASA planetary quarantine requirements. Consequently, these preliminary data, including those from the JPL EASL facility, indicate that use of a clean room environment for assembly of space hardware has merit.

The world of micro-organisms is one of tremendous numbers. A basic characteristic of microbial life is rapid reproduction. During a certain phase in the growth of micro-organisms, cell growth and division becomes exponential. Thus, a single cell that weighs 10^{-12} gram and has a generation time of 20 minutes would produce, in 48 hours of exponential growth, approximately 2.2×10^{43} cells, or some 2.4×10^{24} tons, a mass approximately 4000 times that of the Earth. Due to factors such as the exhaustion of available nutrients and space, as well as to the accumulation of toxic metabolic waste

products, the full reproductive potential of a microbial cell is never realized.

A cup of ordinary garden soil usually contains more microorganisms than there are people inhabiting the Earth. Some 1600 species of bacteria have been isolated, identified, and classified. Similarly, there are thousands of known species of other microorganisms such as algae, molds, and protozoa. Terrestrial microorganisms have been found wherever they have been sought, including sources such as fresh water, stagnant water, the oceans, hot springs, the Arctic, soil, dust, air, and on and in humans.

Medicine was one of the first sciences to recognize a need for the control of microbial contamination, especially in operating rooms. During the past decade a rise in hospital-acquired infections has led to detailed studies to determine the microbial profile of institutional environments (refs. 1–10). Such studies have shown that vast numbers of micro-organisms can exist on surfaces and in the air of intramural environments. However, if proper control measures are continuously employed, the level of microbial contamination can be held low. An even more pertinent observation is that proper control measures can exclude or drastically reduce the number of pathogenic micro-organisms present. It is important to realize that the measurement of microbial contamination in any environment with humans present is a recently developed art. Procedures available for the microbiological sampling of air have been reviewed by Wolf et al. (ref. 11). Techniques for the microbiological sampling of surfaces also have been reviewed (refs. 12 and 13). Taken collectively, the qualitative and quantitative studies of microbial contamination on surfaces and in the air of intramural environments constitute the field of environmental microbiology.

Industry has recognized a need for the control of particulate (fine dust, fibers, etc.) contamination. The need for such control became apparent when it was discovered that fine particulate dust caused seizure of small gears and bearings used in precision instruments. Controlled areas were developed to eliminate gross contamination associated with general manufacturing areas. These controlled assembly areas have been referred to as "clean rooms." Conventionally, a clean room is classified into one of several classes; either Class II, III, or IV as defined in the Air Force Technical Order OO–25–203 (ref. 14), or Class 100, 10 000 or 100 000 as defined in Federal Standard No. 209 (ref. 15). Classification of clean rooms is done according to set limits of tolerable

particulate matter of specific sizes. Particulate contamination is not differentiated into viable (microbial-bearing) and nonviable matter.

As stated, the planetary quarantine requirement of the National Aeronautics and Space Administration (NASA) requires the control of microbial contamination on board planetary orbiting or impacting space hardware (refs. 16 and 17). The use of clean rooms for the assembly of space hardware has been suggested as a step in the development of sterile space hardware. The objective in using this approach is to reduce both the number and species of micro-organisms on the hardware so that a low level of microbial contamination is obtained. A requirement for reduced microbial contamination is basic to any sterilization plan, because the probability of obtaining sterility by any sterilization process is increased as the number of micro-organisms to be sterilized is decreased.

A number of preliminary reports have been made on the microbiology of environmentally controlled areas (refs. 18–24, and the paper entitled "Control of Microbial Contamination: The Clean Room Concept" by J. J. McDade, W. Paik, and M. Christensen, in press). Yet at present, data on the levels of microbial contamination that may exist within industrial clean rooms are only beginning to become available. As the results from such investigations become available, it will be possible to firmly estimate levels of microbial contamination that exist within areas employing various degrees of environmental control (both physical and procedural). Based on this information it should be possible to make a judicious choice of the type of controlled assembly environment that will be necessary to produce space hardware containing a level of microbial contamination at or below that specified for a particular dry heat sterilization cycle.

Experimental

The microbiological sampling procedures used were similar to those described in a manual prepared by NASA entitled "Standard Procedures for the Microbiological Examination of Space Hardware." A brief recapitulation of the procedures used is presented below (commercial names are used for identification only):

1. *Microbiological Sampling of Air.* — Volumetric air samples were collected with Reyniers slit samplers (fig. 1). All samplers were equipped with a 1-hour clock motor and each was calibrated to operate at a sampling velocity of 1 cubic foot of air per minute. Individual sampling periods never exceeded 60 minutes. Trypticase Soy agar was used as the collecting and incubation medium. All samples were incubated at 32° C for 72 hours.

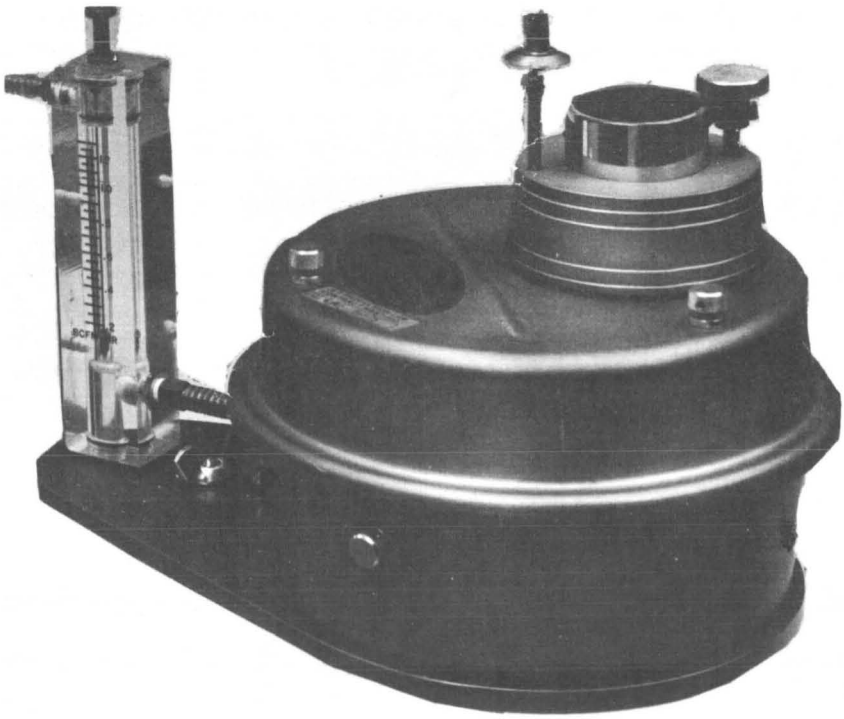

FIGURE 1.—Volumetric air sampler with inline flowmeter.

2. *Microbiological Sampling of Surfaces*

a. *Rodac plate method* (ref. 25).—Sterile, Rodac plates were filled with 15.5 ± 0.2 milliliters sterile Trypticase Soy agar. After sampling, the Rodac plates were incubated at 32° C. Colony counts were made after 44 to 48 hours of incubation.

b. *Microbial contamination accumulating on surfaces.*—Sterile stainless steel (SS) strips (fig. 2) were used to measure the degree of airborne microbial contamination that accumulated on surfaces within each of the areas sampled. The details of SS strip preparation and sterilization have been described in the NASA Standard Procedures for Microbiological Examination of Space Hardware. Trays containing SS strips were placed at work height. After exposure, the SS strips were returned to the laboratory in sterile bottles. Sterile 1.0 percent peptone water (Bacto, Difco) was added to each bottle and the bottles were shaken mechanically for 5 minutes. After shaking, portions of the peptone water were plated in Trypticase Soy agar. Other portions of the peptone water were heat shocked (80° C for 15 minutes) and plated in Trypticase Soy agar. All samples were incubated at 32° C for 72 hours.

3. *Assessment of Microbial Contamination Resulting From Human Handling.* — Sterile SS strips prepared as above (2b) were handled by each person to be tested. Following handling, the SS strips were assayed by shaking in 1.0 percent peptone water and assayed as just described.

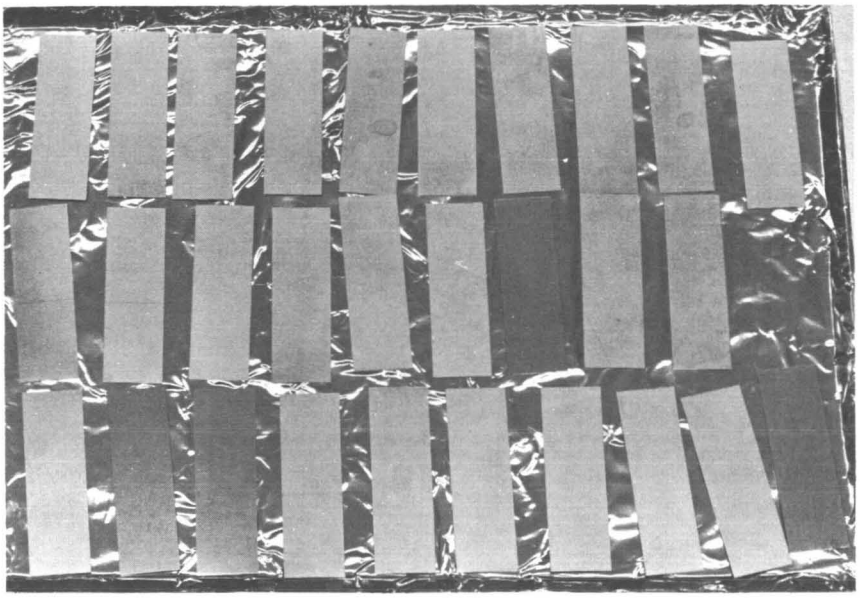

FIGURE 2.—Microbial fallout sampling strips (stainless steel) on carrier tray.

4. *Effect of Environmental Exposure on the Survival of Micro-Organisms.*— To determine the death rate of naturally occurring microbial contamination that accumulated on SS surfaces, the following procedure was used:

   a. *Die-away on surfaces.* — Following exposure to a given environment for a specified period of time, SS strips were either covered with sterile aluminum foil or individually placed into separate sterile bottles having loosely capped tops. Exposed SS strips were held in this static situation for periods up to 6 weeks. Bottles containing test SS strips were kept in the same environment where surface sampling studies were being conducted. The SS strips were assayed according to the procedure described in section 2b.

   b. *Human handling experiments.* — After being handled by a subject, each SS strip was placed into a separate sterile bottle having a loosely capped top. Handled SS strips were held in this static condition for periods up to 28 days. The bottles containing test SS strips were kept in the same environment where handling studies were being conducted. Assays were performed according to the procedure described in section 2b.

5. *Environmentally Controlled Areas Surveyed.*

   a. *Jet Propulsion Laboratory (JPL) Study*

      (1) Very little environmental control
         (a) Surveyor spacecraft assembly facility
         (b) Mariner spacecraft assembly facility
      (2) Class II clean room
      (3) Class III clean room
      (4) Class IV clean room

(5) Experimental Assembly and Sterilization Laboratory (EASL)
(a) Vertical laminar airflow clean room
(b) Horizontal laminar airflow clean room

b. *U.S. Public Health Service (PHS) Study (Phoenix)*
(1) Manufacturing Area A — open factory area
(2) Manufacturing Area D — open factory area
(3) Clean Room — operated as a Class 100 000 clean room
(4) Clean Room B — operated between a Class II to III clean room (closer to Class III)
(5) Clean Room C — operated between Class II to III clean room (closer to Class II)
(6) Clean Room D — horizontal laminar air flow

c. *University of Minnesota (UM) Study*
(1) Company No. 1
(a) Room E — open factory area
(b) Room D — operated as a Class 100 000 clean room
(2) Company No. 2
(a) Room A — operated between a Class II to III clean room (closer to Class III)
(b) Room C — operated between a Class II to III clean room (closer to Class II)

Results

It must be emphasized that most industrial clean rooms are operated for assembly of specific products. For a number of reasons, including operating economy and personnel considerations, it may not be necessary to fulfill all the requirements imposed by a specific class for a clean room. Product quality and reliability are the overriding considerations. Consequently, it is possible to construct and operate a facility between the Class II to III clean-room requirements, with the facility being classified as closer to a Class III than a Class II clean room (UM Company No. 2, Room A). Several of the clean rooms sampled in the studies being reported fall into the in-between category. Thus, it will not be possible to make direct comparison of the results obtained in the three geographical areas. However, general comparisons and suggestions of trends are definitely detectable.

Results of Air Sampling Studies

Areas Containing Very Little Environmental Control

Figures 3 and 4 contain the results of air sampling studies conducted in spacecraft assembly facilities. Both facilities are high bay areas and operating personnel are required to wear head covers, smocks, and gloves. Figure 3 contains the results of air-sampling studies conducted in the JPL Spacecraft Assembly Facility (SAF). The environmentally controlled area of the SAF is

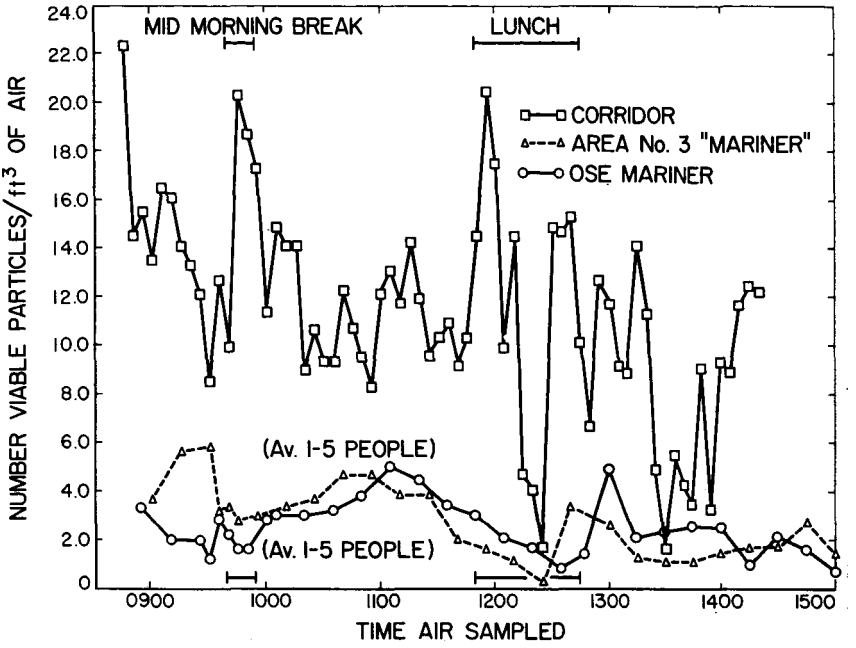

FIGURE 3.—Results of JPL air sampling studies conducted in the Mariner spacecraft assembly area.

a high bay area. A Mariner Proof Test Model (PTM) spacecraft was in the controlled area and tests were in progress throughout most of the day. In figure 3, a relationship between the number of airborne viable particles and the degree of personnel activity can be seen. As traffic increased in the corridor outside the controlled area, a marked increase in the number of airborne viable particles was detected. Airborne contamination levels of 20 to 23 viable particles per cubic foot of air were detected during periods of heavy corridor traffic. High levels of airborne microbial contamination were particularly apparent during the midmorning break and at the beginning and end of the lunch period. However, during lunch, corridor traffic was very light and a sharp drop was detected in the level of airborne microbial contamination (approximately two viable particles per cubic foot of air). Inside the controlled area of the SAF, the highest number of viable particles recovered was approximately six per cubic foot of air. Fluctuations in the levels of airborne viable contamination occurred within the controlled area, but the rises and falls that were noted in samples collected in the outside corridor were not too apparent. During lunch, the test technicians left the high bay area and a drop was noted in the degree of airborne microbial contamination. As

operating personnel returned from lunch, a rise in the level of airborne microbial contamination was detected. Then, as operational activity stabilized, the level of airborne viable contamination seemed to remain fairly constant.

Figure 4 contains the results obtained during 8 hours of continuous air sampling in the Surveyor spacecraft System-Test

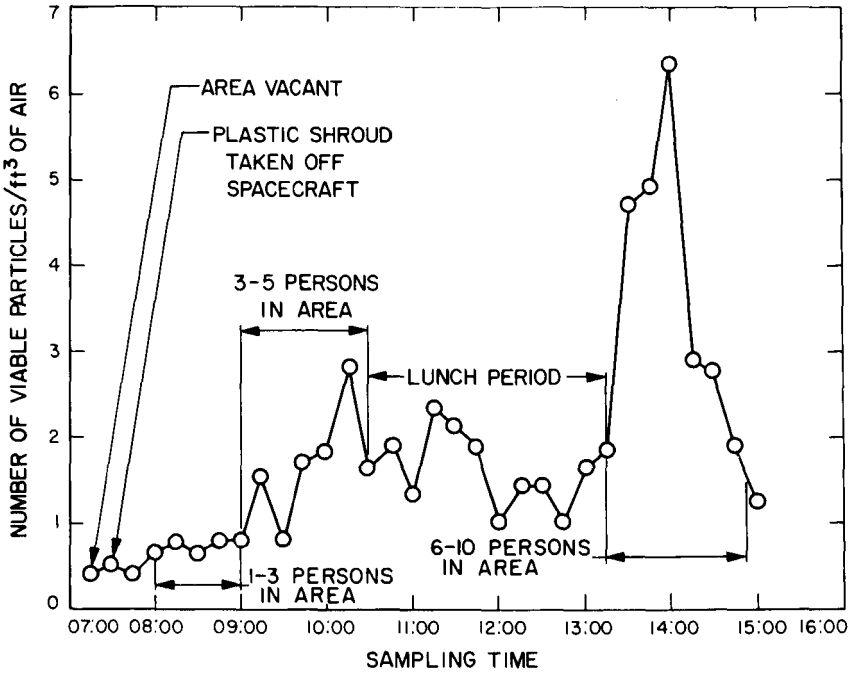

FIGURE 4.—Results of JPL/PHS air sampling studies conducted in the Surveyor spacecraft assembly area.

Equipment Assembly (STEA) area. A Surveyor spacecraft was present in the area and, with the exception of the lunch period, the area was occupied by test technicians throughout the day. As seen in figure 3, the highest number of airborne viable particles recovered was approximately 6.5 per cubic foot of air. Also, as noted in the JPL SAF (fig. 3), the number of airborne viable particles appeared to be related to the amount of personnel activity in the area.

Conventional Clean Rooms

Figure 5 contains the results of the JPL air sampling studies conducted in Class II, III, and IV clean rooms in the Los Angeles area. The Class II clean room is operated three shifts a day

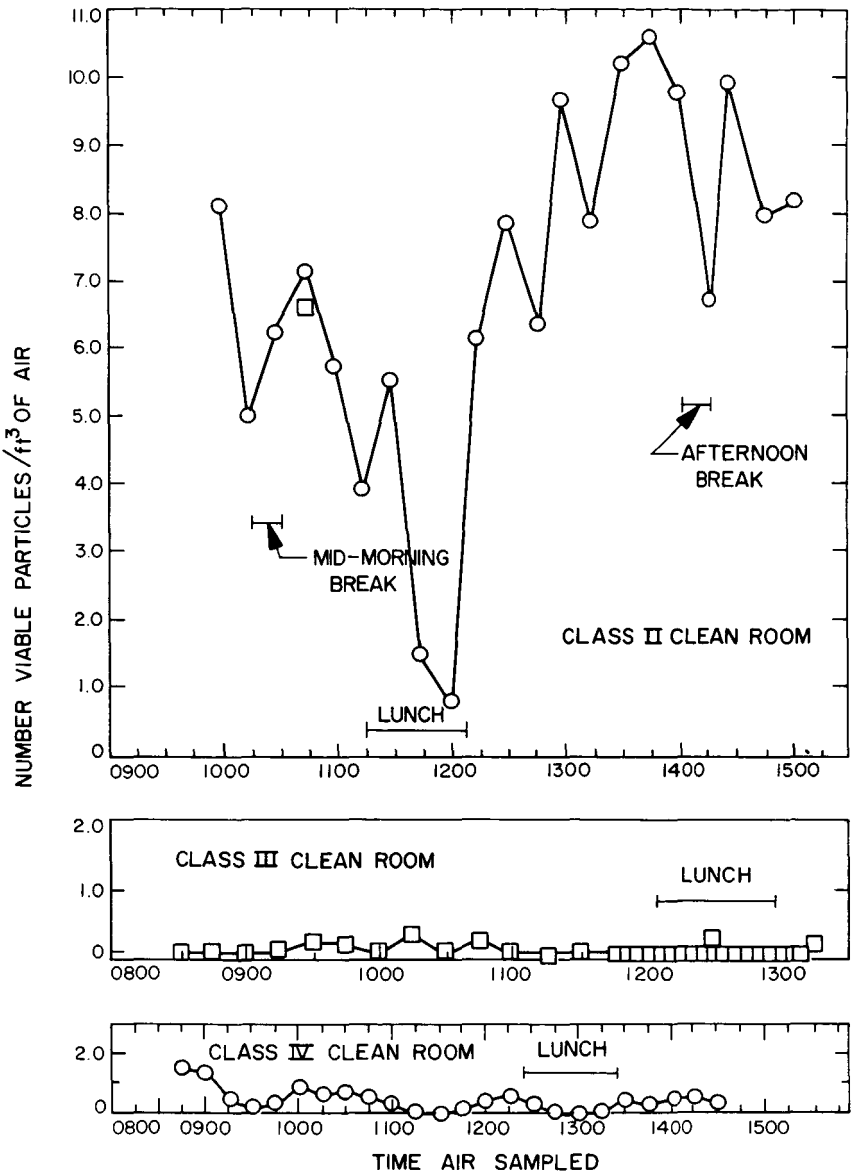

FIGURE 5.—Results of JPL air sampling studies conducted in conventional clean rooms.

(personnel occupancy, 25 to 40, first shift; 10 to 20, second and third shifts), 7 days a week. As seen in this figure, the highest number of airborne viable particles recovered in the Class II clean room was approximately 10.5 per cubic foot of air. Microbial showers of 20 to 25 viable particles per cubic foot of air have been

recovered within this clean room. During lunch, the room was vacant and a sharp decrease in the level of airborne viable particle contamination occurred (1.0 viable particle per cubic foot of air). When the operating personnel returned, another peak in the airborne microbial contamination occurred. Thus, a relationship between personnel activity and the number of airborne viable particles was again observed.

The results contained in figure 5 also indicate a low level of airborne microbial contamination within Class III and Class IV clean rooms. In fact, the level of airborne viable contamination rarely reached a level of 1 viable particle per cubic foot of air in the Class IV clean room and did not exceed a level of 0.5 of a viable particle per cubic foot in the Class III clean room. Results such as those shown in figure 5 have been obtained in repeated samplings in all these clean rooms.

Figure 6 contains the results of PHS air sampling studies conducted in two clean rooms and two manufacturing areas in the Phoenix area. Though not shown in this figure, air sampling

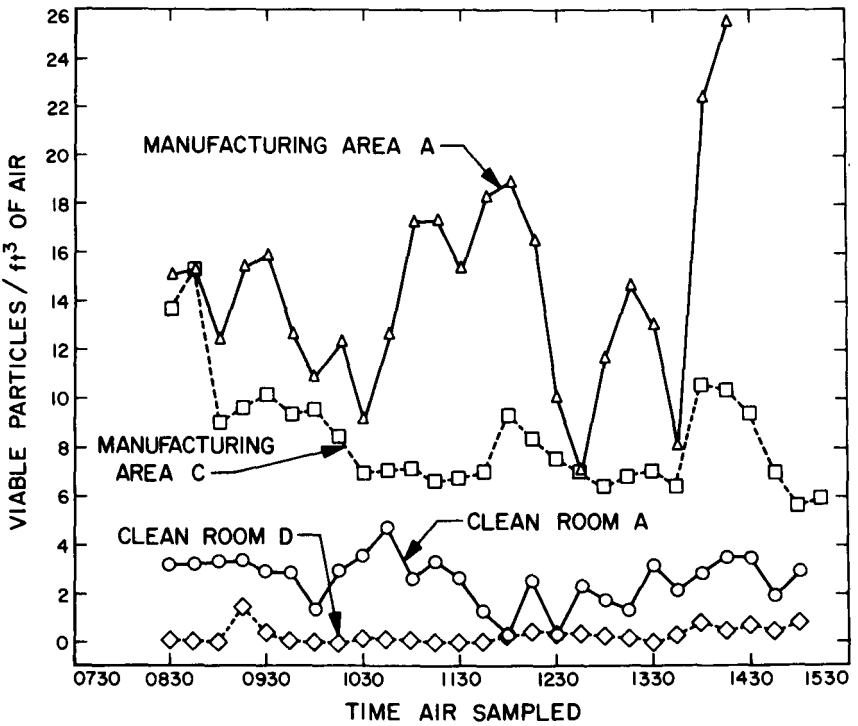

FIGURE 6.—Results of PHS air sampling studies conducted in several different environmentally controlled areas.

results in clean rooms B (0 to 2 viable particles per cubic foot of air) and C (0 to 2 viable particles per cubic foot of air) were similar and somewhat lower than those presented for clean room A (0 to 5 viable particles per cubic foot of air). The data presented in figure 6 afford a good comparison between the levels of airborne viable particle contamination that exist in manufacturing areas (6 to 25 per cubic foot of air), in conventional clean rooms (0 to 2.5 per cubic foot of air), and at the exhaust wall of a horizontal laminar airflow clean room (clean room D, 0 to 1.8 per cubic foot of air) in the Phoenix area. Results similar to those shown for the manufacturing areas in figure 6 also have been obtained by JPL in the Los Angeles area.

Air sampling studies conducted by the University of Michigan in clean rooms in the Midwest have produced results similar to those shown in figures 5 and 6.

Laminar Airflow Clean Rooms

Recently, JPL constructed and is operating an Experimental Assembly and Sterilization Laboratory (EASL). The floor plan of the EASL facility is shown in figure 7.

Figure 8 contains the results of an air sampling test conducted during a normal working day in EASL. As seen in this figure, the

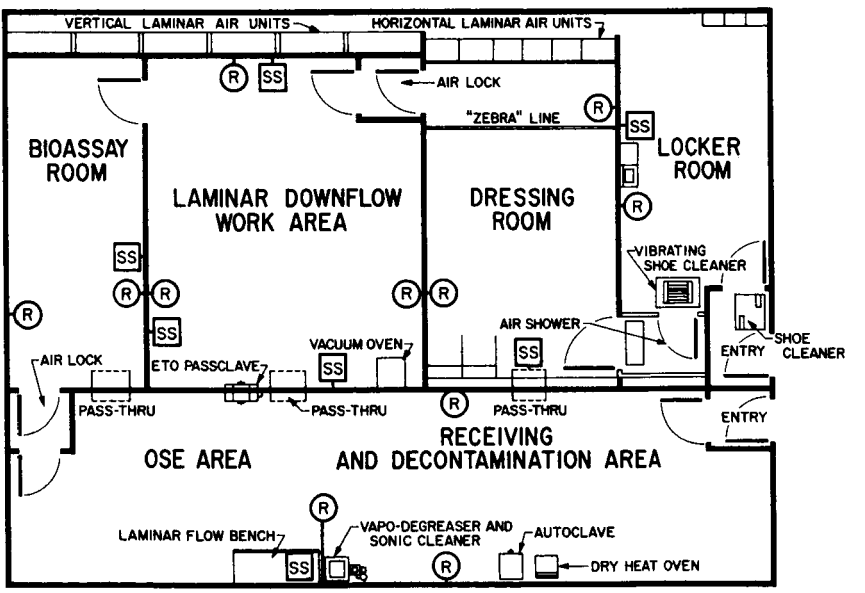

FIGURE 7.—Floor plan of the JPL Experimental Assembly and Sterilization Laboratory (EASL). Air samples collected at ®, surface samples collected at SS.

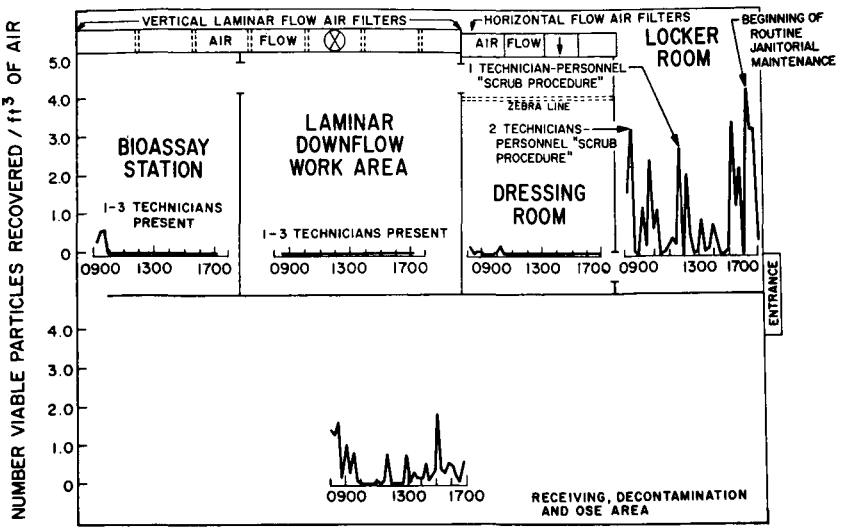

FIGURE 8.—Results of JPL air sampling studies conducted in the EASL facility during a normal working day.

levels of airborne viable particle contamination within the receiving, decontamination, and operational support equipment area and the locker room fluctuated with the degree of personnel activity. The level of airborne microbial contamination in the vertical laminar airflow (bioassay station and laminar downflow work area) of EASL is extremely low. Frequently, periods of 3 to 4 hours elapse without recovery of a single viable particle from any of three air sampling sites within the laminar flow work area. This represents sample volumes from 540 to 720 cubic feet of air. Results similar to those shown in figure 8 have been obtained in repeated air samplings in EASL.

Figure 9 presents the results of other air sampling studies conducted in a horizontal laminar airflow clean room (PHS Room D). These data indicate that the air leaving the filter wall was extremely clean. However, as the filtered air moved downstream past operating personnel to the exhaust wall, a corresponding increase in the level of airborne viable contamination was detected. The relationship between operating personnel and levels of airborne viable contamination is clearly indicated in this figure. During three breaks, personnel left the room and the detectable level of airborne viable particles fell to zero. When the operating personnel returned, after each break, the level of airborne microbial contamination roughly paralleled the degree of personnel activity.

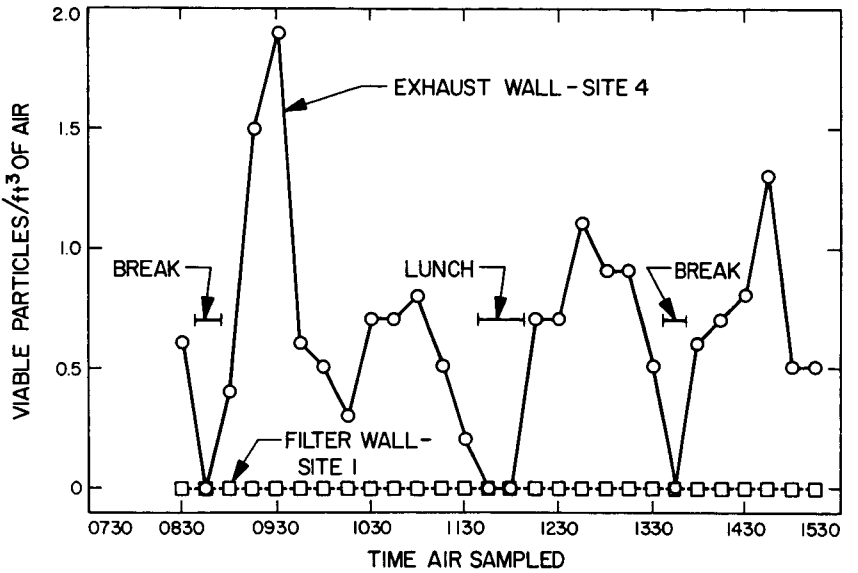

FIGURE 9.—Results in PHS air sampling studies conducted in a horizontal laminar airflow clean room.

Results of Surface Sampling Studies

Microbial Contamination Accumulating on Surfaces

Figures 10 through 14 contain the results of studies to determine the levels of microbial contamination that accumulate on SS strips during environmental exposure for periods up to 25 weeks. Figure 10 presents the results obtained from each of three sampling sites

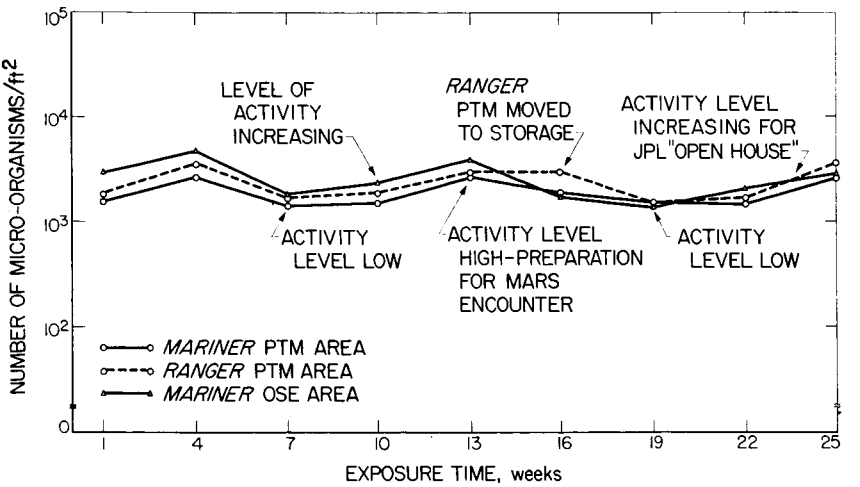

FIGURE 10.—Results of surface sampling studies on stainless steel strips conducted in the JPL SAF.

within the environmentally controlled area of the JPL SAF. Each plot represents the average value from six SS strips. As shown in figure 10, the microbial contamination on SS strips exposed in this area seemed to stabilize between 1000 to 9999 (10^3) viable micro-organisms per square foot of surface. Results similar to those obtained with SS strips have been obtained with glass and Lucite strips. In fact, significant differences were not noted in the numbers of micro-organisms recovered from stainless steel, glass, or Lucite surfaces. The number of viable micro-organisms ranged from about 1200 to 4500 per square foot throughout the study period. This leveling off, or "plateau," in surface contamination has been observed by a number of investigators (refs. 18–24). Results similar to those shown in figure 10 have been obtained during surface sampling studies conducted in Surveyor spacecraft assembly areas (refs. 18 and 22).

Table I contains the results of surface sampling studies conducted on a Mariner PTM spacecraft. It is interesting to note that the results obtained on SS strips placed in close proximity to the Mariner PTM (test 1) are similar to those results obtained with Rodac plate samples taken from horizontal surfaces of the spacecraft (test 1). These data lend support for use of the SS-strip-sampling method to determine the level of microbial contamination on space hardware.

TABLE I. — *Comparison of Methods to Assess the Level of Microbial Contamination on Surfaces of a Mariner Spacecraft*

Test No.	Number viable aerobic mesophiles recovered per square foot of surface		
	SS strips placed near spacecraft — simulated sampling (horizontal surfaces) [a]	Rodac plate samples from spacecraft surfaces	
		Horizontal surfaces [b]	Vertical surfaces [c]
1	1450	[d] 1150	160
2 (3 weeks later)	1700	[e] 750	30

[a] Average of 6 SS strips.
[b] Average of 12 Rodac plate samples.
[c] Average of 6 Rodac plate samples.
[d] Spacecraft uncovered.
[e] Spacecraft covered with plastic shroud for 2.5 weeks after test 1.

Figure 11 presents the results obtained from surface sampling studies conducted in Class II, III, and IV clean rooms in the Los Angeles area. Each plot represents the average value from six SS

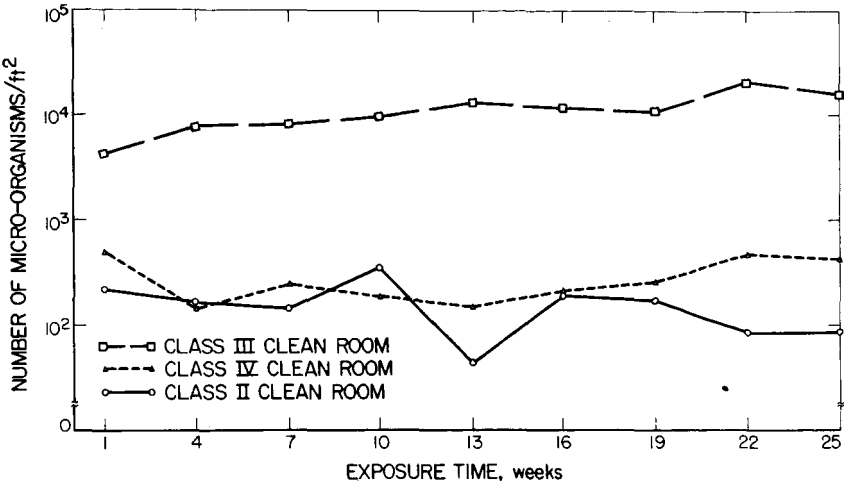

FIGURE 11.—Results of JPL surface sampling studies conducted in conventional clean rooms.

strips. From the data presented for the Class II clean room, it would appear that the level of surface contamination has risen progressively over the first 13-week sampling period. Then a plateau in surface contamination between 10 000 to 99 999 (10^4) micro-organisms per square foot of surface occurred on SS strips exposed within this clean room after the 13th week of sampling. Results for the Class III and IV clean rooms would indicate that a plateau in surface contamination occurred within 100 to 500 micro-organisms per square foot of surface in both rooms. Over a 25-week sampling period, the levels of microbial contamination on surfaces in the Class III clean room ranged from 40 to 400 micro-organisms per square foot of surface. During a similar exposure period in the Class IV clean room, the levels of microbial contamination on surfaces ranged from 140 to 480 micro-organisms per square foot of surface.

Figure 12 contains a summary of the results of surface sampling studies conducted by the PHS in the Phoenix area. From this figure, it appears that the microbial contamination on surfaces within clean room A appeared to stabilize around a level of 10 000 organisms per square foot. With the exception of the data obtained during the 15th week of exposure, the microbial contamination on surfaces within clean rooms B and C was approximately the same as the level observed in clean room A. The level of microbial contamination on surfaces exposed in the general manufacturing area seemed to be consistently higher than the levels of contamination accumulating in the clean rooms.

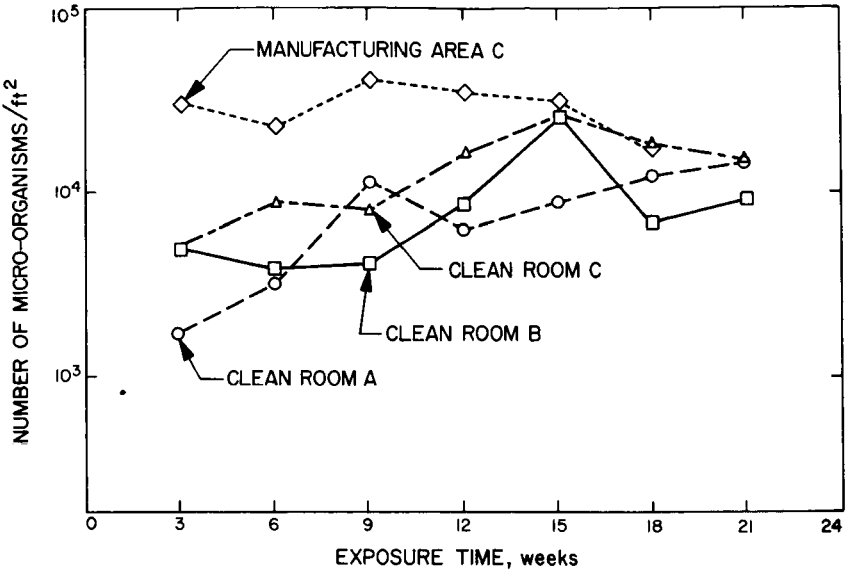

FIGURE 12.—Results of PHS surface sampling studies conducted in conventional clean rooms and in a factory area.

Results similar to those shown in figure 11 and 12 have been obtained in surface sampling studies conducted by the University of Minnesota.

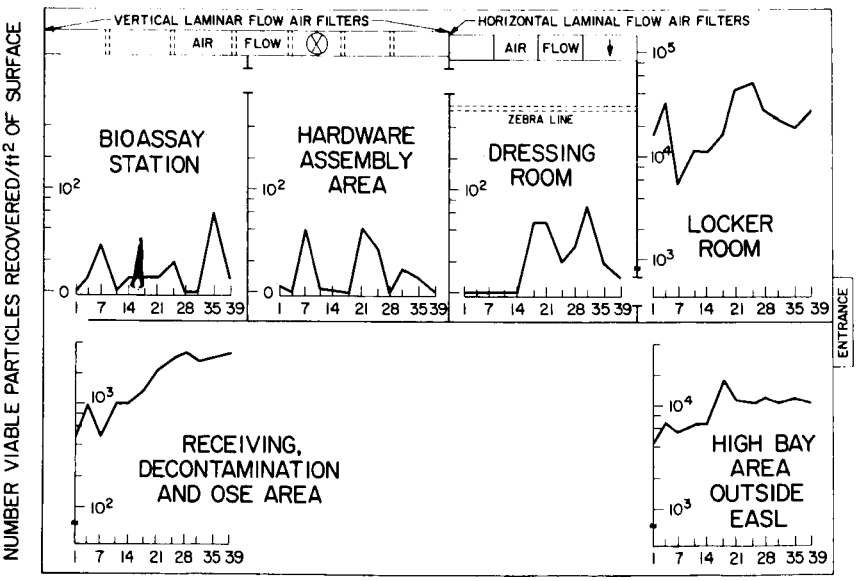

FIGURE 13.—Results of JPL surface sampling studies conducted in the EASL.

Microbial Contamination Accumulating on Surfaces Exposed Within Laminar Airflow Clean Rooms

Figure 13 presents the preliminary results obtained from surface sampling studies conducted in the JPL EASL facility. Each plot for the laminar downflow work area is the average value from 14 stainless-steel strips. For all other areas, each plot is the average value from six SS strips. A summary of the data given in figure 13 would indicate the following ranges in the levels of surface contamination per square foot of surface over a 39-day exposure period: laminar downflow work area, 0 to 40; bioassay station, 0 to 60; dressing room, 0 to 70; locker room, 5700 to 52 200; operational support equipment area (OSE), 500 to 3350; and the corridor outside of the EASL facility, 4500 to 17 600.

Figure 14 contains the results of the PHS surface sampling studies conducted in a horizontal laminar airflow clean room. Each

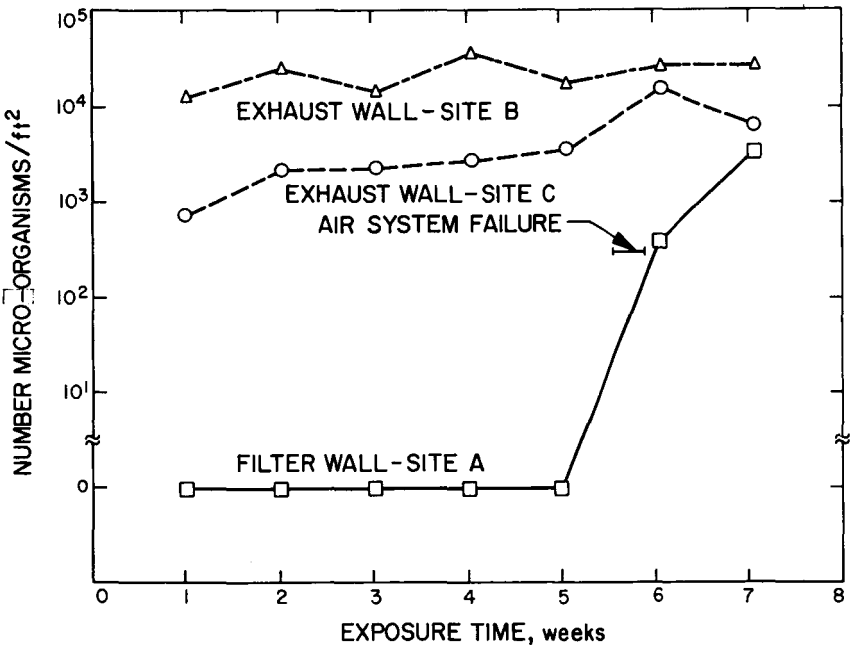

FIGURE 14.—Results of PHS surface sampling studies conducted in a horizontal laminar airflow clean room.

plot represents the average value from six SS strips. Site A was at the filter wall. Site B was near the exhaust wall and represented a sampling area downstream from three workbenches. Air leaving the filters passed the operating personnel at each of the three workbenches and was exhausted over site B. Site B also had the

highest level of surface contamination (10 000 to 40 000 micro-organisms per square foot of surface). Site C was also at the exhaust wall of clean room D, but the airstream did not directly pass by operating personnel. For the first 5 weeks of the study, no viable micro-organisms were recovered from SS strips placed at site A. Between the fifth and sixth week of the study, the air handling system failed. Following this failure, a 3-log (greater than 10 000 micro-organisms per square foot) increase in the level of microbial surface occurred at site A. This observation points out the sensitivity of the SS strip sampling method and also the need for routine surveillance and maintenance of air handling systems for laminar airflow clean rooms.

TABLE II. — *JPL Surface Sampling Studies Conducted in a Horizontal Laminar Airflow Clean Workbench*

Exposure time, weeks	Number micro-organisms per square foot	
	Average [a]	Range
2	64	0 to 288
4	24	0 to 96
6	[b] 144	96 to 288
8	8	0 to 48
10	16	0 to 48

[a] 6 SS strips assayed to form average.

[b] SS strips assayed immediately after 3 hours of continuous work in the clean workbench.

Table II contains the results of surface sampling studies conducted by JPL in a horizontal laminar airflow clean workbench. Stainless steel strips were placed near the filter wall of the workbench and some of the strips remained exposed for 10 weeks. During this period the bench was used to conduct normal operations such as dilution and plating of bacterial cell suspensions, assay of environmentally exposed SS strips, and routine filling of Rodac and Reyniers plates with sterile Trypticase Soy agar.

Qualitative Studies of Airborne and Surface Microbial Contamination Within Clean Rooms

Tables III and IV contain a qualitative breakdown of microbial contamination recovered from air and bench top samples in the four clean rooms surveyed by the University of Minnesota. It appears from these data that some differences do occur among rooms in the same building and also among buildings. These data indicate that the great majority of contaminants in all rooms were

TABLE III.—*Types of Micro-Organisms Isolated From Air and Surfaces in Company No. 1 Clean Rooms — University of Minnesota Study*

Type of micro-organism	Percent of micro-organisms, Company No. 1 clean rooms [a]			
	Room D		Room E	
	Air	Bench top [b]	Air	Bench top [b]
Gram-positive cocci	68.5	53.7	49.6	38.1
Bacillus spp.	3.2	2.6	2.3	3.2
Miscellaneous Gram-positive rods	28.1	42.5	43.2	52.4
Gram-negative rods	.1	.2	1.3	.4
Gram-negative cocci and coccobacilli	.1	.3	.3	.1
Actinomycetes	0	.1	.6	.5
Yeasts	0	.4	.2	.8
Molds	0	.2	2.5	4.5

[a] Based on 8 sampling days.
[b] Sampled with Rodac plate.

TABLE IV.—*Types of Micro-Organisms Isolated From Air and Surfaces in Company No. 2 Clean Rooms — University of Minnesota Study*

Type of micro-organism	Percent of micro-organisms, Company No. 2 clean rooms [a]			
	Room A		Room C	
	Air	Bench top [b]	Air	Bench top [b]
Gram-positive cocci	77.3	74.4	74.8	69.6
Bacillus spp.	2.8	1.0	4.1	.5
Miscellaneous Gram-positive rods	13.0	22.1	19.9	15.4
Gram-negative rods	6.1	1.5	.6	2.0
Gram-negative cocci and coccobacilli	.5	.4	.2	.1
Actinomycetes	0	.1	0	.1
Yeasts	.3	.5	.2	12.1
Molds	0	0	.2	.1

[a] Based on 8 sampling days. [b] Sampled with Rodac plate.

of human origin (Gram-positive cocci and diphtheroids). It also appears that the percentage of "sporeforming" bacteria (those showing evidence of spores after Gram staining) remained relatively constant among all four rooms (0.5 to 4.1 percent).

Tables V and VI contain the qualitative breakdown of microbial contamination from clean rooms A and B in the Public Health Service study. Table V shows the types of micro-organisms found in the intramural air and upon the bench-top surfaces in clean

TABLE V. — *Types of Aerobic Mesophilic Micro-Organisms in the Intramural Air and Surfaces in Clean Room A — Public Health Service Study*

Type of micro-organism	Air, percent of micro-organisms	Surface,[a] percent of micro-organisms
Staphylococcus epidermidis	41.7	20.5
Staphylococcus aureus	8.3	15.4
Sarcina spp.	8.3	0
Gaffkya spp.	2.8	0
Micrococcus spp.	5.5	25.6
Bacillus spp.	0	7.7
Corynebacterium spp.	2.8	0
Flavobacterium spp.	2.8	15.4
Pseudomonas Achromobacter spp.	13.9	15.4
Yeasts	2.8	0
Molds	8.3	0
Unidentified	2.8	0

[a] Sampled with Rodac plates.

TABLE VI. — *Types of Aerobic Mesophilic Micro-Organisms in Intramural Air and on Surfaces in Clean Room B — Public Health Service Study*

Type of micro-organism	Air, percent of micro-organisms	Surface,[a] percent of micro-organisms
Staphylococcus epidermidis	70.2	54.2
Staphylococcus aureus	5.9	0
Micrococcus spp.	3.6	12
Sarcina spp.	0	1.2
Gaffkya spp.	1.2	0
Streptococcus spp.	1.2	0
Bacillus spp.	0	3.6
Corynebacterium spp.	9.5	16.9
Neisseria catarrhalis	0	1.2
Pseudomonas-Achromobacter spp.	3.6	1.2
Yeasts	0	0
Molds	1.2	2.4
Actinomycetes	1.2	1.2
Unidentified	2.4	6

[a] Sampled with Rodac plates.

room A. In both cases the predominant micro-organisms isolated were those associated with skin, nose, mouth, and hair of humans. The occurrence of organisms associated with soil was relatively low. Few members of the genus *Bacillus*, the aerobic sporeforming bacteria, were isolated. Table VI shows that the types of micro-organisms found in the intramural air and upon the bench top surfaces in clean room B were also associated with the skin and respiratory tract of humans. Few sporeformers and other micro-organisms associated with soil were isolated.

Table VII contains the types of micro-organisms that accumulated on SS strips in three clean rooms and two manufacturing areas in the PHS study. The predominant types of micro-organisms that accumulated in clean room A and the two manufacturing areas were *Bacillus* spp. and molds, micro-organisms commonly found in soil. Clean rooms B and C were more rigidly controlled and the predominant types of micro-organisms isolated in these rooms were those species commonly associated with humans.

TABLE VII. — *Types of Aerobic Mesophilic Micro-Organisms Which Accumulate on Stainless Steel Surfaces (1- × 2-inch Strips) Exposed to the Intramural Air of Three Clean Rooms and Two Manufacturing Areas — PHS Study*

Type of micro-organism	Clean room A, percent	Clean room B, percent	Clean room C, percent	Manufacturing area C, percent	Manufacturing area D, percent
Staphylococcus aureus	0	0.5	0	0	0
Staphylococcus epidermidis	1.9	47.2	22.1	12.7	14.6
Micrococcus spp.	4.3	6.4	8.6	1.6	6.1
Streptococcus spp.	0	.9	2.9	0	2.3
Bacillus spp. (sporeformers)	55.5	10.1	17.9	30.9	21.5
Miscellaneous Gram-positive bacilli[a]	14.3	28.4	35.7	10.3	23.8
Gram-negative micro-organisims	0	0	0	[b] 15.1	0
Yeasts	1.9	.9	.7	1.6	.8
Molds	12.9	2.7	6.4	25.4	23.8
Actinomycetes and streptomycetes	5.7	1.4	2.1	2.4	6.9
Unidentified or lost upon subculture	3.3	1.4	0	0	0

[a] Predominant genera: *Brevibacterium*, *Corynebacterium*, and *Lactobacillus*.
[b] *Pseudomonas* spp.

Contamination Due to Human Handling of Sterile Objects

Table VIII contains the results obtained from a PHS study to determine the levels of microbial contamination deposited on SS strips by personnel performing actual assembly operations. There was much variation from person to person, but the levels from each person were usually consistent, especially in the case of those persons who deposited low levels.

TABLE VIII. — *Levels of Microbiological Contamination Deposited on Stainless Steel Strips (1 × 2 inches) by Personnel Performing Assembly Operations in Clean Room A — PHS Study*

Subject	Number of micro-organisms	
	Average per strip [a]	Range
A	0.8	0 to 2
B	23.8	1 to 77
C	.8	0 to 2
D	34.8	18 to 69
E	.8	0 to 1
F	10.1	6 to 16
G	1.2	0 to 3
H	104.0	41 to 207
I	2.0	0 to 4
J	1.4	0 to 3
K	12.2	2 to 24
L	.3	0 to 1

[a] Average of five strips.

Similar results were obtained from personnel also performing actual assembly operations but half of whom wore finger cots (table IX). The use of finger cots reduced the levels of both nonsporeforming organisms and spores deposited on electronic components.

A similar handling study was conducted in the JPL survey. Table X contains the results obtained when five individuals in the Class III clean room handled 10 SS strips in sequence. It may be seen from these data (table X) that the microbial contamination from two of the subjects (nos. 4 and 5) was randomized, whereas the other three individuals in the study showed a gradual reduction in contamination with each strip handled.

Effect of Environmental Exposure on the Survival of Micro-Organisms

The results of microbial survival studies performed by the PHS in clean room B are presented in table XI. Immediately prior to

TABLE IX. — *Levels of Microbiological Contamination Deposited on Transistors by Personnel Performing Assembly Operations (Manufacturing Area A)*

[Average of Five samples]

Subject	Number of micro-organisms		Number of spores	
	Average per/component	Range	Average per/component	Range
	Ungloved personnel			
A	15.8	5 to 21	13.2	0 to 21
B	87.8	31 to 269	5.2	1 to 12
C	51.4	3 to 223	.2	0 to 1
D	1.2	0 to 4	10.8	2 to 26
E	54.2	18 to 122	33.6	2 to 152
	Personnel wearing finger cots			
F	11.2	2 to 22	2.2	0 to 6
G	4.7	0 to 19	.4	0 to 2
H	13.2	6 to 16	.8	0 to 2
I	13.7	8 to 27	.4	0 to 1
J	15.8	12 to 25	1.6	0 to 4

TABLE X. — *Microbial Contamination Resulting on Stainless Steel Strips Handled by Class III Clean Room Personnel — JPL Study*

Strip	Micro-organisms per 1- × 3-inch strip				
	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5
1	72	241	61	46	25
2	12	135	35	43	52
3	16	93	26	98	7
4	1	16	24	31	46
5	3	57	17	79	11
7	3	22	5	98	22
6	9	16	12	29	12
8	2	20	6	36	104
9	4	0	9	67	16
10	1	11	18	60	37

covering, the level of aerobic micro-organisms was approximately 9000 per square foot. The majority of these organisms were

TABLE XI.—*Types of Micro-Organisms Surviving on Stainless Steel (1- × 2-inch) Surfaces Initially Exposed to the Intramural Air of Clean Room B for 21 Weeks and Then Covered With Sterile Aluminum Foil—PHS Study*

Type of micro-organism	Micro-organisms immediately before covering,[a] percent	Micro-organisms 3 weeks after covering,[b] percent
Staphylococcus aureus	0	0
Staphylococcus epidermidis	61.1	5.2
Micrococcus spp.	11.1	17.9
Bacillus spp. (sporeformers)	13.9	48.7
Miscellaneous Gram positive bacilli [c]	5.6	7.7
Gram negative micro-organisms	0	0
Yeasts	2.7	0
Molds	5.6	17.9
Actinomycetes and streptomycetes	0	2.6

[a] Level of aerobic micro-organisms was 9237 per square foot.
[b] Level of aerobic micro-organisms was 4557 per square foot.
[c] Predominant genera: *Brevibacterium, Corynebacterium,* and *Lactobacillus.*

Staphylococcus spp., *Micrococcus* spp., and other nonsporeforming bacteria. The total population of aerobic micro-organisms decreased by 51 percent after 3 weeks. Organisms surviving storage were mostly *Bacillus* spp. (sporeformers) and molds.

Similar results were obtained from PHS experiments in which SS strips were exposed to the intramural air of a laboratory for 3 weeks and then covered. Microbiological assays were performed immediately prior to covering with sterile aluminum foil and at intervals up to 8 weeks after covering. At each assay period, 30 to 40 colonies were picked randomly and subsequently identified. The results are presented graphically in figure 15. The population of aerobic micro-organisms decreased by 50 percent after 2 weeks and then remained at a constant level. Identification studies, however, showed that this seemingly significant decrease was due primarily to the death of nonsporeforming bacteria. Aerobic sporeformers (*Bacillus* spp.) as well as molds and actinomycetes were hardly affected. The relative humidity and temperature, which were recorded continuously, ranged from 42 to 49 percent and from 71° to 76° F, respectively.

Micro-organisms deposited on SS surfaces by handling decreased significantly during sterile storage. Table XII (PHS study) shows that approximately 80 percent of the initial population failed to survive after 2 weeks of storage. As determined by qualitative studies, the vast majority of micro-organisms deposited

TABLE XII. — *Survival of Micro-Organisms Deposited on Sterile Stainless Steel Strips by Handling and Subsequently Stored in a Sterile Environment*

[Average relative humidity throughout test period, 45.5 percent (39 to 50 percent); average temperature, 73° F (70° to 77° F)]

Subject, average, and percent reduction (average of 5 tests)	Number of micro-organisms per 2- × 2-inch SS strip					
	Immediately after handling	24 hours after storage	7 days after storage	14 days after storage	3 weeks after storage	4 weeks after storage
L-2-W:						
Average	69.4	8.6	3.2	2.8	1.0	0.2
Reduction, percent	-----	87.6	95.4	96.0	98.6	99.7
F-1:						
Average	43.3	18.0	2.8	9.2	2.2	.4
Reduction, percent	-----	58.4	93.5	78.8	94.9	99.1
F-2-W:						
Average	178.4	15.2	3.2	1.4	4.2	1.4
Reduction, percent	-----	91.5	98.2	99.2	97.6	99.2
P-1:						
Average [a]	83.7	25.5	5.5	.8	.3	-----
Reduction, percent	-----	69.5	93.4	99.0	99.6	-----
C:						
Average	44.2	20.0	16.5	2.2	1.6	2.2
Reduction, percent	-----	54.8	62.7	95.0	96.4	95.0
L-1:						
Average	6.4	1.4	1.8	1.2	.8	.6
Reduction, percent	-----	78.1	71.9	81.3	87.5	90.6

[a] Average of 4 tests.

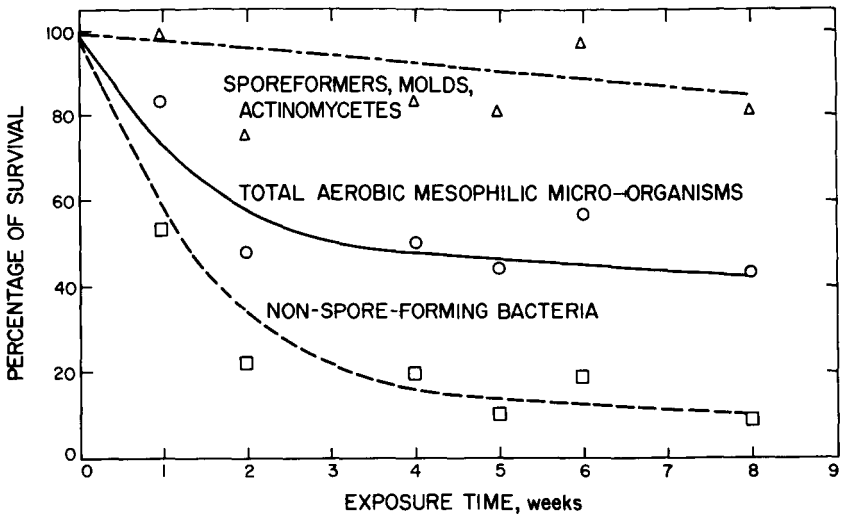

FIGURE 15.—Survival of airborne naturally occurring micro-organisms on stainless steel surfaces (PHS study).

on these surfaces were nonsporeformers. The levels of aerobic spores deposited by handling were consistently so low that dieoff rates could not be measured reliably. Table XIII contains typical results of the latter type of experiment.

TABLE XIII. — *Levels of Aerobic Spores Deposited on Stainless Steel Strips by Handling and Their Survival in a Sterile Environment*

Subject	Average number [a] of spores per 2- × 2-inch SS strip					
	Immediately after handling	Number of days after storage				
		1	7	14	21	28
C	3.4	0	0.2	0.2	0.4	3.8
P	2.3	5.8	.5	.8	0	....
O	4.0	1.0	....	2.0	5.0	....
M	.8	....	.3	.5	.5	....

[a] Average of five strips.

Table XIV contains the results of microbial survival study conducted by the University of Minnesota. With the exception of the results after day 2, all the covered SS strips show evidence of a microbial die-away throughout the 14-day test period.

TABLE XIV. — *Levels of Micro-Organisms Accumulating on SS Strips (1 × 2 inches) Exposed in a University of Minnesota Laboratory*

Exposure period, days	Average[a] number of micro-organisms per square foot	
	A[b]	B[c]
1	655	302
2	720	5220
3	598	720
4	1562	417
5	3477	417
6	3182	59
7	1022	122
8	1562	598
9	2937	122
10	2642	417
11	3002	777
12	3182	144
13	2217	417
14	2462	475

[a] Average based on assay from five 1- × 2-inch SS strips.

[b] Total mean count for strips plated immediately after exposure period.

[c] Total mean count for strips covered and stored for a 2-week interval after exposure period, then plated.

Figure 16 contains the results of microbial survival studies conducted in the JPL SAF. As seen in figure 16, there was an approximate reduction of 1 log of microbial contamination on SS strips during the covered storage period. During this storage period, personnel activity in the SAF was considerably increased and simultaneously an increase in the level of microbial contamination was detected on the uncovered SS strips.

Figure 17 contains the results of JPL studies to determine the survival rates of micro-organisms artificially inoculated onto SS surfaces (1.0 square centimeter) and exposed to controlled environmental conditions (50° C and 49 percent relative humidity (RH)). The test cultures included vegetative species such as *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris* and *Staphylococcus aureus,* as well as *Bacillus subtilis* var. *niger* spores. This spectrum of test cultures was considered to be representative of soil-type micro-organisms (spores) and vegetative cells most of which are commonly associated with the hair, skin, respiratory tract, and the gastrointestinal tract of humans. A set of environmental conditions was selected to provide the worst case situation for the exposed vegetative cells (refs. 25 to 35).

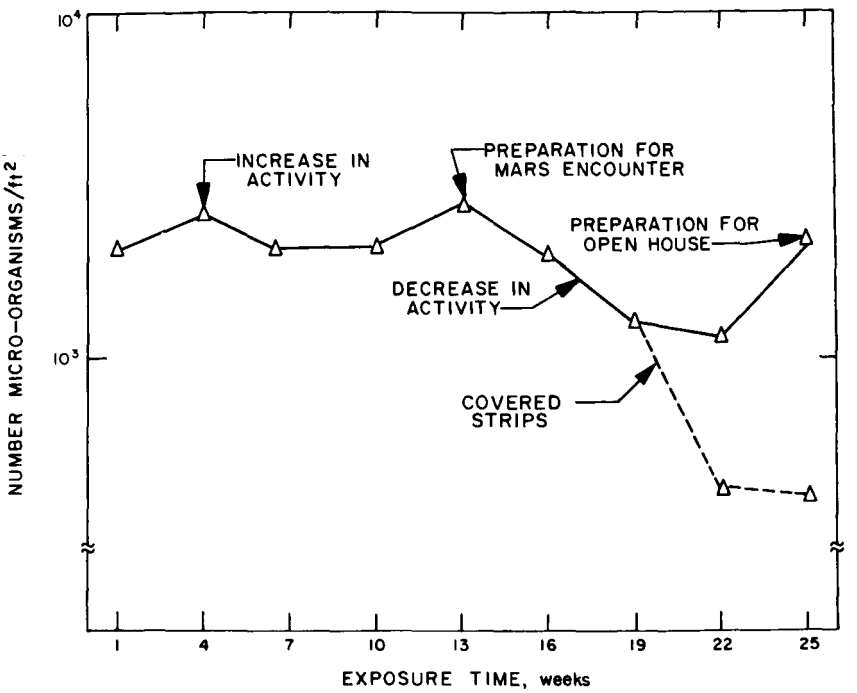

FIGURE 16.—Results of JPL studies to determine microbial survival rates in the Mariner spacecraft assembly facility.

Desiccators containing saturated salt solutions were used as static RH chambers (ref. 29). From the results shown in figure 17, it can be seen that a temperature of 50° C and an RH of 49 percent was rapidly lethal for all species of vegetative cells. Viable microorganisms were not recovered from any of the surfaces inoculated with vegetative cells after 4 to 12 hours of exposure to the test conditions. The survival rate for spores of *B. subtilis* var. *niger* was quite the opposite. It appears as though there may have been a slight drop in the number of viable spores after 4 to 24 hours of exposure to 50° C and 49 percent RH. After this, the level of viable spores remained virtually unchanged for the remainder of the test (1 week). These data are in support of the well-documented evidence concerning the resistance of bacterial spores to adverse physical and chemical conditions.

Prediction of the Level of Microbial Contamination on Space Hardware

Data obtained from all aspects of environmental microbiology (intramural levels of microbial contamination, assays from hardware piece parts, microbial contamination from human handling,

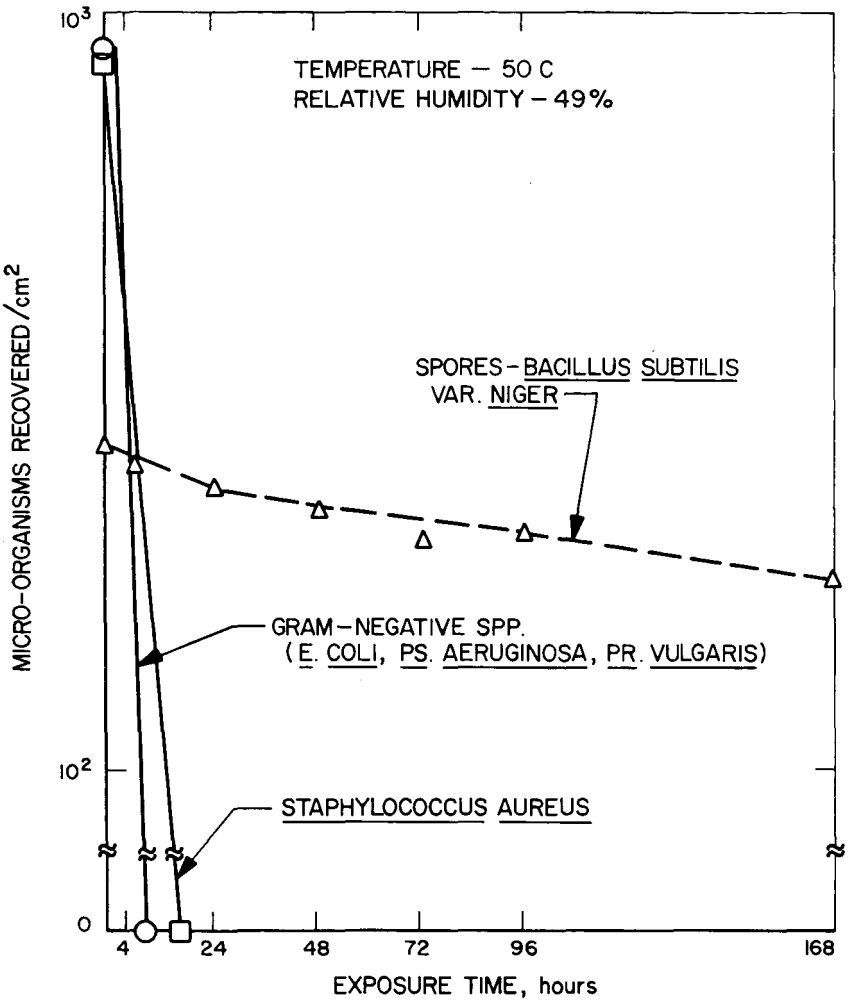

FIGURE 17.—Results of JPL studies to determine the effects of environmental conditions on surface-exposed vegetative cells and bacterial spores.

etc.) must be coordinated with other data (results of visual assays for procedural breaks, records of facility parametric measurements such as temperature, pressure, relative humidity, maintenance procedures, etc.) to predict the total level of microbial contamination on space hardware immediately prior to the terminal sterilization cycle. A system to handle all aspects of data relating to the microbial contamination level on space hardware has been developed and this approach is discussed in a subsequent paper by Jack D. Johnson.

Discussion

It is evident from these preliminary results that as the environment of a clean room was controlled in a more positive manner with respect to the reduction of particulate contamination, the numbers of contaminating micro-organisms were also reduced. The chief sources of microbial contamination were associated with the clean room personnel. This was evidenced by the fact that total numbers of airborne viable particles increased or decreased depending on the presence of personnel in the areas tested. In addition, most of the micro-organisms isolated from the air and surfaces were associated with the skin, nose, and mouth of humans.

Comparison of the results obtained from air-sampling studies with the data obtained in the surface sampling studies is not possible. It must be realized that the two methods of sampling microbial contamination are different. The volumetric air sampler measures the number of viable particles that are suspended in air during a specific sampling interval. The SS strips measure the number of viable particles that sediment from airborne suspension, as well as the subsequent survival and accumulation of such sedimented particles. Furthermore, the methods of microbiological assay do not produce the same type of results. If, for example, large particles containing many viable micro-organisms were shed into the environment by clean room personnel, such particles would, if large enough, rapidly settle out of airborne suspension. If such a large particle was collected with a volumetric air sampler, only one colony would result, even though the particle contained many viable micro-organisms. However, if the same particle landed on an SS strip, subsequent analysis by shaking the SS strip in a liquid medium might break the large particle into clumps of smaller particles, each containing viable micro-organisms. After plating and incubation, an increased number of colonies would be detected from the SS strip sample. Therefore, the air sampling data are expressed as the number of viable particles per cubic foot of air and the SS strip data are reported as the number of viable micro-organisms per square foot of surface.

In general, preliminary results indicate that volumetric sampling of air proves to be a good indication of the levels of microbial contamination when the number of airborne viable particles is high (figs. 3, 5, and 6). Differences in the levels of airborne microbial contamination could be sharply noted and correlated with concurrent activities. But, when the degree of airborne microbial contamination was low (figs. 8 and 9 — site 1), the

sensitivity of the volumetric air-sampling method was quickly lost. In fact, the available sampling devices do not appear to be adequate to insure a representative sample when these instruments are used to sample vertical laminar airflow clean rooms. Volumetric sampling of air is of value in laminar airflow clean rooms when procedural conditions or operations cause interference or interruption in the laminar air stream. Under such conditions, eddy currents may cause microbial contamination to accumulate on, or to be deposited onto, the surface of an item being assembled under such conditions.

The level of environmental contamination recovered is also determined by the microbiological sampling method used. As personnel density increased in the vicinity of a volumetric air sampler, "clouds" of microbial aerosols may have been generated. Collection of such aerosols would result in proportionately high levels of airborne viable particles. However, the deposition of such airborne viable particles onto SS strips might yield an entirely different set of results. Environmentally exposed micro-organisms are subjected to a number of conditions that may have an effect on the survival of a given species. A number of studies (refs. 26–33) have shown that variables such as temperature and relative humidity, desiccation, exposure time, species, age, and number of exposed micro-organisms, as well as the availability of nutrient materials, all play a role in the survival or "die-away" of micro-organisms exposed in the environment.

The level of microbial contamination deposited on SS surfaces as the result of handling varied among different subjects. Separating the skin from the surface being touched by a finger cot reduces deposited contaminants. Alcohol rinses appear to be effective in decontaminating hands (ref. 20). Although the use of hexachlorophene soap reduces the level of contamination deposited, it does not appear to be as effective as alcohol rinsing. The use of a conventional soap is practically worthless if not undesirable. In most instances the level of contamination increases 1 to 2 logs after washing (ref. 20). These results agree with studies concerned with increased shedding of micro-organisms after showering (refs. 34 and 35).

Studies of the fallout and accumulation of airborne micro-organisms on SS strips showed an interesting phenomenon. It must be emphasized that the results from the SS strip method represent a range of microbial contamination per unit of surface (square foot). Such data are extrapolated from results obtained with 1- $\times$ 2-inch or 1- $\times$ 3-inch SS strips. The value per each SS strip is multiplied by a factor of 72 or 48, respectively, in order to convert

it to the number per square foot. It is important to realize that three colonies developing from the assay of a single 1- × 3-inch SS strip would be expressed as 144 viable micro-organisms per square foot. While there may be better ways to express these data mathematically, the fact remains that for each environmental area sampled, a plateau in the level of microbial contamination on surfaces was observed. Preliminary data indicate that this plateau is reached within the first week (ref. 24) and that the plateau then remains for periods up to 1 year (ref. 24), the longest period studied to date. The reason for this plateau is not clear. Earlier work would indicate that an original inoculum of vegetative cells would not survive extended periods of exposure to environmental conditions (refs. 26 to 33). Furthermore, preliminary results from the heat-shocking studies indicate that a large spore population is not deposited onto SS strips in the early weeks of environmental exposure. Consequently, an original inoculum of spores does not seem to be a factor in the occurrence of a plateau in surface contamination. The most plausible explanation for the presence of a plateau would be that the number of micro-organisms deposited onto, or surviving upon, surfaces is roughly balanced by the number of micro-organisms that are dying on the same surface (fig. 18). Such a system is dynamic in nature and is influenced by multiple factors. In any case, more work is necessary to define and elucidate the reason(s) for the leveling off of microbial contamination on surfaces.

Finally, results of the surface sampling studies to date indicate that the SS-strip method is quite reliable. In fact, SS strips have proven to be fairly sensitive in detecting changes in low levels of microbial contamination in the laminar-flow areas of the JPL EASL facility as well as in some of the clean rooms included in the PHS studies. Presently, use of a surface sampling procedure such as the SS-strip method seems to be the only reliable tool to measure the level of microbial contamination within vertical laminar airflow clean rooms. Furthermore, use of a surface sampling technique such as the SS strips is quite logical, for the primary interest in monitoring the level of intramural microbial contamination is to determine what level and species of micro-organisms fall onto and accumulate on space hardware. As presented in table I, a comparison of the SS-strip "simulation" method with actual sampling of spacecraft hardware has shown the SS-strip method to be quite adequate in predicting the level of microbial contamination on spacecraft surfaces. Thus, it would seem that the SS-strip method is a valuable tool for use in monitoring the level of microbial contamination that accumulates on surfaces within

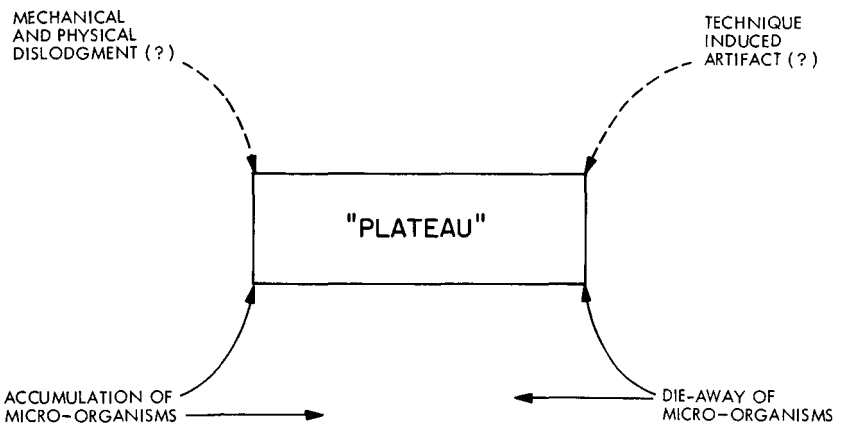

FIGURE 18.—Interplay of possible factors involved in the "plateau" observed in microbial contamination on surfaces.

facilities involved in the assembly and/or test of space hardware.

The results obtained in studies concerned with the survival of micro-organisms on surfaces were most interesting. It appears from the preliminary data that the death rates for micro-organisms on SS strips depends, to great extent, on the species involved. Nonsporeforming species exhibited a definite die-away during exposure to environmental conditions. Aerobic bacterial spores, molds, and actinomycetes do not appear to be significantly affected during environmental exposure. From these and other data (refs. 26–33) it is apparent that vegetative bacteria are quite susceptible and die off quite rapidly during exposure to most environmental conditions. Microbial spores tend to be considerably more resistant to such conditions and show extended survival rates.

Storage of space hardware in a sterile or even a "bioclean" environment may cause significant reductions in the levels of nonsporeforming micro-organisms on surfaces. However, it appears doubtful that the level of microbial spores would be simultaneously reduced. Consequently, any general conclusions applicable to space-hardware decontamination should be based on future experiments designed to measure dieaway of both naturally occurring aerobic and anaerobic spores. Such experiments should be

augmented by artificially inoculating other similar surfaces with known numbers of aerobic and anaerobic spores and measuring subsequent die-away under controlled environmental conditions. Both relative humidity and temperature should be rigidly controlled during such studies.

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Skin Carriage of Bacteria in the Human

A variety of techniques is required to assess the bacterial population on and in the skin. Areas of high and low density of bacteria on the skin are found. The pattern of spread is common in all normal individuals and no sex differences have been observed. Each person maintains a level of population within a limited range and climatic changes appear to have little or no influence on the level. Methods such as scrubbing and flushing and the use of germicides can cause a temporary reduction in numbers, but there is a rapid return to normal levels. Mechanical barriers still remain the best method to handle sterile objects.

Human skin serves as an excellent site for the growth and survival of bacteria (refs. 1–3). The products of excretion may serve as nutritive sources for some micro-organisms, while other excreta may be inhibitory in nature. Bacteria are found not only on the skin surface but are found throughout the *stratum corneum* and also in the ducts of some of the excretory glands.

The skin flora covers all parts of the body, but some areas consistently carry denser populations than others. A variety of methods have been employed to qualitate and quantitate the bacteria of the skin. These methods fall into four main categories or combinations of them (refs. 4-6). They are swabbing techniques, contact plate methods, scrubbing and flushing (Price technique), and tape-stripping methods. No one method can give the full picture of the distribution of bacteria on or in the skin. The last three listed methods have given the most consistent results. Each method determines a specific part of the skin population. The contact plate method is the simplest and most readily performed (fig. 1). The plate containing a nutrient medium is momentarily put in contact with the skin, incubated, and observed for both the kinds and number of bacteria present. It gives results that are reproducible, but measures only the organisms that are on the surface and readily removed.

The Price technique (fig. 2) consists of a consecutive series of scrubbings and flushing into a series of basins followed by a quantitative plate count of the micro-organisms in each basin. This method measures the surface bacteria and those that can be

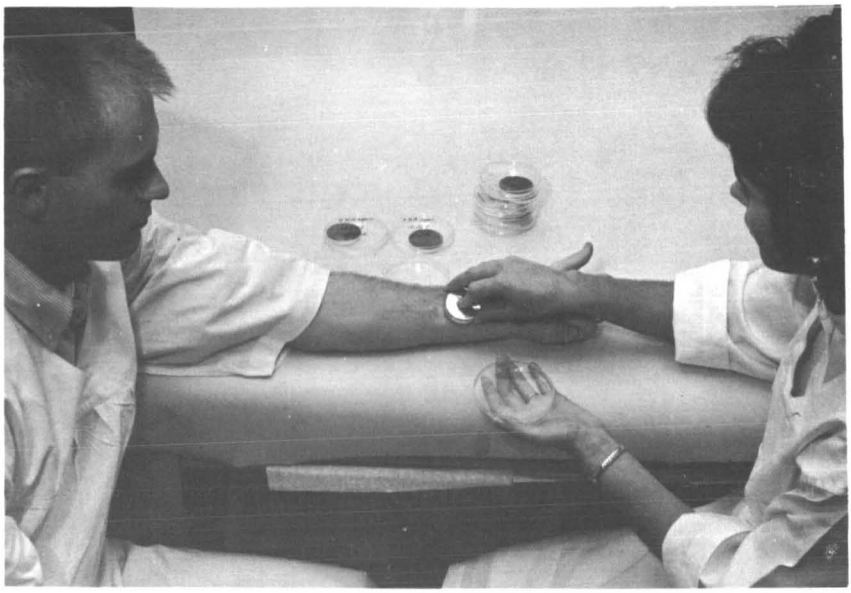

FIGURE 1.—Contact plate method.

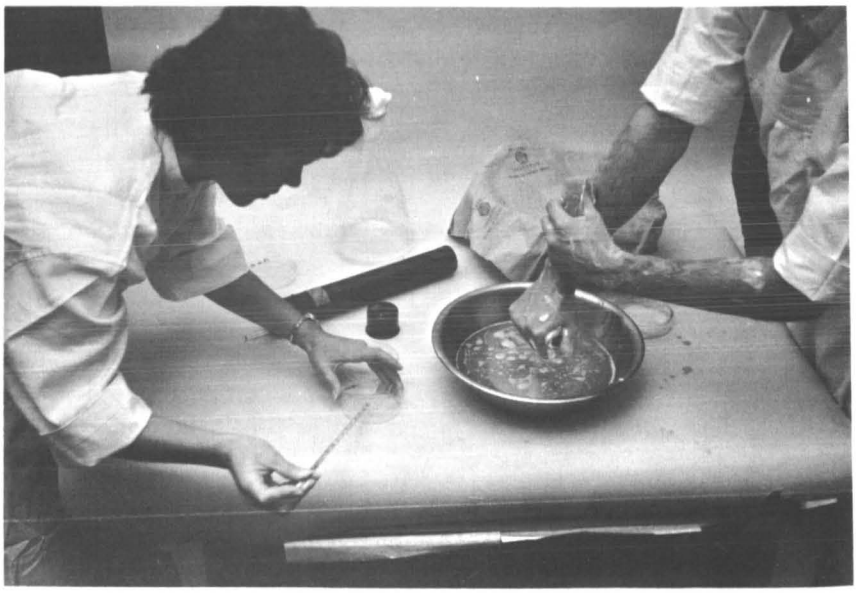

FIGURE 2.—Price technique.

removed from deeper sources such as the ducts of the excretory glands.

The bacteria in the skin can be determined using the tape-stripping method (fig. 3). A transparent adhesive tape is applied to the test area, peeled off, and covered with a nutrient agar in a Petri plate. Serial applications to the same site each remove a layer of skin cells with the bacteria present. Twenty layers of cells have been consistently removed in test subjects with little difficulty and no serious sequelae other than a slight reddening and tenderness after the first series.

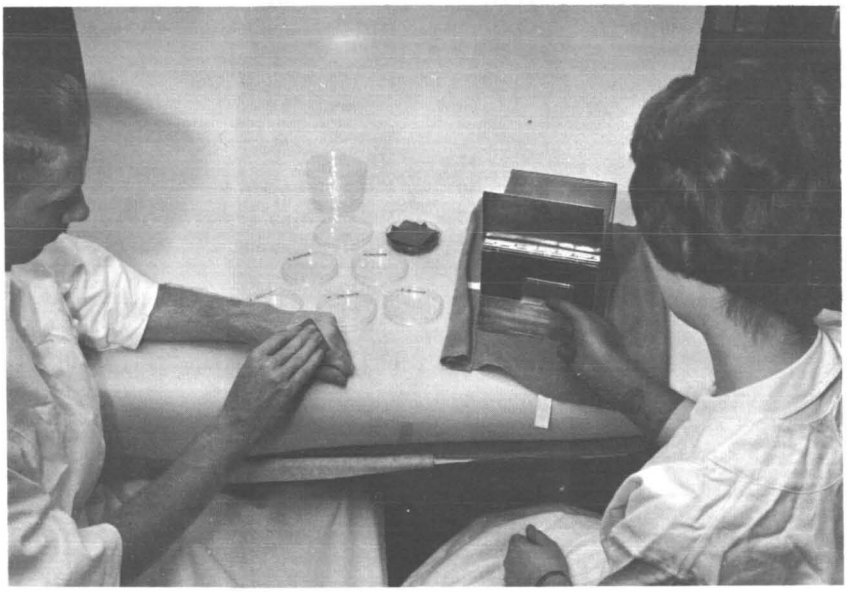

FIGURE 3.—Tape-stripping method.

The distribution of bacteria over the skin surface varies greatly from site to site. The results of full-body testing using the contact plate method are compiled in table I. The data, from one subject, indicate the general type of distribution in individuals with normal skin. The head and neck, wrists and hands, navel, groin, buttocks, perineum, inner aspect of the thighs, and the feet maintain high bacterial populations. The axilla are normally high, but in many test subjects the counts appear low because of the use of underarm deodorants which commonly contain antibacterial substances. The shoulders, arms, and trunk normally carry moderate to low bacterial populations.

TABLE I.—*Bacterial Skin Populations Using Contact Plate Method—Right Side Only*

[TNTC indicates too numerous to count]

SITE	BACTERIAL PLATE COUNT	SITE	BACTERIAL PLATE COUNT
Forehead	348	Lateral forearm	41
Temple	560	Lateral elbow	8
Under eye	TNTC	Volar elbow	24
Nose	TNTC	Lateral wrist	TNTC
Upper lip	TNTC	Volar wrist	53
Cheek	548	Palm — hand	*Pseudomonas*
Angle of jaw	TNTC	Lateral chest	15
Behind ear	TNTC	Lateral waist	15
Under chin	TNTC	Navel	TNTC
Back of neck	211	Groin	TNTC
Right side — neck	316	Perineum	TNTC
Shoulder	43	Subscapular	128
Subclavicular	83	Buttock	TNTC
Nipple	41	Hip	104
Breast	50	Lateral upper leg	232
Axilla	106	Inner upper leg	TNTC
Lateral upper arm	42	Popliteal space	241
Volar upper arm	8		

TABLE II.—*Comparison of Bacterial Skin Populations in Different Individuals—Contact Plate Method*

[TNTC indicates too numerous to count]

Site	Subject and sex			
	I–M	XIII–M	IV–F	V–F
Angle of jaw	TNTC	TNTC	137	TNTC
Breast	31	37	11	278
Axilla	28	TNTC	3	47
Arm (above elbow)	6	95	17	[a]
Abdomen (midline)	17	31	32	121
Groin	155	75	40	113
Leg (above knee)	46	85	51	125
Ankle	169	186	185	[a]
Neck (back)	145	179	32	158
Back (midline waist)	63	50	4	62
Buttock	TNTC	213	16	52
Popliteal space	63	67	11	25
Foot sole	TNTC	TNTC	TNTC	TNTC

[a] Sporeformers.

The distribution of bacteria on the skin of individuals without lesions follows the pattern just discussed, but the level of the bacterial population is peculiar to the individual. Table II shows some of these variations using the contact plate method. Both males, subjects I and XIII, are considered to carry intermediate levels of micro-organisms, whereas subject IV, a female, has a low level of carriage. Subject V, also a female, has a high bacterial skin population. Although the limited data of this table do not indicate the range of variation for men and women, examination of a large number of subjects indicates that there is no consistent difference of range between the sexes.

TABLE III. — *Average Bacterial Counts per Month on Hands and Arms — Contact Plate Method*

[Each value is an average of 32 determinations]

Month	Subject and sex			
	XIII–M	XIV–M	XV–F	XVI–F
January	56	217	40	13
February	45	92	54	25
March	41	68	21	75
April	44	70	33	23
May	18	....	26	17
June	40	35	16	16
July	58	....	....	15
August	42	....	....	....
September	39	....	....	42
October	20	....	40	35
November	44	....	60	53
December	46	146	34	23
AVERAGE	41	105	36	32

The data in table III show the average variations by month and also indicate the range of variation for each subject. Subjects XIII, XV, and XVI are considered moderate-range carriers, with the respective ranges being 18 to 58, 16 to 60, and 13 to 75. Subject XIV is a high-range carrier and extends from 35 to 217 for his average monthly counts. Although a subject may show variations that extend into the range of either side, there is no tendency to remain at the extremes of his range for any length of time.

The data in table III also give no consistent correlation with climatic changes. The average monthly variation appears to be

independent of seasonal changes. This does not imply, however, that local and immediate conditions do not have an effect. Evans (ref. 2) has shown that increased sweating may temporarily cause a slight increase in the numbers of skin bacteria.

Many attempts have been made to remove bacteria from skin, and even to sterilize it using a variety of substances and methods. Surgeons have long relied upon the technique of scrubbing and flushing to reduce bacterial skin flora. Price has developed a standardized method to test the effectiveness of antiseptic and germicidal preparations.

Data gathered using the Price technique are presented in table IV. Castile soap without bacterial inhibitors was used in these

TABLE IV. — *Removal of Bacteria From Hands and Arms, Using the Price Technique*

[Value in parentheses is 2 times the standard deviation of the mean]

Basin	Bacterial count per basin (subject, sex, and number of tests)			
	XIII–M–43	XIV–M–18	XV–F–25	XVI–F–29
1	40 (12)	465 (179)	194 (39)	196 (46)
2	64 (20)	295 (65)	182 (34)	219 (44)
3	71 (17)	214 (38)	156 (23)	210 (54)
4	65 (15)	183 (40)	133 (21)	175 (47)
5	62 (16)	154 (27)	129 (22)	170 (47)
6	44 (11)	147 (38)	124 (34)	151 (39)

studies. As noted in the contact plate method, individuals fall into ranges. Those in the very high or low ranges, using the contact plate method, remain in these ranges with the Price technique, but those in the intermediate contact plate range may fall in either the low or high range with the scrubbing and flushing method. Subject XIII is placed in the intermediate contact plate group, but obviously fits into the low-range group with the Price technique.

The data in table IV are of interest in that they indicate that even after 6 minutes of scrubbing and flushing, considerable numbers of organisms are still present on and in the skin. The number of bacteria continues to fall with each subsequent scrub and flush, but sterility cannot be obtained by this method.

The fingertips are of especial interest because this is the area that most commonly makes contact in mechanical procedures. Table V contains data on the removal of bacteria from the

TABLE V. — *Removal of Bacteria From Fingertips, Using the Price Technique*

[Value in parentheses is 2 times the standard deviation of the mean]

Basin	Bacterial count per basin (subject, sex, and number of tests)			
	XIII–M–10	XIV–M–10	XV–F–10	XVI–F–10
1	17 (6)	128 (51)	50 (19)	120 (109)
2	27 (10)	98 (32)	58 (20)	164 (90)
3	20 (4)	74 (26)	43 (12)	117 (60)
4	20 (6)	62 (25)	55 (23)	109 (45)
5	23 (13)	51 (17)	47 (17)	65 (27)
6	16 (7)	52 (20)	34 (11)	59 (21)

fingertips. As with the whole hand and arm, the number of bacteria removed from the fingertips continues to be reduced with each succeeding basin. Relatively large numbers of bacteria still remain after 6 minutes of the procedure.

Germicides are commonly employed along with soaps or detergents to help suppress bacterial skin populations. They do help hold down bacterial increase for a time, but there is a slow eventual rise in bacterial numbers to the level normal for the individual. Tables VI and VII compile data on the use of surgical scrubbing preparations containing hexachlorophene and an iodoform, respectively. A typical surgical scrub with a brush was performed for 10 minutes, with flushing every 2 minutes. Immediately after the 1-minute test, the hands were put into sterile

TABLE VI. — *Bacterial Skin Populations Following 10-Minute Hand Scrubbing With a Hexachlorophene Preparation, Contact Plate Method*

[TNTC indicates too numerous to count]

Time, min	Bacterial colonies per plate for subject —			
	IV		VI	
	Test 1	Test 2	Test 1	Test 2
Before	210	12	100	103
1	0	3	53	77
15	8	36	TNTC	150
75	0	6	23	13
170	20	0	36	—

TABLE VII. — *Bacterial Skin Populations Following 10-Minute Hand Scrubbing With an Iodoform Preparation, Contact Plate Method*

[TNTC indicates too numerous to count]

Time, min	Bacterial colonies per plate for subject —			
	IV		VI	
	Test 1	Test 2	Test 1	Test 2
Before	8	7	89	TNTC
1	0	1	42	38
15	0	19	25	7
30	1	2	38	---
75	17	9	59	40

surgical gloves which were temporarily pulled back for each subsequent test. Note the rise in population at 15 minutes, especially with the hexachlorophene preparation. This is most likely due to the surfacing of bacteria from sources in the skin; for example, the sebaceous gland ducts. The residual germicide destroys them, but the secondary rise which begins in 20 minutes to 2 hours for normal individuals cannot be held down. Subject VI had a continuing eczematoid-type reaction of the skin and it was not possible to produce negative cultures even after scrubs of 30 minutes.

The scrubbing technique emphasizes the point that not all skin micro-organisms are on the surface and readily removed. The tape stripping technique is employed to determine the deep skin population of bacteria. The data gathered in table VIII are typical. The population tends to drop with successive strips. Note that with strip 11 there is a greater than usual reduction. This is the result of a six-basin Price technique being carried out between the 10th and 11th stripping. It is of interest to note that even after 20 strippings and 6 series of scrubbing and flushing, demonstrable numbers of organisms still can be found.

It is quite likely that the deep-lying bacterial population is well protected against killing concentrations of germicide and against mechanical removal. These organisms most likely form the nidus which produces the eventual return to the bacterial skin level normal for each individual.

There appears to be no known method at present by which human skin can be sterilized, and as bacteria can be almost

TABLE VIII. — *Deep Bacterial Skin Populations Determined by Tape-Stripping Method*

Tape strip	Bacterial colony counts per tape (subject and sex)			
	XIII–M	XIV–M	XV–F	XVI–F
1	72	157	167	35
2	36	145	182	36
3	31	167	260	88
4	51	115	201	46
5	23	160	215	41
6	20	42	132	27
7	29	39	86	10
8	29	56	101	42
9	23	58	61	43
10	43	55	57	37
11	4	24	5	10
12	1	20	3	7
13	5	12	5	11
14	14	8	6	13
15	3	8	8	6
16	8	6	7	12
17	8	10	5	8
18	5	3	7	6
19	6	10	5	7
20	5	6	5	15

constantly shed or removed from the skin, mechanical barriers such as surgical gloves appear to be the best method of control.

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Quantitative Aspects of Shedding of Micro-Organisms by Humans

To date, only limited proportionate or semiquantitative studies of shedding of micro-organisms from people have been reported. British and American investigators have used small rooms and sampled the air to determine relative quantitative amounts of microbial shedding (refs. 1–6). Small plastic chambers have also been used, again with air sampling, to investigate the relative amounts of shedding (refs. 7 and 8). Several of these studies were on shedding of staphylococci into the environment, with application to the hospital setting. Only relative, not total, quantitative analysis of organisms shed by an individual was possible under the conditions of these studies.

In order to study shedding of *all* micro-organisms from people, a stainless-steel chamber large enough to accommodate a standing adult was designed and constructed (unpublished paper by G. W. Sciple et al., from the Communicable Disease Center). This chamber, called a microbiotank, is 7 feet high and 3 feet in diameter, constructed of ¼-inch stainless steel. There is a 3-foot door on one side to permit a subject to enter and leave; a 6-inch viewing port is installed on the opposite side of the tank to observe the subject during experiments.

The tank is designed to permit it to be sterilized by steam prior to occupancy by a subject to be evaluated for shedding. A tubular cotton filter with a ball check valve is installed on the tank so that uncontaminated air will enter during the cooling period following sterilization.

The interior of the microbiotank is designed to facilitate easy recovery of organisms shed into it. The inside wall is polished to a No. 7 luster and has no seams. The tank is fitted with a circular, horizontal, 312-hole wash ring attached to a vertical pipe running through an orifice in the top of the tank.

Immediately after a subject leaves the microbiotank, sterile fluid is sprayed into the unit from the wash ring to collect the micro-

organisms shed. Washing is carried out with approximately 20 liters of a sterile solution of 0.25 percent peptone water. The peptone water is prepared, sterilized, and cooled to 4° C in a pressure media tank. Just prior to washing, the pressure media tank is changed to 75 psi with gaseous nitrogen. Then this media tank is connected through a sterile ball valve and sterile neoprene hose to the vertical pipe attached to the wash ring. With the wash ring drawn to the top of the microbiotank, a valve on the pressure tank is opened for 15 seconds, while the wash ring is slowly lowered to the bottom of the microbiotank. The rinse fluid is allowed to collect in the bottom of the microbiotank, after which it is drained into a sterile carboy. A schematic diagram of the microbiotank, together with the nitrogen and media tanks and the carboy, is shown in figure 1.

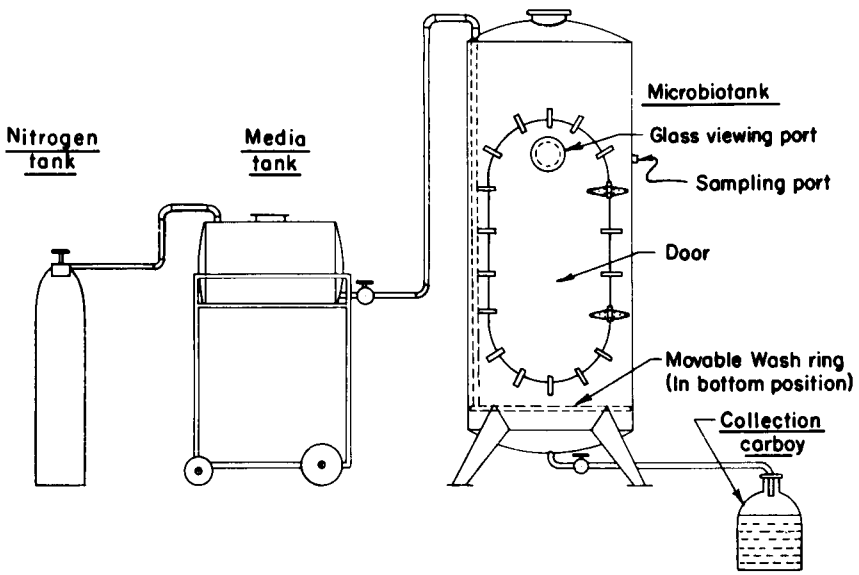

FIGURE 1.—The microbiotank.

After collection in the carboy, the rinse fluid then is processed through an aseptically assembled, continuous-flow, refrigerated centrifuge operated at 34 800 g. The flow rate is 200 milliliters per minute. The operating temperature is 4° C. After centrifugation, the fluid is shaken on a wrist-action shaker, diluted 10^{-1} to 10^{-8}, and plated in triplicate using pour plates of trypticase soy agar (TSA). The plates are incubated at 37° for 48 hours and the number of viable microbial particulates counted.

For qualitative studies, 1 milliliter in 0.2-milliliter aliquots of each dilution is flooded onto TSA plates. Identification of organisms is made after a 48-hour incubation period.

The entire system is checked for sterility at approximately monthly intervals. After the tank is sterilized and allowed to cool overnight, the entry door is opened and closed without a subject entering, and then the tank is rinsed with sterile peptone water. The rinse fluid is processed by the technique given above. On several such sterility checks, the calculated viable particles in the system has been less than 100.

To check the recovery rate of organisms seeded into the microbiotank, *Staphylococcus epidermidis* and *Escherichia coli* have been used as test organisms. One milliliter of 18-hour stock cultures was inoculated into the upper sampling port and allowed to settle in the tank. After a 5-minute interval, the tank was rinsed and the rinse fluid collected. The fluid was processed in the usual manner. The results are shown in tables I and II. It can be seen that the numbers of organisms recovered were of the same log magnitude

TABLE I.—*Percent Recovery of Known Numbers of* Staphylococcus epidermidis *Introduced Into Microbiotank*

Number of experiment	Number of organisms introduced into chamber	Number of organisms recovered from chamber	Percent recovery
1	217×10^7	81×10^7	39
2	133	107	80
3	85	59	69
4	113	62	55
5	111	91	82

TABLE II.—*Percent Recovery of Known Numbers of* E. coli *Introduced Into Microbiotank*

Number of experiment	Number of organisms introduced into chamber	Number of organisms recovered from chamber	Percent recovery
1	179×10^7	69×10^7	39
2	351	45	13
3	136	36	26
4	111	57	51
5	284	70	25
6	138	40	29

as those introduced into the tank. The numbers of staphylococci recovered were higher than those of *E. coli.*

Figure 2 is a drawing of the microbiotank complex showing the room for undressing, the shower, and the area for donning sterile clothing or other apparel desired for a given study. After the

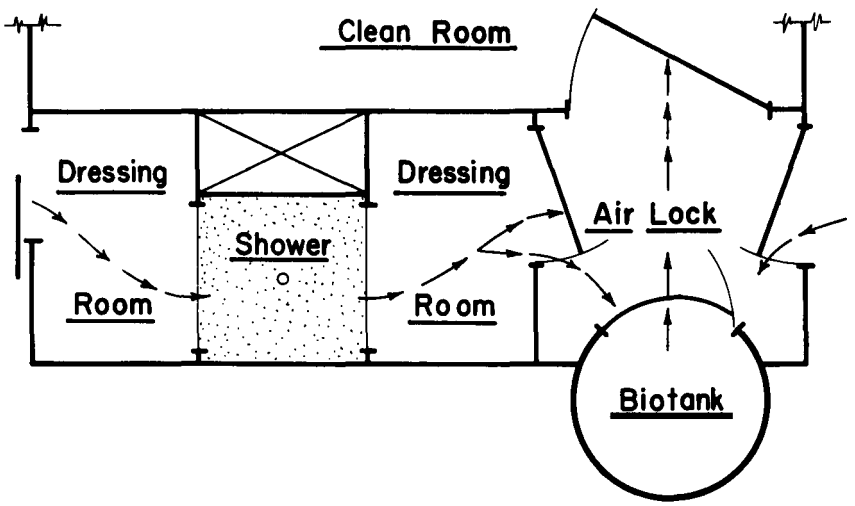

FIGURE 2.—Microbiotank complex.

subject progresses through these rooms, he enters an airlock that has an absolute filter as a ceiling to allow the lock to be purged with uncontaminated air. The subject enters the microbiotank through the airlock and closes the door. The door is then secured. The subject remains in the tank for the desired period of time, during which he engages in moderate physical activity while taking care not to contact the sides of the tank. Measurements of temperature and relative humidity in the occupied tank are made during some runs. Immediately after the subject leaves the microbiotank, the door is again closed. The tank is rinsed, and the fluid is processed as previously described.

In early studies, three subjects were studied at weekly intervals for 3 weeks. The results are shown in table III. Subjects wore street clothing for these experiments.

Because of the variations in these data, it was decided to study individuals on consecutive days to see if there was a close comparison in a day-to-day shedding study. Two subjects shaved and showered approximately 2 hours before entering the microbiotank. Just prior to entering the tank, the subjects donned a sterile surgical scrub suit, cap, and socks and stayed in the tank for a 30-

TABLE III.—*Total Viable Particles Shed by Individuals at Various Exposure Times in Microbiotank*

Subject	Time in tank, min	Total viable particles recovered per minute
A	10	62 000
	20	5 800
	40	46 000
B	15	6 500
	30	55 000
	30	3 300
C	15	47 000
	30	19 000
	30	28 000

minute period. The results from this study are shown in table IV. It can be seen from these results that there is very little difference between daily runs of these individuals. There has seldom been more than one-log variation between daily runs to determine shedding of the same individual under similar circumstances.

TABLE IV.—*Consistency of Microbial Shedding From Individuals on Consecutive Days*

[Subjects wearing sterile scrub suit, socks, and cap]

Subject	Viable particles shed per minute		
	Day 1	Day 2	Day 3
D	3660	3030	3500
E	2170	2130	1870

TABLE V.—*Consistency of Microbial Shedding From an Individual Studied After a Yearly Interval*

[Subject wearing street clothes]

Date	Viable particles shed per minute	
	Day 1	Day 2
Dec. 1963	6500	3300
Dec. 1964	2300	2560

Subject E was again studied, in street clothing, after a 1-year period. These results are shown in table V. These results show that this individual remained a constantly low shedder of micro-organisms.

Subjects F and G were studied in comparisons of the effects on shedding of bathing with conventional soap and with germicidal detergent. The subjects showered each evening using one of the two cleaning agents. After showering, no deodorant was used. Clean street clothes were donned, and normal activities were

TABLE VI.—*Comparison of Microbial Shedding After Showering With Conventional Bath Soap and With Germicidal Detergent*

[Subject wearing sterile scrub suit, cap, and socks]

(a) *Subject F*

Time of sampling	Viable particles shed per minute	
	Counts for soap	Counts for detergent
Initial 5-day period	5 070	83
	3 330	203
	6 100	800
	.....	160
Final 5-day period	29 300	66
	2 200	220
	2 830	.....
	1 670	.....

(b) *Subject G*

Time of sampling	Viable particles shed per minute	
	Counts for soap	Counts for detergent
Initial 5-day period	1600	533
	1370	2230
	373	123
	130	566
	1310	1100
Final 5-day period	2700	133
	843	366
	5430	287
	7270	4030
	787	3600

carried out until the individual was placed in the microbiotank. Approximately 12 hours after showering, the subject was dressed in a sterile surgical scrub suit, cap, and socks and confined in the microbiotank for 30 minutes. Shedding for each individual was studied for 5 consecutive days. The subjects continued for 9 days to use the same cleaning agent used for the initial 5 days; however, they were not placed in the microbiotank for determination of shedding during this period. Then, during the following 5 days, recovery procedures for assaying shedding were again carried out. The results are given in table VI. The number of experiments are too few to make comparisons of these latter data at this time. The counts obtained when the germicidal detergent was used for showering were somewhat lower than those obtained with conventional soap.

Measurements of environmental conditions in the occupied microbiotank have indicated that temperatures generally vary from 70° to 75° F and relative humidities are above 80 percent.

In summary, equipment and techniques have been developed for quantitative investigations of shedding of micro-organisms from people. Good personal hygiene plus the wearing of sterile clothes reduces the numbers of micro-organisms shed into the environment.

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Microbiological Barrier Techniques

A microbiological barrier is a device or system that, to a degree, will prevent the passage or migration of microbiological contaminants. In the case of the food container, the can, the jar, or the plastic bag prevents micro-organisms from coming in contact with the food. The plugged culture tube, on the other hand, prevents the micro-organisms inside from escaping. This paper defines and illustrates the types of microbiological barrier techniques and equipment that could be useful in solving spacecraft sterilization problems.

The essence of any microbiological barrier is the degree of isolation that is achieved. Isolation is the keyword because it is the result that the barrier should provide. But isolation is also a magnanimous concept in that absolute isolation may not be achievable and may have more philosophical than practical importance. Luckey (ref. 1), in illustrating the difficulty of complete isolation, suggested that a small fraction of the air we breathe was in the lungs of Christ, Mohammed, and Buddha. According to some theories, absolute isolation may not even exist on a planetary level. Thus we find that it is necessary to specify the level and degree of isolation required for a microbiological barrier. For example, we may need a barrier for the maintenance of sterility where sterility is defined as the absence of life, based on our present understanding of what life is and present state-of-the-art methods of detecting life. One problem with regard to sterility is that it is essentially a negative quality in which the assumption of a state of sterility is derived from the negative results of microbiological tests. This means that there can always be a question or a suspicion regarding tests showing the absence of micro-organisms, although a result based on the positive recovery of micro-organisms might be accepted without suspicion.

Historical Aspects

It is interesting to trace the early development of mechanical barriers and methods for microbiological isolation. Bacteriological barriers such as the flasks of Schulze, in 1836, and Schwann, in

1837, did much to invalidate the theory of spontaneous generation and heterogenesis. Tyndall's chamber, used in 1868 to show the relationship between the light-scattering ability of aerosols and the ability of airborne organisms to initiate growth in various infusions, was an example of an early microbiological barrier. The laboratory isolation apparatus used by Davaine in 1870, by Lister in 1878, and Koch in 1881 enabled these men to develop the pure culture techniques that put the science of bacteriology on a sound footing. Starting in about 1885, workers interested in germ-free life developed many types of mechanical barrier or isolation systems. These workers started by copying the apparatus of the early gas chemists. Some of the earliest barriers were nothing more than sterile flasks and bell jars used by plant physiologists during the controversy over the means by which nitrogen is fixed into plant tissue. One such apparatus was a 4-liter jug used by Berthelot in 1885 (ref. 2) in an attempt to grow plants on sterile soil. Eleven years later, Nuttal and Thierfelder (ref. 3) published the results of their experiments with germ-free animals using a modified bell jar. Modified bell-jar barrier systems have been used by Cohendy (ref. 4), Balzam (ref. 5), and others. One of the most elaborate cabinets of that era was devised by Kuster (ref. 6) for rearing germ-free goats. Kuster's germ-free cabinet was the first to use arm-length rubber gloves. It contained essentially all the features of modern-day germ-free isolators, including an entrance airlock, air supply filters, and operation at a positive pressure. This apparatus was improved by later workers such as Glimstedt (ref. 7) and Reyniers (ref. 8). Today a variety of mechanical barrier apparatus is used for research with germ-free animals. These have been adequately reviewed by Luckey (ref. 1). The three predominant types are the heavy-walled stainless-steel isolator of Reyniers (ref. 8), and thin-walled stainless-steel tank of Gustafsson (ref. 9), and the flexible plastic isolator of Trexler (ref. 10).

Five Stages of Contamination Control

The use of any microbiological barrier is an exercise in microbiological contamination control. As such, the five stages of microbiological contamination control developed by the Biological Contamination Control Committee of the American Association for Contamination Control (paper by G. B. Phillips et al. entitled "Microbiological Contamination Control, A State of the Art Report" to be published by the Journal of the American Association of Contamination Control) are pertinent to this discussion on barriers. These five stages are shown in table I.

TABLE I.—*Stages, Approaches, and Techniques of Microbiological Contamination Control*

STAGE 1	Recognize and Define the Problem				
STAGE 2	Establish Contamination Control Criteria				
	Maximum number of organisms allowed, types of organisms, where located, how detected, and other criteria.				
STAGE 3	Employ Approaches and Techniques of Control				
	Facility design features	Use of containment equipment	Management functions	Use of correct techniques	Use of sterilizing and decontaminating agents
STAGE 4	Microbiological Testing and Surveillance				
	Air sampling	Surface and component sampling	Physical and chemical tests and measurements	Testing of filters, incinerators, sewage, water	Gas-tightness testing
STAGE 5	Analysis of Results and Certification Procedures				
	Recording results, statistical tests, use tests of items, formal or informal certification.				

For adequate analysis and surveillance of microbiological barrier systems, stages 2, 4, and 5 are particularly important. The criteria or standards for any microbiological barrier and its operation must be established (stage 2). In many cases the criteria will be that sterility be achieved and maintained. In other instances the objective may be to limit, control, or reduce the number and types of micro-organisms on or in specific components during assembly and testing. For example, one such criterion might specify the number of bacterial spores allowable per unit area of surface. The criteria may also specify the allowable airborne microbial contaminants and their particle sizes. It is also necessary to specify the exact assay techniques and other tests and procedures to be used. The specific methods and number of replicates to be used in determining sterility must be indicated. Types of culture media and culture apparatus should likewise be specified.

In the fourth stage, the specified testing and surveillance procedures are carried out. In any barrier system, one or more of these techniques is needed to assess whether the techniques

employed (stage 3) achieved the microbiological control that meets the criteria established (stage 2).

A partial list of the microbiological barrier control tests in common use includes:

1. *Microbial air sampling.*—Air impaction samplers, liquid impingers, and settling plates are used most frequently. The results of impaction and impinger samples are given in terms of viable particles per cubic foot of air and/or micro-organisms per cubic foot of air. The results from settling plates are expressed as viable particles per square foot per hour.

2. *Particle size sampling.*—Liquid impinger samples with preimpingers offer some particle-size selectivity. The Andersen cascade sieve sampler is frequently used to discriminate the airborne viable particles in a microbiological aerosol into six particle-size ranges.

3. *Surface sampling.*—Cotton swabs or Rodac plates are usually used. Results are expressed as micro-organisms per unit area of surface.

4. *Surface contamination accumulation tests.*—Small sterile strips of stainless steel, glass, or plastic are placed in the environment. After various exposure periods, strips are collected and assayed for viable micro-organisms. Results are usually expressed as micro-organisms per square foot.

5. *Component surface testing.*—Small components in systems under microbiological contamination control may be tested by complete immersion in an appropriate nutrient fluid or by washing the component in sterile saline that is then quantitatively assayed for viable microbes.

6. *Internal testing of components.*—Obviously more information on methods for determining internal sterility of components is needed.

7. *Special culture tests.*—Special microbial detection and assay tests may be devised for other materials such as oils, greases, powders, and so forth.

8. *Filter and incinerator testing.*—Periodic microbiological testing of all air filters and incinerators used in contamination control systems is required. Testing must be done in such a manner that a break of sterility is not involved.

9. *Gastightness testing.*—A barrier system can be evaluated for microbiological tightness by determining its gastightness. The ability to contain gas molecules is prima facie evidence that the system will contain microbiological particulates. Gastightness leak detectors include (*a*) thermal conductivity detectors, (*b*) combustible gas detectors, (*c*) infrared absorption detectors, (*d*) argon

differential "sorption" detectors, (*e*) differential pressure transducer detectors, (*f*) ultrasonic leak detectors, (*g*) mass spectrometer detectors, and (*h*) halogen detectors. In high-vacuum systems, the Pirani hydrogen and helium ionization gage leak detectors may be used or radioactive gas detectors may be suitable. The halogen leak detector is probably the most commonly used detector because it is relatively inexpensive, rugged, reliable, simple to operate, and has a sensitivity of 1×10^{-9} cubic centimeters of gas per second. The mass spectrometer leak detector is one of the most sensitive detectors available. Units are available that can detect vacuum leaks as low as 1×10^{-14} cubic centimeters per second. However, the mass spectrometer is expensive and is a complex instrument that requires a skilled operator as well as trained maintenance personnel.

10. *Miscellaneous measurements.*—To insure maximum potency of the decontaminants used, it is important that chemical titrations be made and records maintained of the concentrations of chemicals such as peracetic acid, ethylene oxide, and chlorine solutions. Records of the temperatures and exposure times should be maintained when materials are treated in autoclaves or dry-heat ovens. The temperatures of air incinerators, incubators, and so forth, should be appropriately observed and recorded. Insofar as possible, temperature readings should be made at the most insulated or protected areas in the material being treated. Ventilation rates should be tested at regular intervals.

The control criteria that are established in stage 2 are the guidelines for the analysis of results and certification of stage 5. Moreover, it follows that corrective actions should be started when a microbiological barrier is shown to be out of control or not meeting the minimum standards.

The choice of the proper microbiological barrier and the techniques for its use depend in large part on the selection of the criterion of control. Thus if one wished only to prevent excessive loading of spacecraft components with microbial spores, a different type of barrier would be indicated than that needed if the criterion of control was the maintenance of sterility. In the latter instance, only an absolute barrier system would suffice.

The control of the micro-organisms in a system is also related to the ability to detect and enumerate the microbial load in the system at any particular point in time. Contamination control is achieved if the microbial load does not exceed the level established as the lowest acceptable limit. Maintenance of control, however, is complicated by the fact that the micro-organisms in a population may be going through simultaneous processes of multiplication and

death. Insofar as these processes are concerned, the most stable condition of microbiological control is that of sterility—the absence of all viable micro-organisms.

Once a sterile barrier system is in operation and under good control, maintenance of the sterile environment during work is more of a mechanical and engineering problem than a biological one. This emphasizes the need for the proper training of personnel in work techniques that will avoid rupture or violation of the sterility barrier.

Verification of the sterility of microbiological barrier systems is a problem of some concern. Within a sterile barrier system, it is possible to employ several direct approaches to micro-organism detection, such as the exposure of quantities of liquid or solid culture media or germ-free animals to the environment within the system. It must be emphasized, however, that the present state of the art is such that the ability to demonstrate the presence of viable micro-organisms within cabinet systems or in spacecraft components decreases as the number of viable micro-organisms becomes smaller. In spite of limitations of this sort, it is significant that Trexler (ref. 11) has been able to maintain a colony of mice and a colony of rats in an apparent germ-free condition within a barrier system for more than 12 years.

Classification and Description of Microbiological Barriers

Microbiological barrier systems can be classified according to purpose, size, and degree of containment, as shown in table II.

TABLE II. — *Classification of Microbiological Barrier Systems*

Classification according to—	Types
Purpose	Product protection or personnel protection.
Size	Room size or cabinet size.
Degree of containment	Absolute barriers or partial barriers.

"Purpose" classification relates to the direction of the barrier system. Thus, germ-free animal barriers prevent contamination from entering, whereas microbiological safety barriers prevent the escape of infectious micro-organisms. The difference is sometimes illustrated by referring to "product protection" and "personnel protection" systems. Obviously, for spacecraft sterilization we are most interested in the product protection systems. Occasionally a barrier system is needed that will operate in both directions at the same time. An example of this is the barrier cabinet to be used in

the Lunar Sample Receiving Laboratory. This cabinet must prevent escape of lunar material during a quarantine period, but also prevent contamination of the lunar samples with Earth microorganisms.

A second method of classification refers to the size of the barrier and its position in relation to the protected environment. Is the work externalized from the worker by placement in an enclosure or cabinet of its own, or is the worker internalized within the environment and separated by protective clothing? It is possible to wrap a barrier around the work or around the worker. Wrapping a barrier around the worker is illustrated by a germ-free room entered only by people wearing sterile, ventilated plastic suits. A lesser degree of isolation would be represented by a worker in a clean room wearing a respirator and sterile garments to provide the microbiologic barrier. Some of the difficulties of maintaining an adequate barrier around the worker are overcome by wrapping the barrier around the work. The use of hoods, cabinets, germ-free isolators, and similar enclosures illustrate the externalization type of barrier.

Finally, by classifying microbiological barrier systems according to the degree of containment, they may be described as either absolute or partial barriers. Absolute barriers allow no interchange of the protected and nonprotected environment and aim at total containment. They usually provide for placement of the material or the work to be controlled within a gastight enclosure, usually a stainless-steel cabinet or a plastic isolator. Humans are separated from the system and the work is done through attached arm-length rubber gloves or by means of remote mechanical manipulators. When work within the barrier is to be protected from outside contamination, the system or enclosure is maintained at a positive air pressure. Conversely, negative pressure is used in the enclosure to prevent escape of contaminants from it. According to the criteria for microbiological control, inlet and/or outlet air may be filtered or incinerated. Prior to use, the enclosure may be decontaminated or sterilized. Airlocks, dunk baths, autoclaves, and other devices may be used to preserve the sterile integrity of the enclosure while materials are passed in and out of it.

The most comprehensive report of absolute barrier devices for personnel protection is that of Gremillion (ref. 12), who described the gastight cabinet systems used at the U.S. Army Biological Laboratories at Fort Detrick, Frederick, Md. The systems included incubators, refrigerators, centrifuges, and balances, and utilized disinfectant dunk baths and autoclaves for the entrance and exit of materials.

Flexible plastic barriers at a positive pressure for the absolute containment of germ-free animals are largely a development of Trexler (ref. 10). Rigid plastic absolute barriers have also been used (ref. 13). Stainless-steel containers in common use with germ-free animals are similar to those originally designed by Reyniers or by Gustafsson. The types of barriers or isolators for germ-free animal experimentation have been adequately summarized by Luckey (ref. 1).

Absolute containment can be achieved in a room-sized environment, but only by the use of ventilated suits or some other enclosure for persons entering the room. A product protection room for sterile assembly work must be closed and sterilized prior to use. Suited personnel enter through a series of airlocks where the outside of the suit is sterilized with chemical agents.

The sterile-room concept derives primarily from germ-free research. In 1899 Schottelius (ref. 14) built the first germ-free room in the center of a large empty room at the Institute of Hygiene of the University of Freiburg. This room was used in an attempt to raise germ-free chicks. Reyniers (ref. 15) described a room-sized tank (2.5 meters in diameter and 5 meters long) used for rearing germ-free animals. The tank was sterilized with steam under pressure. Before entering the sterile tank, the operator wearing a plastic diving suit submerged himself for 30 minutes in an entrance dunk bath filled with a 2-percent formaldehyde solution. Trexler (ref. 16) converted a laboratory room into a sterile room by covering the walls and ceilings with asbestos flexboard and covering this with polyester-resin-impregnated glass cloth. He covered the cement floor with a plastic paint. The room was chemically sterilized before use. Workers wore plastic suits that were chemically sterilized in an airlock as they entered the room. Luckey (ref. 1) described a sterile building about 14 meters by 8 meters (46 by 26 feet) at the Department of Virology, Rega Institute, University of Louvain in Belgium. This facility apparently is used for the large-scale production of germ-free animals.

A recent use for sterile plastic isolators of roomette size is the application to special problems of hospital patient care (ref. 17). Patient isolators, or "life islands," that actually enclose the hospital bed have been used with patients who, because of the nature of their injury, illness, or treatment, have little resistance and high risk of severe infection. Some room isolators are as large as 8 by 8 feet and are of sufficient size to allow the patient to get out of bed and walk up and down beside the bed. The plastic room isolators are gas sterilized. Doctors and nurses have access to the patients through attached arm-length rubber gloves.

Absolute barrier rooms for product protection have been successfully employed. One such room that I worked in some years ago housed a nontight spray-drier machine used to process hazardous biological materials. The room had plastic-coated walls, ceilings, and floor. After the drier had operated, sprays of peracetic acid were used to sterilize the contaminated room. Suited individuals working in the room sterilized the outside of their suits as they left the room. At several infectious disease laboratories, similar rooms, operated at a negative pressure and entered in ventilated suits, are used to house large animals infected with highly contagious disease agents.

Classification of a microbiological barrier as a partial barrier indicates that something less than absolute containment or isolation is to be achieved. Open-panel ventilated cabinets and hoods, clean benches, specific-pathogen-free animal-rearing facilities, bioclean rooms, laminar-flow benches, and laminar-flow rooms are examples of barrier arrangements that provide less than absolute microbiological isolation or containment.

Before considering specific examples of partial barriers, I should like to emphasize the importance of the dichotomy between absolute and partial barriers. The principal point is that there is no rational basis for believing that partial barrier systems that allow uncovered or unprotected workers within the protected environment can ever be improved to the point where they can substitute for absolute barriers. As long as workers in a protected environment are capable of shedding micro-organisms, the barrier can hardly be called absolute. To be more specific, I believe that the principles of minimum turbulence airflow (frequently called laminar airflow) and other engineering developments can do much to improve partial barriers, but that such systems can never substitute for the mechanical separation of environments provided by absolute barrier systems. There are three specific reasons:

1. Partial barrier systems are difficult if not impossible to design to be fail-safe.

2. Airflow patterns, even those in minimum turbulence airflow systems, are easily disrupted by people and equipment in the path of the air.

3. The velocity or speed of movement of people and equipment often exceeds the velocity of the air being moved in the barrier system. Most partial barrier systems utilize air velocities between 50 and 150 linear feet per minute, but a person walking at the very modest speed of 2 miles per hour is moving at 176 linear feet per minute.

These comments are in no way intended to minimize the

developments that have taken place in the last 5 years in regard to clean rooms, clean benches, and the like, but the intent is to create a clear distinction between barrier systems that are capable of maintaining sterile conditions or an absolute separation of environments and those that functionally cannot perform in this manner.

The partial-barrier principle uses enclosures, cabinets, or rooms that are not gastight and that are not completely closed. Containment depends on an inward or an outward flow of air through an open working panel, through open glove ports, or through or across a room area. The inlet or outlet air, or both, may be filtered. For cabinets, since only the hands and arms of the operator extend into the environment, the chance of spreading contamination to or from humans is minimized. A great number and variety of partial barrier product protection and personnel protection devices are in use today. The chemical-fume hood finds its biological equivalent in the inward-flow biological safety cabinet.

Microbiologists handling infectious disease agents have long realized the need for mechanical barriers to externalize hazardous procedures. Safety cabinets were in use in German laboratories early in the century (ref. 18). Shepard, May, and Topping (ref. 19) at the National Institutes of Health developed a wooden cabinet for hazardous laboratory operations. In England, Van den Ende (ref. 20) developed similar cabinets for use during large-scale production of scrub typhus vaccine. The first stainless-steel microbiological barriers for infectious disease work were described by Wedum (ref. 21). Other types and improvements have been described by Reitman and Wedum (ref. 22), Phillips et al. (ref. 23), Gremillion (ref. 12), Blickman and Lanahan (ref. 24), Wedum and Phillips (ref. 25), and Phillips (ref. 26).

Cabinets with outward flow for the dust-free assembly and packaging of components undoubtedly reduce microbial contaminants. In the pharmaceutical field, such cabinets are frequently used during the filling and packaging of biologicals.

The principles of the construction and operation of sterile rooms have been modified and slightly downgraded for partial microbiological barriers. Animal breeders have been able to improve the quality of laboratory research animals by closed-colony techniques wherein all animals derive from a nucleus disease-free breeding colony that is maintained in a thoroughly decontaminated room. All supplies and materials entering the room are sterilized and personnel working in the room wear sterile garments and respirators. A slightly different use for room-sized barriers is that for holding infected experimental animals the size of monkeys and

larger. In this case such rooms are made essentially airtight and are maintained at a negative air pressure. Entrance to the room is restricted to personnel wearing ventilated suits that are chemically disinfected before leaving the room. These animal rooms are treated with gaseous germicides after each experiment.

The clean room may also be classified as a type of microbiological barrier, although it is obvious that the presence of unsuited human occupants limits the degree of microbiological isolation that can be achieved. Applying the principles of minimum turbulence airflow has improved the degree of isolation possible in clean rooms but, as pointed out previously, the human in the system still represents an unpredictable variable and makes absolute microbiological isolation impossible to achieve.

Sterilization and Decontamination of Microbiological Barriers

We have previously defined sterilization as that negative state in which the absence of life is indicated by the failure of the test procedures to produce a positive result. The agent bringing about this condition is a sterilizing agent. For the purpose of the following discussion, we also identify a decontaminating agent as one that is effective in destroying or eliminating microbiological contamination but not necessarily to the degree of producing sterility.

Sterilization and decontamination agents have three general uses

TABLE III. — *Sterilization and Decontamination Agents for Use in Microbiological Barriers*

Use of sterilization or decontamination agents	Recommended sterilizing or decontaminating agents
Sterilization or decontamination of barrier systems before use.	1. Steam under pressure 2. Ethylene oxide gas 3. Peracetic acid 4. Steam formaldehyde 5. Beta-propiolactone
Treatment of supplies and equipment moved in or out of barriers.	1. Steam under pressure 2. Dry heat 3. Ethylene oxide gas 4. Dunk bath solutions 5. Peracetic acid passthrough 6. Ultraviolet airlock
Maintenance of microbiological conditions inside barrier during its use.	1. Atmosphere of germicidal gas 2. Irradiation with ultraviolet 3. Periodic washdown with liquid decontaminants

in microbiological barrier systems. Table III shows recommended agents for each barrier application. For sterile assembly procedures, or those where protection of the product or operation is desired, the barrier system and all its components should be sterilized or decontaminated before use. For barrier systems to protect the operator, as in the case of infectious disease laboratory work, decontamination would follow rather than precede use of the barrier. The second use relates to the treatment of supplies and equipment that must be moved in or out of the barrier while it is in use. The third use relates to decontaminating procedures used within the barrier while it is in operation to maintain its sterility or microbiological state. It is obvious that not all the sterilizing and decontaminating agents shown in table III will act with equal efficiency and reliability. All have some advantages and disadvantages. The following discussion covers pertinent points concerning sterilization and decontamination agents classified under four main headings: (*a*) heat, (*b*) vapors and gases, (*c*) liquid decontaminants, and (*d*) radiation.

Heat

Heat is the most effective and reliable method of inactivating micro-organisms and should be used whenever possible. The exposure temperatures and times required for sterility are known and can be readily controlled. Dry-heat ovens containing air or an inert gas can be used while passing some materials and supplies in and out of sterile barrier systems. Steam sterilizers also are recommended for passing materials in and out of barrier systems; moist heat is faster and more reliable. Except for relatively small chambers, heat sterilization of entire barrier systems is usually not possible.

Vapors and Gases

Ethylene oxide (ref. 27), formaldehyde (ref. 28), beta-propiolactone (ref. 29), and peracetic acid (ref. 30) are chemicals used in gaseous, vapor, or fog form. When these chemicals are employed in closed systems and under controlled conditions, excellent decontamination and even sterility can result. However, the properties and limitations of each should be thoroughly understood in relation to the barrier system.

Ethylene oxide is a highly penetrating and effective sterilizing gas, convenient to use, versatile, noncorrosive, and effective at room temperature. However, the gas is slow in killing microorganisms and must be mixed with other gases to avoid explosion hazards. Ethylene oxide is widely used to treat many items not suitable for heat sterilization. It has been used in mixtures with

carbon dioxide or nitrogen, which requires that it be used under pressure. Its most extensive use today is in the form of a low-pressure mixture with chlorofluorohydrocarbons (Freons) in disposable cans or cylinders. In this form it is a highly practical and convenient tool for increasing the usefulness of the laboratory autoclave. A steam autoclave can be inexpensively converted to its use without interfering with the use of the autoclave with steam. A definite limitation to the use of ethylene oxide is the required exposure time. In concentrations practical for use, a minimum of 6 hours is required to sterilize materials contaminated with bacterial spores. Longer (overnight) exposures are recommended for routine use. Another limitation is that neoprene gloves, clothing, footwear, or other plastic, rubber, or leather wearing apparel that have been treated with ethylene oxide must be thoroughly aired for 24 hours before use because of the irritating action of absorbed ethylene oxide on human tissues. Ethylene oxide gas mixtures can be used to sterilize microbiological barriers prior to use or to treat certain materials passed in or out of the barrier.

Almost any method of dispersing formaldehyde into the air in suitable quantities is satisfactory for the use of this chemical as a space decontaminant. Because they are most efficient at higher temperatures and humidities, steam ejectors or steam vaporizers are most conveniently used for small areas. Although formaldehyde has a rather irritating odor, it is relatively noncorrosive to metals, and it can be generally assumed that any equipment or apparatus that will not be damaged by the necessary humidity will not be damaged by the formaldehyde. For decontaminating room-sized barriers, mechanical vaporizers are used. The formaldehyde solution is introduced in a concentration of 1 milliliter per cubic foot of space. In making this calculation, any airflow through the space must be taken into account, and additional formaldehyde added to obtain the above concentration. A hold period of 8 to 10 hours is recommended for room-sized barriers. Formaldehyde fumes are persistent, and a room may require two to three water washes of the floor and 2 to 3 days' ventilation before normal entry.

For smaller ventilated and closed barrier systems such as gastight cabinets, the formaldehyde solution is vaporized at a rate of 1 milliliter per cubic foot of airflow, plus 1 milliliter for each cubic foot of space within the barrier. Thus, if the barrier contains 50 cubic feet and the airflow is 20 cubic feet per minute (cfm), then 650 milliliters of formaldehyde solution ($20 \times 1 \times 30 + 50$) must be vaporized in 30 minutes. In small barriers the formaldehyde may be vaporized with a steam ejector or a mechanical vaporizer. In the latter case, care must be taken to raise the

humidity by boiling water or by injecting steam into the barrier. This technique will decontaminate the entire barrier system; that is, cabinet, exhaust filter, exhaust duct, and blower.

Beta-propiolactone has several advantages over formaldehyde as a vapor disinfectant:

(1) Its vapors are lacrimatory but less irritating than those of formaldehyde.

(2) It does not readily polymerize on surfaces, so that there is little or no residue.

(3) It acts more rapidly. However, in the liquid state, beta-propiolactone is more toxic than formaldehyde and, in handling it, care must be taken that it does not contact the skin.

The technique for disseminating beta-propiolactone is similar to that for formaldehyde. However, more care must be exercised to make certain that the BPL is well vaporized. The chemical must leave the disseminator as a vapor or in particles small enough so that they vaporize rapidly. Otherwise, the liquid droplets settle or impinge on surfaces and dissemination is not effective. Liquid beta-propiolactone is injurious to rubber items and painted surfaces if it is not immediately washed off. After a hold period of 2 to 3 hours, ventilation of the barrier may be resumed. At this point, treated areas should be entered only with protective clothing and respiratory protection. Proper airing will generally allow normal entry after 2 to 3 hours.

Peracetic acid is an excellent bactericide. As a 2-percent solution it can be sprayed as a fog to decontaminate enclosures or other areas. Because peracetic acid is extremely corrosive, it should be used in contact only with plastics, plastic-coated materials, or stainless steel. Its wide use for the treatment of isolators used in the rearing of germ-free animals is adequate proof of its sterilizing ability.

Liquid Decontaminants

There are many misconceptions concerning the use of liquid decontaminants. This is largely due to a characteristic capacity of such liquids to perform well in the test tube and to fail in a practical situation where such factors as temperature, contact, pH, concentration, and the presence of organic material at the site of application are not considered or controlled. Small variations in these factors may make large differences in germicidal effectiveness. Hundreds of decontaminants are available under a variety of trade names. Most may be classified as halogens, acids or alkalies, heavy metal salts, quaternary ammonium compounds, phenolic compounds, aldehydic compounds, and other organic preparations.

None is equally useful or effective under all conditions, and only a few are effective against bacterial spores.

Liquid decontaminants serve in barrier systems as the fluid for dunk baths and as germicides for periodic washing of surfaces and items within microbiological barriers. For dunk baths it is important to use solutions such as formalin or sodium hypochlorite that are active against bacterial spores. Even though many decontaminating agents are not sporicidal, some, especially those with a reduced surface tension, will do much to lower the microbial count on surfaces.

Radiation

When used correctly, germicidal ultraviolet (UV) radiation is an effective means of decontaminating air and surfaces. It can be used in transfer airlocks and within microbiological barriers. But proper use of UV as a decontaminating agent requires an understanding of its limitations. The radiation has limited penetrating power and thus is effective only on exposed surfaces or in air. Proper concentration, contact time, and maintenance are also critical. Phillips and Hanel (ref. 31) have adequately described the use of UV for practical decontamination applications.

Table IV summarizes the recommended conditions of use for the

TABLE IV. — *Recommended Conditions of Use for Sterilization and Decontamination Agents in Microbiological Barriers*

[Based on maximum effectiveness against bacterial spores]

Sterilization or decontamination agents	Condition of use (temperature, concentration, exposure time)
Moist heat (autoclave, high vacuum)	127° C, 2–3 min [b]
Moist heat (autoclave, no vacuum)	121° C, 15–30 min
Dry heat	160° C, 2 hr [b]
Ethylene oxide gas	25° C, 300 mg/liter, 8–16 hr
Peracetic acid spray	25° C, 2% with 0.1% surfactant, continuous for 20 min
Steam formaldehyde vapor	25° C, 1 milliliter per cubic foot in air with RH above 80%, 30 min (cabinets) or 10 hr (rooms)
Beta-propiolactone vapor	25° C, 200 mg/cu ft in air with RH above 80%, 30 min
Dunk-bath formalin (37% HCHO)	25° C, 10%, 10 min
Sodium hypochlorite solutions	25° C, 500–5000 ppm with 1% surfactant, 5 min
Quaternary solutions [a]	25° C, 0.1%–0.5%, 1 min

[a] Not sporicidal but good cleaning agents. [b] Not including comeup time.

sterilization and decontamination agents most commonly used with microbiological barriers.

Desirable Features of Microbiological Barriers

Irrespective of the shape and material used for microbiological cabinet barriers, there are certain desirable minimum features:

1. Proper airflow or air balance within the barrier. For an open-panel barrier, this means low turbulence of inward or outward flow of air sufficient to prevent the entrance or escape of airborne particulates. The minimum air velocity is usually 100 linear feet per minute. For a closed, gastight barrier, this means operation at a positive or negative air pressure of 0.5 to 1.0 inch of water. Internal ventilation patterns for gastight barriers should be determined by the nature of the operation. Some procedures may require ventilation with inert gases or accurate control of temperature and humidity within the barrier.
2. An efficient means of sterilizing or decontaminating all interior surfaces of the system.
3. Appropriate filters or incinerators for the air supply or exhaust, or both.
4. A glass or clear plastic viewing panel between the operator and the operation.
5. Internal surfaces that are resistant to chemical corrosion and free of cracks or crevices that would interfere with sterilization and decontamination.
6. Proper arrangement for handling materials within the barrier. For gastight cabinets, this means attached arm-length neoprene gloves. For open-front cabinets, a panel should be available to close the unit during decontamination. A detachable front panel containing ports for arm-length gloves ideally serves both types of cabinets.
7. Appropriate airlocks, dunk baths, autoclaves, gas chambers, and other devices attached to gastight barriers so as to allow passage of essential supplies and materials.
8. Ample amount and arrangement of working space within the barrier to minimize the need to transfer material in and out of the barrier before completion of an operation.
9. Appropriate services such as electricity, gas, vacuum, air, light, ultraviolet irradiation, water, and drains.

Many of the above features also apply for room-sized barrier units.

Examples of Microbiological Barriers

Figures 1 and 2 show microbiological barriers made of flexible

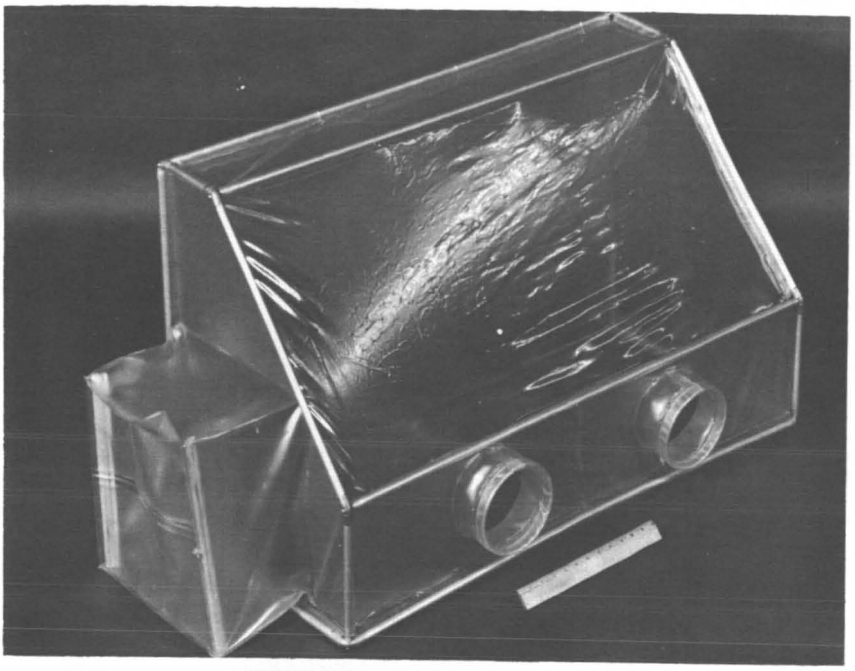

FIGURE 1.—Cabinet made of flexible plastic and supported by aluminum tubing.

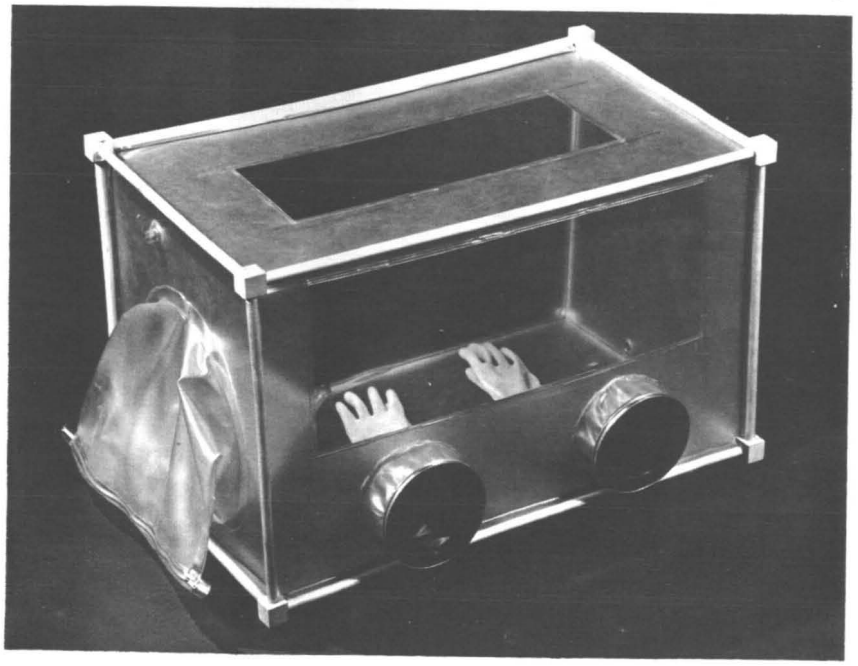

FIGURE 2.—Cabinet supported by wooden doweling.

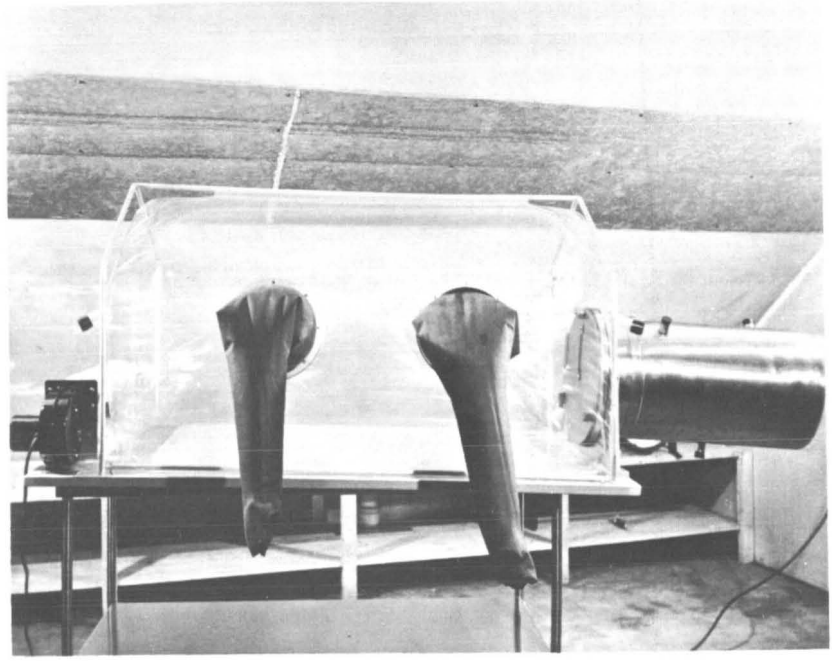

FIGURE 3.—Plastic isolator for germ-free animals.

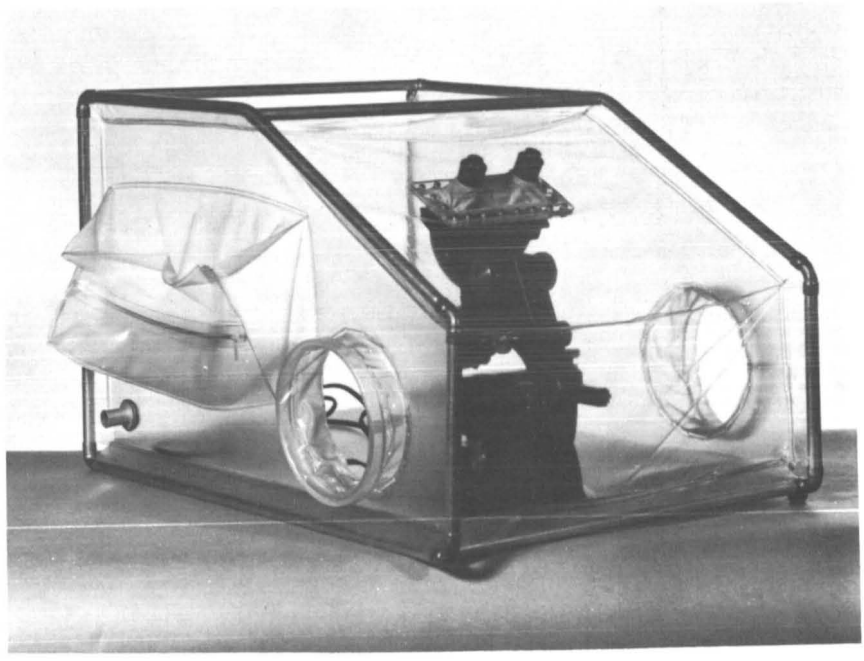

FIGURE 4.—Small plastic cabinet for binocular microscope.

20-mil polyvinyl chloride sheeting supported by an outer frame of aluminum or wood. These cabinets have airlocks with zipper closures and tubes for attaching air inlet and outlet filters. Ventilation is achieved by attaching the cabinet into a laboratory vacuum or compressed air line. Also, an atmosphere of inert gas can easily be admitted to the cabinets via the tubes on the side.

Figure 3 shows an isolator for germ-free animals made of flexible polyvinyl chloride film. This chamber is operated at a positive pressure, with the incoming air sterilized by passage through multiple layers of spun-glass filter media.

Figure 4 illustrates the adaptability of flexible plastic for microbiological barriers. Here a small enclosure has been adapted for an operation requiring the use of a binocular microscope. Ports for gloves are provided on each side of the enclosure and a small zipper airlock is located on the left side for passing materials. A connection for ventilation can be seen at the lower left.

Figure 5 shows a somewhat larger microscope enclosure that was ventilated by attaching it to the glove port of a stainless-steel ventilated cabinet.

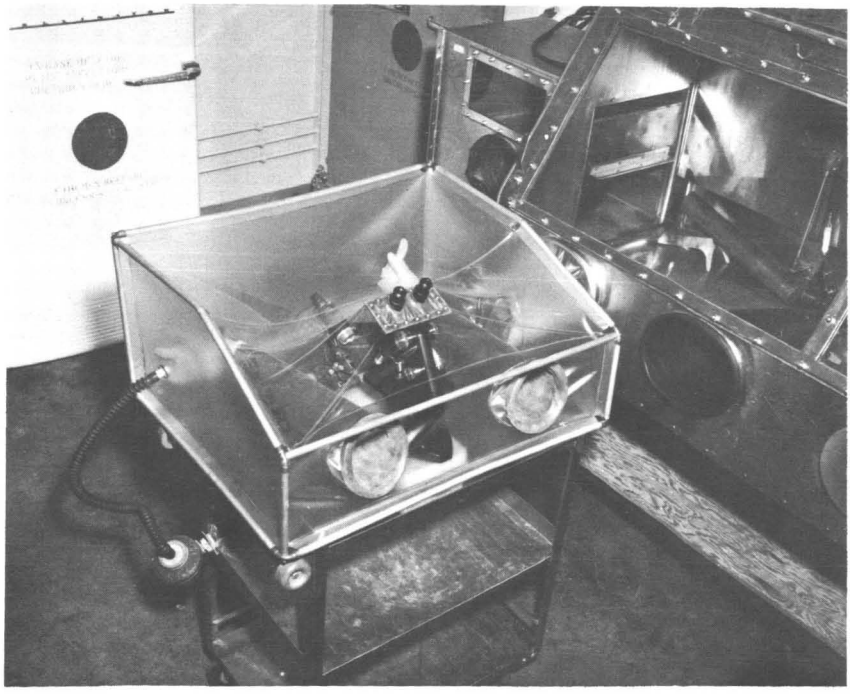

FIGURE 5.—Microscope cabinet of flexible plastic attached to a stainless-steel cabinet.

FIGURE 6.—Plastic enclosure for an animal cage rack.

Figure 6 again illustrates the adaptability of a plastic enclosure. In this case an enclosure has been formed around an animal cage rack and attached by tunnel to a stainless-steel cabinet.

Figure 7 shows the "life island" concept that utilizes an inflated flexible plastic chamber around the bed of a hospital patient. Arm-length rubber gloves are provided for the nurses and doctors in treating the isolated patient.

The use of polyvinyl chloride or other flexible sheeting provides barriers that are economical and can be constructed in almost any size and shape in minimum time. However, these plastic barriers

FIGURE 7.—"Life island" isolator for a hospital patient.

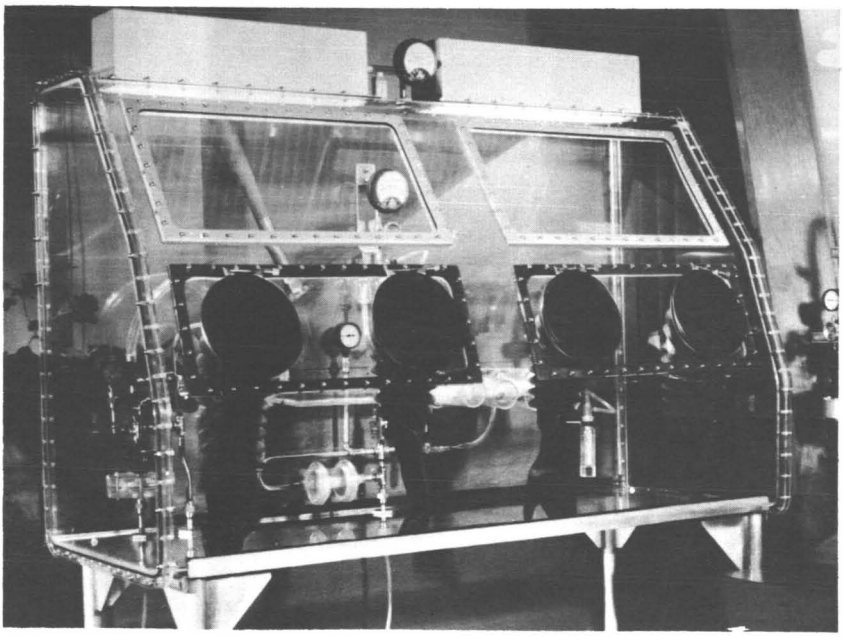

FIGURE 8.—Cabinet made of rigid plastic material.

FIGURE 9.—Open-panel microbiological safety cabinets.

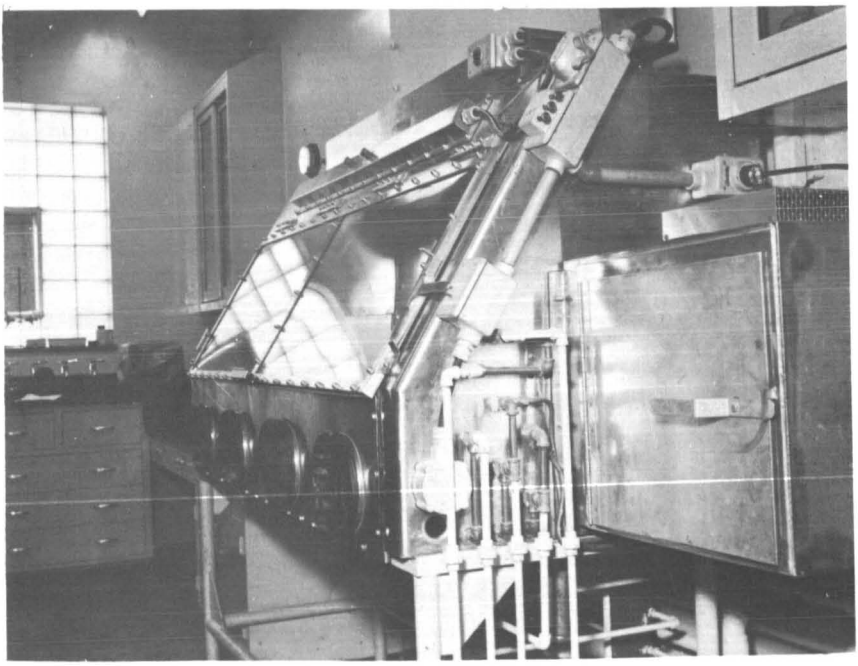

FIGURE 10.—Microbiological safety cabinet with glove panel and gloves attached.

have the disadvantage that they can be easily punctured. Therefore, in general, when the penalty, in terms of dollars or time, for the failure of a barrier system is high, the use of flexible plastics is not recommended.

Figure 8 shows a microbiological barrier made of rigid plastic material, in this case Plexiglas. Enclosures of this type are not easily punctured, but the plastics used are easily scratched and often lack resistance to ultraviolet radiation, heat, and other environmental factors.

Figures 9 through 18 show various types of stainless-steel en-

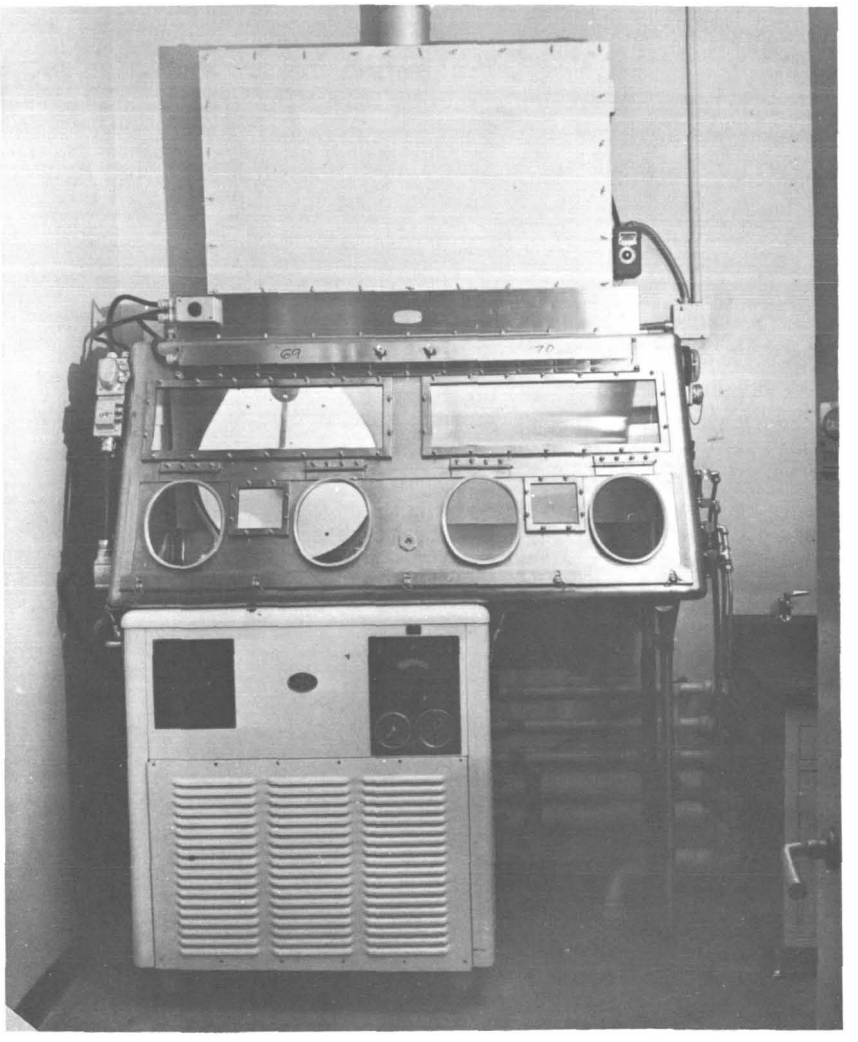

FIGURE 11.—Microbiological cabinet for centrifuge operations.

closures. Figure 9 shows the simplest type of microbiological safety cabinet used in infectious disease laboratories. An inward flow of air sweeps hazardous materials away from the operator and onto the exhaust filter located above the cabinet. The same type of cabinet can be used with outflowing air in product protection systems.

Figure 10 shows similar cabinet with the panel closed and arm-length gloves attached to the glove ports. In this case an ultraviolet airlock is provided on the right side for passage of materials. Cabinets of this design can be used for personnel protection or product protection, but they are not considered absolute barriers because they are not constructed to be gastight.

Figure 11 illustrates that cabinets of this type can be adapted to many types of operations. In this case a microbiological barrier cabinet has been built onto a refrigerated centrifuge.

For absolute microbiological barrier requirements, that is, when

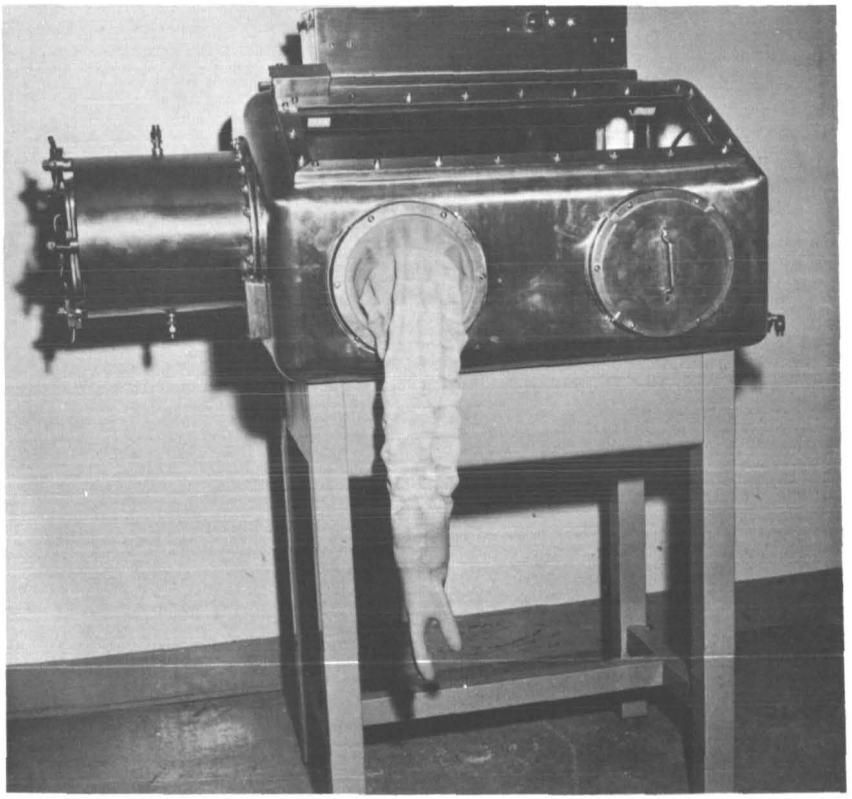

FIGURE 12.—Small gastight microbiological barrier.

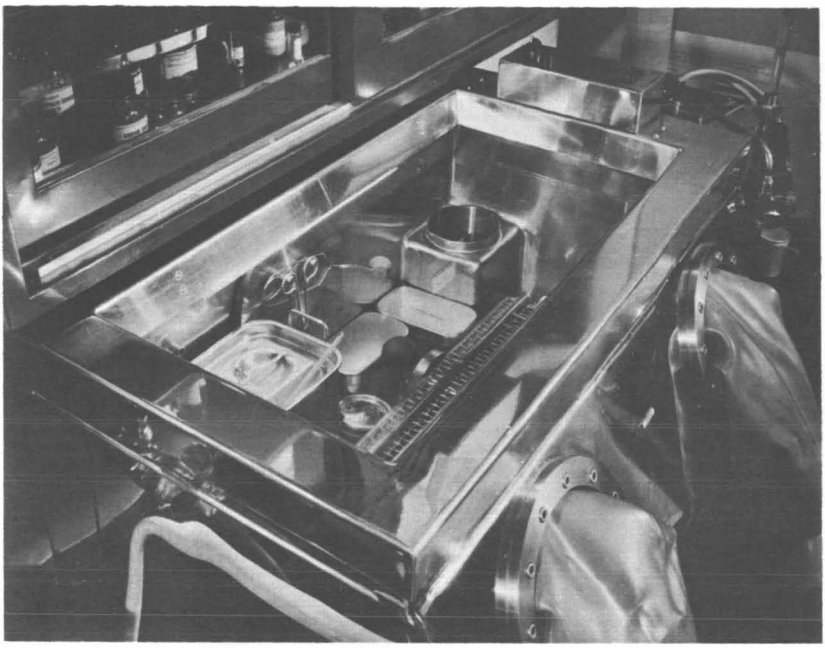

FIGURE 13.—Gustafsson germ-free animal chamber.

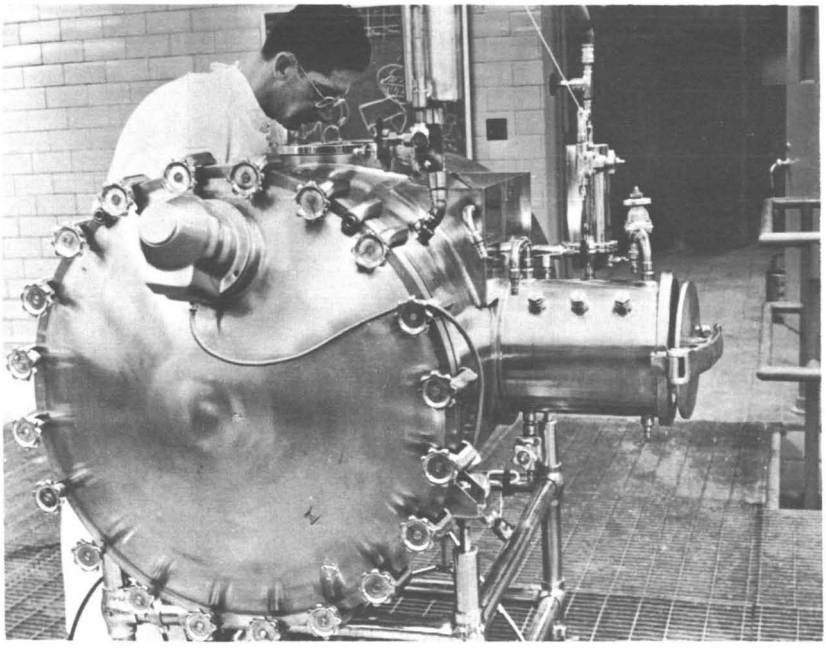

FIGURE 14.—Reyniers-type germ-free animal chamber.

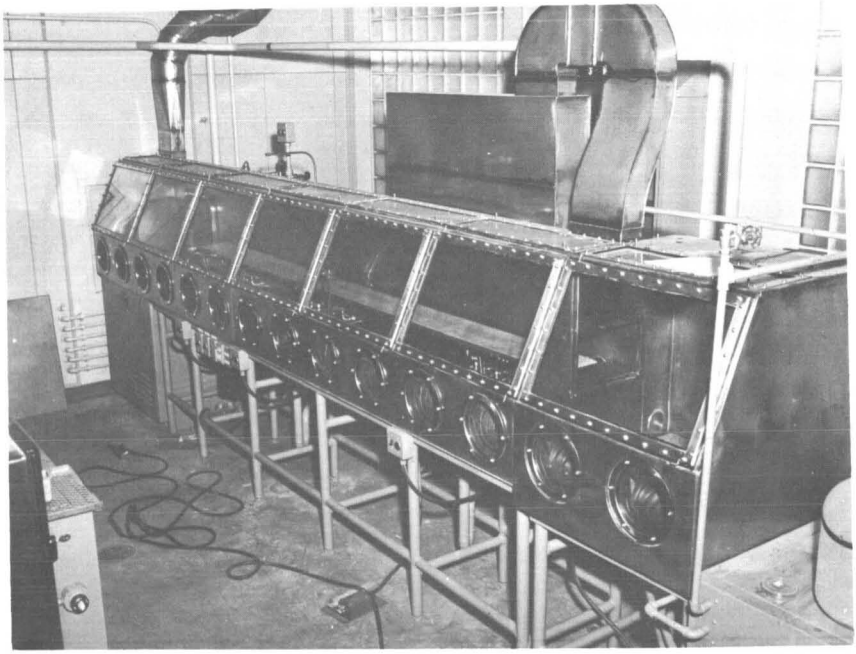

FIGURE 15.—Gastight system with endless belt.

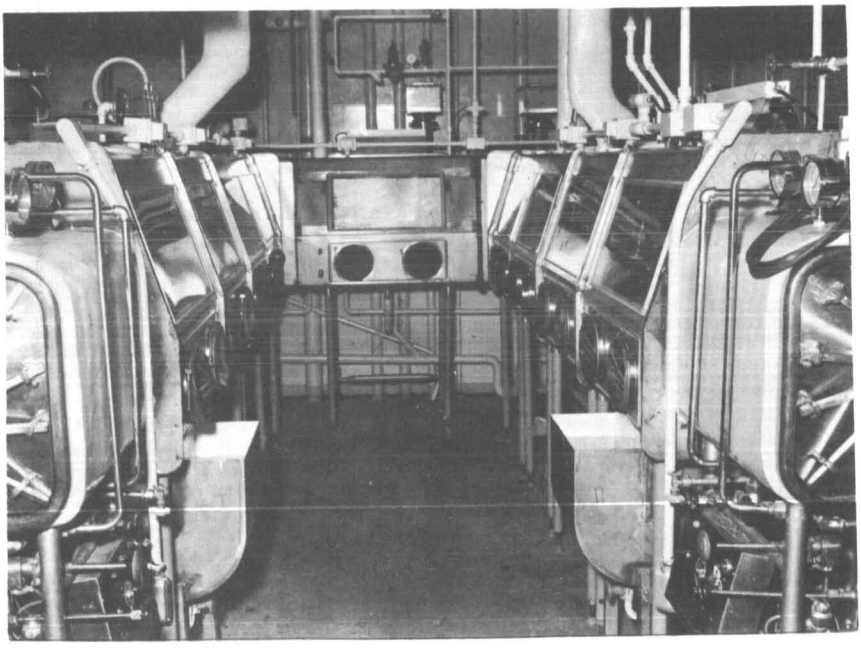

FIGURE 16.—Gastight cabinet system with autoclaves and dunk baths.

FIGURE 17.—Large gastight cabinet system for laboratory operations.

FIGURE 18.—Large gastight cabinet system for laboratory and animal research.

FIGURE 19.—Ventilated suit.

complete containment or sterility must be preserved within a barrier, closed and gastight cabinet systems are often required. Figure 12 shows a small sealed unit designed for one specific operation. This unit can be presterilized and a single operation done under maximum barrier conditions. Units of this size, however, are not particularly efficient because of the limited amount of working space within them.

Figure 13 is a stainless-steel germ-free chamber used in Lund, Sweden, by Gustafsson.

Figure 14 shows a Reyniers-type germ-free animal chamber in use at the National Institutes of Health.

Figure 15 shows a line of gastight cabinets that will accommodate seven operators. Notice that a continuous belt is provided in the cabinets for movement of materials.

Figure 16 shows a larger gastight cabinet system arranged in a U-shape around three walls of a laboratory room. Each leg of the system terminates in an autoclave. Disinfectant dunk baths for passage of materials into the cabinet system are also provided.

Figure 17 shows a larger gastight cabinet system. This system has an autoclave, a dunk bath, and an endless belt for movement of materials within the system.

Figure 18 perhaps illustrates the maximum in complexity that has been achieved to date with gastight cabinet systems. The system shown here contains working space for laboratory operations, including enclosed incubators, refrigerators, deep freezes, and so forth, and attached autoclaves and disinfectant dunk baths, as well as cabinets at three levels for housing animals being used in infectious disease research.

Figure 19 shows one type of ventilated suit adaptable for use in sterile assembly rooms. The external surface of the suit can be sterilized with peracetic acid as the man enters a previously sterilized room. The air supply and air exhaust lines to the suits have to be regulated to maintain a negative pressure inside the suit in relation to the room. For personnel protection applications, a positive pressure should be maintained within the suit. As with the flexible plastic cabinets, a major disadvantage of this type of system is the easy rupture of the suit during use.

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Visual Monitoring as an Assay Technique

Visual monitoring is defined to be the direct or indirect visual observation of an area or process and the evaluation of the activities performed in the area or by the process. Visual monitoring and inspection techniques are used in a multitude of applications to provide close control of products and processes. In general, visual monitoring is not sufficient in itself, but is used to augment specific quantitative testing procedures.

Visual monitoring may be performed by direct observation or indirectly, using television or photography. To establish a complete monitoring system, a combination of techniques is desirable.

In comparing monitoring systems for spacecraft operations and for typical commercial processes, it must be recognized that while a commercial process may produce several hundred or thousand identical items, a typical spacecraft assembly series produces three to five units. Second, any mechanism for monitoring the assembly and testing of a spacecraft must be capable of rapid modification to reflect changes in procedures and in the final product.

To assemble a sterilizable spacecraft, visual monitoring is needed both to assure operational reliability and to assure control of microbiological contamination. Efficient monitoring will require coordination of monitoring systems used to assure sterilizability with those used to assure operational reliability; the coordination is required to prevent duplication of effort.

The microbiological monitoring of milk production is discussed to illustrate the place of microbiological visual monitoring in a successful commercial operation.

The applications of visual monitoring to microbiological contamination control during spacecraft assembly, test, and launch are discussed in the context of a complete monitoring system. The needs for exchange of information between functional groups are indicated. Mechanisms are indicated by which microbiological problems can be solved and the solutions reflected in future operations.

Microbiological visual monitoring in an integrated system of quantitative monitoring, documentation, and microbiological expertise is one required function in the assembly, test, and launch of a heat-sterilizable spacecraft. Most of the monitoring can be performed by nonprofessional personnel if they are provided with detailed monitoring

criteria and adequate job-specific training. To provide safeguards and to assist the interpretation of monitoring data, a professional Microbiologist Visual Observer should be present in the spacecraft handling area.

Visual monitoring is defined to be the direct and indirect visual observation of an area or process, and the evaluation of the activities performed within the area or by the process. The evaluation is normally limited to determination of conformance to a set of established requirements. Visual monitoring and inspection are not new techniques; they are used in a number of industrial processes. Monitoring specifically directed toward the control of environmental microbiological contamination is commonplace in the dairy, food, and pharmaceutical industries, and in hospitals. In general, the visual techniques employed in these situations are not sufficient to evaluate fully functions and products, and are used in conjunction with quantitative laboratory measurements and automated controls. Visual monitoring is also used in the spacecraft assembly industry where it forms an integral part of present quality assurance systems. The monitoring has, however, been limited to nonbiological applications. The modification and expansion of existing spacecraft visual monitoring systems to assist in the control of microbiological contamination appears feasible and the remainder of this discussion will be directed toward: (*a*) Reviewing the general monitoring techniques employed for the control of microbiological contamination in the dairy industry; (*b*) reviewing the monitoring methods employed in spacecraft operations in the light of their application to microbiological contamination control; and (*c*) indicating which microbiological visual monitoring functions may be fitted into existing inspection programs, and which will require new functional groups.

Visual Monitoring in the Dairy Industry

When an engineer or scientist develops a product, he does not normally produce it himself, whether the product is a newly formulated food, an automotive innovation, or a spacecraft; rather, the developer establishes a set of specifications and guidelines designed to tell another group how to do the job. The developer will usually maintain cognizance of production operations, especially in their early stages, in order to provide guidance and expertise where needed, but the major part of his time is freed for new tasks.

To assure that the job is carried to completion in a proper manner, a monitoring organization is often required. This group

watches the people who do the actual work to insure that the work is carried out according to specification and, if tests are required, that such tests are carried out at the proper time and in the proper manner. Frequently the tasks of the monitoring organization include the establishment of such tests as are necessary to carry on a monitoring operation properly.

The monitoring procedures used in the dairy industry serve as an example. Similar monitoring techniques are used throughout the food industry; the example is chosen because milk production is carried out under a very tight and extensive system of controls from the cow to the consumer. Additionally, the requirements for the handling of milk prior to and after the terminal heat treatment relate closely to the requirements faced by the constructors of heat-sterilized spacecraft. In neither case is the heat-exposure cycle sufficient to kill an unlimited number of micro-organisms; the time and temperature are limited by the heat lability of the product. Therefore, protective handling techniques are required which reliably insure both a low level of contamination at the time of heat treatment and protective handling after heat treatment.

A cow used in milk production is regularly tested for disease. Before milk is taken, the milking equipment is decontaminated and the cow's udder is washed. All collection and storage tanks are kept in a clean and decontaminated condition. Periodically throughout its handling, the milk and milk contact surfaces of equipment are tested to determine the bacterial count, tank trucks are washed and sanitized after each trip, and so on. All the cleanings and decontamination treatments serve a single purpose: to insure that when the milk reaches the pasteurizer, it is free of filth, and the numbers of pathogenic and spoilage bacteria are sufficiently low that the limited time-temperature exposure of the pasteurization process will yield a safe and palatable product to be marketed.

The U.S. Public Health Service has been the central organization primarily responsible for the presently observed high levels of sanitation control in the dairy industry. Through intramural programs and cooperative research with industrial, agricultural, and public health agency laboratories, the Service has developed a model Milk Ordinance and Code which is adopted by State and local health agencies for the regulatory control of milk and dairy products. In essence, the document is a manual of procedures intended to assure the quality of Grade A pasteurized milk.

Throughout the considerable time period that preceded the present 1965 Grade A Pasteurized Milk Ordinance, experience has shown that the success of such an ordinance depends on the following factors: (*a*) Development of adequate procedures for

maintaining clean cows in a clean environment and for decontaminating milk contact surfaces; (*b*) development of practical but adequate refrigeration and pasteurization procedures; (*c*) development of tests by which laboratory evidence is obtained to determine the efficacy of the decontamination, refrigeration, and pasteurization processes which are used; (*d*) maintenance of documented records to indicate the frequency and manner in which control procedures and testing programs are performed; and (*e*) the bringing into being of a trained corps of sanitarians or "visual monitors" whose function is to make certain that milk is produced and processed in accordance with recommended practices.

Experience has also indicated that the final quality and safety of the finished product depends on the use of all the above mechanisms, including documentation supported by visual monitoring; to depend on any one to the exclusion of the others results in product failure. The basic philosophy is then to keep the product free of filth and extraneous matter and to provide safeguards against the entry and buildup of pathogenic and spoilage micro-organisms which could cause pasteurization failure or product deterioration.

Visual Monitoring for the Control of Microbiological Contamination in Spacecraft Operations

While the basic philosophy of Grade A milk production is applicable to the production of sterile spacecraft, certain important differences should be noted. Milk is a product which is mass produced by a thoroughly proven, relatively slow changing process; therefore, the functions of process development and process monitoring are only casually interconnected.

On the other hand, only a very small number of spacecraft, often fewer than five, are produced according to a given design; also, during the assembly of a spacecraft a number of changes may be incorporated into procedures before the spacecraft is completed. The implications of the facts are far reaching. In a typical mass production system, a uniform manufacturing process is established and qualified to a certain confidence level; then a monitoring system is established relying primarily on statistical sampling of the product, but not the process. The success of the process is judged by the attributes of the product. Clearly, if five or fewer spacecraft are produced to a given design, a statistical monitoring system based on the characteristics of the product would be meaningless, and so would the control of microbiological contamination; therefore, two conclusions are established: First, a spacecraft assembly and test system must include close monitoring of

the assembly processes for contamination control in addition to testing of the engineering characteristics of each product; close monitoring will assure that the individual characteristics of each spacecraft are identified and documented so that general tests performed on any unit will give data which can be applied to the evaluation of all similar units. The individual characteristics of each unit can then be applied to give a complete analysis.

Second, the system must include feedback mechanisms so that problems engendered by procedural change or ambiguity are solved rapidly and by means which are scientifically sound. Because of the need to assimilate rapid change during the assembly and testing of spacecraft, a microbiological contamination control system is needed, containing the same basic elements of testing and inspection as are used in production processes but having in addition a formal system for collection and dissemination of information about contamination problems which are encountered.

Throughout the flow of information and documents, from the conception of the spacecraft through the issuance of detailed assembly, testing, and inspection procedures, and actually through the completion of the mission, continuous liaison is maintained between all cognizant organizations. While this liaison may be minimal in large-scale manufacturing operations, the production of spacecraft requires excellent intercommunication between all groups to assure that each completed spacecraft, including the first, is a flightworthy unit.

Many of the visual monitoring operations could be carried out by the existing Quality Control Inspectors. Their duty would be to verify that specified requirements for personnel, facility, decontamination, parts handling, and so forth, are followed; to witness tests; and to perform such quality control tests and verifications as are required of them. All duties should be spelled out in detailed procedures. If a discrepancy is noted, whether by the Quality Control Inspector or by any other person, it is recorded. The recorded discrepancies may include any violation or suspected violation of procedure as well as commonsense value judgments. Discrepancy reports would be audited by a review board consisting of representatives of the microbiologists, manufacturing organization, quality engineers, facility operator, cognizant engineers, and other interested groups; the review board makes disposition of each reported problem. Information on important problems would be disseminated widely to assure the maximum use of available technical talent. In addition to directing correction of the immediate problem, the work of the review board could serve a

"feedback" function by continually making higher level personnel aware of the problems being met on the working level, thereby assisting them to eliminate such problems in future operations.

Many of the functions mentioned here are contained in various present quality assurance systems; they are mentioned here to show that they are applicable to microbiological contamination control as well, and because certain microbiological visual monitoring functions may be incorporated into the duties of the Quality Control Inspectors.

Methods for Visual Monitoring

Visual monitoring operations may be conducted directly by observation of the monitored process by inspectors familiar with the correct conditions and able to distinguish improper conditions. Visual monitoring may also be performed indirectly, using television or photographic cameras. Certain advantages accrue to each technique; for a complicated and critical operation such as assembly and test of heat-sterilizable spacecraft, a combination of all three methods is desirable.

Direct visual observation is most efficient for close work, both in monitoring of very critical procedures and in inspection of products and equipment. Direct observation is almost a requirement for inspection work which requires microscopes or other visual aids.

For monitoring of noncritical areas and for general gross observation, television may be used. Conventional closed-circuit systems lack color and detail, but have the advantage that one person can monitor a number of areas simultaneously. In addition, use of television may allow a reduction in the number of monitoring personnel present in critical areas, thereby providing a bonus in contamination control.

Photographic monitoring has the two major advantages of providing a permanent visual record and of providing a record which can be examined at leisure by a number of people. The commonest method of photographic monitoring is with time-lapse motion pictures, using a number of cameras sufficient to provide full coverage of the area to be monitored. If pictures are taken at a rate of one every 2 seconds and reviewed at 16 frames per second, an 8-hour shift can be reviewed in 15 minutes. The method, while providing thorough coverage of a given area, does not lend itself to monitoring of fine detail. On the other hand, careful review of a thorough set of photographic records provides a high degree of assurance that major procedural breaks or accidents have not occurred.

The Integration of Microbiological Visual Monitoring Into a Complete Spacecraft Monitoring System

Implementation of NASA spacecraft sterilization policy places a set of microbiological cleanliness constraints on the assembly and testing of spacecraft in order that the numbers and types of microorganisms on the completed spacecraft will permit the attainment of the required sterilization assurance level. To illustrate the proper position of microbiological visual monitoring in the spacecraft production program, one possible functional scheme for overall microbiological monitoring is presented in figure 1; the implementation of microbiological visual monitoring is illustrated in the light of the overall scheme. In the interest of efficient monitoring, certain microbiological visual monitoring activities may be carried out by the Quality Control Inspectors; the required interfaces with the quality assurance program are indicated.

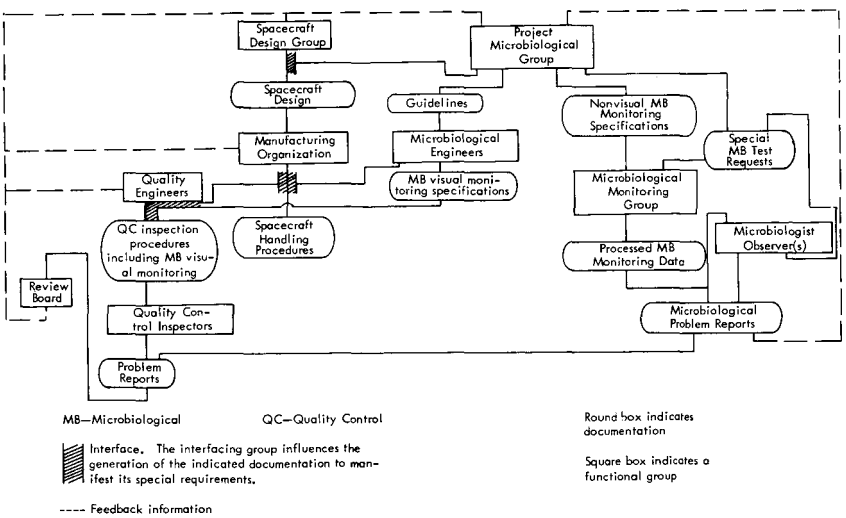

FIGURE 1.—A possible functional scheme for overall microbiological monitoring.

The Project Microbiological Group is the functional center of all microbiological operations related to the project. Its tasks are to publish microbiological monitoring specifications of all types including visual monitoring specifications, to provide liaison with spacecraft design and manufacturing personnel as necessary, to generate guidelines and provide consultation for the microbiological engineers, and to operate a laboratory to conduct such research as is needed to define and allow solution of day-to-day problems

and to allow application of new techniques. Based on their researches, the Project Microbiological Group would decide on the specific visual monitoring techniques to be used and would provide guidelines to the microbiological engineers concerning the uses and limitations of such visual techniques.

The Microbiological Monitoring Group is responsible for collection and processing of routine microbiological samples and for the publication of concise periodic reports of the microbiology of the facility and of the spacecraft according to specifications set down by the Project Microbiological Group. In addition, the Monitoring Group is responsible for emergency microbiological monitoring of procedural breaks and other accidents including those detected by visual monitoring. In general, the Microbiological Monitoring Group would be responsible for all nonvisual microbiological monitoring.

The microbiological engineers would use guidelines set down by the Project Microbiological Group to establish detailed visual monitoring procedures. One task of the microbiological engineers would be to create an effective and efficient balance of all visual monitoring methods.

The microbological engineers would also work in close conjunction with both the Manufacturing Organization and the Quality Engineering Organization; their responsibility would be to insure that all assembly and quality-control procedures reflect the need for microbiological contamination control as well as the need for operational reliability of the spacecraft.

To achieve efficient microbiological visual monitoring, it should be recognized that even though a procedure is conceived for the purpose of microbiological contamination control, it is not necessarily true that monitoring for adherence to the procedure requires microbiological knowledge. For example, microbiologists recognize that microbiological contamination is present on the human face; therefore, a possible requirement is that a handler of flight equipment who is wearing gloves should change the gloves if he touches his face. Adherence to the glove-change requirement may be verified by any Quality Control Inspector. Consideration of a number of similar potential requirements yields the important result: Microbiological knowledge is required to generate spacecraft visual monitoring requirements, but in general not to implement them. Most microbiological visual monitoring may be performed by Quality Control Inspectors, provided that (*a*) some job-specific training is supplied to make the inspectors aware of the proper handling techniques, and (*b*) microbiological requirements are translated into well-defined visual monitoring specifications.

The development of precise visual monitoring specifications is one of the functions of the microbiological engineers.

The problem reporting and disposition function may operate as it has in past spacecraft projects, but the review board should have, in addition, representatives from the cognizant microbiological groups.

This system accounts for the routine microbiological visual monitoring activities and establishes a problem reporting and disposition mechanism in which procedural breaks are documented by nonprofessional personnel but are evaluated by professional microbiologists. The system requires the availability of laboratory facilities for the precise evaluation of reported problems and of suggested solutions in addition to such laboratory facilities as are required for routine and emergency microbiological monitoring. In addition, a feedback loop is provided so that problems and their solutions will be reflected in future procedures. A mechanism is still needed, however, to accomplish the following functions:

(1) To interpret anomalies in the microbiological monitoring data

(2) To provide a direct source of information for the Project Microbiological Group so that changing conditions in the assembly areas may be reflected in procedural guidelines and monitoring specifications

(3) To make microbiological expertise immediately available to meet emergency situations

(4) To serve as a doublecheck on routine microbiological monitoring operations.

One way to implement the functions is to have present in the spacecraft operations area a Microbiologist Visual Observer. The person should be a professional microbiologist with experience in environmental microbiology; the background would allow him to perform the following tasks to implement the functions given above:

(1) Review the microbiological monitoring data. If unusual contamination is noted in some area, determine possible sources by inspection and by reference to monitoring data. If necessary, order experiments to be carried out to pinpoint the contamination source. Suggest possible solutions.

(2) Observe spacecraft-handling operations and verify the adequacy of procedures on a spot-check basis.

(3) Be cognizant of planned changes in activities and notify the Project Microbiological Group in detail so that appro-

priate monitoring and handling specifications and guidelines may be generated.

(4) Conduct periodic inspections of the facility and document conditions not covered by specification which may compromise microbiological cleanliness.

(5) In case of a serious procedural break, order such microbiological tests as are necessary to determine the extent of the break.

(6) Review visual monitoring records, both documentary and photographic, and verify the continuing adequacy of both.

(7) Be cognizant of the activities of the problem review board.

Concluding Remarks

In conclusion, microbiological visual monitoring is a required function in the assembly, test, and launch of a heat-sterilizable spacecraft. Visual monitoring is not sufficient, however; it is only part of a complete monitoring operation and must be supported by an integrated system of quantitative monitoring, documentation, and microbiological expertise.

Most visual monitoring can be performed by nonprofessional personnel, provided that detailed monitoring procedures are published and that adequate job-specific training is administered. This portion of the microbiological visual monitoring can probably be performed by existing Quality Control Inspectors.

To assist the interpretation of visual monitoring data in the light of microbiological knowledge, professional microbiological talent should be present in the spacecraft-handling area. The direct availability of technical knowledge will improve the efficiency of microbiological monitoring operations and will aid the proper and prompt resolution of anomalies.

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Survey of Microbiological Techniques for Recovery From Surfaces

Standardized methodology is being developed for several applications of microbial surface sampling in the spacecraft sterilization effort. Surface evaluations will include: (1) room surfaces in assembly areas (Rodac and swab methods), (2) clothing and packaging materials (Rodac method), (3) stainless-steel strips to determine cumulative contamination over an extended period in assembly areas (rinse method), (4) piece part evaluation (rinse and direct agar plating methods), and (5) subsystems evaluation (rinse and direct agar plating methods on detachable coupons designed into the system).

Thus, while no universal sampling method is currently available, considerable progress has been made toward developing techniques, which give us a realistic appraisal of surface contamination on space hardware. In particular, the combination rinse and direct agar plating technique appears to be a valuable tool for quantitative and qualitative evaluation of microbial loading prior to terminal sterilization.

Hand in hand with the task of sterilizing space hardware without impairing its ability to function is the task of determining microbial loading at various intervals prior to launch. In practice, the two problems are inseparable, for the ultimate choice of a sterilization technique must depend in part on the degree of microbial loading which must be overcome by the sterilization cycle.

While the final presterilization loading determination will ultimately be the most important application of microbial surface sampling in the Space Sterilization Program, there are other applications with which we are concerned.

Monitoring of the Component Assembly Environment

As part of the philosophy of minimizing total microbial loading through assembly in ultraclean environments, it has been necessary to establish baselines of microbial cleanliness in such facilities. The criteria for these baselines include several measurements of microbial surface contamination: (1) working bench-top surfaces, (2) clothing and packaging materials, and (3) assessment of contamination on stainless-steel strips which, by exposure over a

long period of time, can simulate total concomitant contamination to which an exposed part might be subjected.

Monitoring of Piece Parts and the Assembled Subsystems

Of greater significance than the assembly environment are piece parts during the course of assembly and assembled subsystems of the spacecraft itself. Monitoring of the parts will be an indication of how successful the "clean assembly" approach is at any given stage of development, while monitoring of the assembled subsystems can be performed before terminal sterilization as an indicator of total contamination or microbial loading which must be overcome.

Historical Development

In order to accomplish these goals, considerable effort has been expended toward the development of suitable sampling technology. The basic problem of quantitatively and qualitatively evaluating viable microbial contaminants on surfaces is not as simple as it might first seem.

Over the years, workers in other fields, particularly in the dairy and food industries, and medical care facilities, have developed methods for such evaluations. Their efforts are well documented in the literature (refs. 1 and 2). These methods can be grouped basically into four categories:

1. *Swabbing Techniques.* — The contaminants are dislodged from the surface, then transferred to a growth medium for incubation and subsequent evaluation. This approach is the basis for the American Public Health Association Standard Method for evaluation of surfaces in the dairy and food industries (ref. 3). It has the advantages of being applicable to irregularly shaped surfaces and of lending itself to dilutions, but has been found to be relatively unreliable for detecting known concentrations of contamination (ref. 4).

2. *Agar Contact Techniques.* — The contaminants are dislodged by direct contact with a solid agar surface which can then be incubated directly (refs. 5 and 6). This method is particularly applicable in the institutional environment because of its greater simplicity and reliability compared to swabbing techniques. It is, however, confined to flat surfaces, plus certain textiles, and has a relatively low recovery efficiency. The most satisfactory example of this method is the Rodac plate, a disposable plastic dish specifically designed for the purpose (ref. 5).

3. *Direct Surface Agar Plating.* — Liquid agar is poured directly over the surface to be tested and allowed to solidify (ref. 7). The

surface can then be incubated and evaluated. It has the advantage of eliminating the need for dislodging and transfer of contaminants, but is very awkward to work with unless confined to small portable objects which can be brought to the laboratory for evaluation.

4. *Rinse Techniques.* — The surface to be tested is placed in a sterile liquid solution and shaken. The liquid can then be plated in agar and incubated. This technique actually arises from the dairy industry Standard Method for evaluating contaminants in bottles, wherein the bottle itself is the subject for evaluation (ref. 3).

Problems concerning percentage of recovery and reproducibility of data have remained as stumbling blocks throughout the development of these techniques. However, considerable progress is apparent in recent years coincident with the application of statistical techniques to surface sampling problems (refs. 8 and 9). The use of large sample sizes has helped to overcome some of the variability problems, and random selection of experimental units has been recognized as necessary for making inferences from data collected. Perhaps most important has been the realization that standardization of technique is vital for any data comparison. Thus, all investigators should recognize that statistical solutions are available for problems in data accumulation and interpretation.

Methods Applicable to the Spacecraft Sterilization Program

Investigators interested in the Spacecraft Sterilization Program have necessarily borrowed and adapted techniques developed for other purposes. The need for standardization of methods has, fortunately, been recognized for some time. With this in mind, NASA has recently appointed a task force to draft procedures for microbial examination of space hardware (paper entitled "Proposed Procedures for the Microbiological Examination of Spacecraft," by J. J. McDade, C. W. Bruch, M. S. Favero, and D. Vesley, in preparation). When these procedures have been finalized, they should prove to be a real asset to the overall program.

Among these procedures are several dealing specifically with microbial examination of surfaces. As part of the policy of standardizing techniques, it was decided to utilize a single broad-spectrum agar and a single incubation sequence for all applicable procedures. It was recognized that no single growth medium and no single incubation sequence was available which could recover all viable micro-organisms. It was decided, therefore, that maximization of counts would be preferable to the use of multiple media and varied incubation sequences which could well make the entire

effort unmanageable. In this regard, Trypticase Soy agar (TSA) was chosen as the medium and 32° C for 72 hours as the incubation sequence to be used. It was also felt that the specific importance of the sporeforming species and the fact that the Martian environment would probably be particularly conducive to anaerobic growth prompted special attention to spores and anaerobes in recovery techniques.

The specific methods currently being considered can be summarized as follows:

(1) For environmental room surfaces, both swab and agar contact methods will be used. For flat surfaces, such as bench tops, the Rodac plate method will be used, while for curved or irregular surfaces the cotton swabs with a 4- by 4-inch sterile template for area standardization are required. In both cases, the standard TSA agar will be the medium and plates will be incubated at 32° C for 72 hours. However, recognizing the possibility that relatively high contamination on such surfaces might make enumeration difficult, counts will also be made at 24 and 48 hours on aerobically incubated plates. Seventy-five percent of the Rodac plates will be incubated aerobically, while 25 percent will be incubated anaerobically. The swab samples will be shaken in peptone water, then plated, 1 milliliter in 20 milliliters of TSA agar. Two such plates shall be incubated aerobically and one anaerobically. Anaerobic conditions shall be achieved by using gaseous hydrogen to remove oxygen catalytically in a Brewer jar or equivalent. Any necessary sampling of clothing or packaging materials shall be carried out with Rodac plates.

(2) For assessment of microbial contamination accumulating on surfaces, a relatively new technique has been decided upon. Originally developed by the Fort Detrick Army Biological Laboratory, the method utilizes a series of 1- by 2-inch stainless-steel strips which can be left exposed in an assembly environment for an extended time (ref. 10). At various intervals, several of the strips can be aseptically removed and evaluated. The method of evaluation is diagramed in figure 1. It is an adaptation of the rinse technique employed in the dairy industry and previously discussed (ref. 3). In this adaptation the strips are placed in individual bottles containing 50 milliliters of sterile peptone water. The bottles are mechanically shaken to dislodge contaminants. Duplicate 5-milliliter aliquots of the diluent are then plated in 20 milliliters of TSA agar and incubated aerobically while two other 5-milliliter aliquots are incubated anaerobically. The remaining 30 milliliters of the diluent are transferred to a test tube and heat shocked at 80° C for 15 minutes. The purpose of this step is to

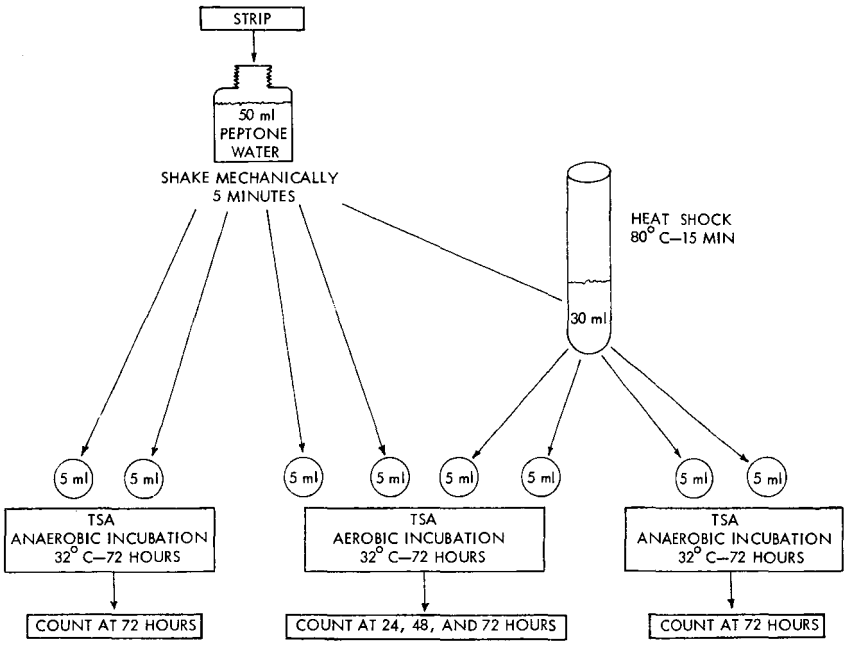

FIGURE 1.—Strip analysis.

destroy all nonsporeforming species so that sporeformers can be separately evaluated. After heat shocking, again duplicate 5-milliliter aliquots can be plated in 20 milliliters of TSA agar and incubated both aerobically and anaerobically. This technique has proved to be quite successful in evaluating accumulated contamination in assembly environments (ref. 11). In addition, it can be used to evaluate strips which have been contaminated through human handling.

One drawback to the method is the possibility that materials other than stainless steel may demonstrate dissimilar properties of microbial retention and survival. The ramifications of this phenomenon are currently under study. The method is, of course, applicable to many materials other than stainless steel if this should prove necessary.

(3) For small piece parts, a technique is suggested which is virtually identical to that described for the strips. This method is outlined in figure 2. The only difference is that the part itself, after shaking, is removed from the diluent and plated directly in liquid TSA agar. After the agar hardens, the plate is incubated aerobically at 32° C for 72 hours. This is actually an adaptation of the previously described direct surface agar plating technique (ref. 7). The embedded part can thus be evaluated directly as a control to

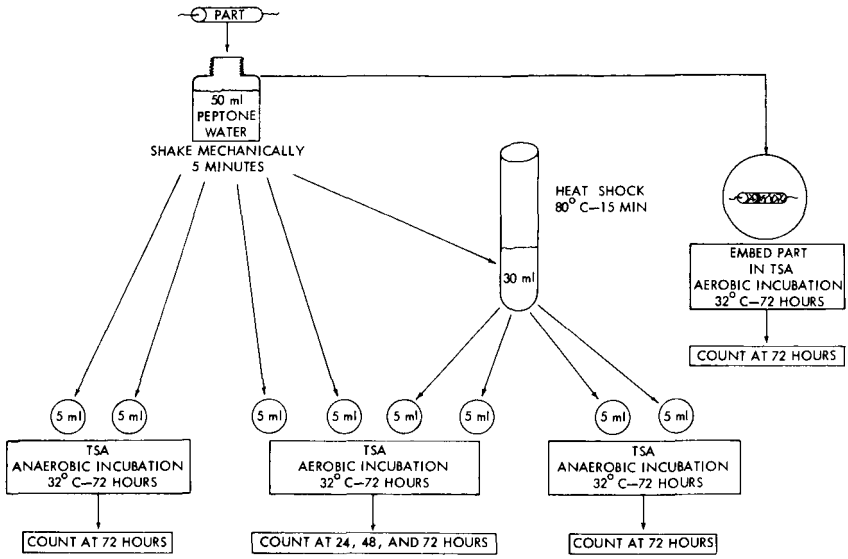

FIGURE 2.—Parts analysis.

determine the number of contaminants remaining following the shaking process. Contaminants remaining on the strip are counted and added to those from the aerobically incubated, non-heat-shocked aliquots for a total count.

(4) The final surface sampling technique to be discussed is perhaps the most important of all. It is the evaluation of assembled subsystems prior to terminal sterilization. The current plan is to utilize again the basic strip-evaluation method. In this instance, all flight hardware other than piece parts will be designed to provide for the inclusion of detachable 1- by 2-inch stainless-steel coupons on the surface of each flight item. The coupons will go through the entire assembly and checkout process until just prior to terminal sterilization. The coupons will then be removed with sterile forceps and evaluated by the identical procedure used for the piece parts.

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Microbiological Techniques for Recovery From Interiors of Solids

Micro-organisms entrapped within solids present unique contamination control problems. Bacterial spores encased in plaster of paris, asbestos plastic cement, various plastic dental materials, and solid rocket propellant are more resistant to dry heat than those residing on surfaces (ref. 1, and unpublished data obtained by E. G. McNall and W. T. Duffy). Because of this increased resistance to the effects of dry heat, it will be necessary to develop assay procedures which will allow the detection of very small populations of micro-organisms within solids. Some solids within the spacecraft may be subjected to temperatures in excess of the sterilization requirement and thus constitute a minimal contamination control problem. There are, however, solid components which are adversely affected by such treatment. It is essential that the microbial population within such solid materials be accurately known in order to assess their contributions to the total contamination load of the entire spacecraft. This final load must be known in order to predict the successful application of the terminal dry heat sterilization cycle. In order to compute a less than 10^{-4} probability of an Earth microbe surviving the terminal heat sterilization cycle, the contamination load must be known.

Not only is it essential to develop adequate pulverization techniques for exposing these micro-organisms, but it is also necessary to develop methodology for overcoming the adverse environments provided by the entrapping solids. At the time of fabrication of spacecraft components, micro-organisms from the environment may exist and be incorporated as spores or as vegetative cells in any of a wide range of physiological states. Obviously then, the effects of the various plastics, propellants, and other solids on the physiological states of micro-organisms are important in the development of sensitive assay and detection techniques.

The ultimate aim of pulverization is to reduce the size distribution of particles to that which statistically will provide for optimum diffusion of nutrients into the micro-organism and thereby permit the outgrowth of micro-organisms from their encased sites within the solid particle. Since bacterial and fungal spores would be best adapted for survival in the interior regions of solids, the pulverization process must be optimized for their detection and culture recovery. In the course of such pulverization, large micro-organisms — fungal spores, mycelia, and large vegetative cells — may be physically damaged. Thus, in order to assure recovery of such large cells, additional samples of the solid should also be pulverized in a manner modified for the recovery of large cells.

A wide range of substances are present within solids which interfere with the growth of micro-organisms. Some plastic solids are polymerized through the use of free radical initiators. These initiators or their toxic products may be present in minute but detectable quantities within solids and must be neutralized in order to provide optimum recovery conditions.

Solid rocket propellant contains large quantities of inhibitory substances which must be leached from the pulverized solid propellant and neutralized prior to the application of cultural techniques. Epoxy resins, coating compounds, potting compounds, and adhesives may contain residual unreacted epoxide groups which inhibit the growth of micro-organisms. Such functional groups should be reacted to prevent their interference with the recovery of the micro-organisms.

This paper discusses some of the techniques developed for the pulverization, leaching, neutralization, and culture of certain solid spacecraft materials.

Methods of Pulverization

The pulverization of solid materials used in the fabrication of spacecraft may be accomplished through the use of high-speed blenders, drills, mortar and pestle, ball and vortex mills, abrasive devices, and saws. (See refs. 2 to 9.) These methods each have their characteristic advantages and disadvantages. Control of the extent and uniformity of pulverization with these methods is difficult and in some cases not possible. The production of aerosols limits the usefulness of the high-speed blenders and drilling techniques. With certain types of solids, explosions occur when the level of pulverization reaches a certain particle distribution range. The recovery of micro-organisms pulverized in solids using high-speed blenders and drills is disappointingly low. It would appear

likely that the lack of adequate control of heat generated in these forms of pulverization as well as the physical trauma incidental to these methods may be responsible for such results. Sawing permits the control of both the heat production (by controlling the sawing rate) and the extent of pulverization through selection of the ideal tooth design.

Various blade-and-tooth conformations produce widely different forms of pulverization. The types of saw blades used in the sawing of the various types of materials are numerous; however, the major parameters of importance in determining the type of pulverization produced are dependent upon the following factors:

(1) Tooth conformation and design
(2) Pitch (number) and size of teeth
(3) Sawing rate
(4) Pressure applied to the solid
(5) Tension of the blade
(6) Type of metal (hardness and lack of toxicity).

The most important factor affecting the effectiveness of pulverization, using saw blades, can be related to the design and configuration of the teeth. The angles of the tooth face, back, clearance, and rake, in addition to the tooth spacing and gullet depth, are illustrated in figure 1. There are several possible combinations of teeth configurations which produce widely different results in the pulverization of solids. The short, nearly vertical angle tooth design, typical of hacksaw blades, appears to be the most useful for the pulverization of rigid plastic solids.

There are a variety of saws in which the teeth are set (fig. 2). The alternate set teeth are set with one tooth to the right, and the next to the left. This type of set is common for some types of plastic-cutting blades and for nearly all wood-cutting blades. The configuration has not proven to be effective in the pulverization of solids, since it produces large cuboidal chips. By adding one straight tooth between the pairs of left- and right-set teeth, the type of chips that are produced differ. If the angle of the cutting blade is acute, with respect to the surface of the solid, a ribbon is produced in place of a chip.

The raker set, which interposes a straight tooth between the pairs of left- and right-set teeth with a hacksaw configuration, is capable of clearing the ribbon and partially disrupting it. The most common blade containing some 22 to 32 teeth per inch employs wavy set teeth. These teeth are formed by alternately bringing two or more teeth to the right and the left in graduated degrees. In this configuration a centerline drawn through the center of each tooth could follow a smooth, low-pitched wavy curve. The blades with 32

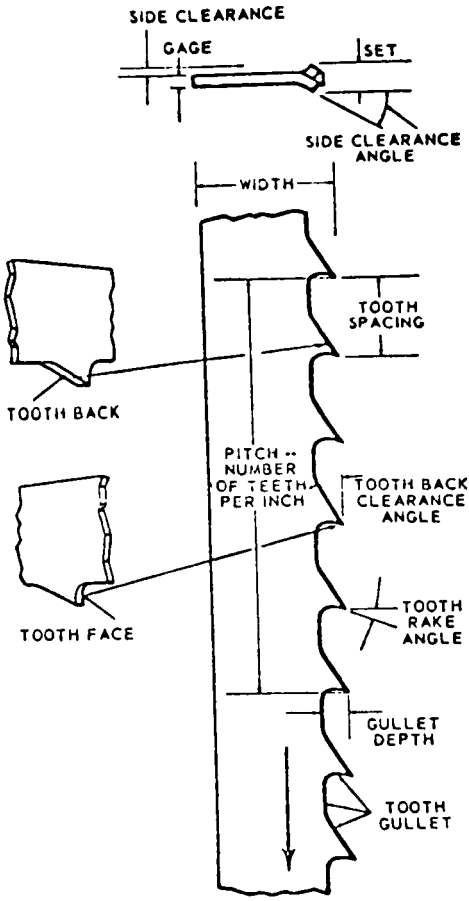


FIGURE 1.—The design features of saw blades

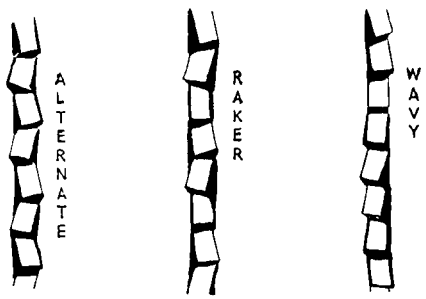


FIGURE 2.—Teeth settings of saw blades.

teeth per inch are generally gang set. In this conformation the tooth-edge portion of the blade is formed with a device which gang-sets six to eight teeth in one direction and alternates the succeeding group of eight teeth so as to form a wavy pattern

throughout the entire length of the blade. These blades are designed for cutting thin metal and hard steel. Among the commercially available blades, the fine-tooth blades show advantages over the alternate or raker-type configuration in the pulverization of solid plastics.

In spite of the fact that hacksaw blades are designed for cutting hard metals, it is desirable to use nickel-molybdenum-alloy blades or tungsten-steel blades in order to minimize the rate of dulling of the blade. Dulling which occurs in using other types of hardened steel alloys may result in poor pulverization. Crosslinked epoxy polymers with fillers cause surprisingly rapid dulling of blades. The types of blades studied included a representative range of metal alloys.

In order to achieve reproducibility, it is important to tension the blade to the specific requirements recommended by the manufacturers. For accurate determination of blade tension, a torque wrench is recommended. Alternately, one may adjust the tension by use of an adjustable blade handle which has been previously calibrated using a tensiometer. It has been noted that in the pulverization of plastic materials, a variable degree of whip occurs when the blade is improperly tensioned.

The pressure applied to the blade during the sawing process is of critical importance. Ideally, this pressure should not exceed 100 grams per inch. The greater the amount of weight on the blade, the thicker the resulting ribbon. The length of blade is significant, since it is convenient to use slow long strokes which utilize the entire length of the blade and thus allow a more even, continuous stroke and increased uniformity of pulverization. The effect of pressure and blade designs on the pulverization of plastic materials studied is shown in tables I(a) to I(c).

Solid rocket propellant is the most difficult spacecraft component material to pulverize. The dremel drill, slicing with a microtome, and other methods which were studied were ineffective because of the peculiar characteristics of this substance. Sawing with appropriate types of hacksaw blades provided the most efficient pulverization. The particles produced were sticky and tended to agglomerate. However, the individual particles, even though agglomerated in the culture media, provided sufficient surface area for effective recovery of micro-organisms. The particles produced by sawing differ depending on the amount of pressure applied and the rate of sawing. Ribbonlike structures produced when the sawing pressure was less than 100 grams per inch were less than 15 μ in diameter and displayed a high incidence of microcracks and imperfections

TABLE I.—*Size of Particles and Intervals Between Cracks and Imperfections*

(a) Star Blades No. 1218–3 (12 × ½ × 0.025 inches; 18 teeth)

Plastic	Vertical pressure, grams/in. (sawing)	Interval between cracks, microns, for particles –							
		<1 μ	1 μ	1–2 μ	2–5 μ	5–10 μ	10–20 μ	20–40 μ	>50 μ
Maraset	19	4	9	11	20	15	26	8	7
	152	2	4	8	22	19	26	10	9
	546	2	3	4	9	13	27	18	24
Stycast	19	5	8	10	19	16	28	9	5
	152	3	5	9	20	22	25	9	7
	546	1	5	7	11	19	30	19	8
Epocast 4D	19	2	5	12	21	17	25	12	6
	152	1	4	7	24	21	26	11	6
	546	2	2	4	13	23	27	20	9
Epocast 4H	19	3	7	11	21	15	28	11	4
	152	1	5	7	21	20	27	10	9
	546	2	4	6	12	22	29	17	10
Epocast 4B	19	1	7	9	23	19	27	11	3
	152	2	5	8	25	17	28	6	9
	546	1	2	5	13	25	28	18	8

(b) Star Blades No. 1218–3 (12 × ½ × 0.025 inches; 24 teeth)

Plastic	Vertical pressure, grams/in. (sawing)	Interval between cracks, microns, for particles –							
		<1 μ	1 μ	1–2 μ	2–5 μ	5–10 μ	10–20 μ	20–40 μ	>50 μ
Maraset	19	17	23	17	11	12	12	4	4
	152	9	14	19	21	17	10	7	3
	546	3	6	10	14	21	12	14	20
Stycast	19	11	13	16	12	19	19	7	3
	152	6	8	9	20	18	20	11	8
	546	3	8	10	16	20	24	11	7
Epocast 4D	19	13	21	18	11	13	9	8	7
	152	10	21	12	15	12	17	6	7
	546	5	11	15	14	17	23	9	6
Epocast 4H	19	9	14	17	10	16	12	7	5
	152	8	10	11	19	17	19	10	6
	546	4	9	12	15	19	23	11	7
Epocast 4B	19	12	22	16	10	16	11	8	5
	152	7	14	11	21	17	18	7	5
	546	4	10	14	14	19	22	10	7

TABLE I. — *Size of Particles and Intervals Between Cracks and Imperfections*—Concluded

(c) Disston Blade Special Alloy Duraflex No. F–1232 (12 × ½ × 0.025 inches; 32 teeth)

Plastic	Vertical pressure, grams/in. (sawing)	Interval between cracks, microns, for particles –							
		<1 μ	1 μ	1–2 μ	2–5 μ	5–10 μ	10–20 μ	20–40 μ	>50 μ
Maraset	19	21	26	16	12	6	10	4	4
	152	11	17	21	23	15	6	5	2
	546	2	7	12	15	20	10	12	22
Stycast	19	13	15	17	14	19	11	5	6
	152	7	10	9	19	18	16	13	8
	546	5	9	12	17	21	17	7	12
Epocast 4D	19	19	25	20	12	7	8	3	6
	152	16	26	19	13	11	10	5	9
	546	7	13	23	17	12	12	6	10
Epocast 4H	19	17	20	19	11	15	9	4	5
	152	9	17	15	13	19	15	5	7
	546	5	9	12	9	25	18	10	12
Epocast 4B	19	13	19	19	18	16	6	6	3
	152	9	20	16	12	22	9	7	5
	546	8	12	14	13	19	14	9	11

radiating in all directions (fig. 3). Care must be taken that the particles are collected and handled in such a way that agglomera-

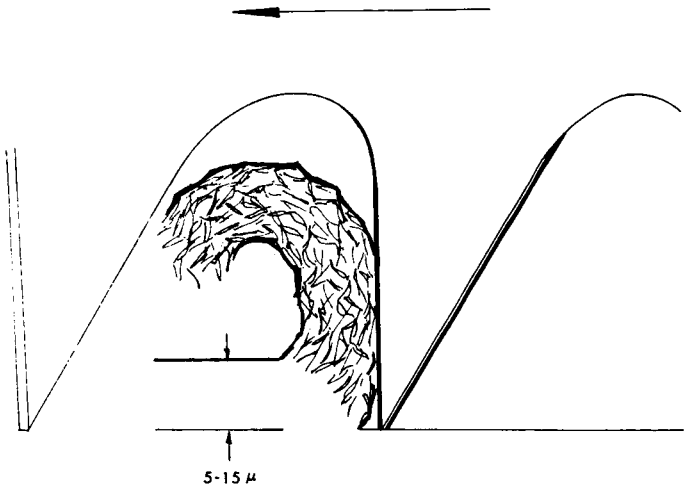


FIGURE 3.—Saw — Solid interaction. Large arrow indicates direction of saw movement. In some instances a ribbon which is highly fractured is produced as shown, whereas with most brittle solids, fragmentation into small particles occurs.

tion of particles is minimized. The typical microscopic appearance of this material is shown in figures 4 and 5.

Polyester and epoxy resins are satisfactorily pulverized by sawing. Ribbons are produced whose thickness is generally 5 to 15 μ when a minimum amount of pressure (less than 100 grams per inch) is applied by the blade to the plastic. The rigid, highly crosslinked or filled rigid plastic tend to pulverize as a powder. The less rigidly crosslinked plastics and semiflexible types readily form ribbons (figs. 6 to 9).

If we assume that a bacterial spore in an idealized model may occupy a space of 1 cubic micron, in the absence of trauma, toxicity, and neglecting dieaway, the solid pulverized to this size range should provide a theoretical recovery of 100 percent. If we further assume that the model for the solid particle is 2 μ on each side, the volume is increased eightfold (fig. 10). Is it reasonable to suppose that the theoretical recovery is decreased by a similar ratio to volume increase? The worst case would require that the spore exist in the center of all planes within the cube. Under these conditions, the maximum distance between the spore and the exterior would only be 1/2 μ. The probability of this worst case

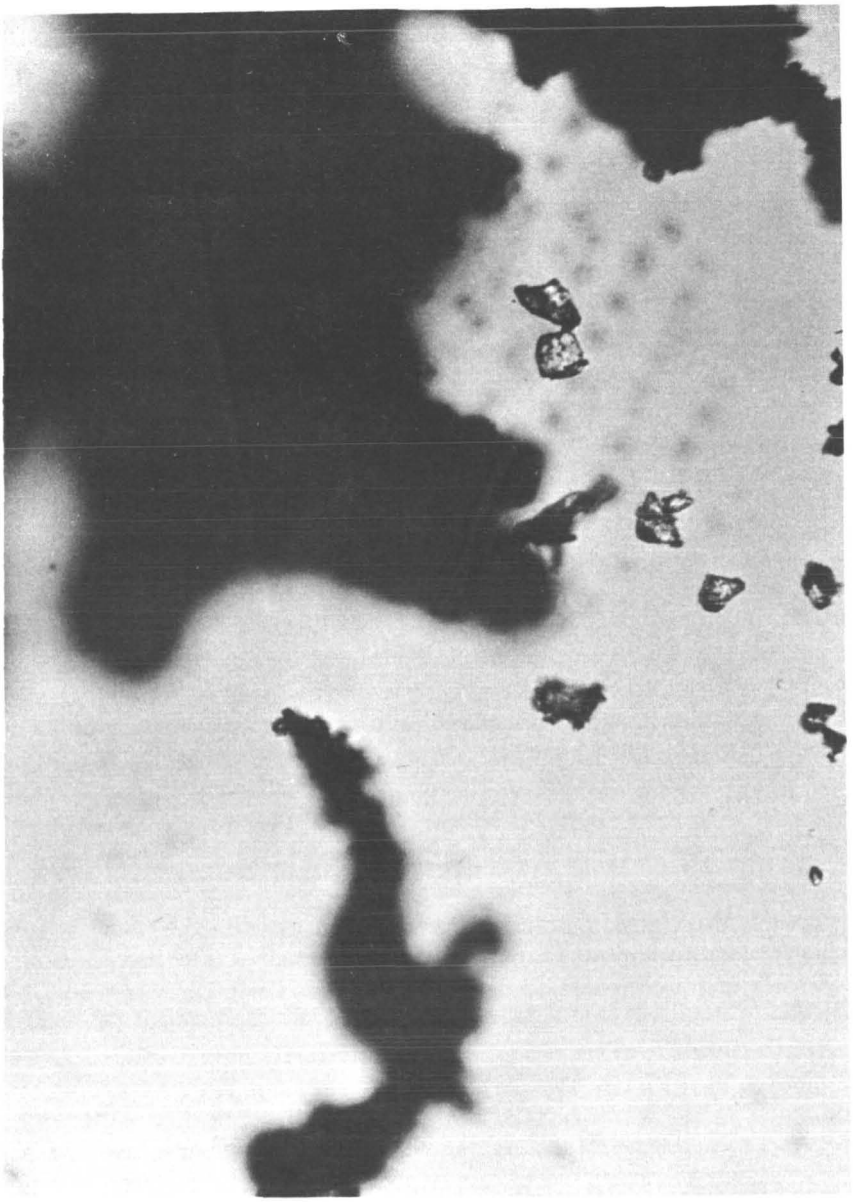


FIGURE 4.—The microscopic appearance of solid rocket propellant particles demonstrating the agglomeration of particles. Note presence of fractured ammonium perchlorate crystals.

would approach zero. The physical constraints of a particle containing the entrapped spore would be less than the theoretical

FIGURE 5.—Microphotograph of solid rocket propellant particles in water demonstrating structure of binder. Most crystals of ammonium perchlorate have been dissolved, exposing structure of binder.

cube case within any particle of 2 μ or less actually observed in a pulverized sample.

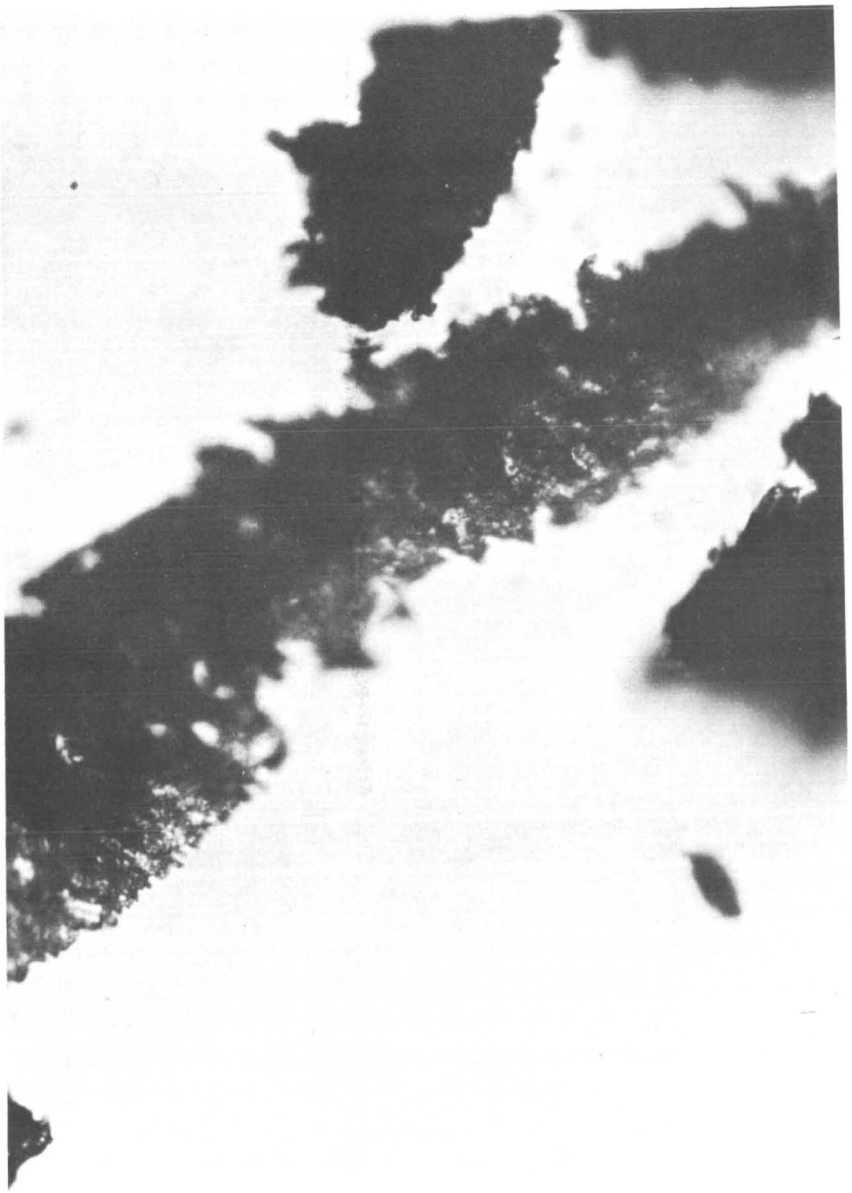


FIGURE 6.—Microphotograph of ribbon produced by Epocast 4H. Note the microcracks, surface irregularities, and imperfections within ribbon.

The worst case situation in such a particle would require that there be no microcracks or imperfections in any plane which would encroach upon the central location of the spore. The probability of

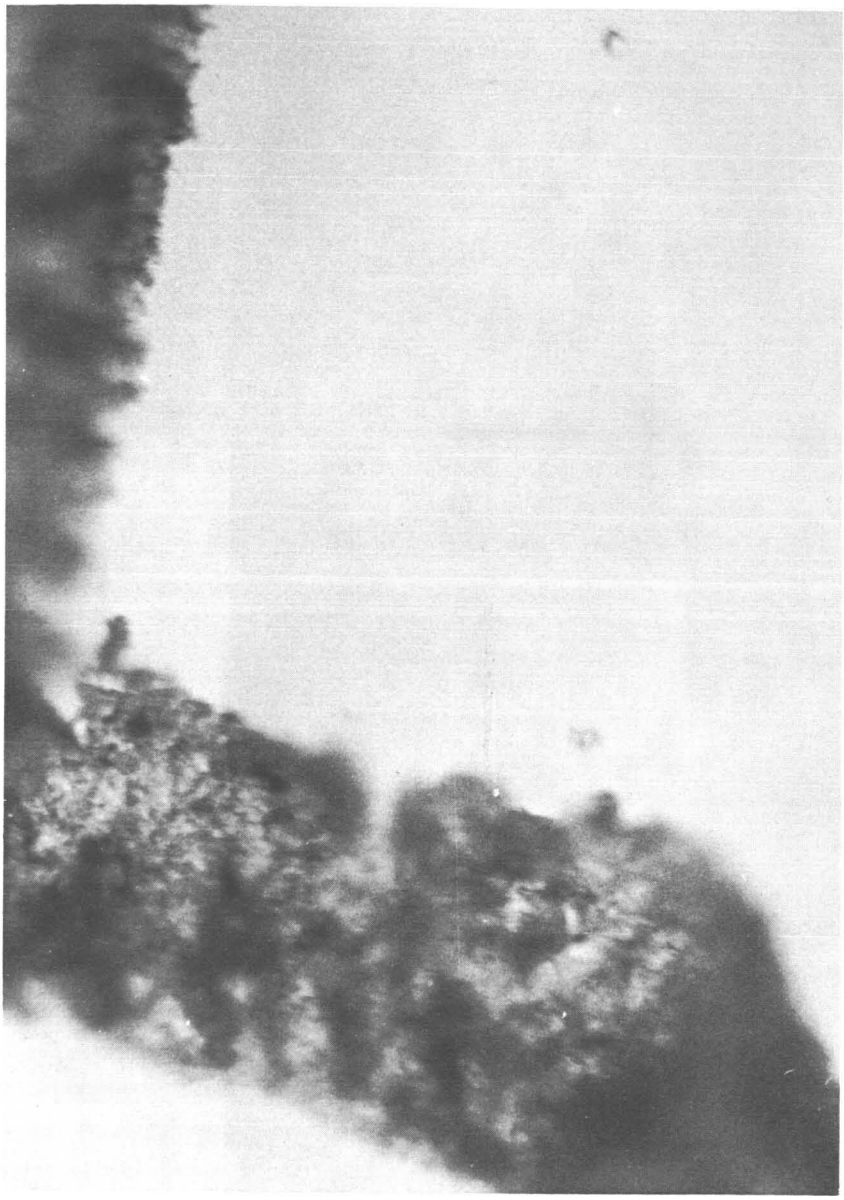


FIGURE 7.—Microscopic appearance of a ribbon produced by Epon 901/B1. Note presence of microcracks and imperfections.

this occurring also approaches zero. Particles resulting from pulverization showing a maximum dimension in any plane are generally sized by that dimension. Nearly all the particle, with the

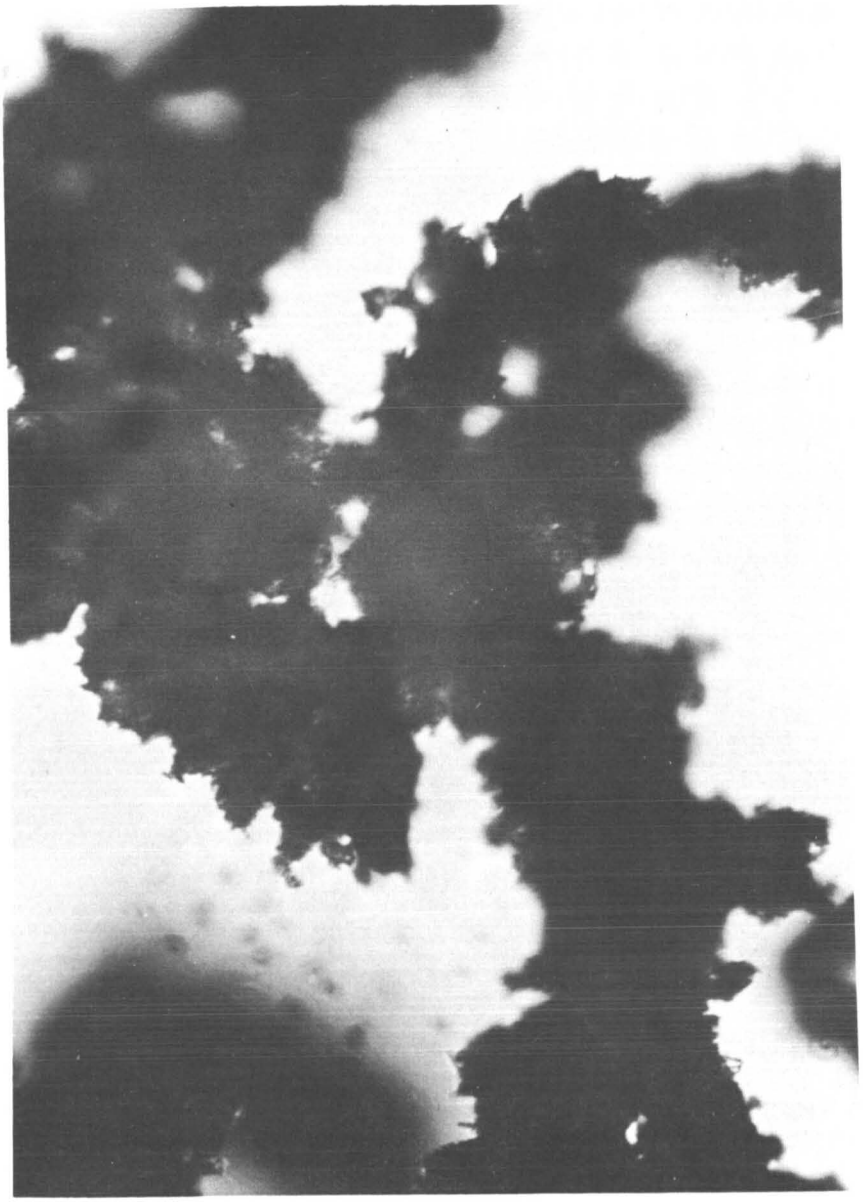


FIGURE 8.—Microphotograph of Stycast 1090 ribbons. Ribbon was produced under 500 grams per inch pressure. Stycast fragments into smaller particles when sawed at 100 grams of pressure, producing a mixture of ribbon fragments and free particles.

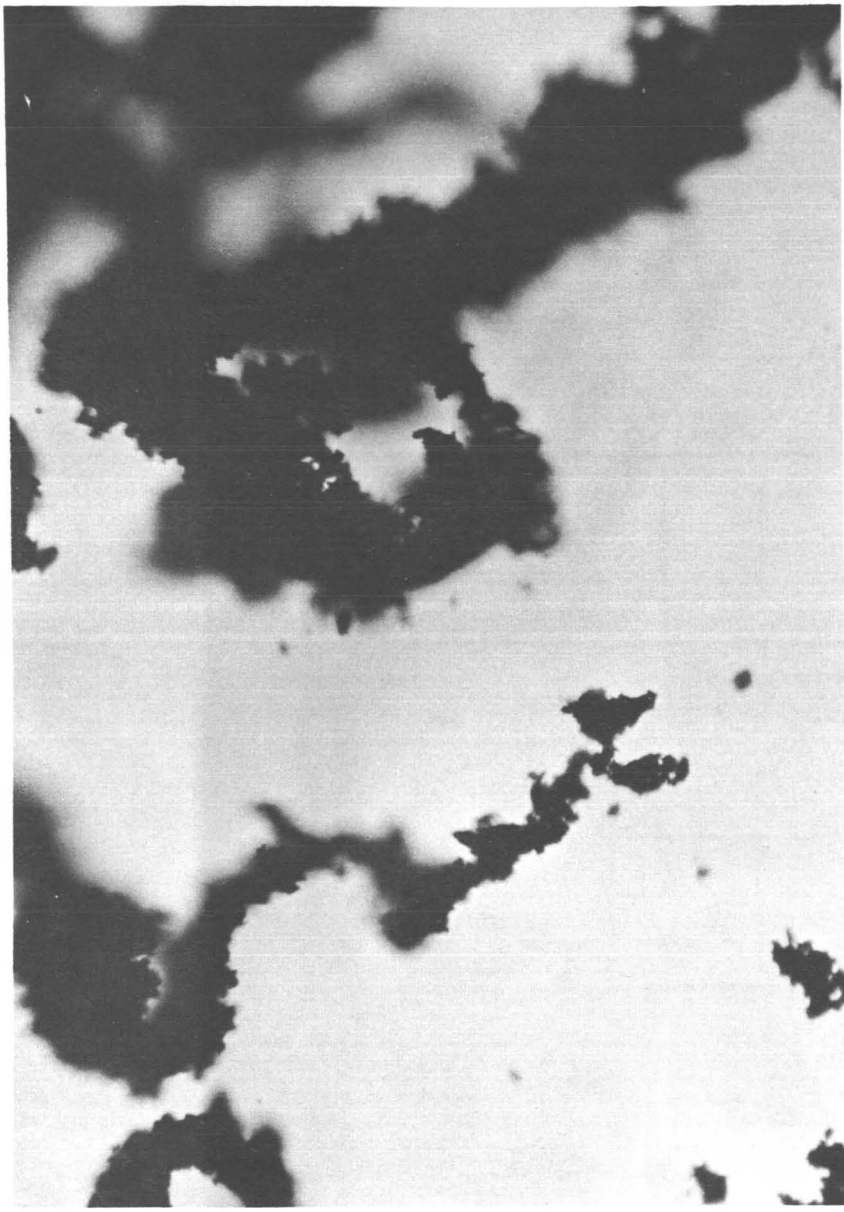
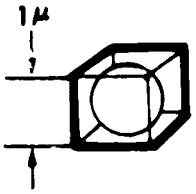
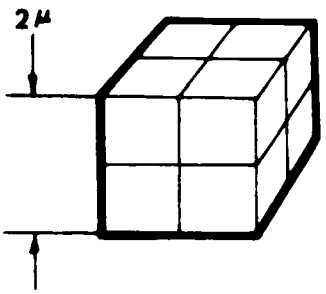


FIGURE 9.—Microphotograph of Epocast 212/951 showing some fragments lying on edge.

exception of the longest plane, may be a small fraction of 5 μ. Statistically, nearly all such particles would certainly have in one plane or another distances much less than 5 μ. In the course of our





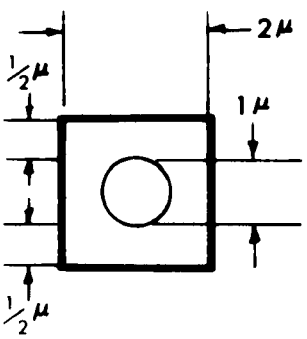


FIGURE 10.—Theoretical model of entrapped spore.

studies on pulverization, we have observed that well over 80 percent of such classified particles contain cross-sectional distances of less than 2μ. It may be noted that in table I(c) when using a 32-tooth blade and light pressure, as much as 70 percent of the particles were less than 5 μ in size.

Leaching and Neutralization

Often soluble toxic substances may be extracted from pulverized solid materials. In some instances, simple dilution of the inhibitory substance is sufficient to allow nearly maximum growth rates in a reasonable final culture volume. Leaching may in some instances be necessary when inhibitors or toxic materials are not neutralizable. Under these conditions the pulverized solid should be extracted with many small volumes of liquid and the liquid phase passed through a suitable membrane filter to recover micro-organisms washed into the leached fluid. The membrane filters then may be cultured and the micro-organisms from the leached liquid entrapped on the filter may be recovered.

Solid plastic materials are usually polymerized by acid, base, free radicals (benzoyl or isobutyryl peroxide), epoxides, formaldehyde, and bifunctional agents (epichlorhydrin). The neutralization of the acid and basic catalysts may be achieved by adjustment of pH. The neutralization of free radicals may be achieved by using reducing agents. Common free radical initiators used in the synthesis of plastics are listed below:

Benzoyl peroxide
Acetyl peroxide
α,α-azodiisobutyronitrile
t-butyl hydroperoxide
Cumene hydroperoxide
Di-*t*-butyl peroxide
Azodicyclohexylcarbonitrile
Dimethyl-α,α-azodiisobutyrate
Succinyl peroxide
Dicumyl peroxide
Dichlorobenzoyl peroxide

The neutralization of benzoyl and isobutyryl peroxide are summarized in table II. Of the various reactants studied, glutathione has proven to be the most effective and the least toxic. The other reductants investigated included: sodium borohydrine, cysteine.

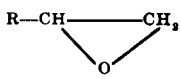
While all these reductants were effective in the adjustment of the redox potential, it was found that cysteine sulfoxide was formed as a reaction product. This compound was toxic to some

TABLE II. — *Neutralization of Benzoyl Peroxide and Isobutyryl Peroxide*

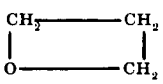
[After reduction of the peroxide, 10^3 spores of *B. subtilis* were added. After exposure for 2 hours at 25° C, the organisms were counted by plate dilution technique subsequent to incubation at 35° C for 24 hours]

Inhibitor	Percent of control counts (average of 5 plate counts)			
	Sodium borohydride 0.1 *M*	Cysteine 0.1 *M*	Glutathione 0.1 *M*	Water
Benzoyl peroxide, 5 percent	73	52	92	0
Isobutyryl peroxide, 5 percent	65	50	88	0
Control (H_2O)	100	100	100	100

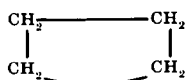
micro-organisms. In addition, cysteine reacts with S—S bonds within proteins. This amino acid can also react with transition state metals and thus interfere with trace metal metabolism of micro-organisms when this amino acid is used in moderate-to-high concentrations. Borate ion produces a low level of toxicity to micro-organisms. While this reductant is effective in the adjustment of redox potential when used in dilute solutions, its slight toxicity limits its usefulness.



OXIRANE



OXETANE



TETRAHYDROFURANE

Virtually all oxiranes (epoxides) and oxetanes (trimethylene oxides) as well as tetramethylene oxides may be used in the synthesis of plastic materials. Most of these compounds require the use of ionic catalysts (in most cases either anionic or cationic). Tetrahydrofurane will polymerize readily only when cationic catalysts are used. Antimony pentachloride is the preferred catalyst for the latter type of plastic. Each of the above compounds show evidence of various degrees of toxicity. The epoxides are the most toxic to both vegetative cells and spores. Tetrahydrofurane displays lesser toxicity.

The most important class of plastic materials utilized in potting compounds, electronic components, and incasement materials are the epoxy resins. A wide range of physical characteristics may be obtained with these substances depending upon the types of compounds utilized and the nature and the configuration of hardeners. A typical type of epoxy resin may be prepared by the

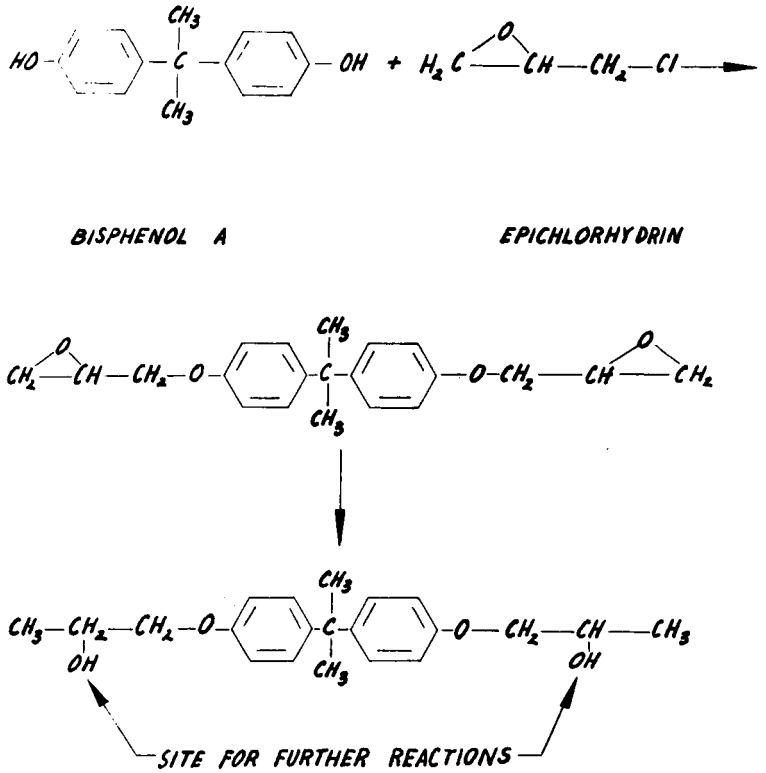


FIGURE 11.—Synthesis of epoxy plastics.

base-induced condensation of a polyhydroxy compound, usually a bisphenol utilizing, in most cases, epichlorhydrin which forms an intermediate of low molecular weight. This compound is essentially a linear polymer, containing terminal epoxide groups and pendant-hydroxyls (fig. 11). An excess of the epichlorhydrin in the reaction results in the termination of the polymeric chains with epoxy groups. These unreacted epoxide groups cause most of the toxicity associated with epoxy resins. The resins may be reacted to achieve maximum crosslinking by using curing or crosslinking agents, examples of which are: amines and carboxylic acids and their anhydrides. In nearly all the currently marketed epoxy resins, epichlorhydrin is used.

Among the various methods of neutralizing this toxicity which were developed in our laboratory are: (1) adjustment of pH to a moderately acidic concentration (pH 3 to 4) to open the epoxide ring, or (2) reacting the epoxide with a suitable compound to form a chemical derivative. Both of these approaches were investigated and the results are summarized in table III. The reaction of

TABLE III. — *Recovery of* 10^4 *Spores* B. subtilis, *Percent of Control*

[10^4 spores of *B. subtilis* were added to a final volume of 3 milliliters of epichlorohydrin, immediately treated as indicated and brought to final volume of 5 milliliters. Recovery of viable organisms was measured as percent of control unexposed to epichlorohydrin. The organisms were counted by plate dilution technique subsequent to incubation at 35° C for 24 hours]

Treatment	5 percent epichlorhydrin
5 milliliters H_2O	0
Adjust pH to 3, hold 2 hours, readjust to 7	42
Adjust pH to 4, hold 2 hours, readjust to 7	28
Adjust pH to 4, hold 15 minutes, presence of 5 percent histidine, readjust to 7	67
Adjust pH to 4, hold 15 minutes, readjust to 7	12

TABLE IV. — *The Effect of Epichlorohydrin on the Amino Acids of Casein*

[2 grams of casein were reacted with 100 milliliters of 0.5 percent epichlorohydrin at 20° C for 30 minutes at pH 7.0. Note that all the histidine was destroyed, while very little reaction occurred with other amino acids]

Amino acid	Amino acid composition	
	Before reaction	After reaction
Arginine	4.3	4.1
Histidine	2.2	0
Lysine	7.5	7.1
Tyrosine	6.9	6.3
Tryptophane	1.2	0
Phenylalanine	4.8	4.2
Cystine	.35	0
Methionine	3.4	3.1
Threonine	3.8	2.1
Serine	7.6	5.2
Leucine	9.8	8.8
Isoleucine	6.2	5.7
Valine	6.6	6.2
Glutamic acid	23.3	21.3
Aspartic acid	6.0	5.8
Glycine	.5	.2
Alanine	5.5	4.1
Proline	7.5	6.2
Hydroxyproline	.2	0

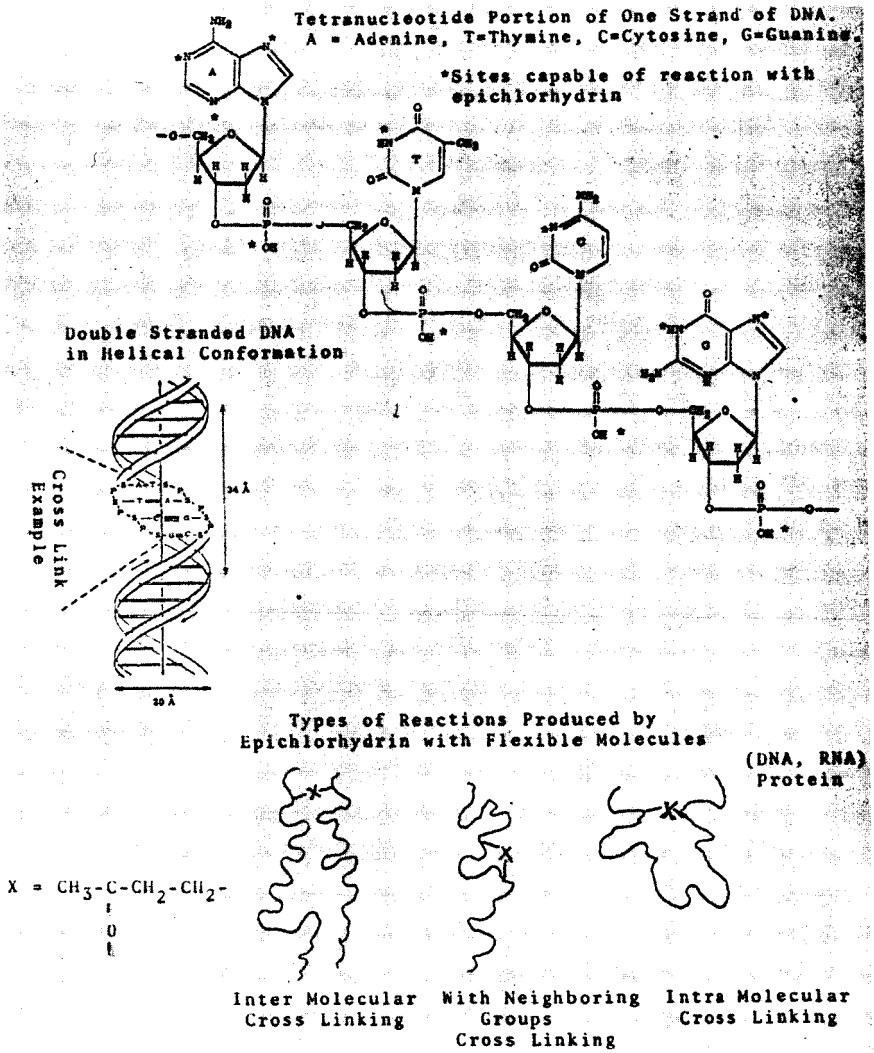


FIGURE 12.—Interaction of epichlorohydrin with nucleic acids and proteins.

epoxides with proteins is complex and thus requires some elaboration. It was discovered that this interaction at physiological pH's involved chiefly the amino acid histidine.

Epichlorohydrin was allowed to react with 2 percent casein hydrolysate for a period of 4 hours at 25° C. The amino acid which reacted most vigorously at pH 7.0 was histidine. The amino groups of some of the other amino acids were also reactive. The extent of reaction of the various amino acids in the mixture are summarized in table IV. Epoxides may react with nucleic acids. The halogen

portion of the molecule may also react; however, it is of less importance. Epichlorohydrin exerts its more profound biological effects on DNA by causing either intermolecular crosslinking, crosslinking with neighboring functional groups within the molecule, or with the nucleohistone present in the vicinity in the DNA or intermolecular crosslinking. The various forms of interaction are summarized in figure 12. Nucleic acids were active in the neutralization of epichlorohydrin.

Certain proteins contain large quantities of histidine. Examples of these are protamine and nucleohistone. The advantage of using such polymeric materials in the neutralization reside in the likelihood that such large molecules would not be easily assimilated into the microbial cell. Further studies are underway with other proteins known to be rich in histidine but whose availability and cost are more reasonable.

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Development of a Biological Indicator for Dry-Heat Sterilization

A microbiological sterility indicator system for use with dry heat is being developed. The indicator system will consist of a spore tablet, a sealed carrier or container, and a handling procedure designed to prevent inadvertent contamination of the space hardware being assayed. It will be capable of discerning, biologically, the attainment of the conditions considered necessary to sterilize space hardware. Sterilization of the indicator will not, in itself, prove that sterility of the lander capsule has been achieved; it will merely verify that the particular process being utilized was successfully applied. The process used to produce sterility must have been previously shown to be reliable and predictable as to the probability of attaining sterility.

One of the principal goals of unmanned exploration of the planets, such as Mars, is the search for life. The search for extraterrestrial life requires the use of life-detection apparatus which must be delivered by a lander capsule. In order to be valid, life-detection experiments on the planets must be conducted in the absence of Earth forms of life. Also, to prevent the transport of life forms from Earth to other planets, it follows that all life associated with the life-detection apparatus and the lander capsule must be destroyed before launch. This constraint obligates us to sterilize all space hardware associated with the capsule and life-detection apparatus.

The stringent sterility requirement imposed by the National Aeronautics and Space Administration has exposed the inadequacies of certain microbiological techniques; namely, recovery, culturing, and counting viable micro-organisms from external and internal surfaces of materials such as spacecraft hardware. The task of developing a sensitive, reliable method of assaying manufactured space hardware for microbial contamination is both monumental and complex because of the vast number of components which must be considered and the probable existence of an unknown number of unperceived and/or uncontrollable variables associated with each component.

Total microbiological assay of space hardware requires the reduction of all parts to a size which will permit all micro-organisms

associated with them to be released or exposed to the sterility test medium being employed. This method of assay leaves much to be desired; as an example, it may be possible, with improved techniques, to assay a single part for survival of micro-organisms of a particular class but it is not possible to assay all the parts for all classes of micro-organisms or even one part for all classes of micro-organisms. Furthermore, the parts assayed would be incapable of operation after the destructive process required for such assay.

By using any known methods of assay, the percent recovery of known organisms present in large numbers from selected materials under ideal, controlled conditions is extremely low. The percent recovery is, in fact, so low that it assures failure of any attempt to recover unknown organisms present in moderate numbers or less from materials with unknown biological properties and under conditions far from ideal. Microbiological assay methods which are sensitive enough to give usable data and are universally adaptable to all materials have not yet been developed. Until such assay is an accomplished fact it will be necessary to use a microbiological sterility indicator to prove the adequacy of the proposed dry-heat sterilization cycle — an indicator which is divorced from the problems associated with direct assaying. After the development of adequate assay methods it will still be advisable to utilize sterility indicators because of the ever-present possibility of a breakdown in the assaying methods or the existence of unknown variables which may influence the results.

Method of Approach

There is a time-temperature population-density relationship which influences any attempt to sterilize any material with dry heat. This relationship furnishes a tool which can be utilized in the development of a biological indicator and subsequently in the effort to certify the sterility of space hardware. Such an indicator must meet the following two criteria:

(1) The indicator organism must have maximum dry-heat resistance.
(2) The indicator system must have an organism population per unit volume greater than any possibly associated with space hardware.

Then, if all the organisms associated with a number of these indicators can be consistently killed by subjecting them to a specified set of conditions, it can be stated with statistical certainty that any space hardware which has been subjected to this same set of conditions has, in fact, been sterilized.

Sterility indicators are universally used whenever it is necessary to prove the adequacy of a sterilization cycle. Indicators are usually made by inoculating an inert substrate with a specific micro-organism which has been shown to be highly resistant to a particular sterilization cycle. The sterility testing of a few of these indicators can produce more meaningful information about a sterilization process than testing a much larger number of production articles.

The method to use is to place an indicator on, within, or wrapped with the article being sterilized. After completion of the sterilizing process, the indicator is removed and cultured. If the culture is negative the article is presumed to have been sterilized. Repeated testing of the sterilization cycle and consistently negative indicators increases the confidence level that the cycle is adequate. If a positive culture occurs, it may be due to process failure or a break in the technique of handling and culturing the indicator. In any event it merely calls for resterilization of the suspect articles which are held in quarantine until the results are known.

Sterility testing of space hardware, however, presents additional problems which are not encountered elsewhere. Three of the more obvious ones are:

(1) Up to the present time no attempt has been made to sterilize, and still leave functional, a piece of equipment as complex and heterogeneous as the lander capsule being considered for the Voyager mission. Because of the difficulties involved, the sampling and sterility assay methods needed have not yet been developed.

(2) Space hardware sterilization will be accomplished at a lower temperature and a longer time period than normally employed.

(3) Contamination of a culture would make the sterilization process suspect and would demand resterilization of the lander capsule, which is, to say the least, inadvisable.

The quarantine constraint has made it necessary for us to concentrate on the development of new methods of handling and culture — methods which are more stringent than any previously required in sterility testing. After retrieval of the indicator from the lander capsule and subsequent culturing we must prevent the inadvertent introduction of viable micro-organisms into the sterility test medium; that is, organisms which were not associated with the indicator or capsule after exposure to the sterilization cycle. This constraint will require the development and use of sterile transfer techniques carried out in a sterile atmosphere, an atmosphere in which the external surfaces of the carrier have been

sterilized prior to opening of the carrier and transfer of the test organism to the sterile culture medium.

When the task to develop a microbiological sterility indicator was initiated, the proposed dry-heat sterilization temperature was specified as 135°+40°–0° C and the time was specified as 24 hours after the materials being sterilized had reached equilibrium with the stated temperature. The possibility now exists that other exposure temperatures and times may be required as more information is gathered on the effects of time and temperature on space hardware. Information is also being gathered in an attempt to determine more accurately the number of organisms associated with space hardware. This information will have a bearing on the ultimate sterilization cycle and the ultimate indicator system.

Up to the present time, there has been no standardized sterility test system or organism which could be used with the proposed dry-heat procedures. Prior to the present study, which is still in progress, sterility indicators were aimed at determining sterility at higher temperatures for shorter periods of time in the case of dry heat and lower temperatures for shorter periods of time in the case of steam, but in all cases for only a fraction of the time required for space hardware sterilization. Also, sterilization in an atmosphere of dry nitrogen was not previously required. This constraint has required the reexamination of existing microbiological kill data. In addition, the necessity for maintenance of a nitrogen or other inert atmosphere required the development of new techniques of manufacture and a test system designed differently from any previously used.

It is well known that considerable variation in resistance of an organism can be demonstrated when different types of substrates are used. Thus, the development of a reliable biological sterility test system for dry-heat sterilization involves both a sufficiently dry-heat-resistant organism and an appropriate substrate.

Determination of space hardware sterility necessitates the use of a biological sterility indicator designed so that it can be subjected to the prescribed sterilization cycle in close proximity to the components while remaining divorced from the influence of variables associated with them.

In order to develop a satisfactory test system for determining the adequacy of the sterilization process, it is necessary to —

(1) Isolate an organsm which produces highly dry-heat-resistant spores.
(2) Develop a suitable substrate and carrier.
(3) Determine the organism's specific dry-heat resistance, both with and without the carrier.

(4) Formulate a system, operating procedure, and functional method for practical usage.

(5) Conduct performance tests to demonstrate the adequacy of the system and the basic sterilization cycle. Such testing will include, but not necessarily be limited to, the accumulation and recording of survival-kill data.

In addition, the test system must —

(1) Show organism survival for 18 to 20 hours and no survival after approximately 23 hours of exposure at 135° C. The time and temperature are subject to change, as previously noted.

(2) Provide stability during a storage period of up to 30 days at room temperature.

(3) Provide simplicity of operation and function and be sufficiently small to allow free and easy placement and retrieval.

(4) Be capable of variations to meet dry-heat sterilization specifications of other time/temperature relationships.

The test organism shall be —

(1) Nonpathogenic.

(2) Cultured in a simple manner from the carrier by use of readily available media. The organism, plus the substrate, must be compatible with the culture medium.

(3) Easily discernible as to growth and readily identifiable.

The carrier and substrate shall be nontoxic to both the user and the test organism, both in the dry state and when inoculated into medium.

To date, the following objectives have been accomplished:

(1) Isolation of an organism resistant to dry heat. Several species of organisms resistant to dry heat were examined as potential indicator organisms. Spores of *Bacillus subtilis* WC 18, one of the most dry-heat-resistant organisms found as a result of this study, were evaluated for use as the indicator organism, but because of inconsistent survival patterns, this organism will be replaced by *B. subtilis* var. *niger* which had been chosen as an alternate organism.

(2) Determination of a proper carrier. A hermetically sealed, easily manipulated Teflon carrier has been designed which will contain a tablet composed of the test organism plus a suitable substrate of pure, freeze-dried, spore powder in an atmosphere of dry nitrogen under positive pressure. The Teflon carrier is sealed in Aclar film, which also contains dry nitrogen under pressure. (See figs. 1 to

FIGURE 1.—Spore tablets compressed at 30 tons per square inch.

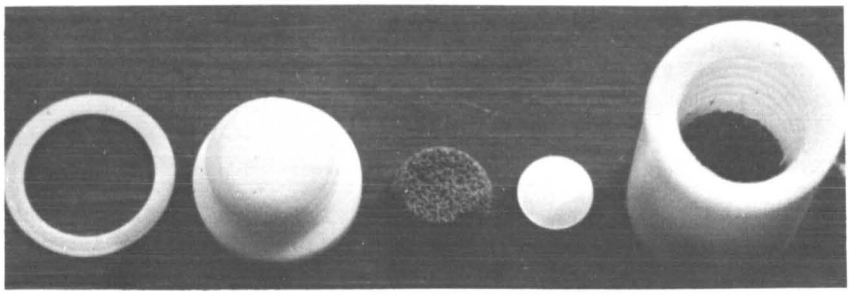

FIGURE 2.—Disassembled carrier for spore tablet.

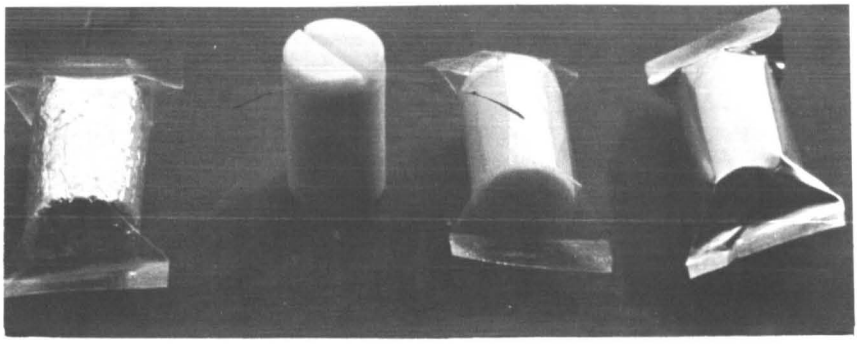

FIGURE 3.—Assembled carriers sealed in plastic films.

3.) This acts as an additional barrier to prevent the escape of nitrogen and an indicator of pressure loss by virtue of collapse. To achieve this, all assembly operations are carried out in a flexible film isolator under a slight positive pressure of dry nitrogen. A variety of thermostable films, metalized films, and tubing have been evaluated for indicator applications.

(3) Determination of the organism's specific resistance with and without the carrier.

(4) Formulation of a system, operating procedure, and functional method for practical usage.

All operations concerning culturing, harvesting, and tableting spores of *B. subtilis* WC 18, assembly of the indicator, and viability determination after thermal treatment were standardized to promote reliability in application of the indicator. This procedure will also be applied to *B. subtilis* var. *niger*, or any other organism under investigation. Biological indicators for dry-heat sterilization are presently being evaluated by determining whether viable spores can be detected on culture after appropriate exposure at 135° C. Currently, the Teflon spore tablet carrier indicator is being critically evaluated in tests at 135° C to determine the adequacy of the system.

Spore tablets are produced in the following manner. Successive spore crops are produced in Roux bottle cultures of tomato juice broth supplemented with 2 percent agar. After harvesting and successive washing and centrifuging at low temperature, the cleaned spores are sharp frozen in a dry ice and Freon 11 bath and then vacuum freeze dried. At this point the spore powder may be mixed with an appropriate substrate or may be compressed directly into tablets at a pressure of 30 tons per square inch. After being formed, the tablets are further dried under high vacuum at low heat before being sealed under dry nitrogen in the carriers. At this time, it appears that a pure spore powder tablet may be required in order to achieve proper thermal resistance. The thermal resistance offered by any suitable substrate tested to date is far outweighed by the dilution factor introduced by the substrate. The desired thermal resistance within the carrier has been obtained with a pure spore powder tablet one-quarter inch in diameter containing 1×10^{10} to 1×10^{11} spores per tablet.

Concluding Remarks

It must be emphasized that no microbiological assaying of the flight hardware as planned after terminal sterilization, which will occur immediately prior to launch. If retrieval of indicators were

attempted, the landing capsule would be open to microbial contamination from outside sources during the retrieval process. The method or process used to attain sterility of the capsule must be proved before terminal sterilization is attempted prior to launch. The actual sterility testing and development of the process to be used will be done on proof test models of the flight capsule and then the proven process will be applied to the flight units.

Session II—Discussion

Stumbo, *University of Massachusetts*: I would like to comment on Mr. Vesley's presentation. It seems to me that the number of spores, rather than the total count, is most important from the standpoint of arriving at a basis of sterilization. The method he devised does not give an estimation of the number of spores present. Also, in devising a technique, I think a certain amount of heat will have to be applied in order to activate the germinating spore to obtain an accurate count. It has been the experience, particularly with resting spores, that one can increase the count 10 to 100 times by applying a sublethal amount of heat. I may have misunderstood Dr. Irons, but I believe he said that the spore tablets should contain more spores per unit volume than the spacecraft. It seems to me that the spore tablet should contain more spores than the entire space unit contains, if you are going to get the same degree of sterilization in the two units.

Vesley: The purpose of the heat shock was to destroy the organisms which were not in a sporeforming state. We do get some indication of the resistant organism in this way. I am not the one who developed this method; it is a consensus method arrived at by many groups.

McDade: In our experience, the percentage of the total population that is recoverable on the stainless-steel strips has been 10 to 20 percent. I think that Dr. Favero finds recovery in the neighborhood of 25 percent. On the basis of our observations we find that the stainless-steel strips have been sensitive indicators as to what is occurring.

Irons: He referred to the degree of sterility and we know it is absolute. If 1×10^{4} organisms are confined to a quarter-inch area and are destroyed, then an equal number of organisms in the same area of a spacecraft is also destroyed. I am not speaking of 1×10^{10} organisms in the total spacecraft. If 100 indicators are placed in the spacecraft, I then have 10^{12} organisms, but they are killed in the same time as 10^{10} organisms.

Bruch, *NASA*: I disagree. Depending on the kill kinetics, what you have worked on before and the kill curve that you are working against, there is a probability associated with those 100 tablets that you have put into the cycle.

Irons: I am saying that each tablet acts as an entity.

Bruch: Yes; but it is the total load of organisms that is going into

the sterilizer which will determine the probability of whether you have a survivor.

Schalkowsky, *Exotech, Inc.*: I would like to pose this same question in another context. Namely, what is the real meaning of this sterility indicator? All the questions we are raising here relate to validating our concepts of the kill mechanisms of micro-organisms. But the purpose of this indicator is to validate the sterility of the spacecraft. Now, suppose we consider the possibility, having applied the required heat cycle, that the indicator shows positive. The indicator signifies that the spacecraft is not sterile. But when one says this, what is he telling the spacecraft designers to do about it? What is happening, in effect, is continuous testing for the possibility that there might be a living micro-organism even after application of this cycle. All these tests can be done right now; we do not have to wait until we actually do the terminal sterilization. The basic question, therefore, relates to the need of a sterility indicator. Or are we really talking about another subject and just calling it an indicator?

Irons: First, we must realize that this indicator is being tailored to a particular cycle, to determine whether the requirements have been met. One does not subject just any organism to this cycle and obtain results indicating we obtained 135° C for 22 hours. Analysis of the *D*-values has established a time and temperature curve. We are trying to prove that this condition has been met in the spacecraft. We are building a better thermocouple. A thermocouple indicates temperature, not biological conditions. It is possible for a thermocouple to indicate sterility falsely.

Hall: Further consideration might suggest that the indicators be exposed to the same heat source used for terminal sterilization of the spacecraft, although isolated from the spacecraft by a thin metal wall. This would also require a thermocouple or similar instrument. Further studies certainly need to be undertaken.

Davis, *Wilmot Castle*: Is there evidence showing that a substantial number of germinating spores are killed during the 80° C/15 minute cycle and, therefore, you are not measuring the true total picture of spores?

Vesley: Theoretically, at least, this level of heat should not be sufficient to both germinate and kill them. If they do germinate at this time, we hope that they will survive to be measured. Whether this is entirely true, I am not sure, and it may be possible that there are some spores which do germinate and are then subsequently killed. There is room for improvement here. Perhaps we can directly heat-shock the strips themselves without the peptone water. I do not think this is the final answer, but right now we have

reasonable confidence in this method. There have been some studies wherein the organisms were recovered and identified. The percentage of spores which survive this treatment seems to be fairly consistent. The method is not perfect, but right now it is the best thing we have.

Silverman, *MIT*: I did not understand whether an aliquot was going to be placed into a heated solution at 80° or whether the solution would be heated to 80° C. There is a calculated risk involved in heating up to 80°, but 80° in some cases can be high. I think it was decided by the panel previously that it is a question of inactivation versus activation. In any case, a little bit more study is called for.

McDade: Douglas performed a clean-room study for us. We had them expose at least 30 strips on different occasions for periods up to a month. After this exposure period, we divided the strips into 3 lots of 10 each and heat shocked each set: one in peptone water, one in distilled water, and one in trypticase soy agar. We did not find any difference in the recovery of the heat-treated organisms. We recovered approximately the same level of spores from these preparations in repeated experiments.

Silverman: I am not an engineer, but I have some feeling for heat transfer. I was wondering what the reasons were for putting the tablet in a Teflon tube, which is a good insulator, and also placing a sponge-rubber cover, which is also a good insulator, over that.

McDade: This very good insulator that you speak of takes 10 minutes to heat up. The heat is transferred through it and to the tablet in 10 minutes.

Silverman: At what temperature?

McDade: At 135° C. If we place the Teflon tube in the heat sink at 135° C, 10 minutes later the tablet is receiving the 135° temperature.

Wald, *Aeronutronic*: Dr. McDade, what was the flow rate in the clean air room? Mr. Riemensnider, I was wondering how you arrived at the decision to use this tank rather than some kind of flow system and whether the restriction of movement by this tank would not affect your recovery considerably?

McDade: At JPL the horizontal laminar flow rate was 110 feet per minute and the vertical laminar flow rate was 85 feet per minute.

Riemensnider: The tank was designed to be a static rather than a dynamic chamber. We could recover our organisms in a wash fluid instead of by air sampling. Also, semiquantitative work has been done in a dynamic chamber which does not give total recovery. It will sample 1 cubic foot of air per minute and the person can be kept in the chamber for an hour. The total volume in

our tank is 50 cubic feet and we can recover all the organisms. When using a dynamic chamber, one is constantly replacing air and losing organisms.

Slote, *Grumman Aircraft Corp.*: Concerning the three concurrent studies on the stainless-steel coupon, was the surface finish standardized on all three or did you just neglect that?

McDade: No; the surface finish was standardized. The University of Minnesota started it first. The surface finish used at JPL and Phoenix was based on a specimen of the Minnesota strip.

Slote: The second question I have concerns the meaning of volumetric sampling versus gravimetric sampling. What does the comparison mean when the mechanisms involved are so complex, especially gravimetric sampling? You are working with a micrometeorological phenomenon. The sampling techniques vary according to the thermal gradients and wind movements, and if one does not specify, people will be doing just gravimetric-type samplings using any type of coupon with a variety of results. In regard to volumetric sampling, I can see why one would be interested in air contaminants from the standpoint of respiratory dosages. But we are dealing with a static vehicle. What real meaning has this volumetric figure?

McDade: As far as volumetric sampling is concerned, we use the air as an indicator of particles in suspension at a given instant and particles that might settle out onto the hardware. In addition, the sampling is an indicator of resuspended surface contamination. As I tried to indicate, volumetric air sampling might be good in conventional clean rooms. The method must be updated if we are going to use it in laminar flow rooms. As for gravimetric sampling, we are measuring viable particles. The sample obtained can have from 1 to 1000 or more viable organisms in it and still yield a single colony. Using the gravimetric method on the stainless-steel strips, one fragments these clumps. The stainless-steel coupons show what is settling onto the hardware. That is why I attempted to show the results with the sampling surface of the Mariner proof test model as well as the tray of stainless-steel strips which was placed in close proximity to a test-model spacecraft. The results indicated that the surface contamination was about the same when compared to gravimetric sampling.

Slote: Another point concerns whether this gravimetric sampling of the coupon is an average or mean number. You are sampling this area, and automatically assuming a homogeneous distribution and then extrapolating these figures to any size vehicle.

Opfell, *Aeronutronic*: What improvement did you find when using

the saw instead of the drill or ball mill in the percent recovery of a prescribed inoculum?

McNall: We found in comparing the saw with the ball mill on rigid-type plastics that we could achieve almost a tenfold increase in effective recovery. The ball mill tends to produce a population of pulverized particles which are extremely large. As fragmentation takes place, there is a variety of fragment sizes. As pulverization leads to smaller and smaller fragments, destruction of organisms results because many of these are below 1 micron in size. The average population may be somewhere in the vicinity of 8 or 10 microns. As you progress, you reach the point where there is an average of 5 microns with a large amount of very fine material within which the organisms are destroyed.

Fisher, *NASA, Marshall:* Dr. Ulrich, what are your subjects doing with their hands during those 170 minutes after scrub time and where do you think those organisms might come from during those 170 minutes? Dr. McDade, you mentioned that there was a difference between your groups handling the little metal tags with the bare hand and those using finger cots. Since there were different people in both groups, you did not tell me anything about the *within*-group variation as opposed to the *between*-group variation. How can you make such a decision?

Ulrich: Concerning the change in the skin condition, hands were encased in sterile surgical gloves immediately after the wash had taken place. The organisms were held in during this period of time.

McDade: I cannot say with a sample size of five that there was a significant difference. It was an intuitive figure derived from the data in the table.

Session III

Microbiological Decontamination and Sterilization

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Survey of Certain Nonthermal Methods of Decontamination and Sterilization

Although thermal inactivation has been a main method for destroying micro-organisms and decontaminating materials, there are many other methods available for achieving this objective. Certain of these, such as gaseous sterilants and filtration, will be discussed in this symposium in detail, but other agents, such as germicides, antibiotics, and ultrasonics, may also be used for specialized and limited applications during spacecraft assembly. This paper will deal mainly with a more recent sterilization technique which has received a great deal of attention within the past 20 years — that of ionizing radiations. Ultraviolet light and two newer methods, the use of lasers and of radio-frequency and microwave energy, will also be considered briefly.

Radiations

The ability of radiations to decrease or eliminate the microbial flora contaminating materials was recognized as early as 1898 in studies employing X-rays. In fact, even before World War II, many of the uses of irradiation that are envisioned today — the destruction of micro-organisms, the destruction of insects and fungi that infect grains and fruits, the preservation of foodstuffs — were postulated. Since World War II, when large quantities of cobalt 60 and other radioisotopes and high-energy electron sources became available, research in the use of radiation for biological applications has increased enormously (refs. 1 and 2). The early promise that radiation would destroy micro-organisms has been verified and elaborated upon in an extremely large number of publications. This presentation makes no attempt either to review the literature or to cover the main subject, that of microbial inactivation by radiation exhaustively, but it will, hopefully, be a guide to the feasibility and limitations inherent in the use of this lethal energy. The subject matter is restricted to those factors which appear to be most applicable to the sterilization of materials and which may be of importance in spacecraft sterilization. The types of radiations of interest for inactivation of micro-organisms are classified into particle radiations and electromagnetic radiations. From a practical consideration, one may limit these to fast electrons, X-rays, gamma rays, and ultraviolet light.

Particle Radiations

Although particle radiations include a wide variety of particles such as alpha particles, beta particles, neutrons, mesons, positrons, and neutrinos, the only particle which lends itself to general applicability is the beta particle or electron. Beta particles are electrons arising from radioactive materials, but electrons can also be produced with high energies from machine sources. Penetration of an electron with a unit charge is finite in matter and depends upon the material being irradiated and upon the electron energy. Most of the machines used produce electrons of 2 to 5 MeV. Some models can produce electrons with energies in excess of 5 MeV, but these may induce radioactivity in irradiated materials. To date, the one disadvantage in using electrons for sterilization is their rather limited penetrating power.

Electromagnetic Radiations

Electromagnetic radiations arise either from radioactive sources, such as cobalt 60, and are known as gamma radiations, or by the bombardment of a heavy target, such as gold or tungsten, with fast electrons and are known as X-radiations. Cobalt 60 gives photons with two main energy levels, 1.17 MeV and 1.33 MeV. X-rays can be produced over a wide range of energies. Soft X-rays have energies in the neighborhood of 0.1 MeV; those with greater energies have greater penetrating power and are known as hard X-rays.

Another type of electromagnetic radiation is ultraviolet radiation. Ultraviolet rays have been used for a number of years to decrease the bacterial load in thin films, on surfaces, and in aerosols. The maximum bactericidal effects of ultraviolet light are in a narrow region of around 2650 angstroms for bacteria, fungi, and viruses, although certain viruses are inactivated maximally at 2350 angstroms. These wavelengths correspond closely to the peak wavelength of 2537 angstroms of a mercury vapor germicidal lamp, and the lethal effect is attributed to the maximal absorption at 2600 to 2650 angstroms by deoxyribonucleic acids. The difficulty with ultraviolet radiation as a sterilizing agent is that it has a very low penetrating power and micro-organisms are easily shielded from it by soil or other materials through which it cannot penetrate. Although it has poor reliability as a sterilization agent, ultraviolet radiation can, by proper application, be utilized to decrease the microbial load on a surface. With modern lamps this decrease can be accomplished in a very short time. Its lack of penetration may, in certain instances, be an advantage, since many materials can be altered by ultraviolet light. It has been shown that

ultraviolet and gamma radiations are synergistic to some organisms.

Units of Radiation

The units commonly employed in radiation are derived from the curie, which is equivalent to 3.7×10^{10} nuclear disintegrations per second. The energy imparted by electrons, X-rays, and gamma rays results in ionizations within the absorbed material. The electron passing close to an atom will be a free electron and a positively charged ion or an ion pair. Photons of X- or gamma radiation will cause the production of ions indirectly by photoelectric effect, Compton effect, or pair production. The energy required to produce an ion pair in tissues is in the neighborhood of 30 eV. One erg equals 6.24×10^{11} eV, and a unit commonly used is the rad, which equals 100 ergs/g. In terms of more familiar units, 1 calorie equals about 4.185×10^7 ergs and therefore the actual temperature rise, even for materials subjected to appreciable doses of radiation, is slight. In fact, radiation has often been called "cold sterilization."

Biological Effects

Ionizing irradiation produces an intensively localized series of disturbances along its track. These disturbances are so local in nature that other portions of a sample being irradiated will not be affected over a given increment of time. Ionizing irradiations can produce free radicals and excitations (fig. 1) in addition to ion pairs. It should also be noted that linear energy transfer (LET),

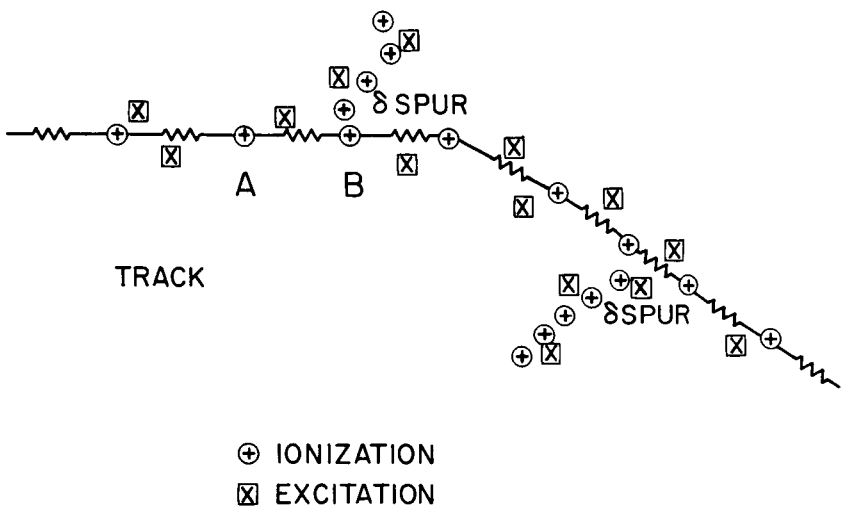

FIGURE 1.—Diagrammatic depiction of events along an ionizing track.

which is a measure of the density of events along a track and is expressed in keV absorbed per micron of track, increases with the square of the charge and decreases as the speed increases.

Ionizing radiation is essentially a random process; an organism on or within a material will be inactivated only if sufficient ionizing tracks actually hit it or come within an effective vicinity (measured in angstroms) to it. Moreover, micro-organisms differ in their resistivity to radiations (table I). When one speaks of sterilization one normally implies the total destruction of microbial spores, but this may not necessarily be the only consideration. There are a number of vegetative species, such as *Micrococcus radiodurans* and a red-pigmented cocci (Nos. 248 and 249 in table I) isolated in the author's laboratory and distinct from *M. radiodurans*, which are equally as resistant as microbial spores. To derive some idea of the radiation dosages under consideration, one can note that vegetative cells have *D*-values (the number of rad necessary to reduce a population 1 log cycle) of less than 100 000 rad and usually of less than 50 000 rad, whereas bacterial spores may have *D*-values of as much as, or more than, four times this amount. The yeast and fungi are intermediate in resistivity. Moreover, by being discrete in nature, ionizing radiations (unlike, for example, heat) may be less effective against clumps of organisms concentrated in a single locality. That is, a finite dose of radiation would be less effective against clumps of organisms than it would be if the organisms were distributed uniformly. In essence, it is easy to waste a portion of the ionizing dosage in this manner.

Even with the assumption that all the above factors are compensated for by increasing the dosage required, there are still a number of additional factors of importance in radiation sterilization. It is assumed that, if the *D*-value is multiplied by the desired number of log cycles (i.e., the number by which one wishes to decrease the microbial population), sterilization will result. This implies an exponential type of destruction or a linear function between the log numbers of micro-organisms and the dose in rads. This is not always the case and a variety of nonlinear curves can be produced when organisms are irradiated in buffers. The most significant of these are those spores which have shoulders on the inactivation curves before an exponential relationship between population and dosage is established. For this type of curve, an additional dosage is required. However, this is not the only difficulty encountered, since the estimation of low numbers of survivors is one of the more difficult aspects of sterilization.

Recent work on *Clostridium botulinum* illustrates the difficulties

TABLE I.—*Radiation Resistivities of Biological Systems of Interest*

Species	D^{10}-values, Mrad	Presence of a "shoulder," Mrad	Irradiation menstruum	Reference
Anaerobic sporeformers				
Clostridium botulinum:				
Type A NCTC 7272	0.12	0.90–1.00	Water	3
Type B 213	.11	.90–1.00	Water	3
Type D	.22	.25– .35	Water	3
Type E Beluga	.08	.25– .35	Water	3
Type F	.25	.25– .35	Water	3
Clostridium sporogenes: PA 3679/S_2	.22	.25– .35	Water	3
Clostridium welchii: Type A	.12	.25– .35	Water	3
Clostridium tetani	.24	.25– .35	Water	3
Aerobic sporeformers				
Bacillus brevis	0.05	.....	Buffer	4
Bacillus subtilis	.06	.....	Saline + 5% gelatin	5
Vegetative bacteria				
Salmonella typhimurium	0.02	.....	PO_4 buffer	4
Escherichia coli	.009	.....	PO_4 buffer	4
Pseudomonas species	.003–0.006	.....	PO_4 buffer	4
Flavobacterium species	.12	.....	PO_4 buffer	4
Lactobacillus brevis NCDO 110	.02	0.02–0.05	PO_4 buffer	6
Staphylococcus aureus	.28	.....	PO_4 buffer	7
Streptococcus faecium	.003–0.006	.....	Dry state	8

TABLE I.—*Radiation Resistivities of Biological Systems of Interest*—Concluded

Species	D^{10}-values, Mrad	Presence of a "shoulder," Mrad	Irradiation menstruum	Reference
Vegetative bacteria — Continued				
Micrococcus radiodurans R.	.22	1.0	PO_4 buffer	9
Micrococci (Smooth Strain 248)	.52	[a]4.0	Water	10
Micrococci (Rough Strain 249)	.41	[a]3.0	Water	10
Yeast				
Saccharomyces cerevisiae	0.05		Saline + 0.5% gelatin	5
Torulopsis candida	.04		Saline + 0.5% gelatin	5
Molds				
Aspergillus niger	0.047		Saline + 0.5% gelatin	5
Penicillium notatum	.02		Saline + 0.5% gelatin	5
Viruses				
Foot-mouth virus	1.3		Frozen at −60° C	11
Vaccinia	.17		In vacuo	11
Process requirements for complete destruction				
Trichina inactivation	0.02–00.05			12
Enzyme inactivation	2.00–10.00			12
Insect deinfestation	.10–00.50			12

[a] No shoulder; dose required for 10^{-4} inactivation.

of insuring sterilization (refs. 3 and 13). After the initial shoulder, death is usually exponential and the dose required will depend upon the initial number of organisms present. Obviously, the higher the initial number of spores, the larger the dose required to destroy them. But, in addition, it was found in certain studies that a small number of *Clostridium botulinum* spores were not destroyed by extremely high radiation dosages (ref. 14). At present this tailing effect is not well understood or verified and appears to require further consideration. It had been the assumption for years past that thermal death is exponential; however, recent work has cast doubt on the universality of this assumption and certainly it has not been shown to be the case for the inactivation of a number of species of micro-organisms during irradiation.

There are many additional factors which must be considered in the sterilization of a material by ionizing radiations. These are:

(1) *The dose rate.* — The data to date are inconclusive as to this variable, but it appears that this may be a minor consideration because the usual dose rates under consideration are sufficient for sterilization.

(2) *The presence of oxygen.* — Organisms are more susceptible to radiation when oxygen is present. For vegetative cells the dose modifying effect varies from 2 to 3 and for spores it is probably 2.

(3) *Presence of organic materials.* — Most of the data existing in the literature for radiation resistivity are for micro-organisms irradiated in buffers. In the presence of organic compounds, resistivity is generally higher and the oxygen effect is minimized or eliminated. In a large part this is due to the neutralization of ions and free radicals by the organic material before they can injure the micro-organisms.

(4) *Protective compounds.* — There are a variety of organic compounds such as aliphatic alcohols, glycerol, and sulfhydryl-containing compounds which can protect an organism against radiation (ref. 15). Not much is known of the effect on radiosensitivity when organisms are embedded on or in polymeric or ceramic materials.

(5) *Physiological state.* — Most studies on micro-organisms are conducted on organisms which are in the spore state or on vegetative cells harvested in the stationary phase. Natural contaminants will be in a variety of physiological states. This can be important, since vegetative cells are least resistant in the logarithmic phase and spores are less resistant after germination (fig. 2). The radiation resistivity of spores may vary with both the sporulation media and the environment into which they are placed after sporulation.

(6) *Water content.* — It might be expected that, for spores, the

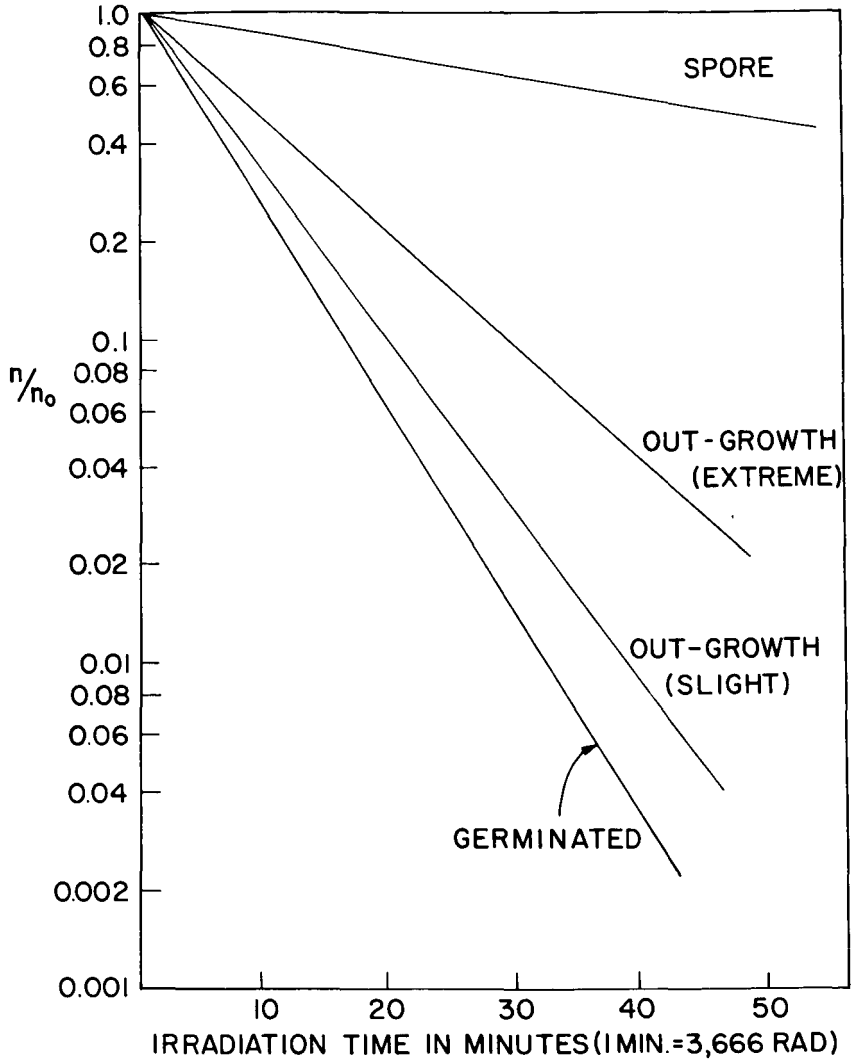

FIGURE 2.—Changes in radiation resistivity with spore germination and out-outgrowth (*Bacillus subtilis* var. *niger*).

absence of water would, by eliminating indirect effects, cause an increase in radiation resistivity. This does not appear to be the case for either spores or vegetative cells (refs. 16 to 19). As seen in figure 3, with gamma radiation spores appear to be more sensitive to radiation when dry, and this is also true for exposure to ultraviolet light (table II). In addition, there is an oxygen effect. It has recently been shown that the radiation sensitivity of spores equilibrated at different moisture levels depends upon whether

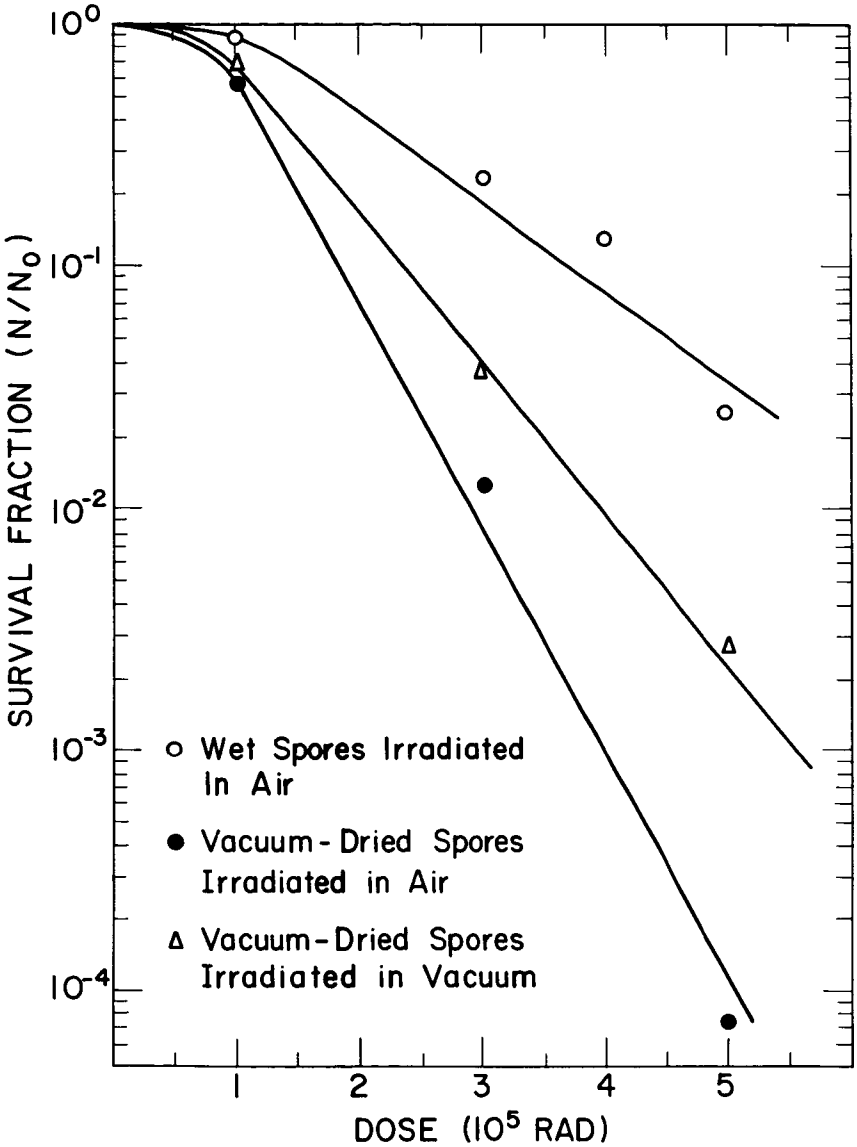

FIGURE 3.—Resistivity of *Bacillus megaterium* spores to gamma radiation. Spores were dried in an ultrahigh vacuum for 5 days prior to being irradiated.

oxygen is present or absent during and after irradiation. The presence of oxygen during rehydration caused increased damage to spores of *Bacillus megaterium.*

(7) *Temperature during irradiation.* — For fully hydrated spores or vegetative cells, extremely low temperatures will protect

TABLE II.—*Percent Survival of Spores of 4 Organisms After Ultraviolet Irradiation in Ultrahigh Vacuum and at Atmospheric Pressure*

[Unpublished data of N. S. Davis and G. J. Silverman]

Organism	Dose, MW/sec/cm²	Survival, percent, in [a] —			
		UVI(100)/DCI	UVF(100)/DCF	VUV(100)/V	DCI(100)/DCF
B. megaterium	500	81	94	1.3	100
	750	83	100	.06	117
	1,000	42	58	.009	109
	10,000	.33	.49	.002	104
B. subtilis var. *niger*	500	55	42	5.0	100
	750	19	15	.39	83
	1,000	6.5	9.5	1.2	105
	2,500	.33	.31	.04	94
	10,000	.03	.02	.001	1.23
B. stearothermophilis	500	38	33	1.0	104
	750	14	15	.05	82
	1,000	1.0	5.9	.23	97
	2,500	.17	.26	.03	90
	5,000	.08	.08	.02	76
A. niger	1,000	.....	83.0	83.0	.....

[a] V, vacuum; UV, ultraviolet; DC, desiccator control; I, initial; F, final.

the cells and elevated temperatures will generally be synergistically lethal when coupled with irradiation. For dried cells, a portion of the synergistic effect of temperature appears to be nullified by a tempering effect which neutralizes the lethal effects of stabilized free radicals and excited molecules upon exposure to an elevated temperature of around 80° C.

It is therefore apparent that the use of ionizing radiations to achieve sterility of a surface may present many problems, especially if the nature and numbers of contaminants are not known. However, the problems are not insurmountable and radiation has had a number of successful applications. In various countries, biologicals such as vaccines and tissues, hospital supplies, foods, pharmaceuticals, and plastics are being sterilized by ionizing irradiations either on a pilot or a commercial scale. From this discussion, it might also be suggested that a limited and supplemental use of ionizing radiations in a sterilization program whose main lethal agent is heat may be beneficial. The temperature and duration of the heat cycle required for sterilization will depend on the initial number of contaminants. Moderate amounts of radiation can be expected to decrease the microbial load on a surface. In addition, radiation appears to sensitize spores to subsequent thermal exposure; therefore, the time that a component has to be heated can be shortened. It has been proposed that components be exposed to gaseous radioactive compounds which will then sterilize the surface. This procedure has the advantage that the gas can penetrate microscopic fissures and, if the isotope is correctly chosen as to energy of the particle emitted, the component will not be damaged. The gaseous isotope can be recovered by a vacuum operation and reused. It is obvious that the dosages employed must be below that capable of damaging a component. At present the tolerances of a wide variety of components to radiation have not been ascertained.

Microwave and Dielectric Heating

Microwave and dielectric heating, which is essentially short radio waves, converts electromagnetic energy into heat (ref. 20). If conditions are optimal, heat arises internally because poorly conducting materials, when placed in a high-frequency alternating field, tend to aline themselves with the electrical field. The molecular dipoles, such as water, in the rapidly alternating field will, by friction, develop heat. The two processes, dielectric heating and microwave heating, are similar in principle and operate on given frequencies controlled by the Federal Communications Commission. These are:

Source	Frequency, Mc/sec	Power, kW
Dielectric heaters	13.56 ± 6.78 Kc 27.12 ± 160 Kc 40.68 ± 20 Kc	Up to 500
Magnetrons and klystrons	915 ± 25 Mc 2 450 ± 50 Mc 5 850 ± 75 Mc 18 000 ± 100 Mc	0.08 to 5.0

Microwave heating has inherent advantages over dielectric heating. The main advantage is in power generation, where $P = 2\pi f \times E^2 e'' \times 0.0885 \times 10^{-12}$ when P is power in W/cm^2; f is frequency in cps; E is the rms field strength in V/cm; and e'' is the loss factor of the dielectric.

The most efficient way to increase the heating rate is to increase the frequency; hence microwave heating appears to be most promising. In this range of radiofrequencies, energy obeys the laws of optics in that it is reflected, transmitted, and absorbed. For this reason limitations are inherent in the use of this technique. Metals reflect this energy; glass, ceramics, paper, and plastics transmit it. A micro-organism on or within a component may or may not be affected by this energy, since it may be protected by a metal or it may be exposed if embedded in or lying on a transparent ceramic or plastic material. It is necessary that the organisms have sufficient water to respond to the rapidly alternating electromagnetic field. Microwaves have been proposed for use in the pasteurization of dairy products and in the sterilization of soil and of nonmetallic medical equipment. The shortcomings which exist in this method can be serious in samples of varying thickness. There is a nonuniformity of heating caused by the nonuniformity of thickness. In the above discussion only the lethal effect of heat produced by microwave heating has been considered. There is evidence in the literature that radiofrequency waves may, in addition to existing thermal effects, influence biological systems by demodulation effects of pulsed signals and by molecular alterations. It has also been speculated that there may be an interaction between magnetic fields and radio waves on biological materials. As mentioned earlier, microbial contaminants on hardware in space vehicles may be in a dried or partially dried state; certainly microbial spores have a low water content. The support material, if nonmetal, would have a lower moisture content. If it is also transparent to microwaves, it will be less affected than the micro-

organisms by the thermal and as the yet unproven biological effects of microwaves.

Lasers

The use of laser technique for destroying micro-organisms on surfaces is a distinct possibility, but as yet no extensive publications have appeared as to its bactericidal effect. A variety of lasers have been developed that will deliver coherent radiations at a variety of wavelengths. Laser light will also obey the laws of optics. This author's rather limited experience to date with lasers indicates that laser beams originating from a ruby crystal and for use upon spores embedded on or near the surface of membrane filters is more efficient against moist spores, that localized splattering occurs, and that the main lethal activity appears to involve thermal destruction. The ability of the support material to absorb energy appears to be necessary. This field is still in its infancy and as more sophisticated lasers are developed and data are obtained on the effects of wavelengths and energy imparted to a biological entity, its potential will be more realistically evaluated. At present one disadvantage lies in the fact that the coherent beam is of a rather small diameter. However, the cost of a laser apparatus is not excessive, especially when compared to that of an electron accelerator. It is conceivable that a battery of lasers could be employed or that technology will develop wide, efficient laser beams.

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Dry-Heat Sterilization for Planetary-Impacting Spacecraft

The dry-heat cycle of 135° C for 22 hours currently being investigated for the sterilization of planetary spacecraft is described herein. A historical approach of how that particular cycle was derived is presented. After an analysis of some of the environmental factors that influence dry-heat sterilization, various mathematical considerations of the available data are examined. By use of activation energy calculations, it is shown that some components might suffer less damage with high-temperature short-time cycles. It is also necessary to consider the integration of the lethality that occurs during the comeup time and cooldown time of the heating process with the lethality that occurs at the desired holding temperature.

The only experience of the National Aeronautics and Space Administration (NASA) to date in the sterilization of launched hardware has been with Rangers 3, 4, and 5. After these Ranger capsules failed in their mission objectives, the sterilization requirement was removed from lunar spacecraft in early 1963; the new policy stated that lunar probes would be decontaminated to the best practical extent. The sterilization requirement remained for all planetary-impacting spacecraft (ref. 1).

NASA policy has fixed no definite time-temperature cycle for spacecraft sterilization, but development efforts have centered around a terminal dry-heat sterilization cycle of 135° C (275° F) for 22 hours. In order to be certain that the spacecraft will tolerate this particular environment, NASA has set a qualification requirement of three cycles at 145° C (293° F) for 36 hours each which all planetary flight hardware must meet. This qualification test is not a biological requirement but an engineering mandate that makes allowance for a flight acceptance (FA) dry-heat test of the subassemblies, the possible need for resterilization following repair, and local differences in temperatures during sterilization (including the comeup and cooldown portions of the cycle).

A fair amount of mythology has accumulated regarding the derivation of the spacecraft sterilization cycle of 135° C for 22 hours. It is my goal to analyze in microbiological terms the ancestry

of this cycle, to evaluate the kinetics of dry-heat sterilization with mathematical formulas that may have meaning to the spacecraft engineer, and to give some approaches on how to obtain flexibility in our time-temperature cycles for sterilization. This paper will update a review of dry-heat sterilization which was presented at the COSPAR meetings in 1963 (ref. 2).

Determination of Adequate Sterilization Cycle

Many microbiologists have different ideas about what is an adequate dry-heat sterilization cycle. It is common practice in the hospital trade to use cycles at temperatures of 160° to 170° C. It has not come to my attention that the hospital trade has based its dry-heat sterilization processes on rigorous tests of the dry-heat resistance of many micro-organisms. The U.S. Pharmacopeia states that containers for pharmaceutical products should undergo dry-heat sterilization at 170° C for 2 hours. In contrast, the British Pharmacopoeia states that sterilization at 150° C for 1 hour is satisfactory for the vessels and containers to be used in parenteral injections. Lastly, the state Pharmacopoeia of the U.S.S.R. indicates that heating in a drying cabinet for 1 hour at 160° to 170° C is adequate for containers to be used to hold various pharmaceutical preparations.

The discrepancies between these various time-temperature cycles is due in part to the practical experience of these various groups with dry-heat sterilization and to differences in types of micro-organisms, environments, and contamination levels used in assaying the adequacy of a particular time-temperature regimen. The food industry began a systematic approach to determining the dry-heat resistance of micro-organisms when that industry embarked on the use of aseptic canning systems (refs. 3 and 4). This was the only group actively working on dry-heat resistance of micro-organisms until about 4 years ago. At that time the NASA decided that dry heat would be the most acceptable agent for the terminal sterilization of spacecraft.

The original cycle of 125° C (257° F) for 24 hours selected for sterilization of the Ranger spacecraft was derived by a spacecraft engineering group at the Jet Propulsion Laboratory (ref. 5). They arrived at this particular value by an extrapolation of time-temperature relations at higher temperatures (140° to 170° C). There are very few data in the literature for lower temperatures (110° to 140° C). NASA then undertook a contract with the Wilmot Castle Co. to verify the adequacy of the 24-hour cycle at 125° C.

At this time the Castle group had just finished a study of the dry-heat resistance of spores of *Bacillus subtilis* var. *niger* and

Bacillus stearothermophilus strain 1518. The published data (ref. 6) showed that *B. subtilis* var. *niger* had three to five times more dry-heat resistance than *B. stearothermophilus* strain 1518, which is exceptionally resistant to moist-heat sterilization. With this knowledge and with a statement from NASA that a solution to the dry-heat sterilization requirement for spacecraft should be reached in 2 years, the Castle microbiologists embarked on a quick survey of the most dry-heat-resistant populations in nature. This survey was initiated by screening various soil samples from diverse environments. The results of these early soil studies (ref. 7) are shown in table I and indicate that a cycle of 125° C (257° F)

TABLE I.—*Thermal Death Times and D-Values for the Mesophilic Bacterial Spore Populations of Two Soils*

[From ref. 7]

Temperature		Soil FG		Soil CO	
°F	°C	Time to sterilize, hr	*D*-value, hr[a]	Time to sterilize, hr	*D*-value, hr[a]
248	120	60	8.9	60	8.7
257	125	28	4.0	24	4.1
266	130	18	2.6	15	2.4
284	140	8	1.1	8	1.1
302	150	3	.41	3	.39
320	160	1	.13	1.5	.19

[a] *D*-values for each soil were calculated from a mesophilic spore count obtained after a heat shock of 10 min. at 80° C. The count for soil FG was 3.1×10^{6} spores per g; the count for soil CO was 1.0×10^{6} per g. Assays for sterility were made in thioglycollate broth.

for 24 hours was not adequate for sterilization of some of the specimens. Thioglycollate broth was used as the recovery medium because the experimenters did not have time to run a screening of the most suitable recovery media for the various microbial populations in soil. It has since been found by using a tryptone-glucose yeast extract recovery broth at mesophilic temperatures that survivors of the treatment from over 100 hours at 135° C (275° F) can be obtained from soil samples (ref. 8). This result also points out the critical role that recovery media play in determining the adequacy of a proposed sterilization cycle.

The technique for assaying when the soils would be sterile depended upon a partial survival endpoint of the population; that is, the group of tubes being exposed at a given time-temperature

cycle would not all be sterile. Out of 10 tubes a few would be positive and the rest would show no growth. From these partial survival data a sterilization endpoint was calculated (ref. 7) and this endpoint (F-value) was verified by laboratory tests. The assumption was also made that the microbial death rate in dry heat was a logarithmic function from which a D-value could be calculated (the D-value is the reciprocal of the slope of the death-rate curve and is equal to the time for a 90 percent kill or a 1-logarithm reduction in count).

From the positive tubes of the partial survival tests, cultures were isolated and grown in the laboratory. These particular isolates were found to have heat resistance similar to that obtained for other mesophilic aerobic sporeformers that were under investigation. In later studies, Koesterer (ref. 8) has been able to isolate from soil a mesophilic aerobic sporeformer that has resistance beyond that found for *B. subtilis* var. *niger* and *Bacillus coagulans*, which were the two most resistant sporeformers in the culture collection of the Wilmot Castle Co.

From a comparison of the data obtained on the heat resistance of the indigenous soil microflora and the heat-resistant sporeformers isolated from soil, it was evident that some factors in the soil were responsible for increased heat resistance. This could be attributed in part to nutritional factors for microbial growth and spore formation and to the physical-chemical environment of soil which is an organic-inorganic colloidal milieu of undefined scope. It appeared that different carriers for spores would affect the heat resistance of the spore populations, and this fact was confirmed by studies with paper strips, glass tubes, sand, and vermiculite. These data have been published (ref. 7) and there is no need to go into an extensive analysis of the D-values obtained. It was found that the spores on sand and vermiculite had more resistance then spores on glass, which in turn had more resistance than spores on paper strips. The reason for this particular carrier effect has not been defined, but it is suspected that the cell is better able to control the environment immediately around the cell and thus affect the transfer of inactivation energy to sites within the cell. Recent reviews of dry-heat sterilization kinetics point to the critical role of moisture levels in this inactivation process (ref. 9). A better hypothesis is that the soil and some of the carriers act as buffers in maintaining proper moisture levels for maximum dry-heat resistance.

After this investigation of the heat resistance of known laboratory sporeformers and the indigenous flora in soil, an analysis of the effect of various environmental factors on pure culture systems

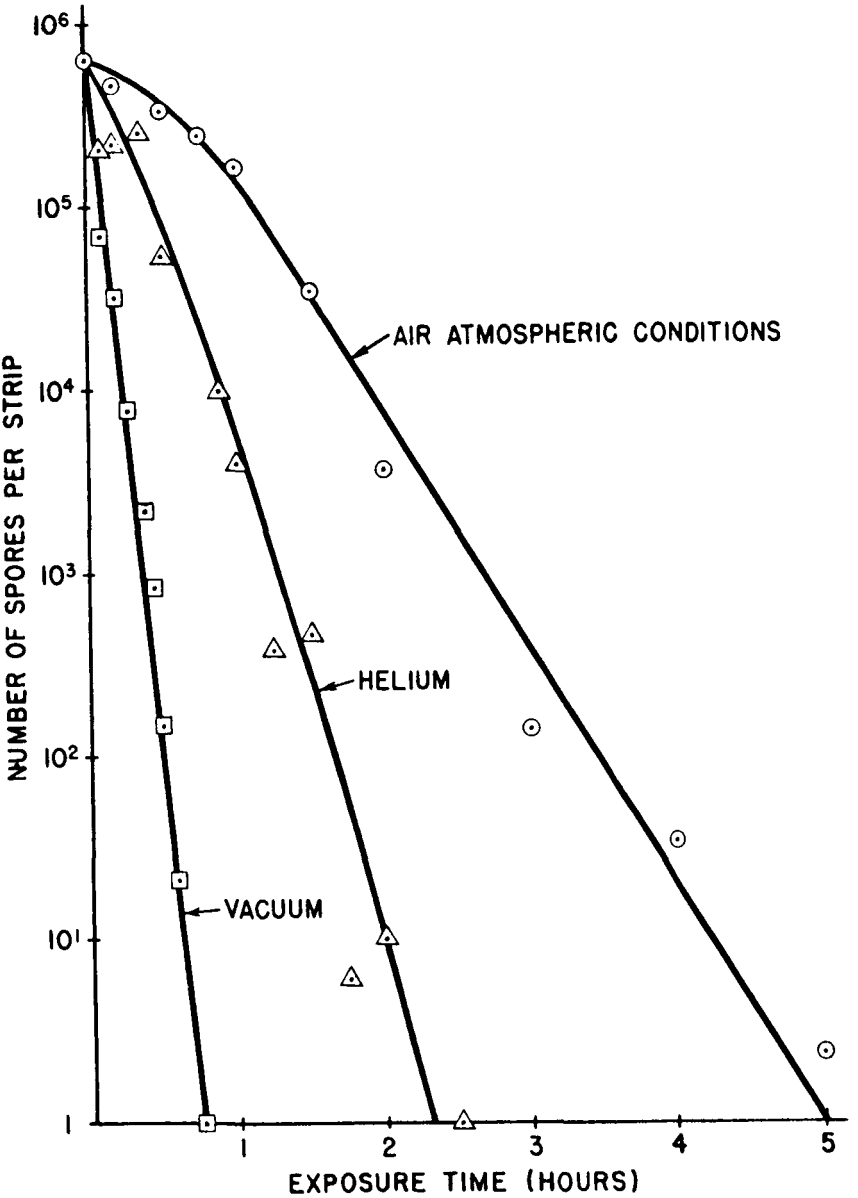

FIGURE 1.—Survival curves at 120° C (248° F) for spores of *B. subtilis* var. *niger* showing effects of vacuum (0.01 mm Hg) and helium and air at atmospheric pressure (reprinted from ref. 10).

was attempted. It was found that vacuums of 0.01 millimeter Hg would significantly decrease the sterilization time for most of our spore populations (ref. 7). The enhanced killing effect of helium

gas or vacuum is shown in figure 1. These plots also reveal that the death rate is a logarithmic function over a substantial portion of the inactivation curve (see ref. 10).

Another environmental condition that was investigated was the entrapment of spores in various solids. The first results at 120° C (248° F) (ref. 7) are given in table II, which shows a wide range of *D*-values for the various preparations. These values are greater than those that are normally found necessary to sterilize various laboratory spore preparations on either paper strips, glass tubes, sand, or vermiculite. A reinvestigation of this work at higher temperatures has been performed by Koesterer (ref. 8) and the

TABLE II.—*Thermal Death Times and D-Values at 248° F (120° C) for Spores of* B. subtilis *var.* niger *Entrapped in Several Solids*

[From ref. 7]

Compounds	Time to sterilize, hr	*D*-value, hr[a]
Solid rocket propellant	24	2.5
Asbestos patching cement	20	2.1
Plaster of paris	12	1.7
Glue-base marble patching plaster	30	4.0
Dental materials:		
Inlay investment A	4.5	.6
Inlay investment B	30	3.2
Inlay die material	30	3.6
Bridge model material	15	1.6

[a] *D*-values were calculated from levels of spore contamination found by assay of solid materials. The weight of samples for a given solid was held constant and was in the range of 0.5 to 1.5 g for all materials. Samples solidified around thermocouples showed that all solids reached temperature in 10 min.

data are presented in table III. In addition, organisms were added to activated carbon and also to kaolin, a very fine colloidal mineral that is a member of the clay minerals in soil. The various solids and the activated carbon increased the resistance of the *B. subtilis* var. *niger* spores to dry-heat sterilization. The same effect was also shown for *B. coagulans* spores entrapped in some of the same solid materials.

As an extension of this particular study, the times necessary to sterilize various natural contaminated materials with dry heat were examined. Table IV shows the time-temperature cycles required to sterilize vacuum cleaner dust, dirty hardware, and airborne contamination that had been trapped out on membrane filters (ref. 8). Unfortunately, *D*-values have not been calculated

TABLE III.—*Thermal Death Times and* D*-Value at Three Temperatures for Spores of* B. subtilis *var.* niger *Entrapped or Added to Various Materials*

[From ref. 8]

Materials	Spores per test sample	Time to sterilize, hr, at temperature, °F, of —			*D*-value, hr, at temperature, °F, of —		
		239	257	275	239	257	275
Paper strips	1.2×10^6	18	8	2.5	2.5	1.1	0.46
Plaster of paris	1.2	28	12	6	3.4	1.4	.63
Glue-base marble patching plaster	2.7	34	16	7	4.7	2.0	.84
Dental material, inlay investment B	2.1	44	22	10	6.1	2.8	1.1
Bridge model material	1	48	22	8	6.4	3.0	.93
Activated carbon:							
Indigenous flora [b]	.001 to 0.01			5			.80
Dry spores added [b]	4.6			8			.74
With spores grown in 0.01-g samples	6			8			.80
Kaolin (fine power), 0.1-g samples	2.2	—	—	2			.21

[a] *D*-values were calculated from levels of spore contamination as found by assay of solid materials on plate count agar. The samples were cylindrical pellets and the weight was approximately 0.33 g for all materials. Samples solidified around thermocouples indicated that all solids reached temperature within 10 min.

[b] 0.1-g samples of 80-mesh carbon were employed.

for these various populations and it is difficult to compare each of the samples because the populations were different in numbers and types. An analysis of the time-temperature regimens for sterilization shows that these values are not very high; that is, the organisms are showing resistance that is similar to that found for known sporeforming organisms on paper strips. An exception is noted for the airborne contamination trapped on membrane filters, where resistance is much less than that found for laboratory sporeformers.

Some attention has been given to whether a synergistic effect exists between dry heat and ionizing radiation. Preliminary data have shown that a synergistic effect can be obtained by certain sequences of dry heat and gamma radiation (ref. 8). Such an effect could be obtained with dry-heat treatment simultaneous with gamma irradiation and with heat treatment prior to irradiation on dry spores of *B. coagulans* on paper strips. In studies with dry

TABLE IV.—*Times To Sterilize Naturally Contaminated Materials With Dry Heat*

[From ref. 8]

Materials	Number of contaminating spores	Temperature, °F	Time to sterilize, hr[a]
Vacuum cleaner dust	10^3 to 10^4	248	8
		257	6
		266	4
		284	2
		293	.5
Dirty hardware[b]	10^1 to 10^2	275	[c]4
		275	[d]6
Airborne contamination on membrane filters	10^1 to 10^4[e]	239	4

[a] All assays for sterility were made in tryptone glucose yeast extract broth, except those for the vacuum cleaner dust which was assayed in thioglycollate broth.

[b] Items such as screws, nuts, bolts, and metal bushings were collected from a manufacturing area of Wilmot Castle Co.

[c] 102/106 items sterile.

[d] 104/106 items sterile.

[e] Number of organisms collected varied, due to several factors influencing the laboratory air.

spores of *B. subtilis* var. *niger* on paper strips, the only synergistic effect with the two agents was obtained when the heat treatment was simultaneous with irradiation.

NASA has held in abeyance programs to evaluate the terminal sterilization of spacecraft with ionizing radiation until more information on the material effects of this radiation is obtained. In the event that the material effects are not so severe as have been predicted, NASA may initiate a program to define further the indicated synergistic relationship between dry heat and ionizing radiation. Enough information exists from radiation research supported by the Atomic Energy Commission and the U.S. Army Quartermaster Corps to limit the need by NASA for new determinations of the radiation resistance of micro-organisms.

The fixation on one time-temperature regimen for dry-heat sterilization seemed unnecessarily rigorous, and it was decided to investigate a range of temperatures from below 100° C up to and including 160° C. Most of the results of these studies (refs. 7 and 8), which were carried out over a period of 3 years, are presented in figure 2. This type of plot is called a thermal resistance curve and gives the effect of temperature on heat resistance in terms of

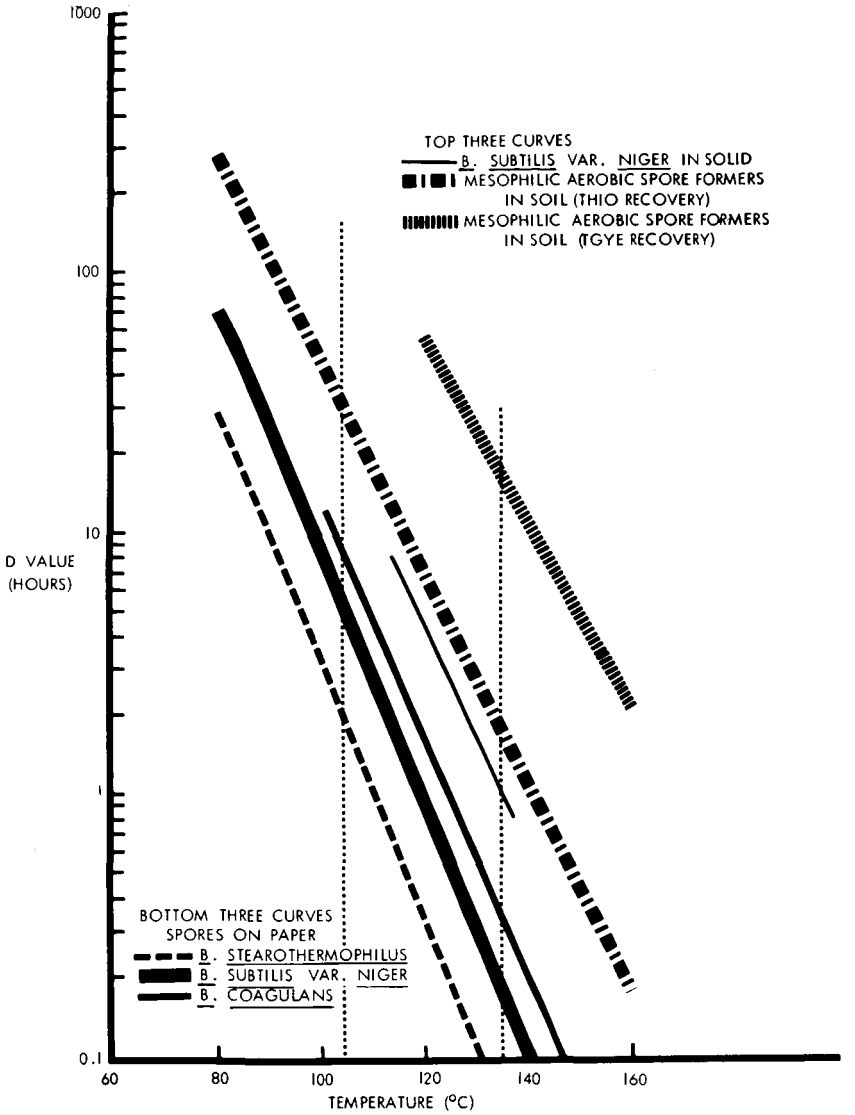

FIGURE 2.—Resistance of several microbial populations in different environments to dry-heat sterilization.

D-values. The bottom three curves represent the thermal resistance of dry spores on paper strips. The most resistant of three bacterial species listed here was *B. coagulans*. The top three curves represent organisms in solids or in soils. The two upper curves are for the indigenous mesophilic aerobic sporeformers in soil and indicate the influence of the recovery medium on the resulting thermal resistance values. For the uppermost curve the heated spores were

inoculated into tryptone glucose yeast extract broth that was able to recover more of the heat-damaged spores. The middle curve represents recovery of the soil organisms using thioglycollate broth. The curve for *B. subtilis* var. *niger* spores trapped in a solid material (dental bridge material) shows that spores trapped in a solid have much greater heat resistance than the same spores exposed to dry heat on paper strips. Many additional curves could be plotted on this same graph, but these particular examples point out the various classifications of resistance.

The slopes of these plots appear to be similar from visual analysis. The slopes measured as z-values (°F to transverse 1-logarithm cycle in time) for the 3 sporeformers on paper strips were 38 to 41, while the organisms trapped in solids or on soil had z-values ranging from 45 to 48. A vertical line has been drawn through the 135° C point and if one reads off the D-value for the curve of the indigenous soil flora recovered in thioglycollate broth (1.8 hours) and multiplies it by 12, he would obtain the length of time that would be required sterilize 10^8 microbial spores in soil with the probability of 10^{-4} of a survivor. By such analysis, a process time of 22 hours at 135° C is obtained ($1.8 \text{ hr} \times 12 = 22 \text{ hr}$). This is a brief description of how the sterilization cycle of 22 hours at 135° C was derived.

It must be understood that the D-values and total process times prescribed for the sterilization of spacecraft are based on the kinetics of destruction for mesophilic spores occurring naturally in agricultural soil. This "worst case" situation has been justified for use in spacecraft sterilization because insufficient data exist on the kinetics of inactivation for spores entrapped in solid materials. If the existing data for dry thermal destruction of *B. subtilis* var. *niger* spores in dental plastics were used to derive a sterilization cycle for spacecraft, the process time would decrease 40 percent from that based on the destruction curve for mesophilic spores in soil. If the scientific community should decide that the probability of release of micro-organisms from solids is extremely small and that the hazard to an extraterrestrial body from these internally trapped contaminants is minimal, a dry-heat process for the destruction of surface and occluded microbial contamination could be considered. Such a cycle would have a process time 60 to 80 percent less than that of a spacecraft sterilization cycle based on time to kill indigenous spores in soil. A comparison of three such cycles for several temperatures is presented in table V. The process times in the column for the spores in soil comprise the list from which the sterilization cycle for the Voyager landing capsule will be selected.

TABLE V.—*Comparison of Sterilization Cycles Based on Destruction of Different Sporeformers in Various Environments*

[Cycles are based on 12 logarithms of spore kill (12D)]

Temperature, °C	Time to sterilize, hr, for —		
	B. subtilis var. *niger* on paper strip[a]	*B. subtilis* var. *niger* in solids[a]	Indigenous mesophilic spores in soil[b]
105	59	228	336
110	35	144	210
115	20	84	132
120	12	54	84
125	6.7	32	53
130	4.0	20	34
135	2.3	12	22
140	1.3	7.5	14
145	.65	4.6	8.8
150	.43	2.8	5.5
155	.25	1.7	3.7
160	.14	1.1	2.5

[a] Recovered in trypticase soy broth.
[b] Recovered in thioglycollate broth.

That the destruction of the natural spore flora in soil is a "worst case" situation is evident from our attempts to develop a biological sterilization indicator to monitor the application of this cycle. The approach to this development problem was the following: Take the most dry-heat-resistant sporeformer currently known, produce a large crop of clean dry spores, and manipulate a large population of 10^9 or more spores (5 to 100 milligrams) into a form that is easily assayable. Each indicator is to yield survivors at 18 to 20 hours, but no survivors at 22 to 23 hours of exposure to 135° C. The data in table VI show that the bulk of the spore pellets were sterile after 10 hours of exposure to 135° C. The remaining fraction of the spore pellets had some survivors for as long as 22 hours. The constant recovery ratio from 10 to 18 hours of exposure at 135° C indicates that some factor contributing to heat resistance was present in these particular spore pellets. It is speculated that a critical moisture level was maintained inside those spore pellets that yielded survivors.

The original reason given for going to low-temperature dry-heat cycles (120° to 135° C) was that the electronic components would be affected at higher temperatures (140° to 180° C). It has not

TABLE VI.—*Effect of Time at 135° C on Survival of Spores of* B. subtilis *WC 18 in Pressed Tablets of 10^9 to 10^{10} Spores Held in Teflon Containers*

[Unpublished data from Wilmot Castle Co.]

Time, hr[a]	Recovery, ratio of positives to total exposed[b]	Percent of tablets yielding survivors
2	22/22	100
4	19/21	91
5	1/3	33
6	9/15	60
8	11/24	46
10	16/65	25
12	3/13	23
14	6/24	25
16	24/77	31
18	24/72	33
20	2/35	6
22	1/9	11
23	0/20	0
24	0/6	0

[a] No correction for lag time; containers reached temperature within 2 min.
[b] Recovery in trypticase soy broth incubated at 37° C for 1 month.

come to my attention that a systematic analysis has been made of whether the damage to components would really be so severe at high temperatures as it has been assumed that it would be. There exists a body of data in the food canning industry which shows that many food products are less injured by high-temperature short-time cycles than they are by long-time low-temperature cycles. The problem of the relative merits of sterilization of heat-sensitive electronic components at various temperatures has not been investigated by an aerospace group.

The data presented earlier in figure 1 show that the inactivation of micro-organisms by dry heat generally follows a monomolecular type of reaction. We can reasonably expect the death rate of micro-organisms to follow first-order reaction kinetics. It has been found in the food industry that the half life of several vitamins exposed to increasing levels of moist heat also follows first-order kinetics. In figure 3 a typical relationship is given between the thermal death time for a heat-resistant microbial population and the half life of a vitamin in a food being exposed to same temperatures. It is evident from the curves that at a low temperature such as A, the half life of the vitamin may be much shorter than the necessary sterilization time for the food. Yet, at a higher temperature B, the food can

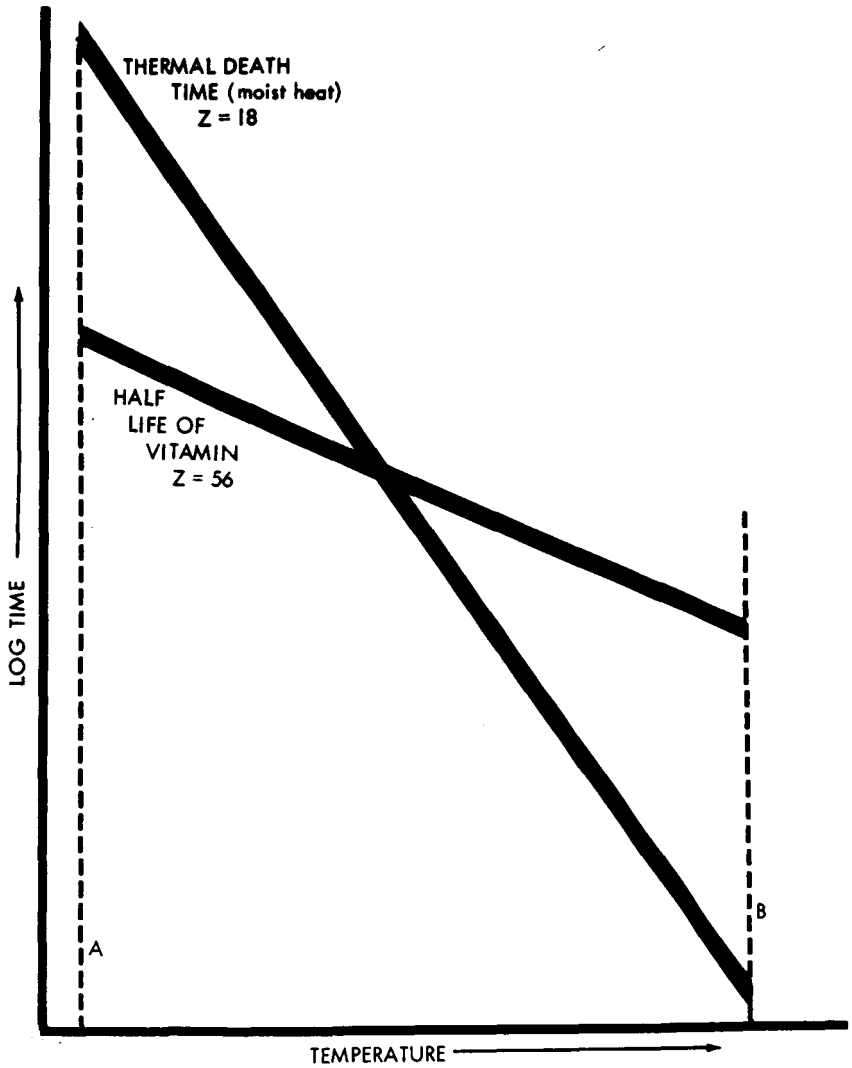

FIGURE 3.—Relationship of thermal death time for heat-resistant microbial population and half life of vitamin exposed to same temperature.

be processed in a shorter time with less decomposition of the vitamin. The determining factor for such a situation is that the thermal death line has a much sharper slope than the line representing the vitamin destruction reaction. Published heat data on thiamine destruction (ref. 11) show that with an 18° F (10° C) increase in temperature, the rate of thiamine destruction is approximately doubled. The thermal death time curves of spores of typical food spoilage organisms in neutral phosphate buffer show

that with an 18° F increase in temperature the rate of destruction of such organisms is increased tenfold. Hence, an increase in processing temperature produces an increase in rate of spore destruction approximately five times that of thiamine destruction.

Kinetics of Dry-Heat Sterilization

The data on dry-heat sterilization have been analyzed and derived in terms of three parameters: (1) the D-value (the time needed to reduce the population of a particular micro-organism by 1 logarithm (90 percent) at a given temperature); (2) the F-value (the time it takes to achieve sterility of a microbial population at a specified temperature); and (3) the z-value (the slope of the F- or D-values plotted with respect to temperature in °F).

The use of D-, F-, and z-values is an example of Q_{10} kinetics which are commonly employed to describe many chemical reactions. There are complications in that three different relationships, namely, Q_{10}, Arrhenius, and Eyring kinetics, have been proposed to correlate death-rate behavior with temperature variations. This has been particularly true with work on moist-heat sterilization. At a low temperature such as 210° to 250° F with moist heat, the difference between these three relationships cannot be distinguished from the normal variances in biological data. However, when extrapolated from the low 200° F range to 320° F, as much as a twofold difference exists between these relationships.

Some mathematical considerations for the temperature relationships in sterilization will be presented. The D-values are related to the temperature of sterilization in the form:

$$z = \text{Slope of } \log D \text{ vs. } T = \frac{-\mathrm{d}(\log_{10} D)}{\mathrm{d}T}$$

Justification for the proportionality relationship of the equation is assumed on the basis that plots of log D against T appear to yield a straight line, thus giving rise to the constant slope parameter z. However, it has been demonstrated from the moist-heat sterilization experiments that a better fit for the data would be obtained by using an Arrhenius rate expression

$$K = \frac{1}{D} = A\mathrm{e}^{-E/RT}$$

where

A constant referred to as frequency factor
E activation energy, cal/g-mole
R gas constant
T temperature, °K

In this situation the value of the constant E/R is obtained from the slope of log D versus $1/T$. The fact that the latter generally gives

a straight line over a wider range of temperature than does the first equation is itself sufficient to commend the Arrhenius rate expression for use in dry-heat sterilization. There is, however, additional justification for its use.

There is a need to evolve a basis for a relative evaluation of time-temperature effects on the viability of micro-organisms on the one hand and on the performance and reliability of equipment on the other. The Arrhenius rate expression has found extensive use in different scientific areas because it is a good approximation to the temperature dependence of many physical and chemical processes. Because of this generality in its applicability and since it also appears to be valid in the sterilization process, it provides the needed link between the two areas of interest, planetary quarantine and spacecraft engineering.

It has been found in moist-heat sterilization work that the activation energy E calculated for destruction of bacterial spores does not vary widely. This had led to the acceptance of values of E in the range of 65 000 to 85 000 cal/g-mole by the food industries in approximating sterilization requirements (ref. 12). There is also good indication that the same relationship is true in dry-heat sterilization.

Samual Schalkowsky, in a personal communication, has analyzed some of the data published by Koesterer (ref. 8) and derived the value of 25 500 cal/g-mole as the activation energy E_a for the dry-heat destruction of mesophilic spores in soil. The author has taken the same data published by himself and by Koesterer (refs. 7 and 8), as shown in figure 2, and obtained the following E_a and z-values: *B. stearothermophilus* on paper strips, $E_a = 33\ 200$ and $z = 38$; *B. subtilis* var. *niger* on paper strips, $E_a = 31\ 600$ and $z = 39$; *B. coagulans* on paper strips, $E_a = 30\ 200$ and $z = 40$; *B. subtilis* var. *niger* in solids, $E_a = 28\ 800$ and $z = 41$; mesophilic spores in soil (thioglycollate broth recovery), $E_a = 27\ 600$ and $z = 45$; and mesophilic spores in soil (TGYE broth recovery), $E_a = 26\ 200$ and $z = 48$. The discrepancies between calculations is due to the difference in interpretation of the plotted (graphical) data by Schalkowsky and the use of actual laboratory data by the author.

This estimated value of the activation energy E for dry-heat sterilization E_{dh} may now be related to a derivation of time-temperature cycles at other specified temperatures. The particular equation that we would use to make such a plot is

$$n = t\, e^{-E_{dh}/RT}$$

where n may be considered to be the sterilization requirement and

t is the sterilization time. It specifies the permissible combinations of sterilization time t and sterilization temperature T which will give the desired probability of contamination for an initial population of micro-organisms with a certain resistance parameter. Figure 4 illustrates the use of this equation; it shows equivalent time-temperature combinations with the assumption that one such

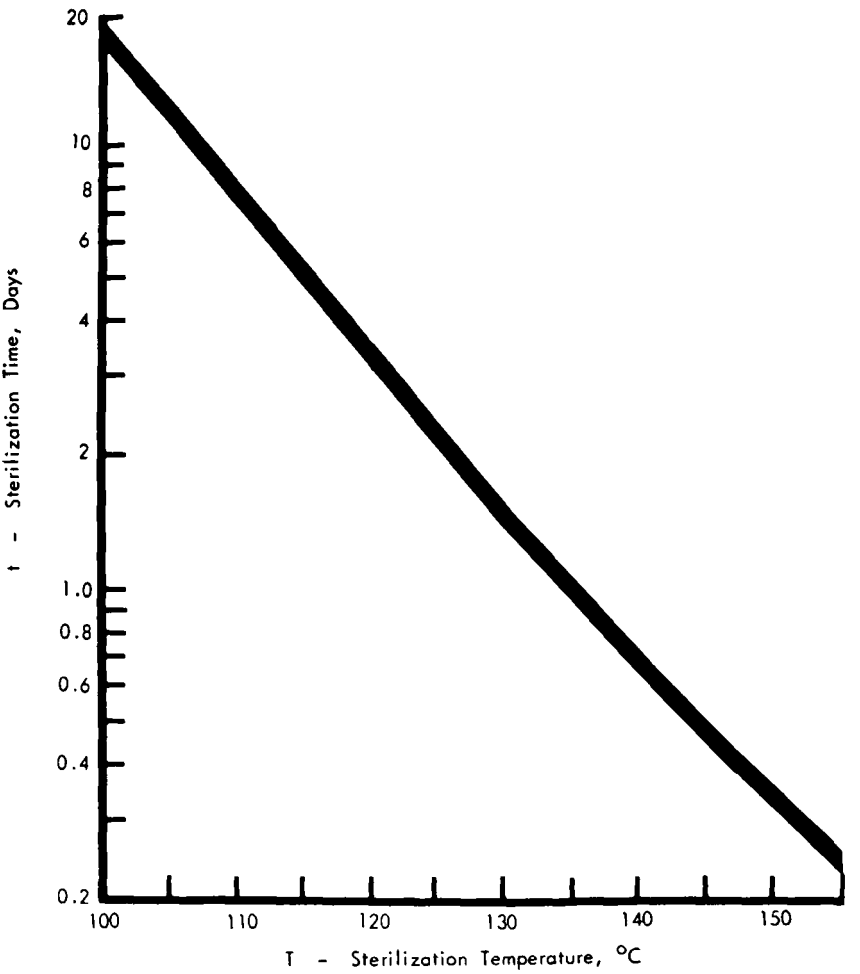

FIGURE 4.—Equivalent sterilization requirements based on a modified Arrhenius equation with 22 hours at 135° C as a base and $E_{dh} = 25\,500$ cal/g-mole, $t = \left[\exp\ 12\,750\left(\frac{1}{T} - \frac{1}{408}\right)\right]$ (Unpublished analysis of S. Schalkowsky.)

combination is 22 hours at 135° C. The plot is based upon an activation energy of 25 500 calories for dry-heat sterilization. At 125° C the equivalent sterilization time would be 52 hours and at 105° C it would be 13 days. With consideration of the plot given previously in figure 2, the suggested sterilization time at 105° C is 14.5 days.

During a previous discussion of figure 3, it was shown at temperature B that the destruction of the organisms was accomplished in much less time than was required for inactivation of half of the vitamin's activity, whereas at temperature A the reverse was true. For a frame of reference, visualize the concept that an electronic component or some piece of the spacecraft hardware takes the place of the vitamin but provides the same kinetic relationship as that given on the graph. Assume that the operational life of a piece of electronic componentry becomes marginal because of exposure to the heat-sterilization cycle. It would thus be desirable to choose a time-temperature combination which will optimize the operational lifetime of such equipment. When the sterilization activation energy E_{dh} is larger than the activation energy for equipment deterioration E_D, sterilization should be at a high temperature for a short period of time. Conversely, when the activation energy of the equipment decomposition is greater than the activation energy of dry-heat sterilization, sterilization should be at a low temperature for a long period of time.

The graph given in figure 5 is reproduced from a report by Reich and Hakim (ref. 13) on reliability effects in semiconductor devices. It shows failure rate (percent failures per 1000 hours) as a function of the reciprocal of absolute temperature in the temperature range of 145° to 307° C. Since the semilogarithmic plot produces a straight line, the failure rate can be put into the Arrhenius form and the corresponding activation energy can be calculated from the graph. This has been done and the activation energy is found to be 6900 calories per gram mole. The activation energy for dry-heat sterilization is about four times larger.

In this situation, because of the relative magnitudes of the activation energies, sterilization at a high temperature for a short period of time would minimize degradation to semiconductor reliability. No attempt should be made to generalize on this conclusion, since the data on which it is based are much too restricted. This actual example, however, does support the type of analysis that should be made of the effects of heat on the microbial sterilization process in relation to the effects of heat on the reliability or operating life of our componentry. It may thus be possible to increase an operating life of a few months to a desired

longer lifetime by suitably choosing the sterilization time-temperature combination.

The Arrhenius equation has had some use in the semiconductor industry because it is a rapid procedure for extrapolating both time and stress. The Arrhenius model is simple and can be employed when aging is produced solely by thermal stresses (ref. 14). The utility of this equation in treating data on accelerated testing of electronic components requires two assumptions:

(1) The degradation of quality or some function of quality is a linear function of time at fixed level of stress.

(2) The logarithms of the slopes of the degradation lines yield

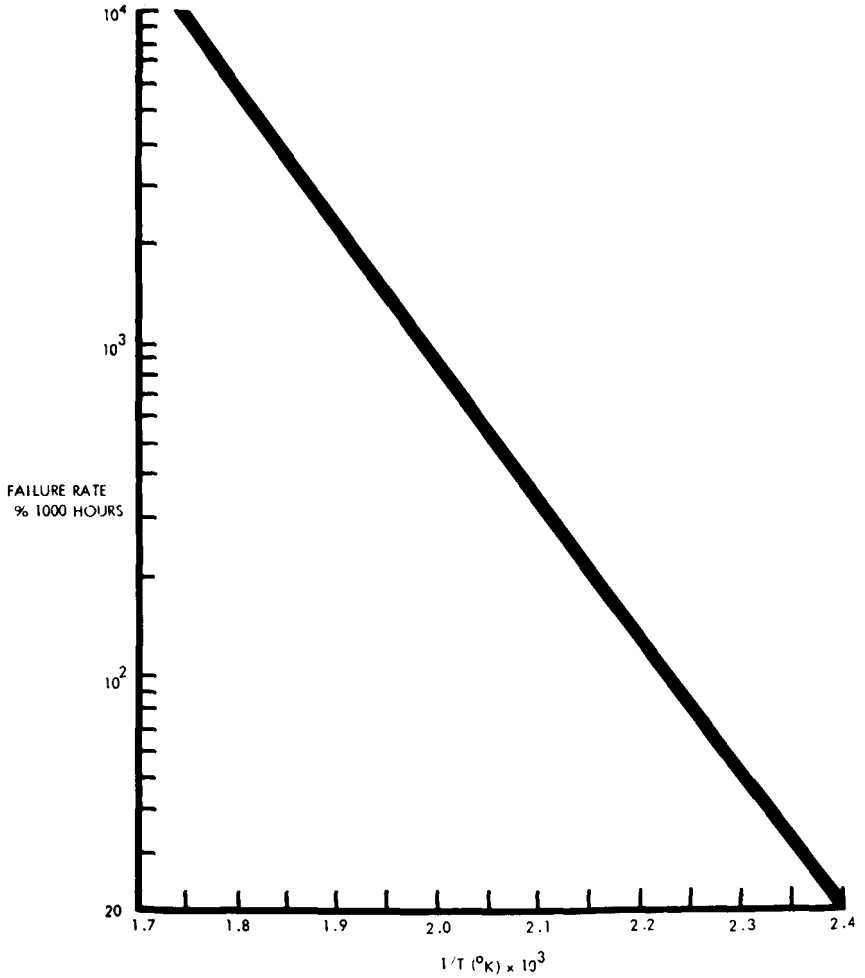

FIGURE 5.—Relationship between semiconductor failure rate and temperature. Activation energy, 6900 cal/g-mole.

a linear function of the reciprocal of the absolute temperature.

Since such data are available in the electronics industry, many more comparisons of the activation energy for dry-heat sterilization against the activation energy for component degradation could be made.

A further example of the use of the Arrhenius model for comparing temperature stress is the analysis of the activation energies for thermal degradation of several pure polymers (see table VII). Although several of the polymers tested are used in solid propellants, their structure as binders would be modified from the pure form. Thus, these activation energies in table VII are indicative only of how these pure polymers would thermally degrade. Because most of the values are higher than the activation energy for dry-heat sterilization, it would be expected that low temperatures for longer periods of time would be less destructive.

TABLE VII.—*Activation Energies for Thermal Degradation of Several Pure Polymers*

Polymers	Activation energies, kcal/g-mole	Temperature range, °C
Polymethyl methacrylate[a]	30-48	110-180
Polystyrene[a]	45-65	335-365
Polyethylene	60-70	315-360
Natural rubber	43	220-270
Polyurethane (urethane bonds)[a]	14-26	110-150
Polyurethane (ether bonds)[a]	48-52	282-305
Polybutadiene[a]	62	380-395

[a] Polymer structure used in solid propellants.

Another aspect of the dry-heat sterilization data is the use of that data to determine the total lethality of a given heat-process cycle. A lethality curve for the sterilization process which involves the integration of heat penetration into a container with the heat resistance of the micro-organisms at several temperatures will be described. Heat penetration curves are themselves of little practical value unless they can be integrated with the resistance of the contaminating bacteria that the heat process must destroy. Bigelow and coworkers in 1920 (ref. 15) conceived the idea of constructing a "lethal rate curve" that related time-temperature measurements in the slowest heating point in a can of food with the destructive effect on the contaminating

bacteria. They first determined the thermal death-time curve for a population of the most heat-resistant organism in that particular food product in the temperature range between 212° and 250° F. From this straight-line thermal death-time plot, it was possible to relate resistance of the spores at any temperature on the curve with their resistance to some reference temperature.

For example, they recorded that after 24.6-minute heating in a retort at 250° F, the center of a No. 2 can of corn had reached 214° F. Their thermal death-time curve indicated that it required 1180 minutes to kill the population of their test organism at 214° F. Expressed in terms of the reference temperature of 250° F, the heating of spores for 1 minute at 214° F was equivalent to heating them for 1/1880th of a minute at 250° F. This reciprocal of the resistance of 214° F is called the lethal rate. By plotting lethal rates at various temperatures recorded in the center of the can against times at which these temperatures were attained, they were able to construct a curve resembling that in figure 6.

This particular product was at 250° C only 13 minutes (area between 30 minutes and steam-off time of 43 minutes), yet destruction of the bacterial spore population in this product required 27 minutes at 250° F. The equivalent of 14 minutes at 250° F was achieved during the comeup and cooldown periods.

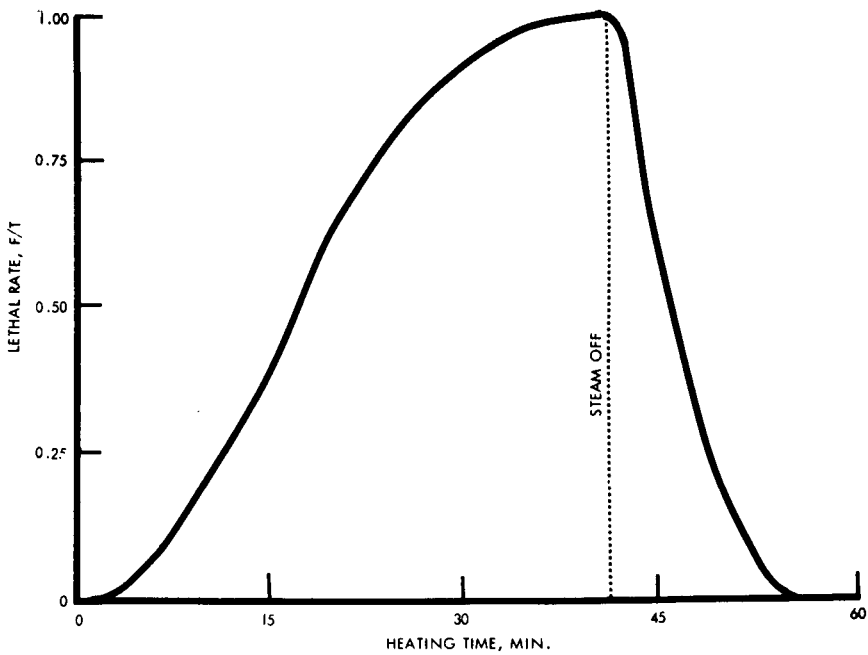

FIGURE 6.—Lethality curve for a moist-heat canning process.

These data also point to one of the difficulties inherent in the concept of biological sterilization indicators. If an indicator is devised in the laboratory to resist 20 hours at 135° C, it is possible that the indicator will be inactive during the long comeup time of days before the desired temperature of 135° C is reached in a spacecraft sterilization process. Table VIII contains preliminary data that illustrate this phenomenon. Under the condition of a 24-hour comeup time to 135° C, the majority of the spore tablets were sterile by the time 135° C was reached. The same effect would occur with the microbial contamination in and on the spacecraft. Our process time should be calculated to destroy a certain level of microbial contamination (a 12 *D*-value process) and should not be based strictly on a fixed number of hours at a stated temperature. Thus, the cycles as listed in table V would be modified depending on the comeup and cooldown times for the total process.

TABLE VIII.—*Effect of Slow Comeup Times to 135° C on the Survival of* B. subtilis *WC 18 Spores in Pressed Tablets of 10^9 to 10^{10} Spores Held in Teflon Containers*[a]

[Unpublished data from Wilmot Castle Co.]

Time at 135° C, hr	Recovery,[b] ratio of positives to total exposed	Percent of tablets yielding survivors
4 hr to reach 135° C		
4	0/3	0
8	0/3	0
10	0/3	0
6 hr to reach 135° C		
4	6/6	100
5	1/7	14
7	1/8	13
12	0/2	0
24 hr to reach 135° C		
0	1/4	25
2	0/4	0
6	0/4	0

[a] Teflon containers are 1.4 by 2.4 cm.
[b] Recovery in trypticase soy broth incubated at 37° C for 1 month.

One potentially useful application of the mathematics relating to activation energies described earlier would be in thermal design of the sterilization process. In a complex piece of equipment, such as a planetary lander, it would be unreasonable to expect the sterilization temperature to be achieved uniformly throughout the assembly. Rather than base the sterilization cycle on an exposure time at a fixed temperature at the innermost portions of the assembly, a less harmful but equally effective procedure might be to base the thermal design on a sterilization requirement defined in the integral form of the previous equation for n. The integral form defining the sterilization requirement

$$n = \int_{t_1}^{t_2} dn = \int_{t_1}^{t_2} e^{-E_{da}/RT}\, dt$$

is more compatible with the temperature gradients and transients in a complex spacecraft assembly. Thus, exterior components will generally have steeper gradients and will reach a higher steady-state temperature than interior components. It may thus be possible to specify the schedule for external surface temperature against time so that the integral of the above equation will be achieved in an approximately uniform manner throughout various components of the assembly. Determination of the degree to which such techniques can be effectively utilized and suitable procedures for their utilization should be among the projects for our future research efforts.

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Gaseous Sterilization[1]

Gaseous sterilization can be considered a relatively new technique since it has come into prominence mainly within the last decade. More and more medical devices contain electronic, plastic, or other easily damaged components, so that the gas sterilizer is becoming common in hospitals alongside the autoclaves. Ethylene oxide is responsible for this new and widespread interest in gas sterilization; however, formaldehyde, with a history going back to the last century, still has present-day uses. These two compounds together with *Beta*-propiolactone only quite recently recognized, account for almost all practical applications in gaseous sterilization today. Many other compounds have been studied for this purpose, however, even if they have not been put to any great use. Some of these compounds will be discussed briefly in the present paper.

There are early activities related to this field that antedate by many hundreds of years the science of bacteriology itself. The incense on altars in many religious rites and the frankincense and myrrh of Biblical fame were associated with concepts of purification. In medieval times, records indicate that during plague epidemics, letters and messages were often held in the smoke from fires before delivery, in the hope that this would prevent the spread of the disease. Spices were sought out not only for their flavor but also because of a belief that their pungent odors somehow reduced food spoilage. Thus, along with belief in the theory that disease was caused by miasma or evil vapors, there were attempts to counteract their bad effects with other vapors.

Probably the first instance of a procedure related to gaseous disinfection with any scientific basis was that of Lister and his spraying of phenol in operating rooms to reduce surgical infections. The real beginnings of gaseous sterilization, however, appeared at the end of the 19th century in the practice of terminal fumigation of sickrooms following their occupancy by someone

[1]The material in this paper will appear as a chapter in the book entitled, *Disinfection, Sterilization and Preservation* (C. A. Lawrence and S. S. Block, eds.), Lea & Febiger (Philadelphia), 1967.

suffering from a contagious disease. Sulfur dioxide and chlorine were the main gases used at first for such practices, but their corrosiveness and damaging effect on the contents of the room certainly exercised a restraint on their use regardless of whether they were effective. A satisfactory agent for terminal fumigation was not found until about 1894, when within a short period of time several investigators showed that formaldehyde vapors could be used for such purposes. The bactericidal activity of formaldehyde had been scientifically established a few years previously, and in the next two decades hundreds of articles appeared discussing its application in sickroom disinfection. An excellent summary of this early work with formaldehyde was made by Nordgren (ref. 1), so no attempt will be made to describe it here in detail. Many of these studies contained either discussions of the relative merits of different methods for generating formaldehyde gas or the results of practical experiments, oftentimes crude, in which various contaminated samples were placed in closed rooms and examined after the room was treated with formaldehyde gas.

Terminal sterilization was a standard public health practice well into this century. It was abandoned not because it was impossible to disinfect a sick room effectively but because of evidence that terminal fumigation was often poorly done in practice, and, more to the point, our major epidemics were gradually brought under control by other means.

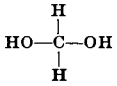
Three quite important facts concerning the action of formaldehyde as a gaseous fumigant were well established during this period, however. These were that the success of the procedure was dependent upon an elevated temperature, that a high relative humidity was necessary, and that the penetration of formaldehyde into porous materials was quite uncertain.

Formaldehyde

Pure formaldehyde gas, CH_2O, is stable in high concentrations only at temperatures above 80° C. At ordinary temperatures the gas polymerizes and condenses out as a white film on any available surface, so that high concentrations of the gas cannot be maintained at room temperatures. There are several types of solid polymers of formaldehyde, some with a cyclic structure such as trioxane, but the most common one is paraformaldehyde, which has a linear structure. This is a white solid; at ordinary temperatures it gradually gives off gaseous formaldehyde, so it possesses the same sharp irritant odor. When heated it depolymerizes rapidly, giving off formaldehyde and a little water vapor.

Formaldehyde gas also dissolves readily in water to produce

about a 37-percent solution at room temperatures. In water solution it exists mainly as the hydrate



with some higher hydrates probably also present. Commercial formalin is a solution that also contains certain stabilizers (8 to 15 percent methanol being the one most widely used) to prevent the formation of solid polymers if the solution is chilled or is kept for too long a time.

The concentration of formaldehyde in air over either paraformaldehyde or 37 percent formalin is about the same, amounting to about 1 milligram of CH_2O vapor per liter at room temperature. Actually, not even this amount can be readily maintained in air unless a large excess of paraformaldehyde or formalin is present. Figure 1 contains unpublished data from this laboratory that show

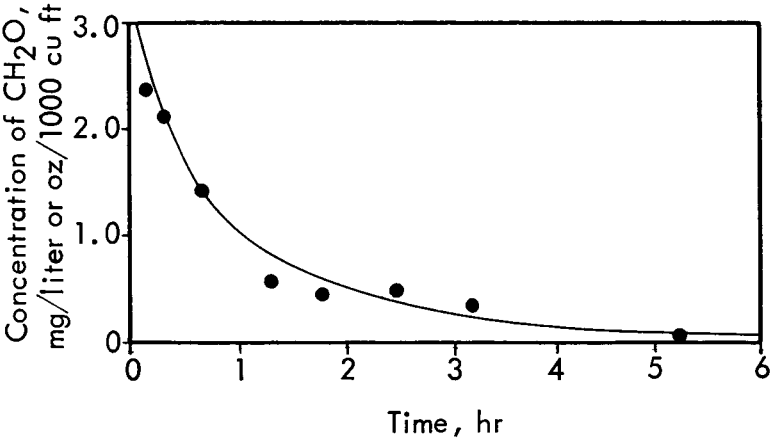


FIGURE 1.—Drop in concentration of formaldehyde vapor in air in an enclosed space with time. Initial concentration, 7 mg/liter.

the typical change in concentration of formaldehyde in air when formalin is vaporized into a small room in amounts of one-half milliliter of formalin per cubic foot of space. If it is all vaporized, this corresponds to an initial CH_2O concentration of about 7 mg/liter. Air samples taken a few minutes following its introduction show the presence of only 2.5 mg/liter and this concentration dropped steadily over a matter of hours. Since the formaldehyde did not escape from the room at rates that would account for this rapid disappearance from the atmosphere and since both the formaldehyde and the water in the formalin solution were intro-

duced in amounts above their equilibrium concentration in air, the moisture and the formaldehyde must both have condensed out on the various surfaces within the room. Thus, gaseous sterilization with formaldehyde is somewhat a misnomer, because introduction of formaldehyde gas into a closed space serves mainly as a mechanism for distributing either moisture films in which formaldehyde is dissolved or solid formaldehyde polymers over all available surfaces within the enclosed space.

Experiments in this laboratory have shown that sterilization of enclosed spaces may be accomplished most readily by vaporizing liquid formalin into the space at a rate of ½ to 1 milliliter for each cubic foot of enclosed space. This gives a theoretical dosage of 7 to 15 mg of formaldehyde per liter. If the relative humidity of the air is below 70 percent, additional moisture above that already contained in the formalin mixture must be vaporized into the space to bring the relative humidity to 100 percent. The temperature of the space is maintained at least 20° C and preferably higher. Within an hour or two, vegetative bacteria will be destroyed, but the space must be kept closed for as long as 12 hours if a high concentration of bacterial spores is present.

This technique results in complete sterilization of all well-exposed surfaces, but it is difficult to sterilize completely surfaces covered in any manner or to sterilize porous materials throughout. One other serious disadvantage of formaldehyde is the fact that prolonged airing, often of several days, is required to remove the adsorbed surface film of polymer, which continues to release formaldehyde gas slowly.

One advantage of this technique is that the space need be enclosed only relatively tightly and does not necessarily have to be hermetically sealed, a condition often impossible to achieve when entire rooms are to be treated.

Formaldehyde fumigation still has practical applications today, even though disinfection of laboratory rooms (Hundemann and Holbrook, ref. 2) and veterinary applications (Stover, ref. 3) are more likely applications than the terminal treatment of sickrooms. Other special uses include the sterilization of certain types of surgical or medical equipment in small cabinets (Goedrich and Schmidl, ref. 4) or of blankets and mattresses in hospitals (Committee on Formaldehyde Disinfection, ref. 5). Raw wool imported into the British Isles from areas where anthrax is indigenous is usually fumigated with formaldehyde. Salle and Korzenovsky pointed out, as other authors have also done, how the typically poor penetrating properties of formaldehyde gas can be improved by utilizing a vacuum chamber (ref. 6).

Ethylene Oxide

The use of ethylene oxide vapor for gaseous sterilization is a much more recent development than the use of formaldehyde. Most interest in the use of this compound has been comparatively recent, although Phillips and Kaye (ref. 7) in reviewing the literature on the bactericidal activity of ethylene oxide found references dating back to 1929. Occasional articles on this subject have appeared from that time on, and some early patents were taken out on sterilizing procedures utilizing ethylene oxide, particularly the series by Griffith and Hall (refs. 8 to 11).

Many of the difficulties encountered in using formaldehyde are avoided with ethylene oxide. It can be readily obtained and released in the pure state; no particular difficulty is encountered from polymerization or condensation on outer surfaces; and residues are quickly dissipated by simple airing.

Ethylene oxide is a colorless gas at ordinary temperatures, liquefies readily at 10.8° C, freezes at −111.3° C. The liquid is miscible with water in all proportions as well as with all common organic solvents. It has the formula

and, as such, represents the simplest cyclic ether or epoxy compound.

The compound has a rather pleasant etheral odor, with a median detectable concentration of about 700 parts per million (ref. 12). The acute inhalation toxicity has been reported as "moderate" and 50 parts per million recommended as the maximum allowable concentration for prolonged human exposure (refs. 12 and 13). Joyner (ref. 14) found no evidence of chronic toxicity among plant workers who had experienced a mean 10-year exposure to concentrations of approximately 5 to 10 parts per million. He also reviewed cases of acute human exposures, with nausea, vomiting, and mental disorientation as the chief symptoms. No deaths were reported. The compound can also act as a skin vesicant, particularly if aqueous solutions are spilled on clothing (Sexton and Henson, refs. 15 and 16) or materials to which it is adsorbed are held against the skin (Phillips and Kaye, ref. 7). Skin burns rather than illness following inhalation appear to be more common in the cases of accidents (Joyner, ref. 14).

The compound, like formaldehyde, is manufactured on a very large scale, largely for use in synthesis of other organic chemicals. It is available in this country either in the pure state as a liquid under mild pressure or mixed with carbon dioxide or the fluorinated

hydrocarbons. The other gases, which are inert biologically, are added to make a nonflammable mixture. As little as 3 percent of ethylene oxide vapor in air will support combustion and will have explosive violence if confined. When 90 percent carbon dioxide is present (Jones and Kennedy, ref. 17), or about the same percent of various fluorinated hydrocarbons (refs. 18 and 19), the resulting mixture can in turn be mixed with air in all proportions without passing through a flammable range. For safety reasons these diluted mixtures are usually used in large chambers, although occasionally a vacuum is first pulled and then either pure (Alguire, ref. 20) or still flammable ethylene oxide mixtures are admitted. Carbon dioxide is a cheaper diluent, but because of its high vapor pressure its mixture must be contained in expensive steel cylinders. The fluorinated mixtures, although more expensive pound for pound, can be kept in inexpensive throwaway metal containers. Also, they do not exert so great a total pressure for the same concentration of ethylene oxide when expanded within an exposure cabinet. The author has suggested that ethylene oxide be considered as closely resembling ordinary diethyl ether insofar as toxicity and flammability of the pure compound is concerned.

The sporicidal action of ethylene oxide vapor is slow, as the data in table I show. The effect of time, temperature, and concentration upon the rate of sterilization of ethylene oxide is quite straightforward, as the data indicate. Doubling the concentration (or vapor pressure) will permit sterilization in roughly half the time. The temperature coefficient is also what one would expect; each 10° C temperature increase a little more than doubles the activity. However, at higher temperatures and concentrations where sterilizing times are short, these factors become less important and the time required to penetrate becomes predominant (Ernst and Shull, ref. 21). The effect of moisture, however, is more complicated (Kaye and Phillips, ref. 22; Phillips, ref. 23; Ernst and Shull, ref. 24; Gilbert, Gambill, et al., ref. 25) and has frequently been a source of trouble (Opfell et al., ref. 26; Znamirowski et al., ref. 27). The high relative humidities needed with the other gases (essentially 100 percent relative humidity for formaldehyde or over 70 percent relative humidity for *Beta*-propiolactone) are not required with this compound. Ethylene oxide sterilization is most rapid at about 30 percent relative humidity and becomes progressively slower as the relative humidity increases to 100 percent. Care must be exercised, however, when the objects to be sterilized (or more correctly, the microorganisms contained on them) are equilibrated to lower relative humidities, or have been previously exposed to extremely desiccat-

TABLE I.—*Time Required for Sterilization of* B. subtilis *var.* niger *Spores on Cotton Cloth With Ethylene Oxide Gas*

[From ref. 28]

Concentration of gas, mg/liter or oz/1000 cu ft	Spores recovered[a] after exposure time, hr, of—									
	½	1	2	4	6	8	10	24	48	72
	Temperature, 37° C									
22.1	----	----	+++	++	+	0	0	0	----	----
44.2	----	----	++	+	0	0	0	0	----	----
88.4	----	+++	+	+	0	0	0	0	----	----
442	0	0	0	0	0	----	----	0	----	----
884	+	0	0	0	0	----	----	0	----	----
	Room temperature, 25° C									
22.1	----	----	+++	+++	+++	+++	+++	+	----	----
44.2	----	----	+++	+++	++	++	+	0	----	----
88.4	----	----	++	++	+	+	0	0	----	----
442	+++	++	+	0	0	----	----	0	----	----
884	+++	++	0	0	0	----	----	0	----	----
	Temperature, 5° C									
22.1	----	----	----	----	----	----	----	+++	+++	+++
44.2	----	----	----	----	----	----	----	+++	+++	+++
88.4	----	----	----	----	----	----	----	+++	++	+
442	----	----	----	----	----	----	----	0	0	0
884	----	----	----	----	----	----	----	0	0	0

[a] Key: +++ Over 1 percent recovered.
++ Between 0.1 and 1 percent recovered.
+ Less than 0.1 percent recovered.
0 No organisms recovered.

ing conditions. Not only is sterilization more difficult at relative humidities below 30 percent, but once micro-organisms have been highly desiccated either chemically or by vacuum they acquire a resistance that is not completely overcome when the relative humidity is again raised to 30 percent. Not all of the organisms are resistant, but a few maintain their resistance until they are essentially rewetted. The phenomenon is not well understood, but it is real and appears as a problem most often when one attempts to sterilize lyophylized material or material that has been previously subjected to high vacuum or heat.

The mechanism by which ethylene oxide kills micro-organisms has been linked to its chemical activity as an alkylating agent (Phillips, refs. 28 and 29). An older theory, which held that ethylene oxide activity resulted from its conversion by hydrolysis to ethylene glycol (Gross and Dixon, ref. 30), was contested by the simple fact that the latter compound had a lesser bactericidal activity (Yesair and Williams, ref. 31; Wilson and Bruno, ref. 32). According to the alkylation theory, ethylene oxide replaces labile hydrogen atoms with hydroxy ethyl ($—CH_2CH_2OH$) groups, thus blocking many reactive groups needed in essential metabolic reactions. Fraenkel-Conrat (ref. 33) showed that with the protein keratin —COOH, $—NH_2$, —SH, and —OH groups could all be attacked in this manner.

Phillips (ref. 29) noted that ethylene imine, methyl bromide, and formaldehyde are also active alkylating agents. Taken as a group, these compounds all have the interesting bactericidal property of attacking bacterial spores almost as readily as they do vegetative organisms. Chlorine, heavy metals, phenol, and quaternary ammonium compounds are 1000 to 10 000 times more active against vegetative organisms than against spores. However, with the alkylating agents the resistance of spores averages not more than 5 to 10 times that of vegetative cells. Toth (ref. 34) states that spores are about only twice as resistant to ethylene oxide as are vegetative bacteria. If the alkylating mechanism is correct, this phenomenon could be explained by other pathways such compounds could use, attacking any reactive group that contains a replaceable hydrogen atom. If the sulfhydryl groups, which could be the main point of attack by the other groups of disinfectants, were protected by spore formation, alkylating agents could still react with groups such as carboxylic or amino, which happen to be free. More information is needed, however, on the internal structure of complex macromolecules and the exact reactions taking place between disinfectants and these molecules before more can be done than hypothesize on mechanisms of action from known facts

in simpler organic reactions. Shull (ref. 35) points out that ethylene oxide treatment also interferes with spore germination.

The advantages of ethylene oxide sterilization lie not in the speed, simplicity, or economy of the treatment but rather in the fact that many types of materials are sterilized with least damage to the material itself when this technique is used. Newman, Colwell, and Jameson (ref. 36), who were concerned about articles made by patients in the occupational therapy room in a tuberculosis hospital, reported that leather, wool, cotton, rayon, silk, felt, nylon, paper, plastics, wood, straw, oil and watercolor paintings, pottery, metals, semiprecious stones, linoleum, cellophane, metallic textiles, and rubber were undamaged by ethylene oxide treatment. Other materials reported successfully treated include soil (Clark, ref. 37; Allison, ref. 38) ; laboratory plastics (Engley, ref. 39; Bruhin et al., ref. 40) ; medical and biological preparations (Griffith and Hall, refs. 8 to 10) ; hospital bedding and books (Kaye, ref. 41) ; plaster bandages (Wilson, ref. 42) ; various types of medical instruments (Skeehan et al., ref. 18; Bekker and Onvlee, ref. 43; Spencer and Bahnson, ref. 44; McCaughan et al., ref. 45) ; finely powdered material (Masci, ref. 46; Mayr and Kaemmerer, ref. 47) ; and surgical transplants (Hufnagel et al., ref. 48). Ethylene oxide has also found application in surface sterilization of interplanetary vehicles in the space program (Phillips and Hoffman, ref. 49).

A note of warning has been sounded in the ethylene oxide sterilization of certain foodstuffs, particularly those used in experimental animal diets. This was first noted by Hawk and Mickelsen (ref. 50) at the National Institutes of Health; they observed that rats failed to grow when fed special purified diets treated with ethylene oxide. Several papers which followed from that laboratory showed that various essential vitamins and amino acids were attacked and it finally appeared that tertiary nitrogen in ring structures was the main point involved (Windmueller et al., ref. 51). Apparently a reduction or destruction of certain essential nutrients occurs when whole diets are treated; it does not appear that any toxicity is imparted to the foodstuffs by the ethylene oxide treatment. In the latter connection, the earliest application of ethylene oxide sterilization was in the foodstuff industry (Hall, refs. 52 and 53; Yesair and Williams, ref. 31), where spices, in particular, have been so treated commercially as to render them "essentially sterile" for over two decades. Gordon, Thornburg, and Werum (ref. 54) using C^{14}-labeled ethylene oxide on dried prunes showed that half of the reacted ethylene oxide was attached by alkylation to undigestible cellulose molecules and that the glycols formed were only about 0.002 percent of the dried weight and so

contributed little toxicity. Microbial culture media, moreover, have been reported to support growth following ethylene oxide sterilization (Hansen and Snyder, ref. 55; Wilson and Bruno, ref. 32; Judge and Pelczar, ref. 56), as have diets for insect larvae (Barlow and House, ref. 57) and tissue culture media (Brown and Fuerst, ref. 58). In these cases, however, two factors may have contributed to the success with the experimental diets. The dietary requirements of the organisms involved were less exacting, and in several cases portions of the diet, such as supplementary vitamins, were not added until after the ethylene oxide treatment.

It is not surprising, with a chemical as active as this, that many compounds will react to form products whose biological activity is lessened or destroyed. For example, Kaye et al. (ref. 59) showed that, although penicillin could be sterilized without apparent change, a loss in potency occurred when streptomycin was treated. Just as the stability of food nutrients and biologicals to heat and other processing treatments must be determined individually, so also must their stability to the action of ethylene oxide. It is not enough in this field to show that no toxicity is produced by the treatment. The absence of any loss in biological activity must also be shown.

A wide variety of equipment has been used for ethylene oxide treatments. The vacuum desiccator has often been used in small-scale laboratory experiments. Any airtight container into which the gas can be admitted is suitable; however, when large amounts of material are to be treated, special equipment has usually been devised. Kaye, Surkiewicz, and Jacobson presented a paper before the American Chemical Society in 1952 (not since published) on various types of pressurized and nonpressurized fumigation chambers and on the use of gastight bags and tarpaulins. The Griffith and Hall patents (refs. 8 to 11) are based on treatment in large vacuum chambers from which air is removed and then pure ethylene oxide is admitted. In pressure chambers, ethylene oxide-carbon dioxide mixtures can be added without prior removal of air. Autoclaves can easily be converted to such use (Newman et al., ref. 36; Skeehan et al., ref. 18; Spencer and Bahnson, ref. 44; Schley et al., ref. 60). General acceptance of ethylene oxide sterilization in hospitals and various industries, however, followed the introduction of commercially available automatic equipment for its use (Perkins, ref. 61; Gewalt and Fischer, ref. 62; McDonald, ref. 63), even though there was some controversy in the literature about the efficiency of certain units (Linn, ref. 64; Walter and Knudsin, ref. 65; Znamirowski et al., ref. 27).

The penetrating ability of this gas is remarkable, the gas being

quite unlike formaldehyde in this respect. Phillips (ref. 66) gave data published in abstract form showing that cotton cloth impregnated with bacterial spores and placed inside unsealed paper envelopes could be sterilized by exposure to ethylene oxide vapor at about 450 mg/liter concentration and at room temperature in 4 to 6 hours of exposure time, even though the envelopes were set between many layers of clothing. Lorenz et al. (ref. 67) reported that the gas penetrated through unbroken egg shells, sterilizing the contents within. With vacuum treatment, penetration is achieved through whole barrels of spice (personal communication with McCormick & Co.).

There are limits to this ability to diffuse and penetrate, however. Phllips (ref. 66) reported a simple experiment in which 5-milliliter samples of a suspension of *B. subtilis* spores in distilled water were placed in a petri dish and in a test tube, respectively, and exposed simultaneously to ethylene oxide vapor. At the end of 4 hours the suspension in the petri dish was sterile, but the count of the suspension in the test tube was reduced only about 50 percent; this indicates that the gas diffused through the shallow layer of water in the petri dish, but only about halfway down through the liquid in the test tube. This ability of the gas to diffuse and penetrate so well through various types of barriers also presents a problem in its practical application; exposure cabinets or chambers must be very tightly sealed if the concentration is to be maintained for the several hours usually required for treatment. It is usually impossible to make an ordinary room tight enough to retain the gas; for this reason, as well as because of cost factors and flammability hazards, formaldehyde or *Beta*-propiolactone rather than ethylene oxide is used in fumigating large interior spaces.

The same ability of ethylene oxide to diffuse and penetrate rapidly results in a very quick loss of any residual ethylene oxide after the objects being treated are removed from its atmosphere. It is quite unlike formaldehyde in this respect. Many solid objects can be wrapped in paper and tied loosely before treatment, much as objects are prepared for autoclaving. Penetration of the gas through the wrapping presents no problem, and when the objects are removed from treatment the residual gas quickly dissipates. The paper covering them allows the object to be handled without becoming recontaminated. Exceptions to the quick loss of residual ethylene oxide following treatment have been found with rubber and certain plastics. Ethylene oxide is soluble to a certain extent in these organic solids and quite some time is required for the gas to diffuse back to their surfaces and out to the atmosphere. The author has found as much as 4 percent ethylene oxide present in

rubber immediately following sterilization. Measurable amounts still remained after 5 hours' airing, but none was present 24 hours following treatment. Laboratory personnel have received chemical burns by donning rubber shoes only an hour or so after they were sterilized. Presumably, certain organic liquids or oils could similarly retain ethylene oxide for appreciable lengths of time. The resistance of plastic films to ethylene oxide penetration has been measured by Dick and Feazel (ref. 68).

Many workers have reported directly or by implication that sterilization is not greatly hampered by the presence of large amounts of extraneous materials present during treatment. The success in sterilizing eggs, biological media, and soil already referred to are indications of this fact. Phillips (ref. 66) mentioned that dry bacterial spores mixed with materials such as raw eggs, feces, vaseline, and motor oil could be sterilized by exposing the mixture to an atmosphere containing ethylene oxide, provided that the depth through which the gas had to diffuse was not too great.

Ethylene oxide is apparently effective against all types of microorganisms. The older literature, containing references to its effect on many types of bacteria and molds, is covered in the review of Phillips and Kaye (ref. 7). Since then Ginsberg and Wilson (ref. 69) have reported on the activity of ethylene oxide solutions against several viral agents, and Mathews and Hofstadt (ref. 70) on the effect of the gas on 15 animal viruses as well as on several bacteria and fungi. Other viral agents reported to be sensitive to ethylene oxide include foot-and-mouth disease virus (Savan, ref. 71; Fellowes, ref. 72; Tessler and Fellowes, ref. 73), Columbia S.K. virus and vaccinia (Klarenbeek and van Tongeren, ref. 74), and Eastern equine encephalomyelitis virus (Bucca, ref. 75). Wilson and Bruno (ref. 32), Newman et al. (ref. 36), and Virtanen (ref. 76) reported on the sensitivity of *Mycobacterium tuberculosis*, as did Kaye (ref. 41) for the BCG strain of that organism. Friedl et al. (ref. 77) reported that five anaerobic and five aerobic spores were destroyed as measured by the A.O.A.C. sporicidal test. All indications to date are that the effect of ethylene oxide is irreversible; that is, bactericidal rather than bacteriostatic.

One application for ethylene oxide, although not exactly gaseous sterilization, should be mentioned. This is the technique, first reported by Wilson and Bruno (ref. 32), of treating liquids in cotton-plugged flasks by adding a small amount of liquid ethylene oxide, usually 1 to 5 percent. This is done in the cold, and when the flasks are removed to room temperatures, or 37° C, the ethylene oxide dissipates as a gas through the plug, leaving the flask and its contents sterile. This technique, rather than a strictly gaseous

procedure, has been used in several laboratories. An interesting application is its use by Polley (ref. 78) and Wolfe and Sharpless (ref. 79) in vaccine production to kill micro-organisms without destroying their antigenic properties, and by Brown and Fuerst (ref. 58) to sterilize tissue culture media.

One interesting development has been the work of Royce and Bowler (ref. 80), who developed an indicator to place in an ethylene oxide sterilizer to tell whether conditions were such that sterilization of the load should have occurred. These indicators are sachets, small sealed plastic envelopes containing a concentrated salt solution and a pH indicator. The ethylene oxide penetrates the plastic envelope and reacts with the salt to produce an alkaline reaction product; this, in turn, causes the indicator to change color. Depending upon the size and thickness of the plastic envelope and the salt concentration, the sachets can be designed so that the color changes only when the ethylene oxide concentration and exposure time are sufficient for sterilization to have occurred. The main advantage of this device over spore strips is that an immediate answer is obtained, rather than one some 24 hours later, after the spore strips have been checked biologically for sterility. However, this is an indirect rather than a direct measure of sterility, and spore strips are still more widely used as sterility indicators.

Beta-propiolactone

The latest compound to achieve importance as a gaseous sterilant is *Beta*-propiolactone:

This compound is a colorless liquid at room temperature, freezes at –33.4° C, and boils, with decomposition, at 162° C. Its saturated vapor concentration at 25° C is only 8.1 mg/liter as opposed to 3140 mg/liter for ethylene oxide at the same temperature. It is denser than water, its specific gravity is 1.1490 at 20/20° C, and it is soluble in water to the extent of 37 percent by volume at 25° C. It hydrolyzes moderately quickly to form *Beta*-hydroxypropionic acid (hydracrylic acid); the half life of a 1-percent aqueous solution is 3 to 4 hours at room temperature. It has a sharp odor not unlike acetic acid, which is quickly accompanied by marked irritation of the respiratory tract, and it is strongly lacrimatory at quite low concentrations. Since about 1950 it has been available commercially in bulk lots; the greater part of the production is used as an intermediary for plastics.

The first published report on the bactericidal and virucidal activity of *Beta*-propiolactone was by Hartman, LoGrippo, and Kelly in 1954 (ref. 81). They were members of a research group at the Henry Ford Hospital in Detroit who were screening a large number of chemical compounds which could be used to sterilize blood or blood plasma. They subsequently published extensively on various medical applications of *Beta*-propiolactone and on its activity in aqueous solution. The U.S. Army Biological Laboratories, which helped to support the screening program at the Henry Ford Hospital for new bactericidal compounds, quickly determined that this compound was also bactericidal in the vapor state. This was followed by several years of investigation which resulted in the publication by Hoffman and Warshowsky (ref. 82) of quantitative measurements of the effect of temperature, concentration, and relative humidity on the antibacterial activity of *Beta*-propiolactone vapor, together with information on toxicity and methods of analysis and some references to practical experiments on decontaminating building interiors. Most of these experimental data were obtained with the chemically resistant spores of *B. subtilis* var. *niger (B. globigii)* but later from the same laboratories Dawson et al. (refs. 83 and 84) showed that the vapor was even more toxic to a series of viral and rickettsial agents. Other practical applications of this compound were reported by Spiner and Hoffman (ref. 85), Woodward and Clark (ref. 86), Husmann (ref. 87), and Bruch (refs. 88 and 89).

The basic findings can be summarized as follows. The compound acts very rapidly in the vapor state at quite low concentrations. The relative humidity should be 70 percent or higher. A 1.5-mg/liter concentration of the vapor in air will sterilize cotton patches containing more than 1 million spores of *B. subtilis* var. *niger* in less than 30 minutes at 25° C. Even at −2° C this can be achieved in 3 hours. This led Hoffman and Warshowsky (ref. 82) to point out that it could be considered 4000 times more active than ethylene oxide, a figure quoted several times since without always mentioning that the authors went on to say that this did not imply that it could be generally substituted for ethylene oxide. They stated that it was more likely to replace formaldehyde as a gaseous sterilant.

Phillips (ref. 90) pointed out that comparisons of relative effectiveness of two sterilants or disinfectants on the basis of one or two measurements, such as speed of reaction, could be quite misleading and he used the comparison of the utility of ethylene oxide and *Beta*-propiolactone as a case in point. These two are scarcely competitive in any of their practical applications although

they have closely similar chemical structures. Both are small-ring cyclic compounds containing one oxygen atom in the ring, and both are effective gaseous sterilants, each one apparently acting as an alkylating agent. Their other physical properties are quite different, however; their volatility and penetrability through porous substances differ greatly, for example. One might expect a three-membered ring to be less stable than a four-membered one, but the latter is the more easily split and the more reactive.

Because of this much faster action of *Beta*-propiolactone, it is necessary to maintain it at a concentration of from 1 to 2 mg/liter in air for only 2 hours at room temperatures to sterilize all readily available surfaces in large enclosed areas. Many disseminators are not entirely efficient, however, and the vapor concentration in air rapidly drops if the space contains a large quantity of highly absorptive material such as rugs, drapes, or overstuffed furniture. Hoffman and Warshowsky (ref. 82) therefore recommended that 1 gallon of *Beta*-propiolactone be disseminated for each 16 000 cubic feet of space, which would produce a theoretical concentration in air of 9.6 mg/liter if none of the vapor was lost. This concentration is a little stronger than a saturation concentration of 8.1 mg/liter at 25° C; however, theoretical concentrations are never reached in practice, as mentioned above. Even if more liquid were disseminated, no more than this could evaporate into the air. It is probable that future recommendations may be the use of 1 gallon of *Beta*-propiolactone per 20 000 or 25 000 cubic feet. At saturation level the vapor concentration is still below the flammable range so that it is not necessary for safety reasons to dilute *Beta*-propiolactone with an inert gas such as carbon dioxide or fluorinated hydrocarbon, as was required by ethylene oxide. Also, since the vapor has very poor penetrating properties, it is sufficient to cover all openings into the room and seal any sizable cracks with masking tape to maintain the required concentration for the 2 hours needed for treatment.

There are several precautions that should be observed. The compound is markedly dependent upon relative humidity, which must be 70 percent or higher if the treatment is to be effective. If the rooms or spaces being treated are drier than this, water vapor as well as *Beta*-propiolactone must be added to the room. No more should be added than necessary, because a relative humidity in excess of 100 percent will cause water and chemical to condense on surfaces. Also, if the area is large and the chemical is disseminated in only one location, fans or some other means must be used to circulate the air or saturation vapor concentrations will be built up near the generator and the excess liquid will not be able to

evaporate, while at remote locations concentrations may not rise to the 1 to 2 mg/liter required for sterilization. Finally, care should be taken that the *Beta*-propiolactone used is relatively pure. Although initial purity as received from the manufacturer is usually about 98 percent or higher, 15 percent or more polymer may build up without any noticeable change in appearance if the liquid stays in its container for a year or so at room temperature. When disseminated as a fine spray this polymer will not evaporate, but will settle as a sticky coating on nearby surfaces. This is difficult to remove because it is not water-soluble. For this reason *Beta*-propiolactone should be kept refrigerated or should be analyzed chemically before old lots are used.

Used in this way *Beta*-propiolactone has been employed to disinfect Army barracks, hospital day rooms, animal housing buildings (Spiner and Hoffman, ref. 85), Army hospital wards (Woodward and Clark, ref. 86), operating rooms, maternity wards, and a vaccine production building (Bruch, ref. 88). The treatment was quite effective in all cases. Available surfaces were almost universally sterile, although areas protected by absorbent material, such as samples taken from under the rug in the hospital day room, were often nonsterile even though the count was greatly reduced. Following treatment it was usually possible to ventilate the space so that the vapor concentration fell below the lowest detectable limit for man (about 0.05 mg/liter; ref. 85) within an hour or so. Even in poorly ventilated quarters from which the vapor did not readily diffuse, it would hydrolyze to the nonirritating and nontoxic *Beta*-hydroxypropionic acid in time.

Thus considerable time is saved in a *Beta*-propiolactone treatment over one with formaldehyde because the period of treatment is shorter (2 instead of 10 hours) and because its faster dissipation rate allows the premises to be occupied in a much shorter time after treatment. Residual formaldehyde is often noticed for days after treatment; the concentrations remain irritating even though they may be tolerable.

Although *Beta*-propiolactone has proved highly satisfactory in the hands of experienced operators, it has not yet achieved widespread routine usage. One reason for this is the unavailability of commercial disseminating devices with complete operating instructions. Spiner and Hoffman (ref. 85) and Woodward and Clark (ref. 86) used insecticide aerosol disseminators that, although not ideal, were adapted to *Beta*-propiolactone usage. One problem with such devices is that they often do not produce aerosol droplets small enough to vaporize before they settle out. If the liquid settles on painted surfaces or plastics, they can be damaged as they are

with liquid droplets of ethylene oxide. The work of Bruch (ref. 88) utilized a generator especially designed for *Beta*-propiolactone dissemination; however, only a few experimental models were made and they were not made available commercially.

A second, and more important factor that has restricted the use of *Beta*-propiolactone is its toxicity and the fact that adequate safety precautions have not been developed that will permit its being used by relatively untrained personnel. It is really lack of agreement on this point that has held back the introduction of commercial disseminating equipment. As mentioned earlier, the compound is not only a respiratory irritant but is a strong lacrimator as well. Furthermore it is a vesicant; droplets spilled on the skin produce large watery blisters in a matter of hours. These problems could probably be tolerated because the rapid and painful effect would cause workers to treat the compound with respect as they do other primary irritants such as strong acids. The main item of toxic concern, however, came from reports that the compound is carcinogenic and produces tumors and papillomas when applied repeatedly to mouse skin (Walpole et al., ref. 91; Roe and Glendenning, ref. 92; Palmes et al., ref. 93). *Beta*-propiolactone probably has the lowest molecular weight of the organic compounds in which this property has been clearly demonstrated. The primary problem is that of determining safe threshold limits for man from animal data only. It is quite possible that the carcinogenic levels for men are above those at which the compound is directly irritating and hence would be naturally avoided, but this cannot be proved. No cases have been reported in the literature of cancer in man directly attributable to this compound, even though quite a large number of investigators and plant workers have had repeated minimal and occasional heavier exposures. Some of these cases extend over two decades, but this negative information does not establish safe limits. The concern is with the repeated exposures of personnel who routinely used the compound, particularly if they are careless or not well trained.

LoGrippo (refs. 94 and 95) in discussing this point has shown that the decomposition products of *Beta*-propiolactone are harmless; certainly, many pints of human blood plasma treated with the compound and containing its hydrolytic products have been injected directly into the bloodstream with no harmful results. There is no reason to suspect that there would be any lingering toxic effect in a treated room once the area has been aired and any residue hydrolyzed. The use of the compound by trained personnel has continued, particularly for treating laboratory areas where immediate hazards exist because of the presence of viable micro-orga-

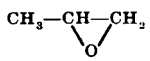
nisms infectious to man. As LoGrippo points out (ref. 94), this compound has unique properties that enable it to be used in certain applications for which no adequate substitute is known. It should be useful in many more routine medical or veterinary applications once safety problems are resolved.

Other Gases

Many other gases have been reported in the literature to possess bactericidal properties. The early use of sulfur dioxide and chlorine has already been mentioned together with reference to their corrosive action, which limited their application. Some of the other gases will now be discussed.

Propylene Oxide

Propylene oxide



was investigated by Phillips (ref. 28) and Kaye (ref. 96), who found it similar in properties to ethylene oxide but less volatile and less active biologically. Its use was overshadowed by that compound until interest in propylene oxide was renewed as a result of the 1958 Food Additive Amendment to the Food, Drug, and Cosmetic Act, under which new applications of ethylene oxide in the foodstuff industry were discouraged because of the toxicity of ethylene glycol, which appeared as a hydrolytic product in small but detectable amounts (Gordon et al., ref. 54). Propylene oxide hydrolyzes to nontoxic propylene glycol and its use was allowed. The compound was systematically studied by Bruch and Koesterer (ref. 97) and the situation was discussed at some length by Bruch in reference 89. It was reported to be like ethylene oxide in that it was more active at moderate (25 percent) than higher (85 percent) relative humidities. These results were questioned by Himmelfarb et al. (ref. 98), but the latter authors desiccated their samples prior to readmitting water vapor to the system in the desired amount, and they may have been encountering the same puzzling rehydration phenomenon mentioned earlier in connection with ethylene oxide (Phillips, ref. 23; Gilbert et al., ref. 25). Alguire (ref. 20) also discussed propylene oxide and ethylene oxide sterilization of foodstuffs, and Masci (ref. 46) was granted a patent on both propylene oxide and ethylene oxide sterilization of crystalline powders.

Ozone

Ozone, O_3, has often been mentioned as a bactericidal agent, and it has found some application in preventing mold formation in

such places as food storage rooms that are inclined to be damp. Elford and Van den Ende (ref. 99) reported that although ozone killed off airborne organisms, it had little effect upon organisms on surfaces or on organisms protected by organic matter. Ingram and Haines (ref. 100) reported upon the inhibition of bacteria in broth or on agar surfaces exposed to ozone vapor. The former authors used very low concentrations, around 0.04 part per million or less than 0.0001 mg/liter, in attempting to learn whether concentrations tolerable to man would exhibit a bactericidal effect. Ingram and Haines reported that concentrations as high as 4000 parts per million, or about 5 mg/liter, are necessary to inhibit growth of established broth cultures in certain cases. Warshaw (ref. 101) reported on the use of ozone at 200 parts per million for 20 minutes to reduce bacteria to noninfectious levels on polarized glasses used with 3-D movies. Germann and Panouse-Perrin (ref. 102) reported that viral aerosols were inactivated by as little as 10 parts per million by volume of ozone in about 5 minutes. They did not investigate surface sterilization. Thomas (ref. 103) reported that a synergistic effect was achieved when ozone was used following a brief ethylene oxide exposure for treating medical equipment such as artificial heart-lung machines. In any case, high concentrations of ozone in air are difficult to generate, and the compound is both a very strong oxidizing agent (particularly damaging to rubber) and quite unstable. To date, the main applications of ozone have been in water purification.

Methyl Bromide Gas

Methyl bromide gas, CH_3Br, was shown by Kolb and Schneiter (ref. 104) to be bactericidal for anthrax spores and was recommended by them for the sterilization of imported wool and hair, in particular. Trickel (ref. 105) and Munnecke et al. (ref. 106) reported that it was effective against fungi. Saiki (ref. 107), who was interested in disinfecting ship compartments, reported methyl bromide to be effective, but less so than chloropicrin or formaldehyde, in destroying intestinal pathogens. He, as others had done earlier, reported no measurable antimicrobiological activity for HCN gas, in spite of its known biological activity against higher forms of life. Unpublished data obtained by D. L. Jones in this laboratory confirm these findings concerning methyl bromide. The activity of the compound was measured as about one-tenth that of ethylene oxide. Methyl bromide has the decided advantage of nonflammability, however, and has about the same order of activity as Carboxide, the diluted form of ethylene oxide that must be used in many applications where flammability is a hazard. Whether it is

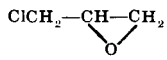
as noncorrosive and nondamaging as ethylene oxide is not fully known as yet because of its limited application.

Chloropicrin Gas

Chloropicrin gas, Cl_3CNO_2, has been mentioned by several authors as having bactericidal properties, and it has found some application as a soil fumigant, where, even if complete sterilization of the soil is not achieved, many phytopathological fungi are destroyed. Saiki (ref. 107) commended chloropicrin over formaldehyde and methyl bromide in the fumigation of ship compartments. Fifteen grams inserted into a 15-cubic-meter compartment sealed with paper destroyed *Vibrio cholera, Shigella dysenteriae,* and *Salmonella typhosa* and *paratyphosa* A in 6 hours. Twenty grams under the same conditions destroyed *paratyphosa* B in the same time.

Epichlorohydrin and Epibromohydrin

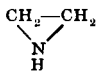
Epichlorohydrin



and epibromohydrin were somewhat more active than ethylene oxide in studies reported by Phillips (ref. 28) and Kaye (ref. 96) on several compounds related structurally, but they have not been studied to any great extent.

Ethylene Imine

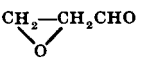
Ethylene imine



was reported in these screening trials to be many times more active than ethylene oxide, and Mayo et al. (ref. 108) reported to the Society of American Bacteriologists on data not subsequently published in detail that under certain conditions (i.e., high relative humidity) it had more than a hundred times the activity of ethylene oxide. It, like formaldehyde, is markedly dependent upon high relative humidity, however, and moreover it is both flammable and corrosive, particularly to many metals. Its high order of activity may perhaps permit it to be used at concentrations low enough so that these latter factors are not particularly objectionable, but to date it has been put to little or no practical application.

Glycidaldehyde

Glycidaldehyde



was reported by Dawson (ref. 109) to be highly bactericidal in the

vapor state. Its effects on materials have been insufficiently investigated, and there are no reports of its being put to practical use.

Peracetic Acid

Peracetic acid

has found considerable application both as an aqueous solution and as an aerosol or vapor in germ-free or gnotobiotic research (Greenspan et al., ref. 110; Barrett, ref. 111; Doll et al., ref. 112), where its corrosiveness as both an acid and a strong oxidizing agent are circumvented by the use of corrosion-resistant stainless steel, glass, and plastics as construction materials for animal isolators and other equipment. Its advantages lie in its quick action, even against resistant spores, and the fact that its decomposition product is the nontoxic and volatile acetic acid. Caution must be exercised against keeping the compound or solutions of it too long in tightly closed containers where the pressure buildup of released oxygen may cause the containers to rupture or explode.

Concluding Remarks

The search for new gaseous disinfectants is continuing, and new compounds or new applications for compounds previously reported as having bactericidal activity in the vapor state may be expected. The truly ideal gaseous sterilizing agent is yet to be found, and it is doubtful that it ever will be. Different requirements call for compounds of differing properties; it is probable that no single compound will possess them all.

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Filter Applications for Spacecraft Sterilization Program

The importance of sterilization for unmanned spacecraft is well illustrated by the fact that at least two scientific societies devoted a half day or more to the subject at their annual meetings this year. The American Society of Microbiology included eight papers on some of the microbiological aspects of spacecraft sterilization at their April meeting, and the American Association for Contamination Control included nine papers on the subject of microbiological contamination at their May meeting. That there is a need for removal of particulates, either biological or inert, is further substantiated by the fact that reference 1 establishes Federal specifications for clean-room and work station requirements. As an example, this specification requires that a class 100 clean room cannot exceed 100 particles per cubic foot of air in the 0.5- to 4-micron range (fig. 1). Every capsule or lander for Mars should be sterilized and handled with procedures to assure that the probability of a single viable organism being aboard is not over 10^{-4}.

The sum and substance of all these sessions and regulations is that a conscientious effort must be made to reduce the number of particles both in the air of assembly room facilities and on the surfaces of spacecraft hardware in order to lessen the microbial load that the sterilization cycle must overcome. It is also appropriate to consider air filtration during manufacture, assembly, and liftoff of the spacecraft for removal of inert particulates which otherwise might cause malfunction of intricate components.

Air filtration is already in use in clean rooms and in the overall missile program to aid in controlling contamination. In Ranger flights 3, 4, and 5, the final surface sterilization of the space vehicle was performed by injecting a mixture of ethylene oxide and Freon underneath the shroud. Because of its heavy weight the gaseous mixture was replaced with nitrogen, a lighter gas, prior to the spacecraft's takeoff. In order to assure that the supply of nitrogen

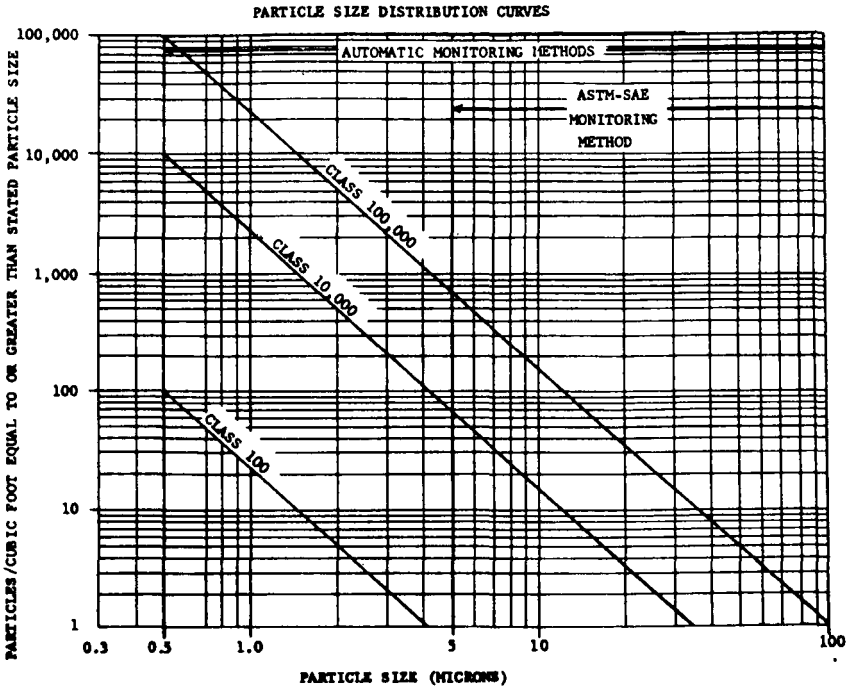

FIGURE 1.—Clean-room and work-station requirements; controlled environment. (Graph taken from ref. 1.)

was sterile, a fiber-glass filter was used to filter the nitrogen before it entered the shroud covering the spacecraft.

Discussion

The discussion of filtration is divided into two phases: air and liquid. Since the authors' experience has been widest in the field of air filtration as a method of removing bacteria from the air, this phase receives greater emphasis than liquid filtration.

Air Cleaning by Filtration

Few practical air filters depend upon screening or sieving action because the interstices of a screening-type filter would have to be smaller than the smallest particles to be removed and the resistance to airflow would be high. As the surface becomes covered with suspended material, resistance rises, and ultimately airflow stops as all of the interstices become plugged. All practical aerosol filters consist of fibers of various materials oriented in such a manner that most of the open spaces or interstices are much larger than the diameter of the particles to be removed. The filtering action

depends upon the particles coming in contact with and adhering to the fibers or collecting surfaces.

There are several collection mechanisms that may cause suspended aerosol particles to impact on the fibers. They are inertial effect, diffusion, electrostatic effect, direct interception, and deposition in accordance with Stokes' law. Direct interception and deposition are of less effect in removing particles by filtration than are the other three mechanisms.

The filters used in biological air cleaning are divided into five categories according to their efficiency and use. These categories are roughing filters, medium-efficiency filters, high-efficiency filters, ultra-high-efficiency filters, also currently classified as high-efficiency particulate air filters (HEPA), and filters for complete filtration. Roughing filters are usually composed of materials such as loosely packed fibers of animal hair, synthetic fibers, or woven metal screens. Medium-efficiency filters may consist of compressed glass, asbestos, cellulose, or other types of fibrous media. High-efficiency filters contain chiefly glass fibers, a good grade of fiber paper, or asbestos fibers, with the diameter of the fiber usually ranging from 1 to 5 microns. Ultra-high-efficiency filters are generally constructed of fire-resistive glass or glass-bearing media. (See fig. 2.) Frames, separators, and cements are incombustible, incorporate fire-suppressing chemicals, or have been impregnated to resist spread of fire. In addition to these properties, filters can be obtained that are resistant to chemicals and high humidities. Filters classified as ultra-high-efficiency achieve maximum removal of small biological particles and are used in pharmaceutical, electronic, and spacecraft industries.

The last category of filters (fig. 3) includes those filters that for all practical purposes can be classified as providing complete filtration. A filter of this type evaluated recently contained a special glass microfiber web with a mean fiber diameter of 0.1 micron.

The data on the performance of filters, filter media, and other air cleaning devices sometimes vary considerably from the performance claimed by manufacturers, usually because of differences in the methods of evaluation. Performance of filters discussed in this paper is rated on the basis of biological test procedures developed by the authors. Most of the filter evaluations in the past have been based on test methods that at times do not correlate closely with the results obtained by using viable bacterial particles.

The system shown in figure 4 is a typical test arrangement that permits accurate determination of the bacterial arrestment of filters or media prior to installation in an air filtration system. The

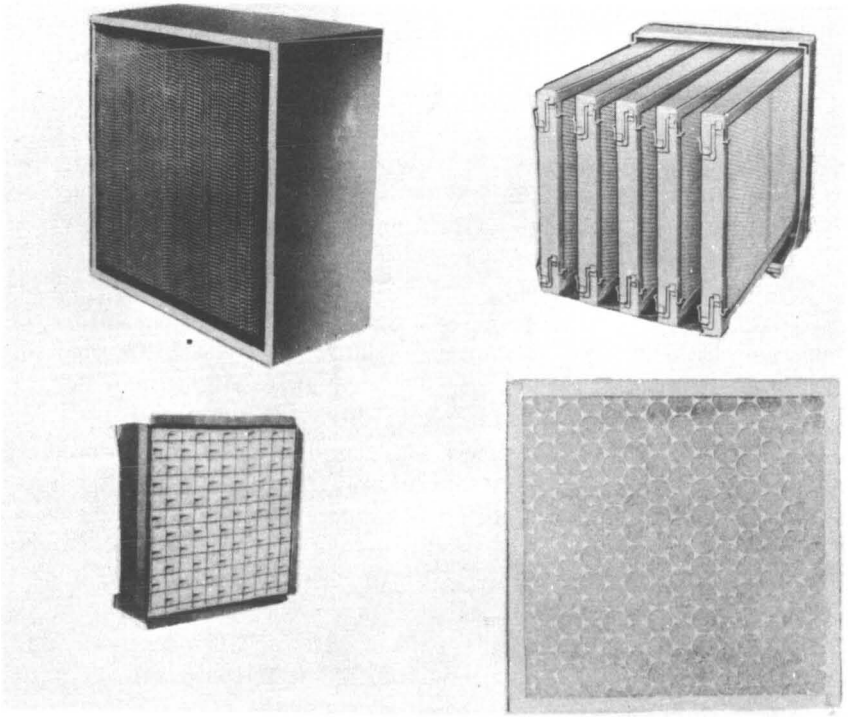

FIGURE 2.—Types of air filters.

test system is simple and can be set up quickly. The bacteria, usually *B. subtilis* var. *niger* spores, are nebulized into a chamber where the cloud of bacteria is mixed with additional air. The aerosol is then drawn into the duct through the filter under evaluation at the rated face velocity and then exhausted through a blower to the outside. Samples of the aerosol are taken before and after passing the filters. If the test filter is not of the ultra-high-efficiency type, it may be advisable to place an ultra-high-efficiency filter in the blower discharge to prevent contaminating the atmosphere with the test bacteria. Results of tests of filters in each of the discussed categories are shown in table 1. As shown in this table, the higher the efficiency, the greater the pressure drop across the filter.

Recently a portable dioctyl phthalate (DOP) test apparatus has been developed for determining the effectiveness of installed filters in removing 0.3-micron particulate material from air. This method is being used by various commercial filter industries and permits an immediate determination of filter effectiveness. However, its chief disadvantage is that it is limited in sensitivity. The DOP test procedure can determine whether a filter system will

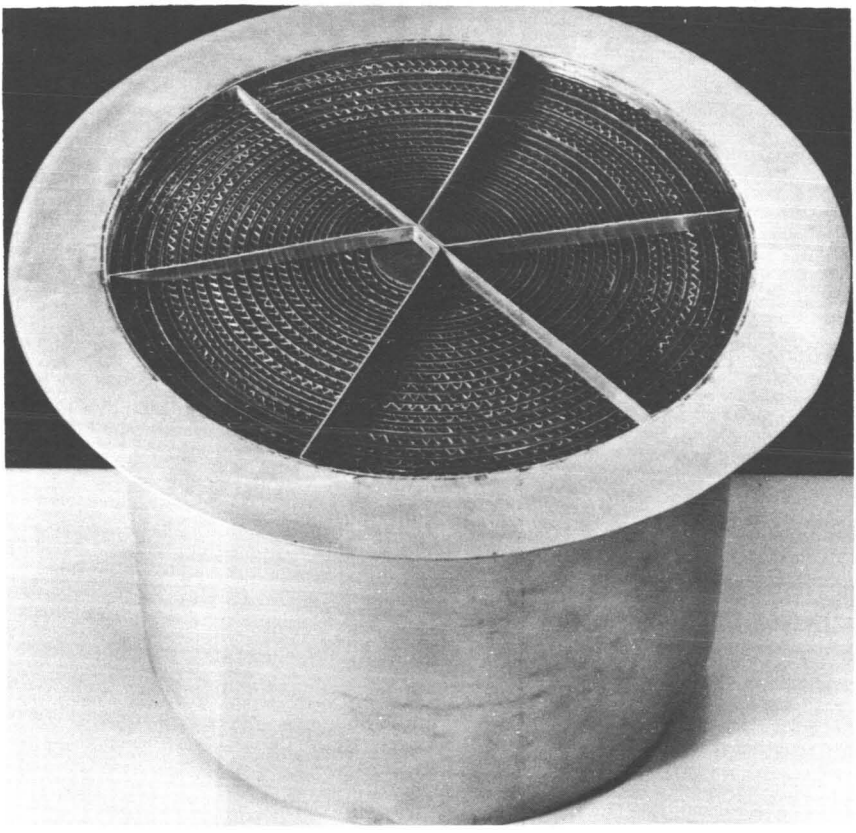

FIGURE 3.—Filter for complete filtration.

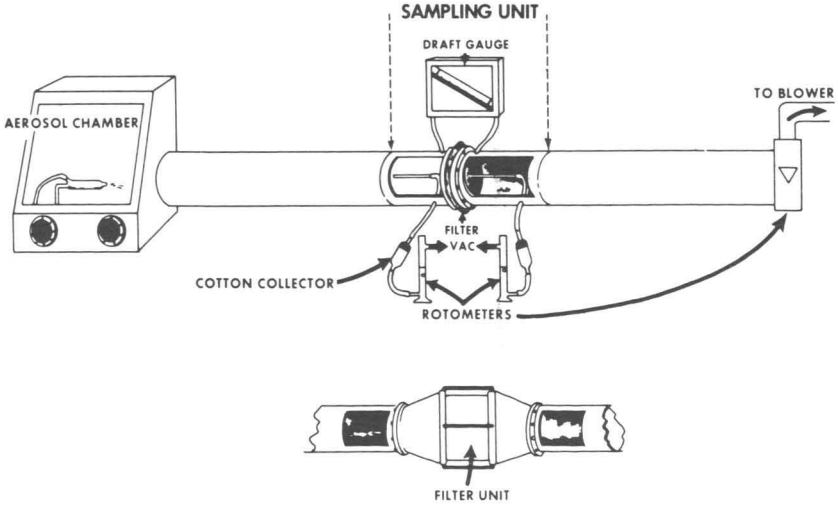

FIGURE 4.—System for determining bacterial arrestment of filters by use of spores.

TABLE I.—*Efficiency and Flow Resistance Range of Devices for Removing Biological Particles (1–5μ) From Air*

Cleaning device	Resistance at rated capacity (in. H_2O)	Bacterial removal, percent
Complete filtration	13.6	99.999999+
Ultra-high-efficiency filter	0.9 to 1.0	99.99+
High-efficiency filter	0.2 to 0.50	90 to 99
Medium-efficiency filter	0.09 to 0.40	60 to 90
Roughing filters: Fibrous, metallic, oiled, and screen types	0.04 to 0.10	10 to 60
Electrostatic precipitators	<0.1	60 to 90
Air washers and scrubbers	<0.1	20 to 90

remove 1 particle per 10 000 to 1 particle per 100 000; however, beyond this level the sensitivity of the system is questionable. In other words, with the DOP test we can determine whether a filter fits into the category of high- or ultra-high-efficiency filter, but we may find it difficult to differentiate between this category and the complete filtration category. The biological test procedure is far more sensitive in this respect, for it can reliably detect differences in low penetration.

The DOP method does have the advantage of providing essentially immediate results contrasted with the 24-hour-or-longer period that is necessary before results of bacterial penetration can be obtained. Its use is indicated in quick checking for possible major leakage resulting from damaged filters or inadequate sealing around filters. These leaks can be readily and quickly detected by DOP and are in good agreement with bacterial leakage measurements.

Air Cleaning by Washing and Scrubbing

In addition to the methods of air cleaning already discussed, there are other industrial processes in use that require comment. Air washing is one of these methods and is chiefly used for removing dust and other particles, although in some instances it has been installed to remove bacteria. Spray towers, zigzag baffles, metal screens, and glass capillary cells are features of some of these cleaning devices. The most efficient air washers are those in which the suspended matter is impinged on wet surfaces and then washed off. However, instances have been reported in which the bacterial content of the recirculated wash water has increased the

contamination in the air. The increase is due to the reaerosolization of the bacteria that accumulated in the wash water.

A similar device is the air scrubber. This device is frequently used in chemical processes to bring the gas into close association with the liquid. One of these devices employs a hygroscopic solution such as lithium chloride to control humidity in the ventilating systems. This arrangement appears to be an improvement over air washers using water because there is no reaerosolization of bacteria.

Air Cleaning by Other Methods

One of the popular air cleaners is the electrostatic precipitator. In this air-cleaning method, air passes through a high-voltage field where the suspended particles are charged and collected on electrodes of opposite charge. Although electrostatic precipitators can remove a high percentage of bacteria and dust from air, they may not be as satisfactory as filters when a constant and dependable supply of clean air is required. In case of power failure it would be possible for contaminated air to pass through the device, a condition that could not be tolerated in some situations. Automatic closures could prevent this occurrence but would increase the cost of the installation. Some units shut off for a time to allow cleaning; during this time there is no protection. It has been our experience that the electrostatic precipitator will exhibit a high collection efficiency only if it receives good maintenance service and will certainly not be as satisfactory as the ultra-high-efficiency filters.

Before leaving the subject of air purification, a quick reference should be made to heat sterilization and ultraviolet light treatment of air. Air incineration, provided that satisfactory temperature is maintained and the retention time is sufficient, destroys biological organisms completely. However, it appears that a system of this nature would not be practical because of the cost of recooling and the complexity of the system required. Ultraviolet air sterilizers have been reported effective for inactivating organisms in the airstream. However, tests of a relatively sophisticated ultraviolet air sterilizer designed for hospital use indicated that ultraviolet light killed only 10 to 12 percent of the vegetative cells in the airstream and none of the spores. In addition, these sterilizers have the disadvantage that the ultraviolet lamps must be cleaned and tested periodically. Furthermore, this light has limited penetrating ability, and those organisms protected by dust may not be killed. Maintenance requirements and operational monitoring are more severe and critical for ultraviolet installations because proper monitoring of ultraviolet energy output is a problem. Furthermore,

this method does not accomplish the removal of particulate matter; therefore no advantage is seen in its use in the spacecraft field.

Concluding Remarks

Efficiency of air-cleaning systems has been established. The consideration now is the determination of the freedom of biological contamination required for any particular given situation. However, maintenance of this cleanliness, particularly in clean rooms or areas where clean assembly is necessary, involves several other factors to insure freedom from biological contamination. First, it is necessary to establish a system of differential air pressurization. Use of a pressurized air system will minimize airflow from contaminated areas to areas where freedom from bacterial count is desired. It is also a good policy in the design of a clean room or clean-room facilities to provide laminar airflow. In addition to being concerned with the efficiency of filters, the contamination contributed by the individuals working in a clean area must be considered. A review of the literature reveals that personnel contribute vast numbers of organisms to the atmosphere, and, if effort is to be expended in freeing the environment of biological organisms, something has to be done regarding organisms contributed by man.

Even the use of a contagion mask will not provide a complete bacteria-free clean room; studies conducted by the Public Health Service have shown that masked personnel continue to shed organisms. The only way this problem could be overcome would be to place the individual in an entirely closed environmental system and to provide complete filtration of all incoming and exhaust air.

In addition to discussions on air filtration, a comment or two can be made regarding the efficiencies of various commercial filters for removing micro-organisms from liquids. It can be foreseen that occasions will arise, such as in certain life detection experiments, where it will be necessary to sterilize liquids that may be heat labile or sensitive to chemical sterilants. Certain fuels might fit into this category, as well as some liquids for biological experiments; these will have to be sterilized by filtration and added to the spacecraft shortly before liftoff. A review of the literature indicates that there have been five main types of filters used to remove bacteria from liquids. These are: (1) diatomaceous earth filters, (2) unglazed porcelain filters, (3) asbestos-pad filters, (4) fritted-glass filters, and (5) membrane filters. Years ago, industries such as the pharmaceutical, vaccine producer, and fermentation industries made use of asbestos-pad and porcelain types of filters, and more recently there has been a conversion to membrane filters. It

is rather surprising that a review of literature shows relatively few comparative tests for removal of micro-organisms from liquids through these types of filter systems. Actually, it has been assumed by many that if any process utilizes such a system it must provide sterility or the entire product is ruined. The validity of this statement certainly cannot be questioned. However, it was foreseen that a hit-or-miss proposition for sterility is rather a poor scientific approach. Some tests have been statistically designed and are being performed at the U.S. Army Biological Laboratories to determine which procedures would yield the greatest reliability of performance. Factors of pressure drop, availability of test equipment, and practical use were the basis of the selection.

Four commercial types of filters and their holders, membrane, Seitz asbestos-pad, fritted-glass, and diatomaceous earth filters, are being evaluated and are shown in figure 5. The tests are still in progress and when they have been completed a report on them will be prepared for NASA. At this time it appears that not all of the various types of commercial filters illustrated can be depended

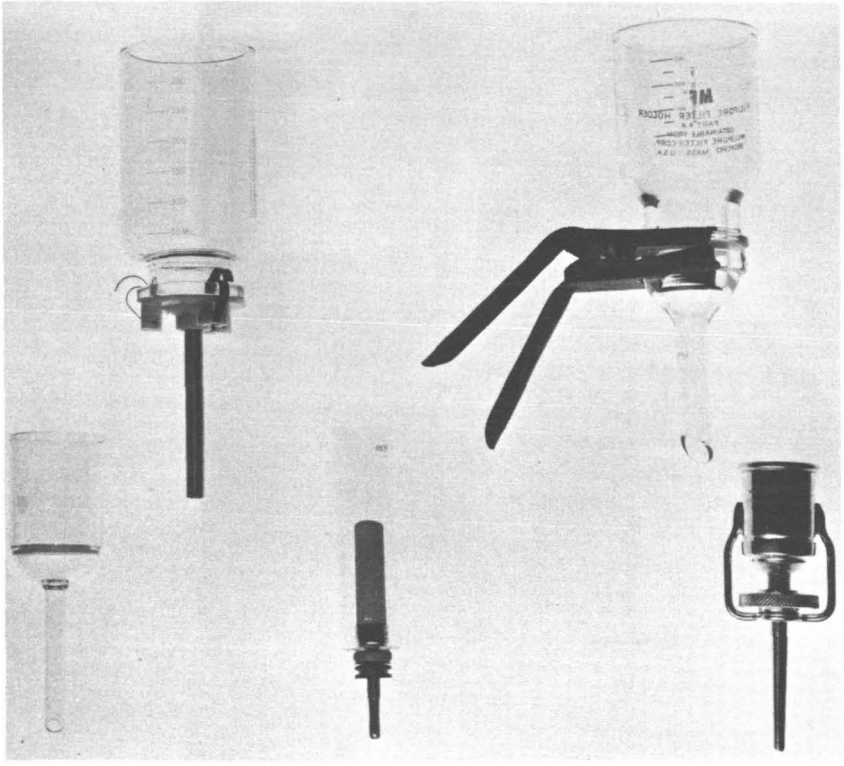

FIGURE 5.—Commercial filters for removal of micro-organisms from liquids.

upon to yield a sterile water filtrate. Tests to date indicate that the fritted-glass and the Seitz asbestos-pad filters yield consistently bacteria-free filtrate, although there has been occasional criticism of these in the literature. Most membrane filter systems show inconsistencies, and no decision can now be made whether these are the result of faults of the filter or holders or of damage to the filters during handling. A few failures also were obtained with diatomaceous earth filters.

Filtration of air for spacecraft sterilization is a feasible method and one that can be easily accomplished with a minimum of effort, since very effective filters are available. Use of some of the types of filters described herein for liquid filtration appears questionable.

Reference

1. ANON.: Federal Standard 209, Dec. 16, 1963.

Session III—Discussion

Saur, *Atomics International:* Mr. Decker, you mentioned the methods used for evaluating the filter. Can you comment on any methods that are used for testing the filters once they are in place to be sure that they are operating correctly?

Decker: As I indicated in the figure, the filters can be evaluated prior to placement or else the procedure can be used of generating an aerosol and collecting samples ahead of and behind the filters in place. It makes no difference; one can use the same procedure in either method of evaluation.

Beuf, *General Electric Co.:* Dr. Bruch and Dr. Silverman, I noticed in several of your kill curve charts, showing survivor curves versus time for radiation and heat, that the rate of survivors fluctuated up and down. Is there a good answer for what is happening? I am an engineer, incidentally, and not a biologist.

Silverman: I take it you mean the tailing effect? The accuracy of assay in that area is so low that I really would not want to say. The author said he obtained values between one and seven spores. I could almost visualize any figure up to 20. I do not think the accuracy there is particularly commendable as it was distributed among a number of replicates. It is not unusual in that range to get this oscillation.

Fisher, *Marshall, NASA:* Dr. Bruch, since the death phenomenon is an inevitable consequence of all living systems, it seems reasonable to assume that a control population exposed to optimum conditions is run concomitantly with the population under stress. Did you run such controls?

Bruch: In terms of die-off in the soil, I did not want to set up a program in soil microbiology when doing the death rate study, so I cannot comment. I think, however, that you would find the phenomenon described by McDade in the clean-room situation. What is present in the soil today may not be there tomorrow, with the exception of spores which are a constant fraction of the soil. Referring to the pure populations used by food microbiologists to calculate death rate, we find that the rate for spores is very slow at ambient conditions.

Comment: Assuming that the bacteria and spores are the organisms tested, have you ever considered the possibility of viral and rickettsial particles as contaminants of the equipment? Heat will destroy viral and rickettsial particles, but will gaseous sterilization?

Bruch: This refers to Dr. Atwood's argument concerning the infective potential of nucleic acids. Anything less than a bacterial cell or an entity that we call a bacterium would not have infective potential for the planetary situation. We do not concern ourselves with the kill kinetics of these particular organisms. I think there are literature references showing that viral particles are destroyed as one destroys the microbial spores. At least this is true with dry heat, but perhaps not with radiation.

Hall: We feel that we are using a satisfactorily resistant organism as our indicator and that other organisms will be susceptible to a lower heat level. As for viral particles, they are not considered a hazard, since they are host specific and we do not expect to find those hosts on Mars.

Hedén, *Karolinska Institute* (Stockholm): Do you not think that the data indicating multiplication of metazoan viruses in bacterial cells are reason for caution? There are indications that DNA and RNA viruses have been multiplying in *B. subtilis* and *E. coli.*

Bruch: My reaction is that I do not feel they would be an infectious hazard to the planet. If *B. subtilis* were there we would have the problem of resolving whether we introduced it or it was indigenous to the planet. If we introduce phage for *B. subtilis* onto Mars, and *B. subtilis* is already there, how disastrous would this be for *B. subtilis* on Mars? It is not that disastrous on Earth. In regard to the metazoan viruses, I do not fully catch the implication.

Hedén: I only wanted to bring up the idea that host specificity breaks down in the cells. This type of replication would require some sort of generic transfer system which might conceivably exist on Mars.

Hall: That is a good point and perhaps ought to be considered further by some of the committees that can give detailed attention to this potential problem.

Price, *Colorado State University*: One thing occurs to me after hearing the last question. We can detect aerosols of virus if they are placed in grids and examined under electron microscopes. Perhaps we are overlooking something that may prove to be a potential problem. Another point concerns the nature of the surface of the material for experimental use. We frequently find that stainless steel is rather specific regarding the nature of the material and varies with the manufacturers' type. Would you comment on that?

Hall: I can assure you that we are not only looking at stainless steel but at quite a number of other compounds or surfaces.

Vesley: We have different types of stainless steel, but we have not any reason to believe that these types would show any wider

differences than the seven different materials we have chosen. We have chosen seven materials that we consider to be somewhat different from each other. I am sure that there are many varieties of each of these seven. I do not think it is feasible to try to find the small differences among varying types of the same material. All of these would probably be within the same log.

Shull, *General Electric* Co.: There are distinct differences in recoverability of micro-organisms from stainless steels of different surfaces. Consequently, the data which have been obtained may be strictly a function of the recoverability of those organisms from that surface. I think that three primary things of extreme importance have come out of this conference so far. First, the engineers now say that we need this heat cycle for qualification in a load reduction situation, especially for internal contamination of components. This makes things a lot easier as far as decontamination for final sterilization. Second, the primary source of contamination of components is the man in the clean room. Elimination of the man in the clean room, topologically, may give us a great advantage in assembling spacecraft under low load conditions. The third thing is the little benefit gained by lowering the microbial load through use of a clean room, thus reducing a population by two to three decades on material which is of relatively minor significance in relation to the total sterilization cycle.

Stern, *The Boeing* Co.: The concept of extending the D-values in the manner that has been described requires the assumption of either uniform heating characteristics of the spacecraft (which is probably invalid) or of the existence of the total number of organisms in the spacecraft at the most heat-sensitive point in the spacecraft. I do not think this is a valid point considering the discussion that has gone before. If we assume that the 10^8 organisms are distributed equally over the spacecraft, then the process time should be reduced accordingly to account only for those micro-organisms present at the most heat-resistant point. For example, if it is assumed that a 10 000-pound spacecraft would have a contamination load of 10^4 organisms per pound and that the most heat-resistant point is a 1-gram sample on a specific part, there would be 200 to 300 micro-organisms at this most heat-resistant point. Using your D-value of 1.8 at 135°, I calculate a process time of 3.6 to 5.4 hours rather than the 22 hours that you recommended. Do you concur with this analysis?

Bruch: I have to agree with you partly, but I cannot agree that we have to calculate the total cycle based only on that fraction at the most heat-resistant point of the spacecraft. If I know the

heatup rate from the surface to the interior, then maybe I can go in that direction.

Stern: In your use of the *D*-value, you are required only to use the most heat-resistant point. Determination of the resistance of the micro-organism does not include the heatup of the spacecraft. The process time you specify is 22 hours after thermal equilibrium of the spacecraft has been reached.

Bruch: Still the fact is that it is the total load of organisms going into the sterilizer. If you are trying to get at the spatial relationship of the organisms, I do not think it is valid. Nor is it valid if they are spread all over or concentrated at one point. It is the amount of heat they get that is the determining factor. I cannot agree with that last calculation of yours.

Session IV

Bioengineering

CHAIRMAN: Lawrence B. Hall
Bioscience Programs
Office of Space Science and Applications, NASA

DONALD VESLEY, ORLANDO R. RUSCHMEYER, AND RICHARD G. BOND
School of Public Health
University of Minnesota

Spacecraft Contamination Resulting from Human Contact

Human beings and micro-organisms have apparently lived in intimate association with each other since the first emergence of anthropoid life on this planet. Throughout his life, man is exposed to myriads of microbial species that are abundant in the terrestrial environment. Of the great spectrum of bacteria, molds, yeasts, and viruses which comprise the natural microflora, a few find the inner and outer surfaces of the human body to be a hospitable ecozone. Here they are able to live luxuriantly and to reproduce prodigiously for long periods of time.

The bacteria and other micro-organisms which are man's lifelong associates are often referred to as his indigenous flora. Most of the time these indigenous forms live on man as saprophytes, commensals, or mildly parasitic species. On other occasions, certain microbes in or on human tissues may cause clinically recognized diseases as a result of increased invasive abilities, the production of toxins, a reduction of the host resistance, or combinations of these (ref. 1).

Thus we observe that man's tissues provide an excellent culture medium for prolific growth of certain micro-organisms. Rosebury's extensive treatise (ref. 2) has pointed out the variety of microbial species that constitute the human indigenous forms. From their habitat on humans these organisms are widely disseminated into the environment by a number of routes such as nasal secretions, sneezes, salivary expectorates of coughing and talking, feces, and urine (ref. 1). Recent studies by Ulrich (refs. 3 and 4) have demonstrated that persons with skin lesions or special skin conditions may also shed large numbers of certain bacteria into the environment.

Another much larger group of micro-organisms with which humans are in daily contact are the numerous transient forms from the environment which adhere to skin and clothing. These species may have widely differing physical, chemical, and physio-

logical requirements for their life processes. Among this group are the sporeforming bacteria of the soil, autotrophic bacteria and algae, many molds, methane bacteria, and the like. For most of these microbes the environmental conditions of human tissues are not generally suitable for growth and reproduction. However, should these micro-organisms become transplanted to a completely different environment, even though it may appear hostile to common animals and plants, as, for example, the Martian surface, certain of the microbes may well grow in profusion.

Therefore, it should be clear that in any attempt to maintain a sterile or near-sterile environment, consideration must be given to the role of personnel as sources of contamination. It appears, in fact, that as greater attention is paid to cleanliness in an area, a greater proportion of environmental microbes is contributed by human sources (ref. 5).

With the advent of plans for interplanetary exploration, the aerospace industry is confronted with the problem of assaying and controlling microbial contamination. Since humans are the principal source of microbial contaminants in clean areas, studies of contamination transferred by workers in assembly processes as well as of the efficacy of control measures are obviously of interest to the aerospace program.

Among the important mechanisms for contact transmission of micro-organisms from the human being to his immediate surroundings are the hands. Because they come into intimate contact with the nose, mouth, body surfaces, and clothing as well as with dust, soil, and a host of contaminated objects, they are vulnerable to exceptionally heavy contamination. Various investigations in the medical field (ref. 3) and in industrial areas (refs. 6 to 8) have confirmed this assumption.

A series of studies now in progress at the laboratories of the School of Public Health of the University of Minnesota is directed toward obtaining additional data about the extent of microbial contamination transferred by the hands of persons. These investigations can be summarized as follows:

(1) Study A: An analysis of the degree of contamination deposited on different component materials by contact and manipulation with hands and fingers.

(2) Study B: Determination of die-off rates of natural heterogeneous microbial populations deposited on surfaces of various materials as a result of human contamination.

(3) Study C: A study of the effectiveness of different control measures as a means for reducing microbial contact contamination in simulated assembly processes for small components.

Test Materials and Methods

Tests of contamination deposited by handling were conducted on 1- by 2-inch strips of the following seven materials: (1) stainless steel of 28 gage and with No. 5 or 6 finish, (2) 1/32-inch-thick aluminum, (3) 1/32-inch-thick epoxy glass laminate, (4) 1/16-inch-thick copper-covered epoxy laminate, (5) 2-ounce rolled copper plated with lead-tin solder, (6) 1/16-inch-thick Lucite, and (7) 1/32-inch-thick Teflon. Simulated component assembly trials were performed with ¼-inch steel machine screws, washers, and nuts.

All materials were thoroughly cleansed by washing, in turn, with detergent solution, hot distilled water, and isopropyl alcohol and were then ether-rinsed and drained dry. Dry-heat sterilization at 180°C for 90 minutes was used to kill any remaining microorganisms (except for heat-labile materials which were steam autoclaved at 120° C for 20 minutes).

Levels of contamination resulting from human handling (study A) were studied using the 1- by 2-inch strips of different materials. All seven types of materials were contaminated simultaneously. The sterile strips were handled according to a statistically designed, randomized protocol. A group of four laboratory workers (two males and two females) handled the strips in each trial. By having each strip handled by four persons, it was hoped that much of the expected individual variability could be counteracted. The individuals were positioned along a laboratory bench to simulate an assembly line. The first person of the group picked up the strip, manipulated it with fingers, turned it over once, and then passed it on to the next individual in line. Strips were ultimately collected in sterile petri dishes and analyzed. A total of four trials, each on 25 strips of each material, was conducted.

Aseptic microbiological techniques were scrupulously observed in the analyses. In random order, each strip was placed in 25 milliliters of warmed (48° C), M/15 phosphate buffer, pH 7, with 0.02 percent Tween "20" and shaken on a shaking machine for 5 minutes. Then strips were removed, embedded in petri dishes containing 15 milliliters of molten trypticase soy agar (TSA), and incubated at 32° C for 72 hours; after the incubation a colony count was made.

The total 25-milliliter buffer eluate for each strip was poured into a 150-millimeter petri dish containing a previously prepared 2-millimeter solidified TSA base. The shaker bottle was rinsed with approximately 25 milliliters of molten TSA which was added to the eluate plate. Lastly, an additional 100 milliliters of molten agar was added to the dish to complete the pour plate. All plating was

done in special cabinets equipped with absolute filters. Colonies were counted with a Bactronic colony counter after 72 hours of aerobic incubation at 32° C.

Total counts of viable particles for each strip comprise the eluate counts, plus the count of residual colonies detected on the embedded strip. Data are reported as viable colonies per strip. With each series, sterile strips were processed as controls to check on the techniques in plating.

Studies of microbial die-off rates (study B) were made on all seven strip materials, but each type of material was assayed in a separate trial. Groups of four persons contaminated these strips by handling procedures the same as those outlined for study A.

Sets of 50 strips were analyzed immediately after contamination. Similar sets of contaminated strips (50 per set for periods through 1 week, 25 per set for longer time intervals) were stored in sterile petri dishes at 72±4° F and 60±10 percent relative humidity. Counts of micro-organisms surviving storage were made after 1, 2, and 3 days and after 1, 2, 6, 12, and 20 weeks following contamination. Analysis of individual strips followed the protocol described for study A.

Effects of special contamination control procedures (study C) were evaluated by simulating component production operations through the assembly of previously sterilized machine screws and washer-and-nut combinations. Four operators, 2 male and 2 female, each assembled 20 units which were collected in sterile petri dishes labeled for each person. Benches where assembly was done were previously cleansed with 70 percent ethanol.

After all "components" were completed, groups of five units from each individual's assemblies were placed in bottles of buffer solution and analyzed by the method described for study A. With this technique, comparative studies were made of the following:

(1) Open laboratory assembly with —
    (a) No hand care
    (b) 2-minute ordinary soap wash
    (c) 2-minute hexachlorophene soap wash
(2) Assembly in a special cabinet purged by an absolute filtered airstream: Units were assembled with hand care similar to that for open laboratory combinations and, also, as an additional experiment, by using a 2-minute hexachlorophene soap wash plus sterile gloves.

Discussion of Test Results

All data reported in this study refer only to the aerobic, mesophilic, heterotrophic micro-organisms. No attempt was made

to enumerate other physiological forms. Data in table I indicate the differences in average numbers of micro-organisms deposited on component materials when handled by different groups of individuals. These results suggest that mean concentrations of microbial deposition varied between groups, which indicates large individual variability, and also varied with the type of material.

TABLE I.—*Microbial Contamination Detected on Component Materials After Handling by Groups of 4 Persons*

Material	Mean microbial plate count per 1- by 2-in. strip[a]				
	Group A	Group B	Group C	Group D	Material mean[b]
Metallics:					
Stainless steel	14.6	7.4	2.2	8.4	8.2
Copper	16.9	3.3	3.8	6.3	7.6
Aluminum	17.1	3.1	6.3	10.9	9.4
Solder	10.2	18.9	2.4	3.8	8.8
All metallics	.....	.....	.....	.....	8.5
Nonmetallics:					
Epoxy laminate	33.0	9.4	16.7	18.8	19.5
Lucite	63.4	10.8	25.9	17.2	29.3
Teflon	17.0	6.5	18.4	16.2	14.7
All nonmetallics	.....	.....	.....	.....	21.1
Group mean	24.6	8.5	10.8	11.6	.....

[a] Represents mean count for 25 strips of each material per group.
[b] Represents mean count averaged over all groups, a total of 100 strips per material.

With the exception of solder strips, the mean levels of contamination deposited by group A were consistently higher than those of group B. Data for groups C and D indicate that contamination from these groups was of intermediate levels. Under the conditions of generally more intense microbial deposition from group A, the greatest accumulated mean contamination occurred on Lucite and epoxy laminate, with approximately 63 and 33 viable particles per strip, respectively. Scrutiny of combined means for all groups provides evidence which suggests that greater numbers of viable particles are retained on nonmetallic materials than on metallic materials under similar conditions of contamination.

TABLE II.—*Survival After Storage of Micro-Organisms on Aerospace Component Materials Contaminated Through Handling*

Storage time, days	Mean microbial plate count per 1- by 2-in. strip[a]						
	Stainless steel	Copper	Aluminum	Soldered copper	Epoxy laminate	Teflon	Lucite
0	239.9	101.4	17.7	25.6	20.1	59.8	55.5
1	253.2	86.5	11.9	6.3	34.9	14.9	39.5
2	140.6	39.6	3.4	7.6	19.2	12.2	30.0
3	28.8	98.2	4.9	8.0	13.6	15.4	27.0
7	36.4	63.7	3.0	26.5	15.4	17.1	35.5
14	5.6	38.4	10.9	4.4	14.5	12.4	54.0
42	1.1	71.6	2.6	3.6	30.8	13.4	26.0
84	1.0	26.9	1.1	2.2	13.6	10.2	-----
140	.9	-----	-----	-----	-----	-----	-----

[a] Represents mean counts of 50 strips for each time interval through 1 week and 20 to 25 strips for subsequent time intervals.

The experimental results obtained from studies of microbial survival on the seven materials demonstrate interesting differences. The mean concentrations of organisms initially deposited on these materials varied widely, as can be seen in table II. Mean values of 240 viable particles were detected on stainless steel, while a mean level of only 17.7 viable particles occurred on aluminum. Presumably these variations resulted from differences in cleanliness of hands, or in shedding from hands, of the persons participating in these studies. Since these materials were handled by different groups of individuals on different days, such variation in concentrations of initial contamination might be expected.

Although this experiment is still in progress, the information collected to date suggests certain trends in microbial survival rates on the materials studied. Highest die-off rates were observed on stainless steel, where mean counts dropped from 239 viable particles to less than 1 over a period of 20 weeks of storage. Thus it appears that simple storage effected a 99 percent reduction in the mean microbial plate count for the steel strips. The aluminum and solder strips yielded mean concentrations indicating a 90 percent reduction from the initial contamination levels after 12 weeks of storage.

Lowest die-off rates were observed on copper strips and on the nonmetallic materials (epoxy laminate, Lucite, and Teflon). Although they yielded lower mean counts following storage, these materials still retained mean levels of 10 to 27 viable particles per strip after 12 weeks of storage. Another point of great potential importance is the fact that preliminary determinations of types of micro-organisms surviving after 12 and 20 weeks on all materials revealed a very high proportion of sporeforming species.

Data from the studies of hand care and special barriers to contamination revealed that certain procedures were effective for reducing the transfer of micro-organisms to component materials (see table III). Washing with ordinary soap yielded erratic results, including in one instance a fourfold increase in counts from the nut and screw assemblies.

Hexachlorophene soap scrubs appeared to be moderately effective in reducing transfer of contamination. The greatest contamination control was obtained with a combination of a hexachlorophene soap scrub and the use of sterile rubber gloves. Use of this procedure reduced mean levels of contamination to less than one organism per assembled unit. These data indicate that even modest precautions in hand care combined with the use of effective barriers such as rubber gloves can reduce microbial contamination considerably below the levels attained under conditions prevalent

TABLE III.—*Levels of Microbial Contamination Detected During Simulated Component Assembly*[a] *Trials Under Different Control Methods*

Method	Microbial colony counts, TSA medium			
	Per 5 assemblies			Per single assembly
	Mean	Median	Range	Mean
Open laboratory:				
No hand care	611.3	432.0	20–1904	122.6
2-min ordinary soap wash	66.5	31.5	11–280	13.3
2-min hexachlorophene soap wash	6.6	2.0	0–37	1.3
Specialaire hoods:				
No hand care	77.1	57.0	12–288	15.4
2-min ordinary soap wash	299.3	46.5	0–3044	59.9
2-min hexachlorophene soap wash	20.8	15.5	5–51	4.2
2-min hexachlorophene soap wash plus sterile gloves	.9	0	0–10	.2

[a] Assemblies of nuts, washers, and machine screws.

in ordinary, uncontrolled circumstances. A variety of additional experiments are planned to investigate these phenomena further.

Summary of Results

Although none of these experiments has been completed and data analysis is preliminary, several interesting trends are apparent. They can be summarized as follows:

(1) As expected, variability in microbial shedding from hands among different individuals is extremely high.

(2) There is an indication that nonmetallic substances such as epoxy laminates, Teflon, and Lucite may be more readily contaminated than metals and may retain viable particles for longer periods.

(3) Sporeforming microbes comprise a very high percentage of surviving species following 12 to 20 weeks of storage.

(4) Relatively simple barrier techniques, such as the use of sterile gloves or even a single hand washing with a hexachlorophene soap, can effect considerable reductions in microbial transfer from hands to component parts.

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Engineering Problems in Sterilization of Spacecraft

The planetary quarantine policy of the National Aeronautics and Space Administration (NASA) affects all aspects of the design, hardware development, assembly, test, and launch of a spacecraft and capsule.

The sterilization requirement has to be considered at all levels of engineering to determine what effect it will have on the various activities of the mission. The present paper presents some engineering problems in which the sterilization requirement must be considered as well as those engineering problems that are unique because of the sterilization requirement. At the present time, many of the problems are the subjects of intensive engineering and microbiological analyses. This paper is a broad survey of the kinds of engineering problems one encounters by requiring sterilization of spacecraft.

Various individuals and professional organizations have investigated the possibility of infecting the planets with Earth microorganisms. There is general agreement that the ecology of the planets should be preserved in order to permit biological observations without interference by organisms introduced from Earth (refs. 1 to 5). In order to distinguish between those parts of a spacecraft that impact a planet, and consequently must be sterile, and those parts of the spacecraft that do not impact the planet, and may be unsterile, the terms "capsule" and "spacecraft" are used, respectively.

The absolute concept of sterility must be translated into a probability of sterility. A particular capsule can be certified as sterile in the sense that there is a certain low probability of viable contamination. Recognition of the probabilistic approach is mandatory for certain types of space engineering problems; it provides an analytical basis on which sound engineering judgment can be exercised in certain types of space engineering problems as, for example, in error analysis of trajectory dispersions of launch vehicles.

The idea of applying probabilistic terms to an absolute concept has a long history in the aerospace industry. When increased emphasis was placed on reliability of military equipment, probabil-

ity statistics expressed reliability in engineering terms. Intelligent judgments could be made without compromising the basic, absolute concept of reliability. Problems of spacecraft sterilization can be dealt with in a similar manner.

A word of caution concerning probabilistic statistics is in order. A review of literature that is concerned with attaching a numerical value to the probability of landing a viable organism on Mars shows that the value varies from $1:10^3$ to $1:10^6$ per launch (refs. 1 and 5). There is a considerable difference, from a statistical and engineering point of view, in performing a certain task with a probability of one in a thousand or one in a million. The obvious judgment must be executed as to whether, if the event occurs, its consequences are irrevocable, critical, or minor. Selection of the more rigid probabilities of sterilization will be eventually reflected in increased cost, operational difficulties, and potential delays in planetary exploration.

The engineering problems of developing a sterile capsule to impact on a planetary surface begin with the imposition of sterilization requirements. One set of requirements proposed is: (1) the NASA policy of prevention of contamination of the Mars planetary surface will be built on the condition that the probability of landing a viable organism on the planet be less than 1 in 10 000; (2) in order to assure that the capsule meets the first requirement, it will be assembled under conditions that will reduce the microbiological count and it will undergo a terminal sterilization treatment; and (3) the sterilized capsule will be microbiologically isolated from any unsterile portions of the spacecraft (ref. 6).

A critical analysis of these requirements reveals that the first is quantitative and probabilistic, while the others are qualitative and place initial constraints on a mission a priori to the formulation of the mission. Ideally the sterilization requirements might be expressed so that all possible exigent situations of a mission can be considered on a probabilistic and quantitative basis because it is presumptuous to suppose that all possible modes of contamination of the planets can be predicted.

A research and development program has been instituted at the Jet Propulsion Laboratory (JPL) under NASA guidance to investigate the spacecraft sterilization problem.

Inhibitions of Sterilization Requirement on Mission Analysis and Overall Systems Design

A large number of mission modes for planetary exploration are possible. We will be concerned with only two. The first of these modes is an impact trajectory with deflection of the spacecraft.

The second mode would place the spacecraft on a flyby trajectory and deflect the capsule. The first of these modes would place the spacecraft and capsule on an impact trajectory toward the planet. Prior to impacting the planet, the spacecraft would be deflected to miss the planet with a probability consistent with the restriction that the probability of landing a viable organism on the planet be less than 1 to 10 000. The capsule would then proceed on an impact trajectory. The second mode would place the spacecraft on a trajectory that would miss the planet by a specified distance. Prior to reaching the planet, the capsule portion would be separated and made to impact the planet. These modes are commonly known as impact and flyby trajectory.

In the first mode, an impact trajectory, it is obvious that if the requirement to not contaminate the planet is 1 to 10 000 then the maneuver to separate the capsule from the spacecraft must have the same probability of success. This requires a complete engineering analysis of the probability of success of the separation maneuver and deflection of the spacecraft. Because of the sterilization requirement, engineering and scientific tradeoffs between an impact trajectory and a flyby trajectory must be evaluated. One feels intuitive prejudices against an impact trajectory because of the sterilization requirements. Other mission modes would require similar consideration of the sterilization requirement.

One solution to the impact trajectory mode would be to sterilize all portions of the spacecraft and the capsule that are to land on a planet. An analysis of this solution requires a systems analysis tradeoff between satisfying the sterilization requirement, increased cost of the mission due to development of sterilizable hardware, and the probability of the success of the mission if the reliability of the hardware is degraded by the sterilization environment. Thus, every mission mode requires a consideration involving the sterilization requirement.

If we assume the particular mission mode is a flyby trajectory of the spacecraft with subsequent capsule deflection, the following engineering problems are apparent. Prior to the development of an overall system design, a method of sterilization must be agreed upon. If the sterilization restraints are not considered early in the systems design, it is questionable whether the restraints can be satisfied. We can perhaps look to the Ranger missions to prove this point. The ideal sterilization method for a capsule would be a treatment yielding sterility without degradation of functional reliability. Of the sterilization methods that are available for destruction of microbial life, dry heat is receiving prime consideration.

Various time-temperature relationships can be developed for a heat sterilization cycle. Under consideration are temperature cycles ranging from 105° C for 336 hours to 160° C for 3 hours. Since a time-temperature heat cycle is a function of the total microbial load, it may be necessary to reduce the total microbial load by a prior decontamination procedure. Decontamination by exposure to a mixture of ethylene oxide and Freon 12 is being studied. The overall system design must be compatible with the selected sterilization time-temperature cycle and decontamination environments. If the sterilization of a capsule is to be accomplished, all parts of the capsule, including engineering hardware and scientific experiments, will have to be capable of withstanding sterilization and decontamination requirements.

The sterilization requirement also affects the systems design in that the overall thermal coefficients of heat transfer of the capsule must be such, or provisions must be made, so that all portions of the capsule receive the sterilization temperature for the specified periods of time. A typical engineering problem in this area is balsa wood. Balsa wood has the excellent property of being able to absorb high shock but has a low coefficient of heat transfer. An engineering tradeoff must be made between the restraint imposed by the sterilization requirement and the choice of a crushable structure to absorb the shock in a planetary landing. Provision must be made so that the length of time needed to attain terminal sterilization temperature is not prohibitive.

Another class of engineering problems is concerned with the interface between the unsterile spacecraft and the sterile capsule. One concept of a planetary capsule is that it it will be sterilized inside of a microbiological barrier and that the barrier will protect the capsule from further contamination. Upon approach to a planet the barrier will be opened and the sterile capsule will be injected toward the planet. Prior to launch, communications will have to be maintained between the capsule and the spacecraft and ground equipment. This will require a sterile electrical interface within the microbiological barrier, since the inside of the barrier will be sterile although the outside will be unsterile. Communications between the capsule and the spacecraft will probably have to be maintained during the interplanetary phase of the mission and will have to be considered during the descent to the planet phase of the mission.

The design and separation mechanisms of the microbiological barrier pose the following constraints:

(1) The microbiological barrier must be impervious to microbial contamination after sterilization during the prelaunch and

launch phase of the mission. Sterile pressure equalization techniques will have to be considered.

(2) The mechanism for opening the microbiological barrier must not contaminate the inside of the barrier that contains the capsule. The problem of effluent materials produced by the separation maneuver and the subsequent deposition of this material (if contaminated) on the sterile capsule will have to be studied.

(3) The mechanism for opening the microbiological barrier, and the microbiological barrier itself, will have to remain with the unsterile, nonimpacting spacecraft.

(4) Temperature control techniques for terminal heat sterilization and interplanetary flight will have to be compatible.

Subsystems Hardware Problems That Are Affected by the Sterilization Requirement

Given the requirements for heat sterilization and ethylene oxide compatibility, the subsystems designer must seek to make his equipment satisfy these requirements. The sterilization criteria used are—

(1) Hardware must be compatible with a mixture of 12 percent ethylene oxide and 88 percent Freon 12.

(2) Hardware must be capable of withstanding three dry-heat sterilization cycles of 145° C for 36 hours in a nitrogen atmosphere.

One cycle of 135° C for 24 hours has been deemed adequate for the terminal heat sterilization cycle; the difference between this cycle and the ones mentioned above insures reliability of equipment, since flight equipment is normally designed and developed to withstand greater environmental stress than that of the expected environment.

The basic objectives of the program are to determine if present capsule hardware can withstand sterilization environments, the effect of sterilization environments on the reliability of hardware, and applicable research for the development of hardware that at present cannot withstand sterilization environments.

In order to provide the basic building blocks for subsystem designs, JPL is investigating and establishing a list of basic electronic components and a list of suitable polymeric products that are capable of withstanding the specified sterilization environments. The electronic components include capacitors, resistors, transistors, fuses, thermistors, relays, inductors, crystals, and microcircuits. Polymeric products are defined to include adhesives, elastomers, laminates, coatings, structural thermoplastics, encapsulating compounds, greases, films, lubricants, insulators, impreg-

nating compounds, thermal sheeting tapes, primers, fluids, and inks.

In addition to the aforementioned studies, the effect of the heat sterilization cycle and the compatibility of the mixture of ethylene oxide and Freon 12 on subsystems is being evaluated. Those subsystems that are not compatible with the sterilization environments are candidates for advanced research and development. Details of this research program are presented in other papers in these proceedings.

Operational Problems Incurred by Sterilization Requirement

Operational problems are defined as those problems imposed by the uniqueness of the sterilization requirement on the assembly, test, transport, prelaunch, launch, and postlaunch operations of a planetary mission. An anticipated requirement of sterilization is performance of the operational sequence of activities necessary to build a spacecraft under conditions that will reduce total microbial contamination, since the efficiency of a sterilization procedure is a function of the total number of micro-organisms present. The effect of this requirement on all of the aforementioned operations has to be determined.

There exist at the present time extensive facilities for the manufacture, test, assembly, and launch of planetary vehicles. If a capsule that is to be launched to a planet must be assembled under environmental conditions that are presently not available, then extensive modifications will have to be provided to existing facilities or new facilities and new methodology will have to be developed for assembly, test, transport, and operations of space vehicles. This, in turn, will necessitate that large expenditures of funds be committed to facilities for planetary spacecraft.

The assembly of the capsule appears to be one problem area. One method of assembly would utilize microbiologically clean rooms and studies of the microbiology of present clean rooms are in progress. Although the aerospace industry has extensive clean-room facilities and intensive background in clean rooms, this knowledge and experience is applicable for only nonviable particulate matter. Limited information existed on the microbiology of industrial clean rooms prior to 1964. The major engineering problem is to attempt to determine what would be the real decrease in microbiological loading if a capsule were to be assembled and tested under a rigidly controlled environment.

In order to minimize contamination during assembly, both vertical laminar airflow assembly working areas and sterile assembly techniques are being investigated. Sterile assembly techniques

of the type used in germ-free animal husbandry and in the manipulation of pathogenic micro-organisms may have particular applicability in a space-vehicle-assembly repair situation or where it is desirable to sterilize individual piece parts and then assemble these piece parts to produce a sterile assembly.

After a capsule is assembled, it may be subjected to a number of environments including vibration, shock, and solar simulation. These systems environmental tests are presently carried out in various and often distant facilities. If these operations substantially increase the total microbial count on the capsule, then some methodology of transport aand maintenance during these environmental tests will have to be developed in order to hold the microbial contamination to an acceptable level.

The prelaunch operations are composed of intensive final tests. Final assembly of explosive materials, pyrotechnics, and propulsion systems is accomplished at this time and matchmate tests are performed with the launch vehicles. The capsule will have to be sterilized. After sterilization, the sterility of the capsule will have to be maintained during the prelaunch and launch phases of the mission.

It appears necessary to develop a quantitative methodology, in the form of a mathematical method of counting micro-organisms, to determine how microbial contamination varies with the imposition of sterilization requirements. As we study the problems of sterilization, we must continuously assess, in a quantitative manner, what is the microbial gain or loss from each operation and determine the value of each sterilization constraint on a particular operation. Planetary missions are now restrained by the immutability of the launch period. A further restraint will be imposed by the sterilized capsule which will decrease alternative choices of action, primarily in the area of last time checks and repairs. Thus, the most meticulous and intensive planning must be instituted.

Concluding Remarks

The imposition of the requirement of sterilization for planet-impacting space vehicles introduces a new dimension in space engineering technology. Translation of the requirement into probabilistic engineering solutions involves the best knowledge and judgment of the microbiological and engineering community. Although formidable, those engineering problems imposed by the sterilization requirement appear soluble. Solutions to these problems are concomitant with increased cost and increased operational difficulties for planetary exploration.

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Contamination Analysis and Monitoring

"Sterilization" is a term applied to any process or procedure that produces a condition of sterility. Sterility is an absolute concept and refers to the destruction or removal of all life. However, when sterility as an absolute concept is applied to the sterilization of space hardware, it ceases to be operational. Absolute proof of sterility of flight hardware is impossible. Hence, for an operational approach to certification of sterility of space hardware, it is necessary to apply the probability concept. The present report is concerned with the applicability of mathematics to predict the probability of achieving sterility in a complex space hardware system. As such, this approach is concerned with the manipulation of microbiological data rather than mathematical rigor.

If the system is to be practical and workable, it is necessary to realize the limitations of existing microbiological sampling procedures and to make allowance for such limitations in the overall approach. The basic assumptions used in this analysis are:

(1) When the total microbial load is known or can be shown to be less than a specified value, there is a dry-heat sterilization cycle that will produce sterility with a calculable degree of probability P_s ($P_s=10^{v-x}$, where 10^v is the microbial load and 10^{-x} is the reduction factor (ref. 1)). This method for determining the probability of achieving sterility is an accepted method (ref. 2); however, it is not based on rigorous probabilistic theory or empirical proof. The fact that it has been accepted is the premise upon which the first assumption is made.

(2) The microbial contamination consists only of the bacterial spore population whose D-value (ref. 1) is the basis for the dry-heat sterilization cycle specified in the first assumption. This assumption is necessary, since different species of micro-organisms will display different resistance to heat. The specified dry-heat cycles are based upon the most heat-resistant spore population that might be expected to accumulate on space hardware. Thus, if it is assumed that the entire microbial load consists only of microbial species that have the heat resistivity assumed in the development of the D-values, other heat-susceptible species will be killed. The assumption is quite valid, although constraining.

Determinants of Microbial Load

Manufacturing Stages To Be Considered in Analyzing Microbial Load

Assembly of a capsule system is a stage-by-stage process to develop electrical and mechanical piece parts beginning with prime materials. These piece parts are assembled into a group of parts which then may be assembled with other parts or groups of parts. This process is continued until all parts are assembled into a capsule system. The capsule may consist of over 50 000 individual parts and 1 000 000 grams of solid material.

Each hardware assembly involves exposure to an intramural environment and human handling contamination that may increase the microbial load on the hardware. The sampling logistics to collect the data necessary to predict accurately the total microbial load for this entire sequence of operations is formidable, if not impossible. However, the task can be simplified if an intermediate stage of assembly, hereafter referred to as a subassembly, could be shown to be sterile. This implies that the microbial load on and in each subassembly is easier to predict than that for the total capsule system. This is not necessarily true; however, if the task is to predict that the subassembly has a microbial load less than, or equal to, a given number and if this number is sufficiently large, the problem is greatly simplified.

At this juncture an operational concept will be stated. Each subassembly will be subjected to a dry-heat sterilization cycle K prior to being assembled on the capsule system. This dry-heat sterilization cycle is defined as that which will produce a probability of sterility P_s when applied to a microbial load less than or equal to a value C_s.

An important part of this operational concept, and one that will be capitalized upon for sterilization, is the relation between the dry-heat sterilization cycles applied to the subassembly and the capsule system. The subassembly will be given at least the same heat environment that the capsule system will receive. This is done for hardware qualification purposes and will be imposed regardless of the microbial load on the subassembly. (See subsequent paper in these proceedings entitled "Plan for Sterilization of Voyager Capsule," by Gordon P. Kautz and Paul Tarver.)

Now the quantification of sterility has two distinct phases: sterilization of the subassembly; and sterilization of the capsule system.

Subassembly Sterilization

The microbiological data required for microbial load prediction during the first phase are as follows:

(1) Determine the number of micro-organisms contaminating the interior of prime materials and parts. (See preceding paper in these proceedings entitled "Techniques for Recovery From Interiors of Solids," by Earl G. McNall, William T. Duffy, and John J. Iandolo.)

(a) Micro-organisms per gram of specific material.

(b) Micro-organisms per specific part.

(2) Determine the contamination due to human handling. (See preceding paper in these proceedings entitled "Environmental Microbiology and Control of Microbial Contamination," by J. J. McDade, George S. Michaelsen, and Martin S. Favero.)

(3) Determine the contamination due to the intramural environment. (See paper in these proceedings by McDade et al.)

It is assumed the data either do exist or can be developed for each item shown. C_w is defined as the "worst case" microbial load prediction, and C_s is defined as the microbial load which, when subjected to sterilization cycle K, will produce sterility with probability P_s. Analyze as follows:

(1) Perform an engineering analysis which provides:

(a) A complete parts list by item and quantity.

(b) A list of materials excluding parts and the estimated total weight.

(c) A prediction of the total surface area, taken part by part and added together along with area of coating materials, chassis, etc.

(2) Predict the worst case of microbial load C_w as follows:

(a) Compute the microbial load contaminating interior of parts.

(b) Compute the worst case of microbial surface contamination from personnel handling and from the intramural environment. (See section following for further discussion of surface contamination.)

(c) Compute C_w, the total microbial load, by addition of the interior and surface microbial load predictions.

(d) If C_w is less than or equal to C_s, then it is unnecessary to impose controls stricter than those utilized for the worst case prediction, and the subassembly can be sterilized without further analysis.

(e) If C_w is greater than C_s, determine which factor is preeminent. For example, if the interior microbial load is predicted to be 10^9 micro-organisms and the surface load is predicted to be 10^5 micro-organisms, the surface load can be ignored for all practical purposes.

(f) If the interior microbial load is the preeminent factor, then reduction of this load can be accomplished by improving the prediction technique or by increasing the number of D-values applied during the sterilization cycle.

(g) If the surface microbial load is the preeminent factor, it can be reduced by improving the prediction or by an ethylene oxide (ETO) decontamination.

(*1*) The analysis assumes that the entire surface area is contaminated by human handling. This is an overstatement of the microbial load. An improved technique would be to analyze the assembly procedures and calculate the surface area handled, and then conduct close monitoring of the actual operation.

(*2*) Similar improvements can be made for the prediction of the microbial contamination resulting from the intramural environment.

(*3*) Engineering analysis can be conducted to determine the proportion of the surfaces that are mated. Such hidden surfaces isolate the microbial load from decontamination by ETO. The microbial load isolated by these mated surfaces is now considered and treated as an internal microbial load.

(*a*) If the internal microbial load is the preeminent factor, the load can be reduced by increasing the number of D-values applied during the sterilization cycle or through stricter intramural environment and personnel handling controls.

(*b*) If the surface microbial load is the preeminent factor, the load can be reduced by decontamination with ETO.

(3) In all cases of this analysis, improvement in the intramural environment or the personnel procedures beyond that required to reduce the microbial load to the specified load C_s is unnecessary. Other factors, such as particulate contamination, may dictate environmental controls that are stricter than those required for sterilization.

(4) It was previously stated that the requirement to subject the subassembly and the capsule system to the same sterilization cycle would be capitalized upon. The following example clarifies this

point: Assume $C_s=10^8$ micro-organisms and $C_w{}^x=10^5$ micro-organisms, where $C_w{}^x$ is a worst case prediction for a specific subassembly x. Through improvement in the analysis, the prediction of the microbial load on subassembly x might be reduced to 10^4 micro-organisms. Nothing has been gained through this improved analysis because subassembly must still be given the K cycle required to kill $C_s=10^8$ micro-organisms. The fact that our C_w prediction is actually one or two orders of magnitude higher than that which probably exists is of no concern if $C_w \leqslant C_s$ and the sterilization cycle K is designed to produce sterility when the microbial load is less than or equal to C_s.

Capsule System Sterilization

Microbial contamination due to human handling is a function of the surface area handled, the contamination rate of the handler, the number of times handled, the die-off rate of deposited micro-organisms, and the time elapsed between contamination and decontamination. (See paper by McDade et al.)

Microbial contamination due to the intramural environment of the assembly facility is a function of the contamination rate from sedimentation of airborne viable particles, the types of surfaces contacted, die-off rate of deposited micro-organisms, the time elapsed between contamination and decontamination, and the surface area exposed to the environment. (See paper by McDade et al.)

The internal microbial contamination on the assembled capsule system is determined in the same manner as that previously described for the subassembly; i.e., compute the microbial load isolated from decontamination by ETO (Occluded surfaces).

In order to predict the microbial load on and in the capsule system, it is necessary continually to monitor the environment and personnel procedures, and from the data collected predict the microbial load. If mathematical rigor is applied to this process, it becomes an almost indeterminable function. This is readily seen if one attempts to develop a model utilizing all the factors mentioned in the three paragraphs above. In order to develop complete rigor, such variables as surface curvature, roughness, and electrostatic forces must be included.

If the predictability of nearly all of the parameters involved is of such questionable value, how then can the microbial load be estimated? A thorough statistical analysis of the microbiological data relative to fallout of airborne viable particles has not been accomplished. However, there are sufficient data and planned experimentation to perform a statistical analysis, and future data

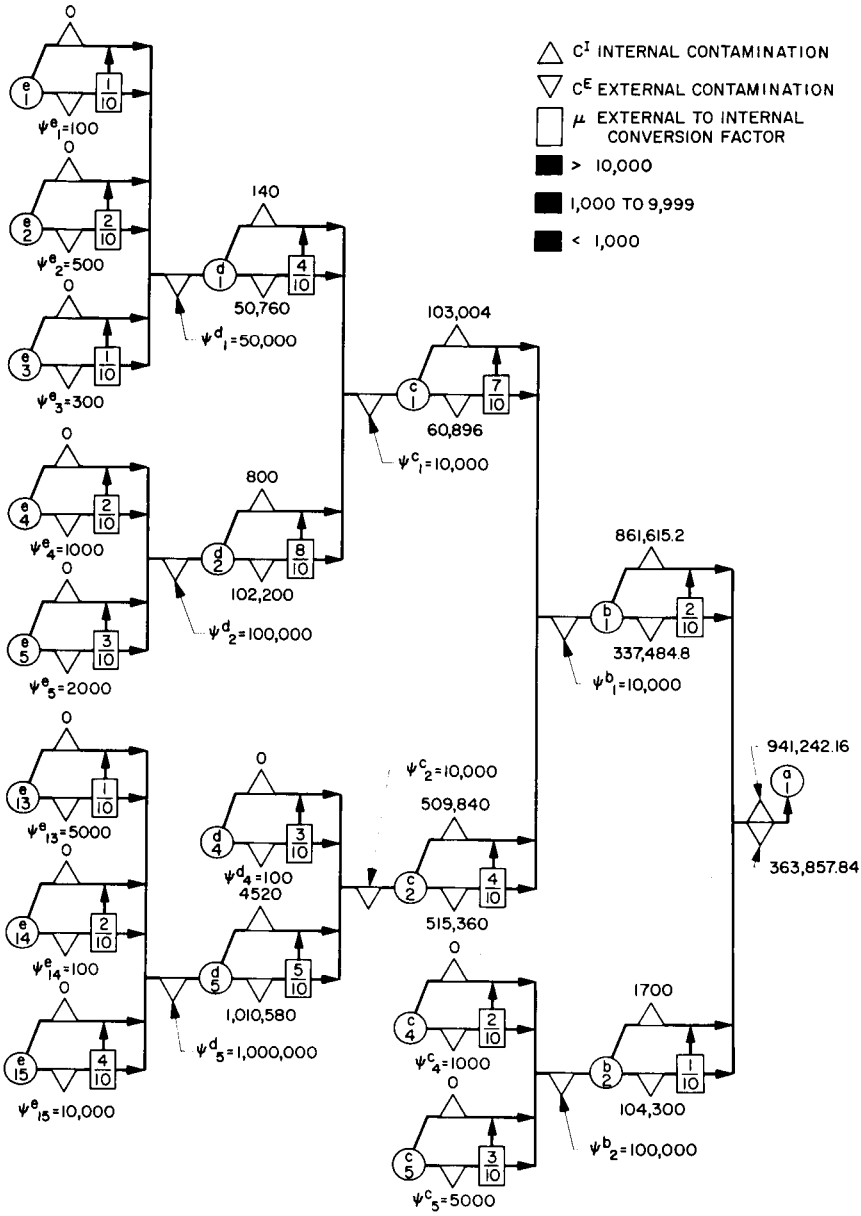

FIGURE 1.—Representative assembly logic for expressing microbial contamination at final assembly.

should be used for a continual refinement of the analysis. The same statement can be made for the microbial contamination resulting from personnel handling procedures.

It is then assumed that the basic microbiological data can be statistically analyzed and that this analysis will provide sufficient knowledge of the surface contamination distribution so that a bound on the contamination level can be stated with a specified significance level. The problem then becomes one of microbiological data acquisition, storage, retrieval, and analysis.

In order to make surface contamination predictions relatively easy, it is assumed that the entire surface area of the capsule system collects microbial contamination at the same rate as that collected by a horizontal flat surface. The next step in the microbial load prediction process is to determine the internal microbial load, which is that portion isolated from decontamination by ETO as a result of mating surfaces together during assembly. Once the values for the internal and surface microbial load are determined, they can be analyzed in the same way the subassembly was analyzed; i.e., determine which microbial load is preeminent and treat accordingly.

A Quantitative Analysis

Although figure 1 is a simplified and hypothetical assembly logic, it is representative. There are several methods of arriving at an algebraic expression of the level of microbial contamination at the final assembly. The method selected factors out the microbial contamination predictor and places within brackets all the conversion factors which operate on the specific microbial contamination. This has the advantage of permitting analysis on each specific contamination.

The following equation describes the total internal microbial load at assembly $^{a}_{1}$:

$$
\begin{aligned}
I_1^a = {} & \psi_1^e[\mu_1^e + (1-\mu_1^e)\mu_1^d + (1-\mu_1^e)(1-\mu_1^d)\mu_1^c + (1-\mu_1^e)(1-\mu_1^d)(1-\mu_1^c)\mu_1^b] \\
& + \psi_2^e[\mu_2^e + (1-\mu_2^e)\mu_1^d + (1-\mu_2^e)(1-\mu_1^d)\mu_1^c + (1-\mu_2^e)(1-\mu_1^d)(1-\mu_1^c)\mu_1^b] \\
& + \psi_3^e[\mu_3^e + (1-\mu_3^e)\mu_1^d + (1-\mu_3^e)(1-\mu_1^d)\mu_1^c + (1-\mu_3^e)(1-\mu_1^d)(1-\mu_1^c)\mu_1^b] \\
& + \psi_4^e[\mu_4^e + (1-\mu_4^e)\mu_1^d + (1-\mu_4^e)(1-\mu_1^d)\mu_1^c + (1-\mu_4^e)(1-\mu_1^d)(1-\mu_1^c)\mu_1^b] \\
& + \psi_5^e[\mu_5^e + (1-\mu_5^e)\mu_1^c + (1-\mu_5^e)(1-\mu_1^d)\mu_1^c + (1-\mu_5^e)(1-\mu_1^d)(1-\mu_1^c)\mu_1^b] \\
& + \psi_{13}^e[\mu_{13}^e + (1-\mu_{13}^e)\mu_5^d + (1-\mu_{13}^e)(1-\mu_5^d)\mu_2^c + (1-\mu_{13}^e)(1-\mu_5^d)(1-\mu_2^c)\mu_1^b] \\
& + \psi_{14}^e[\mu_{14}^e + (1-\mu_{14}^e)\mu_5^d + (1-\mu_{14}^e)(1-\mu_5^d)\mu_2^c + (1-\mu_{14}^e)(1-\mu_5^d)(1-\mu_2^c)\mu_1^b] \\
& + \psi_{15}^e[\mu_{15}^e + (1-\mu_{15}^e)\mu_5^d + (1-\mu_{15}^e)(1-\mu_5^d)\mu_2^c + (1-\mu_{15}^e)(1-\mu_5^d)(1-\mu_2^c)\mu_1^b] \\
& + \psi_1^d[\mu_1^d + (1-\mu_1^d)\mu_1^c + (1-\mu_1^d)(1-\mu_1^c)\mu_1^b] \\
& + \psi_2^d[\mu_1^d + (1-\mu_1^d)\mu_1^c + (1-\mu_1^d)(1-\mu_1^c)\mu_1^b] \\
& + \psi_4^d[\mu_4^d + (1-\mu_4^d)\mu_2^c + (1-\mu_4^d)(1-\mu_2^c)\mu_1^b] \\
& + \psi_5^d[\mu_5^d + (1-\mu_5^d)\mu_2^c + (1-\mu_5^d)(1-\mu_2^c)\mu_1^b] + \psi_1^c[\mu_1^c + (1-\mu_1^c)\mu_1^b] \\
& + \psi_2^c[\mu_2^c + (1-\mu_2^c)\mu_1^b] + \psi_4^c[\mu_4^c + (1-\mu_4^c)\mu_2^b] \\
& + \psi_5^c[\mu_5^c + (1-\mu_5^c)\mu_2^b] + \psi_1^b\mu_1^b + \psi_2^b\mu_2^b
\end{aligned}
$$

The symbols used in this equation are defined as follows:

I_k^i internal contamination of kth component in ith level assembly

ψ_k^i prediction of microbial load on surface of kth component in ith level assembly

μ_k^i factor defining amount of ψ_k^i microbial contamination which is converted to internal contamination during next level assembly operation

Although the equation appears formidable, it is really longer than it is complicated. Its length is a result of there being so many contamination predictions, each of which must be operated on by the conversion factors. The value ψ_1^e is selected as a typical contamination, and the operation on ψ_1^e is investigated in detail.

The factor

$$\psi_1^e[\mu_1^e + (1-\mu_1^e)\mu_1^d + (1-\mu_1^c)(1-\mu_1^d)\mu_1^c + (1-\mu_1^e)(1-\mu_1^d)(1-\mu_1^c)\mu_1^b]$$

represents the internal microbial load at the $^a_1$ assembly level resulting from the ψ contamination only. In order to determine the internal contamination at assembly level $^d_1$ resulting from ψ_1^e, the ψ_1^e contamination is multiplied by the μ_1^e conversion factor:

$$\psi_1^e \mu_1^e = (100)(0.1) = 10$$

and the surface contamination is

$$\psi_1^e (1 - \mu_1^e) = (100)(1-0.1) = 90$$

Now since $\psi_1^e (1 - \mu_1^e)$ is the surface load at $^d_1$ assembly, this must be multiplied by μ_1^d to determine the internal microbial load at the next level,

$$\psi_1^e (1 - \mu_1^e) \mu_1^d = 90(0.4) = 36$$

and the surface load at $^c_1$ is

$$\psi_1^e (1-\mu_1^e)(1-\mu_1^d) = 90(1-0.4) = 54$$

The internal load at $^b_1$ is then

$$\psi_1^e (1-\mu_1^e)(1-\mu_1^d)\mu_1^c = 54(0.7) = 37.8$$

and the surface load at $^b_1$ is

$$\psi_1^e (1-\mu_1^e)(1-\mu_1^d)(1-\mu_1^c) = 54(1-0.7) = 16.2$$

and, finally, the internal load at $^a_1$ is

$$\psi_1^e (1-\mu_1^e)(1-\mu_1^d)(1-\mu_1^c)\mu_1^b = 16.2(0.2) = 3.24$$

Now to obtain the total, each contamination prediction must be added:

$$I_1^a = 10+36+37.8+3.24 = 87.04$$

or

$$\begin{aligned} I_1^a &= 100\,[0.9+(0.9)(0.4)+(0.9)(0.6)(0.7)+(0.9)(0.6)(0.3)(0.2)] \\ &= 100\,(0.8704) \\ &= 87.04 \end{aligned}$$

Since it is a lengthy, cumbersome, and unnecessary process to show the substitution of all the ψ and μ values from figure 1 into the equation, this will not be done. Rather, the results of such a substitution will be shown:

$$\begin{aligned} I_1^a = {} & 100(0.8704)+500(0.8848)+300(0.8704)+1000(0.9616) \\ & +2000(0.9664)+5000(0.7804)+100(0.8080) \\ & +10\,000(0.8560)+50\,000(0.8560)+100\,000(0.9520) \\ & +100(0.6640)+1\,000\,000(0.7600)+10\,000(0.7600) \\ & +10\,000(0.5200)+1000(0.2800)+5000(0.3700) \\ & +10\,000(0.2000)+100\,000(0.1000) = 941\,242.16 \end{aligned}$$

A similar equation and solution can be shown for the surface load; however, since this solution is trivial and serves no particular analytical purpose, it will not be shown. It is absurd to carry the computation to 941 242.16 micro-organisms. In the example this was done to complete the arithmetic. In actual practice this would be rounded off to two or three significant figures.

The microbial load prediction values range from 100 to a million micro-organisms. These extremes were chosen to approximate the actual ranges expected in the development of a complex system. A planetary lander assembly can be expected to contain connectors, cables, transducers, etc., which will have total surface areas of a few square inches as opposed to items such as solar panels, thermal shields, sandwich structures, and parachutes which may run into thousands of square feet of surface area.

If the microbial load predictions in figure 1 are summed, it is found that the total microbial contamination is 1 305 100 micro-organisms. Approximately 941 000 are internal and the remaining 364 000 are the surface micro-organisms which can be reduced by decontamination with ETO. It is apparent that in this particular case decontamination with ETO would be of questionable value. The reduction from 1.3 million to 0.94 million through the application of ETO (the 0.94 million is isolated from ETO) is less than a one D-value reduction in the terminal sterilization cycle. It is possible that the manufacture of subassemblies will yield internal microbial contamination ratios considerably higher than those shown in this example. The opposite will be true for the assembly of the subassemblies into the capsule system. Probably less than 5

percent of the subassembly surface area will be mated during the final assembly.

It is interesting to note the computational error when the lower ψ values are ignored. Table I shows this error when values less than 1000 and 10 000 are ignored. In order to indicate this relationship further, the second column excludes the ψ_5^d=1 000 000.

The implications of such an analysis are often overlooked in discussions relative to sterilization. In other words, when building a wooden workbench it is absurd to try to saw the wood to the nearest ten-thousandths of an inch; such accuracy is beyond the state of the art and is not required, even if it were possible.

TABLE I.—*Microbial Load From Fig. 1*

	ψ_5^d= 1 000 000 included for total load of 1 305 100		ψ_5^d= 1 000 000 excluded for total load of 305 000	
ψ values ignored in computation	Internal load only	Error, percent	Internal load only	Error, percent
None (all ψ included)	941 242		181 242	
All ψ less than 1000	940 304	0.01	180 304	0.5
All ψ less than 10 000	931 360	1.0	171 360	5.5

This general philosophy is good, but the problem is that the example used is incomplete. For example, if there were 10 000 parts, each contributing 1000 micro-organisms, the total microbial contamination would be 10 000 000 micro-organisms. This would dwarf a single part which contributes 500 000 micro-organisms. Does this mean that the single part with its half million micro-organisms should be ignored? Of course not, but it does provide a rationale which can be used to determine where effort needs to be placed, and further, it provides a system for reevaluation and shifting of emphasis.

Conclusions

Basic Microbial Load Prediction

Many inaccuracies exist in the determination of the microbial load on a capsule system. However, it appears that quantification of the microbial load is amenable to a statistical analysis. The following items indicate why the computational results can be stated with confidence.

(1) *Surface area:* This parameter can be increased by a safety factor which will assure adequacy of the prediction.
(2) *Internal conversion:* Same as above.
(3) *Uniform contamination:* Assumes that all surfaces, vertical, inverted, curved, etc., collect contamination at the same rate as a horizontal flat surface.
(4) *Type of contamination:* Assumes that the entire microbial load consists only of the bacterial spore population whose D-value is the basis for the dry-heat sterilization cycle.

The basic mathematical effort which must be accomplished in order to make this microbial load analysis is the determination of the statistical techniques to be used. Several approaches should be investigated, such as the use of extreme value theory, standard logarithmic distribution, and Poisson distribution. Whatever technique is found appropriate can be utilized to set either ranges or maximums on the microbial load predictions.

It should be noted that the example used did not statistically manipulate the data; it merely analyzed the synthesized results expected if such manipulation were conducted. Thus it does not represent the statement of contamination at a significant level, which is the desired output.

Analysis of Environmental and Handling Predictors

As noted earlier, it is expected that the microbial contamination collected on surfaces within the space hardware assembly facilities will be measurable. Much evidence exists to support the so-called "plateau" which indicates that the microbial load increases rapidly and reaches an asymptotic value which varies with different environments. This plateau effect will simplify the analysis required to develop microbial load predictions. (See paper by McDade et al.) However, any attempt to develop a mathematical model describing the process which creates this plateau leads to frustration. The process is not completely understood by the microbiologists; however, they are continually obtaining data to verify this, and, possibly, when the process is better understood it will lead to a mathematical description.

Microbial contamination resulting from human handling is in the early stages of investigation. It is not known how the plateau effect described above will affect microbial contamination by human handling. However, it is generally expected to influence the prediction, since most of the contamination resulting from human handling will be vegetative micro-organisms and can be expected to die away fairly rapidly.

Areas of Research Required to Improve Microbial Load Prediction

Areas of research needed to improve analysis include:

(1) Both areas mentioned above; namely, improved prediction of handling and environmental contamination. (See paper by McDade et al.)

(2) The homogeneity of the microbial population needs to be investigated. The *D*-value could be appreciably altered if it were possible to determine more accurately species of micro-organisms to be found on a spacecraft.

(3) A statistical analysis to determine a system of predicting the probability of having achieved sterility within a confidence interval or limit.

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Session IV—Discussion

Orhon, *Aerojet-General Corp.*: Being a biologist, not a designer, I would like to ask what happens after a capsule is sterilized and sent into outer space. I imagine that it would have to be protected from the Earth's contamination. If so, at what point would the separation between the clean surface and the contaminated surface come into view? And what is the danger during separation of recontamination of the clean surface?

Magistrale: I touched upon the concept of a microbiological barrier and posed a number of attending problems that this would entail. The microbiological barrier containing the capsule is mounted in the spacecraft. At some point separation occurs, and the capsule is projected toward its destination. Where this sequence of operations will occur is under study at the present time; there are a number of alternate choices. I think Dr. Craven has mentioned that the guidance and control people would like separation at about 10 days out, while there are other people who feel that the barrier could serve as a meteorite cover that would protect the capsule.

Comment: One of the problems in spacecraft sterilization involves final checkout and the possibility of trouble occurring while the capsule is in the spacecraft on the pad. How does one go about replacing defective parts through a biobarrier without causing recontamination?

Magistrale: That would be a situation to avoid, since the magnitude of the problem of final checks and repairs would be overwhelming. Your point is well taken, however, and it is essential that techniques of sterile insertion and repair be developed.

Hall: The technique of sterile insertion and repair is the subject of newly engaged research and we sincerely hope it will be successful.

Session V

Sterilizable Capsule Components and Subsystems

CHAIRMAN: Frank E. Goddard
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Spacecraft Sterilization Program

The activities of the Bioscience Programs Division (BPD) of the National Aeronautics and Space Administration (NASA) have been discussed in the foregoing papers. The spacecraft engineering activities performed as part of the Supporting Research and Technology Program of the Lunar and Planetary Programs Division (LPP) of NASA are to be discussed in the following papers. This paper will introduce and provide some background for the following papers, all of which deal with our efforts to create the technology necessary to develop sterilizable spacecraft for planetary landings. The term "sterilizable" is here defined as "serviceable and reliable after heat sterilization preparatory to a trip to and a landing on Mars."

The following tasks are the responsibility of Lunar and Planetary Programs Division:

(1) Develop sterilizable materials, components and spacecraft
(2) Propose sterilization facilities and procedures to BPD for approval
(3) Develop, build, and operate the facilities in which the spacecraft is manufactured and sterilized
(4) Perform all sterilization operations
(5) Deliver items of spacecraft hardware to BPD or to its contractors for biological assay to determine the effectiveness of the sterilization procedures
(6) Assemble the spacecraft
(7) Take the necessary precautions to insure that the unsterile spacecraft will not inadvertently impact and contaminate the planet

Since much of the work in the sterilization program is directly for and applicable to Voyager, some parts of the current program are now in the process of being transferred from the Supporting Research and Technology Program to the Voyager Program; this transfer will be completed in 1966. Thereafter, "mainline" activities of Voyager will be handled by Voyager people; the Supporting Research and Technology effort will move on in its traditional

role of providing alternatives and backup technologies for current missions, including Voyager, and for a long-range advances in the technologies of spacecraft hardware to enable the planning of future missions that would not otherwise be possible.

Implementation of Sterilization Program

At the start of the program we adopted the approach of giving first priority to parts, materials, and subassemblies (such as photomultiplier tubes) that will not change greatly from mission to mission. A list of the items likely to be required for the first missions requiring sterilizable hardware was prepared, individual tasks were assigned, and work was started. Those tasks and many others added since are mentioned in the following papers. In some cases our work was primarily a job of proving that current state-of-the-art items are reliable after heating; at other times we had to develop a totally new item. Throughout we have had to prognosticate what the flight missions might need so that we could have the technology on the shelf by the time the project people needed it. Systems work, especially our procedures tasks, was based on "typical" equipment, since it was not known what specific missions or configurations would be flown.

The following papers will give a broad view of the amount of work now underway in many different places. These tasks will be reported in breadth rather than in depth so that more of them can be covered; our aim is to inform the reader as fully as possible of the work that is being performed, of the people doing the work, of the present status of work now underway, and of present plans for future work.

Background of Sterilization Requirements

A word is in order about the history of the sterilization program. Early in 1963, the Associate Administrator of the Office of Space Science and Application (OSSA) directed that a program to develop sterilizable spacecraft be started. Many planning sessions resulted; these sessions were attended by representatives of several groups, including BPD, and LPPD, and several NASA centers. A philosophy and plan of action were formulated and many tasks were outlined.

We were advised by the biologists that the planetary landers (capsules) would have to be assembled in clean rooms. We were told that the "aerospace" clean rooms we use to eliminate dust particles for reliability reasons would be satisfactory if we would install biological filters for the air and if our step-by-step procedures were approved in advance by BPD and meticulously fol-

lowed. We understood that the capsule and major subassemblies would be assembled in this clean room. What was to be done about the internal cleanliness of small piece parts was not, until recently, resolved. We understood that ethylene oxide would be satisfactory for surface decontamination, and so our tasks call for the hardware to be compatible with this gas. We understood that BPD and its representatives would monitor every step we took which might affect sterility of the flight hardware.

We were advised by the biologists that one cycle of heat of 135° C for 24 hours applied to the spacecraft manufactured in the clean room by approved procedures would satisfy the 10^{-4} requirement for sterilization. We decided that a margin of safety was needed, and so we selected three cycles, each of 36 hours at 145° C, as the requirement for qualifying hardware. All our work to date has been carried on with this as a minimum requirement.

Later on, a document entitled "Interim Requirements for Clean Room" listed considerably more rigorous clean-room requirements. A considerable amount of our work was adjusted to fit these new requirements. Very recently we have been told that a class 100, laminar downflow clean room will be required for assembly. This requirement is both more rigorous and more expensive than the previous ones.

In our early planning, the biologists of BPD instructed us to furnish them with samples of the flight hardware, "black boxes" and subassemblies as well as piece parts, for their assay, so as to assure that there had been no "slips." We have recently learned, however, that it is not possible for biologists to assay a typical black box. We have now been advised that the alternative to total clean-room manufacture, from mine to manufactured subassembly, is for us to apply an additional 10 D-values of heat decontamination. This would amount to, for example, 18 additional hours at 135° C for flight items. The work we have done to date, based as it is on 24 hours at 135° C, will not yield reliability data upon which to design flight hardware that will be subjected to 42 hours at 135° C.

A great deal of time and money has been invested in this program to date, and we hope to use the data we have already obtained as far as possible. We hope that a way will soon be found to quantify the biological parameters so that adequate sterilization requirements can be determined from objective measured data and so that we will not commit ourselves to costly and unnecessary overobligations.

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Electronic Parts Sterilization Program at the Jet Propulsion Laboratory

This report documents the electronic parts sterilization program at the Jet Propulsion Laboratory (JPL). The program is geared to reflect current NASA sterilization policy. The major effort of the current JPL program is concerned with heat sterilization. The primary objective is to establish an approved list of heat sterilizable electronic parts. The secondary objective is to establish maximum information in the new era of higher operating temperatures and longevity of electronic parts.

The NASA sterilization policy requires that all Martian spacecraft activity be conducted in such a manner as to reduce the probability of contamination of Mars with viable Earth organisms to less than one chance in 10 000. Both the mission trajectory, that is, the possibility of impact, and the degree of spacecraft sterility are facets to be considered. However, only the hardware element, particularly the relationship of the sterilization policy to electronic parts, will be discussed in the present report.

Whereas the necessary degree of spacecraft sterility can be achieved through thermal, chemical, or radiation techniques, the thermal approach appears to be the most attractive possibility in the electronic parts. This is not meant to indicate that radiation and chemical methods, particularly the use of ethylene tetraoxide gas (ETO), are not of interest; indeed, ETO-sterilized electronic parts studies of significant proportions will soon be initiated by JPL. However, the major effort in the current electronic parts sterilization program at JPL has been concerned with heat sterilization.

Primary Objective

The primary objective of the electronic parts sterilization program is to establish an approved list of heat-sterilizable electronic parts. This program is intended to consider and study the effects of the thermal sterilization environment on electronic parts, specifically in relation to the reliability of the devices. The sterilization environment has been accepted as a biologically

established constraint; that is, a given temperature for a certain time duration is required to produce the required sterile conditions. Current requirements detail 135° C for 24 hours as a satisfactory and sufficient sterilization environment for flight equipment. It should be understood that the equipment is in a nonoperational storage condition during this sterilization cycle. Application of the sterilization requirement to electronic parts during type-approval testing results in the following increase in the demands: the parts shall be capable of meeting the operational reliability requirements after being subjected to three 36-hour periods of nonoperational storage at 145° C. The proof and assurance that the electronic parts of interest will indeed meet the type-approval demands rests in the parts sterilization program. The program has been designed to meet and fulfill a JPL need; however, it should be obvious that the results of the effort will be applicable to all spacecraft operating within the given engineering constraints.

JPL has been studying sterilization effects resulting from 135° C for 24 hours for more than 2 years in accordance with the acceptance by NASA, in 1963, of this temperature-time relationship as adequate for sterility of flight equipment. In July 1965, NASA expanded the acceptable temperature-time relationship into a range beginning at 105° C for 336 hours, including 135° C for 24 hours (actually 22 hours), and extending to 160° C for 3 hours. As of the date of this report, no official action has occurred to change the sterilization requirement from 135° C for 24 hours for flight gear. However, JPL has given careful technical consideration to the results of a change to a different temperature and time.

The parts sterilization program will produce a variety of documents describing techniques, processes, procedures, and test results. The primary results will be contained in a summary document which outlines electronic parts sterilization candidates for spacecraft applications. This document will present, in tabular form, the part candidates, a brief description of each part, a listing of the specific part's supporting procurement document, and the status of the test program applicable to the part in question.

The listings in the summary sterilization parts document have a close direct relationship with the tabulation of parts in the JPL Preferred Parts List, in that candidates for the sterilization program must have a preferred rating. Therefore, a discussion of the major policies and philosophy surrounding the Preferred Parts List will at this point assist one in understanding the intent of the Sterilization Parts List.

The JPL Preferred Parts List (PPL) is a compilation of high-reliability electronic parts applicable to all JPL electronic space-

craft equipment. Listing of an item indicates that it is known to be of interest to Laboratory design engineers. Rigid controls govern the addition and deletion of entries from this document. Parts are removed only for one or more of the following reasons: (1) actual test data indicate the failure of source process controls, (2) a qualified replacement part results in listing obsolescence, (3) the detail part specification is canceled or (4) the part design standard drawing is canceled.

Parts are added to the list only after (1) a JPL need has been verified, (2) the part has been qualified under the Laboratory's rigorous qualification requirements, (3) appropriate support specifications have been prepared, and (4) the support documents have been cleared (accepted) by the manufacturer of the part.

It is the intent of the PPL to provide a list of high-use standardized items which have been appropriately qualified. It is not the purpose of the PPL to list all electronic parts employed on any given spacecraft. The number of part types listed in the preferred list are maintained at a minimum consistent with the nominal spacecraft-demonstrated usage and with the ability to justify all listed items through appropriate qualification information.

The PPL document format includes, in addition to the parts listings, recognition of parts reliability levels (i.e., Hi-Rel, Preferred, and OSE), definitions of the involved reliability levels and their basic qualification and acceptance test requirements, and general derating consideration statements.

The PPL has received major emphasis and expenditures of effort at the Laboratory for several years. Therefore, the sterilization parts list and the PPL are interrelated to the maximum degree possible to capitalize on the PPL experience and information. Indeed, a part is not generally considered a candidate for the sterilization test studies unless listed on the PPL. Special low-usage parts or new state-of-the-art parts are expected to cause unusual situations. Therefore, future part requirements will necessarily modify this policy; however, qualification will remain a major requirement in the parts sterilization effort.

The discussion of the sterilization parts list has been pursued particularly with the thermal sterilization environment as the predominant mode of achieving sterility. However, considerable attention is also being given to ETO thermal compatibility as a portion of the sterilization program. The ETO studies, utilizing the available literature information on parts and materials and subsidized by test effort as required, will seek to answer the basic question, "Is ETO, when employed as a gaseous sterilant, less than

fully compatible with all categories of electronic parts?" Information produced by the ETO studies will be included in the sterilization parts list.

Secondary Objectives

The secondary objective of the parts sterilization program is to establish maximum information in the new era of higher operating temperatures and longevity of electronic parts. This includes the studies of part wear-out phenomena, derating in the matrix-type tests, the relationship of piece-part parametric ratings to the part's true ability, and the overall reliability of parts over long periods of time. Special consideration is being given to the production of data in real (lapse) time to serve as control or reference information for accelerated life studies. The secondary objective also includes the production of figures-of-merit information relating certain similar parts within a given parts category. The data are also expected to produce strong support for parts-screening efforts. The failure-analysis portion of the program will result in product improvement through close cooperation with the parts manufacturer.

Parts Sterilization Program

At the very outset of the JPL parts sterilization program, a basic philosophy was established. This included two major points, one a byproduct of the other, but certainly nonetheless important. Primarily the program was designed to establish the previously discussed approved list of heat-sterilizable electronic parts. This includes the production of appropriate assurance and/or proof that the itemized parts can indeed survive the sterilization environment. Secondly, the general reliability characteristics of the parts in question must be ascertained as they relate both to the sterilization environment under consideration and to the requirements relating to long operational life.

In cooperation with NASA sterilization policy and the established basic philosophy, the Laboratory is pursuing an extensive electronic parts sterilization program to fulfill the primary and secondary objectives. The current parts program, utilizing heat as the sterilant, was initiated in May 1963. However, a small capacitor sterilization investigation was conducted as early as March 1962. The earlier effort explored certain of the monitoring concepts employed in the current program.

Comparison of the test program existing in 1962 and early 1963 with the present test effort forcefully illustrates the magnitude of the current undertaking. The early tests included about 500 capacitors made up of 15 part types. The present program involves

42 814 parts made up of 262 part types (see table I). The presently planned and implemented test phase will produce about 418 million part test hours of data. The testing will be completed in 1967 (excluding follow-on considerations). As of July 1965, approximately 70 percent of the part testing effort was implemented. The remainder of the effort has been involved with advanced stages of software. In addition to the planned and implemented tests, certain follow-on tests are anticipated. These will involve new parts not available at the initiation of the given test. The follow-on tests will also include parts proposed as substitutes for unapproved devices (parts failing the test criteria). Certain tests will also be performed to provide additional data and information of greater depth where special problems and difficulties exist or are discovered.

TABLE I.—*Current Electronic Parts Sterilization Test Categories*

Part category	Number of part types	Total number of parts
Capacitors	28	4 200
Resistors:		
Fixed	82	14 294
Variable	24	2 640
Diodes:		
General	36	3 856
Varactor	4	384
Fuses	8	7 120
Thermistors	8	800
Transistors	32	3 200
Crystals	5	150
Relays	9	1 800
Microcircuits	7	700
Inductors	19	3 040
Total	262	42 814

The major portion of the parts sterilization test program is being accomplished under contract with industry. Only the more complex state-of-the-art items remain at JPL as inhouse endeavor.

The scope of the parts sterilization program is further compounded by three other related studies of particular significance. These include the previously discussed ethylene tetraoxide gas (ETO) sterilant tests, the electronic-part packaging considerations, and the studies to produce appropriate sterilization screens. The intent of the ETO effort has been discussed and needs no further comment.

The packaging studies are seeking to establish methods of handling sterile electronic parts from the end of the production line to component fabrication. Many parts are sterile as they leave the production line as the result of manufacturing processes. Adequate methods of handling, shipping, storing, and control must be established to maintain this sterility, or sterilization techniques must be needlessly reapplied. Existing information indicates that the number of thermal sterilization cycles which can be used is limited. The effects of ETO are in question; therefore, every effort must be made to maintain sterility at source.

The screening studies must produce screens capable of separating "maverick" parts from a given lot without expending unnecessary sterilization cycles. Simple sampling techniques are not suitable because of the demands for maximum reliability. This problem is severe and has no current acceptable solution.

Program Approach

In order to fully understand the parts sterilization program, some degree of comprehension of the test requirements and test approach is required. As will be recalled, the previously discussed basic philosophy of the program established two concepts: (1) the program is to produce sterilization information, and (2) the program is to produce general parts reliability data.

The procedures to implement and achieve success under the basic philosophy must necessarily vary in certain of the minor points depending on the specifics of the part in question. However, in general, the part types experience the procedure which is discussed below.

The electronic parts to be tested are selected from the Preferred Parts List on the basis of anticipated usage. All manufacturers supplying parts for the sterilization test program are contacted prior to the procurement of test specimens. The intent of the program is carefully explained. The manufacturers are encouraged to modify, improve, screen, or select the proposed test specimens in any manner which seems prudent to the manufacturer. The Laboratory requires only that similar parts be available for future procurement. Part procurement specifications are employed in the purchasing of the specimens to assist in uniformity and quality. Where feasible, more than one manufacturer is included for a particular part type. In this way, a part type evidencing significant degradation can be compared between manufacturers; that is, if the basic part type is susceptible to change, all of a manufacturer's parts of that type should evidence degradation; otherwise, degradation may be due to a particular manufac-

turer's quality control or design. Each manufacturer's part type is normally divided into four groups. Group A is the control group; these part types are not temperature cycled (simulating heat sterilization). Group B is temperature cycled three times (nonoperational), first to 145° C for 36 hours in an inert atmosphere and then to 25° C for 24 hours. Groups C and D are temperature cycled as above, except for six cycles. The method of simulating heat sterilization was selected so that it would be compatible with a JPL environmental test specification for a compatibility test for planetary dry-heat sterilization requirements.

The purpose of the four groups is as follows:

(1) Group A is a control group (no sterilization environment applied) and is compared with groups B and C during the 10 000-hour life test.

(2) Groups B and C are the heat-cycled groups. Three and six cycles are used in an effort to determine the effects of a different number of heat cycles both initially and during the 10 000-hour life test.

(3) Group D is stored nonoperational at maximum rated temperature for 10 000 hours; then the parts are life tested for 250 hours at maximum rated temperature and voltage along with the group C parts (which serve as a special control group). Group D is intended to simulate a mission where specific equipment would be nonoperational during the flight and then turned on at or near its destination.

All groups (except group D) are subjected to a 10 000-hour dynamic life test, with parts operating at maximum-rated electrical and temperature conditions following the sterilization environment.

The data will be analyzed statistically at the 95-percent confidence level by using the statistics outlined in a JPL specification for a general specification computation and submittal of component-test statistics in addition to those specified in the test documents.

Basically, all of the measurements for a specific parameter within a group are combined, and the mean (average) and the variance (variability of the measurements) are computed after each test step measurement. The means and variances of the before-and-after temperature cycling measurements for groups B, C, and D will be statistically compared. This procedure will seek to determine if the temperature cycling has a significant effect on the measurements; that is, if the mean or average of the parameters shifted or if the variability of the individual parameter measurements changed. This same before-and-after comparison technique is used at the 100, 250, 500, 1000, 2000, 4000, 6000, 8000, and

10 000-hour life test steps to test for changes in parameter measurements. In addition, the group A (control group) mean is compared with the applicable group B and C mean at each of the life test steps. This determines if the temperature-cycled parts react in a significantly different way during life testing than the non-temperature-cycled parts. During life testing, catastrophic failures will be recorded, and this information will be used to determine failure data for the final analysis. Upon completion of life testing, the following additional statistics will be computed:

(1) The final analysis of the means and variances included within group comparisons and between group comparisons.

(2) Graphs of the mean of the measurements for each parameter against time during life test.

(3) Exponential failure rate at 90 percent confidence for each group.

(4) Failure rates (a Weibull distribution will be used to indicate whether the failure rate is increasing, decreasing, or constant, or a combination of the three failure modes).

(5) The history of the previous parameter measurements of each failed part, compared with the previous parameter measurements of the unfailed parts from the same group. From this information the feasibility of predicting failures from early nonconforming parameter measurements will be investigated.

(6) Summary of test program including sterilization parts recommendations.

Approach Philosophy

There are three major portions to every given test project. There must be a test design resulting from a hypothesis, the test must be performed in accordance with the design, and there must then be appropriate analysis of both the specimen failures and the produced data. In addition to these general requirements, it is also prudent in most cases to provide a feedback loop from the portions of the test concerned with data reduction and failure analysis to the test design, so that the test method can be improved and perfected.

With these concepts in mind, the previously discussed elapse-time test approach will be compared with the compacted-time or accelerated test approach. First, a description of a nominal accelerated test approach is in order. This plan, commonly referred to as the step-stress test, consists of a method of subjecting a given sample of test specimens to a certain stress P_1 for a specific time interval t_1. At the end of the test interval the stress is removed, and the specimens are allowed to stabilize to standard test conditions. Parametric measurements are performed and recorded. The failed

devices are removed from the test. The remainder of the samples are then subjected to a higher stress, P_2, for the selected time interval. Again the stress is removed and the specimens are allowed to stabilize to the standard test condition, with the attendant parametric measurements being again performed and recorded. This sequence of events is continued until all or some specific percentage of all specimens fail. Concurrently with the described test action utilizing the time interval t_1, other similar test efforts involving a number of selected test intervals t_2, $t_3 \ldots t_n$ are occurring.

The data are assembled in graphic form. A possible presentation of this information is shown in figure 1, where a function of stress $f(S)$ is plotted against time. (The abscissa of these plots is normally a logarithmic expression.)

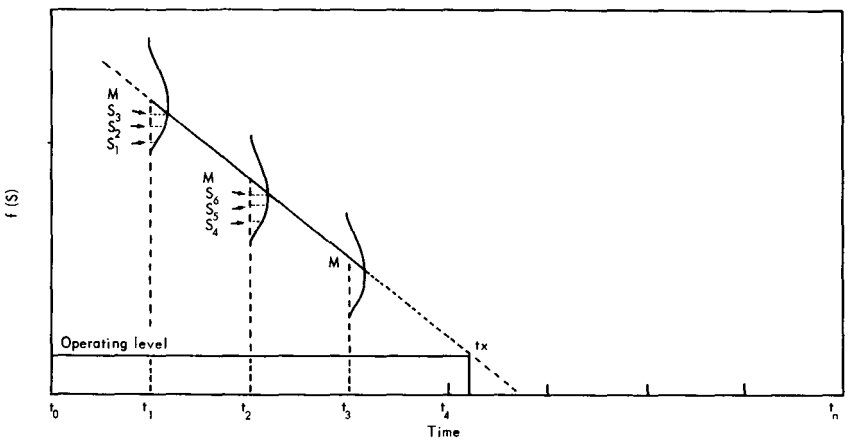

FIGURE 1.—Presentation of step-stress information.

The elements of the distribution curves in figure 1 (examples $S_1 \ldots S_6$) lying on any ordinate t_1, $t_2 \ldots t_n$ are made up of failure data obtained at the various stresses resulting from P_1, $P_2 \ldots P_n$. The slope intercept is drawn through any chosen point, say the median M, of each distribution curve. This intercept is extended by extrapolation to any chosen operating level and yields a predicted failure rate at time t_x at that operating level. Time t_x will usually be from 30 to 50 times above the longest test time interval involved in the actual test.

The step-stress accelerated test is only one approach for achieving high stress loading in the interest of compacting test time. Another method employs high constant stress for defined time

periods or until a given percentage of the test specimens fail. This method also allows a slope intercept to be drawn which relates measured failure distributions and a predicted failure rate at some grossly extended time by means of extrapolation. These methods, if expediently performed, should yield similar predicted values as end results.

The major advantage of the accelerated test is obviously one of time compaction during the specimen screening. The major disadvantages relate to (1) a questionable accuracy of the required extrapolation methods, (2) the unknown anomalies resulting from the assumption that the required temperature cycling associated with the parameter measurements per se is insignificant, (3) the assumption that the failure modes throughout the stress range are constant and similar to those modes encountered in practical application, and (4) in the case of step-stress techniques, the assumption that prior steps do not affect subsequent test levels.

Comparison of the elapse-time test and the accelerated-test time techniques center around two major issues: time and acceptable proof. The accelerated approach has a tremendous time advantage. However, the accelerated approach does not provide a test-proven hypothesis. Therefore, an implemented program with the accelerated approach must in general depend upon other correlating elapse-time information for substantiation of the produced accelerated data. The corollary of these statements indicates that the elapse-time approach is slow, but the test will produce factual evidence in accordance with its test design. For these reasons, it is obvious that the elapse-time approach can support the accelerated studies with solid basic information in order that the accelerated technique may be examined, compared, and improved. The reverse statement is not true, in that the accelerated approach does not necessarily produce baseline information. The elapse-time approach has been employed to study the effects of sterilization. There are enough unknowns within the program without introducing problems through questionable measurement techniques.

These comments should not indicate that there is no interest in accelerated testing. Indeed, the advantages and disadvantages of accelerated testing techniques are being explored at the Laboratory. The analysis of failed test specimens is important information as is the study of the produced test data. Together, this information describes modes of failure. However, the most important aspect of the current work is the feedback of the failure mode information into the test design. Proof that the hypothesis is correct must be obtained prior to any extensive use of the technique.

Program Status as of July 1965

The bulk of the parts sterilization test phase, based on selection criteria in the Preferred Parts List, has been implemented. This does not mean the testing has necessarily been initiated; however, action to involve electrical tests is in process (see table II). Tests of one category of parts, the capacitor, are well advanced toward completion. Whereas it is the intent of this document to consider only the general aspects of the parts sterilization program, it is appropriate to examine briefly some of the broader aspects of the capacitor test, it being the first parts category to complete 10 000 hours of test. A total of 4200 capacitors representing 28 distinct types of dielectrics were selected for this test program. The dielectric types were:

(1) Ceramic (Medium K style)
(2) Porcelain
(3) Glass
(4) Mica (dipped style)
(5) Solid tantalum
(6) Foil tantalum
(7) Paper/plastic
(8) Mylar

In accordance with the general plan, prior to ordering the units for test the purpose of the sterilization test program was explained to the manufacturer of each capacitor selected for evaluation. Therefore, each manufacturer had the same opportunity to submit premium capacitors with the best capabilities of meeting the objectives of this test program. In addition, the manufacturers were required to burn-in each capacitor for a period of 250 hours at maximum-rated direct-current voltage and temperature prior to shipment.

The 10 000 hour capacitor phase of the program has been completed. However, because of certain wearout-type observations made in the later part of the 10 000-hour phase, a considerable portion of the earlier capacitor phase will continue on to 16 000 hours of test time. In addition, a follow-on test has been initiated to provide specific supplemental capacitor information.

The dielectrics most significantly affected by sterilization temperature cycling were the ceramic and solid-tantalum types as summarized below.

Ceramic Dielectric

The capacitance and dissipation factor of the ceramic dielectric increased as a result of sterilization. However, during the 10 000-hour life test at rated direct-current voltage and temperature, the

TABLE II.—*Electronic parts sterilization program schedule (as of July 1965)*

Test		1965									1966											1967											Completion
Type	Number	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	
Capacitors		5			3	Follow-on test → 5																												Dec. 1965
Fixed resistors					4	9950 hr → 5																												Oct. 1965
Trimming resistors					4	8300 hr → 5																												Jan. 1966
Varactor diodes					4	7120 hr → 5																												Aug. 1966
General diodes			1		4	5				5																5								May 1967
Transistors				4	4	5																5												Dec. 1966
Microcircuits				4	4	1000 hr → 5																5												Nov. 1966
Fuses						1		2		3							5										5								June 1967
Crystals							1			2		3			5*				1			2		3										Aug. 1968
Relays						1		2		3																		5							July 1967
Magnetics						1		2		3																		5							July 1967
Thermistors						1		2		3							5										5								June 1967
Capacitor follow-on						1		2		3							5										5								June 1967
Temperature gradient							1				5																								Jan. 1966

1 Start of test

2 Sterilization and environment, start

3 Life testing, start

4 Present status (July 1965)

5 Complete

* Phase 1 completion date

capacitance dissipation factor decreased gradually and approximated the values observed before sterilization.

The temporary increase in capacitance and dissipation factor resulting from sterilization is probably acceptable, providing the circuit applications permit a maximum capacitance increase of 20 percent and a maximum dissipation factor value of 2.5 percent at 1 kilocycle.

Solid-Tantalum Dielectric

As a result of sterilization, the insulation resistance of certain test specimen groups decreased by a factor of 10. After 100 hours of life test at rated direct-current voltage and temperature, the insulation resistance of these specimens generally increased to the values observed prior to sterilization. However, at this same period of time, several catastrophic failures occurred in one of the test groups whose insulation resistance was affected by sterilization, whereas the first catastrophic failures of the test groups unaffected by sterilization occurred after 4000 hours. After 8000 hours of life test, the test groups affected by sterilization had only a slightly greater number of catastrophic failures.

Although this evidence is inconclusive, it would appear that solid-tantalum capacitors, whose insulation resistance is significantly decreased as a result of sterilization, are more likely to become catastrophic failures in a shorter period of life when stressed at rated direct-current voltage and temperature. However, test groups whose insulation resistance was unaffected by sterilization produced catastrophic failures after 4000 hours of life and became progressively worse through 8000 hours, probably due to wearout caused by stressing at maximum rated direct-current voltage and temperature. In an attempt to reduce the number of catastrophic failures and to isolate the effect of sterilization from normal wearout, a retest of solid tantalum has been initiated using a voltage/temperature-type matrix.

The specific details of the capacitor test are contained in another document. All other parts categories, at the appropriate time, will be similarly documented.

Conclusions

The early information obtained from the first phase of the capacitor test, coupled with the initial data returns from certain other operating test categories, can be utilized to support several interesting observations as early conclusions.

First, the technical data produced will certainly satisfy the prime objective of the effort; that is, to produce a list of approved sterilizable parts. In order to preclude obsolescence, the steriliza-

tion list and its supporting test endeavor must be a dynamic effort capable of accepting, within certain restrictions, new or improved parts. This is particularly significant in the state-of-the-art-type devices including semiconductors and microcircuits.

Secondly, special attention must be given to the maintenance of identity of the test specimens. It is certainly desirable for product improvement by the manufacturers to upgrade a given part. However, changes in materials and processes may totally negate existing sterilization data. This is a particularly serious problem because of the long test time required to produce the longevity information. Proper and complete identification will help preclude this loss. JPL will soon initiate a rigorous parts identification program to meet this need.

The third observation relates to the distortion of information due to the presence of multiple degradation factors. A prime example of this problem can be observed when the information produced by the sterilization environment is compared with the data resulting from the long-life portion of the studies at elevated (rated) temperatures. The degradation factors occurring as a result of sterilization tend to become obscure with time during the life-test portion. This is an undesirable situation; however, the total value of the test is not materially distorted even with the loss of identity of the specific degradation. The rationale for this statement can be understood if it is remembered that the intent is to produce information about approved parts. One of the major requirements for approval is that the part be capable of demonstrating long, reliable, life characteristics.

A fourth general observation indicates the secondary objective may well be so copious as to partially overshadow the primary objective results. Early indications point to the following as types of information envisioned from the tests: (1) the potential ability to produce wearout data in certain parts, (2) the relationship of true part ability to its rating information, (3) derating data, and (4) figure-of-merit information.

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Polymers for Use in Sterilized Spacecraft

Polymeric materials for construction of planetary landing spacecraft require careful evaluation to insure compatibility with ethylene oxide decontamination and dry-heat sterilization. The problem of determining which polymers are sterilizable is not an easy one. Information on compatibility with the present sterilization and decontamination environments is generally lacking. What data are available concern mostly pure polymers which are usually considerably modified in the final manufactured form. For these reasons a test program was initiated to evaluate polymer products for potential application on the Voyager landing capsule.

The program consists of exposing products to two environments: (1) 300° F for three cycles of 40 hours each, and (2) 12 percent ethylene oxide and 88 percent Freon 12 mixture for two periods of 24 hours. Property measurements are made before and after exposure to determine changes in strength, hardness, volume, electrical resistivity, dielectric strength, tear strength, compression set, and adhesion. The tests applied to each class of products vary depending on product application. The classes of materials tested in the program included adhesives, coatings, encapsulating compounds, elastomers, reinforced and unreinforced plastics, films, and tapes. Approximately 160 products are presently under test. Results indicate heat sterilization to be a much more damaging environment than ethylene oxide. About 20 to 25 percent of the materials evaluated are considered unacceptable after heat sterilization. Only two have been appreciably degraded by ethylene oxide.

When the requirement to sterilize planetary spacecraft was established in 1963 there was anticipation of problems ahead with the polymeric materials then in use. There was good reason for this concern; only 2 years earlier when the Ranger Lunar Spacecraft was to be sterilized considerable difficulty was encountered. If similar problems are to be avoided on the Voyager Program, polymers compatible with sterilization must be identified. In order to accomplish this, a test program is underway to evaluate these materials after exposure to the sterilization environments. This paper reviews some of the highlights of this program.

The procedure developed for sterilizing spacecraft is accomplished in two steps. The first is a decontamination process to lower the microbiological population, and the second is a thermal

sterilization cycle that kills the remaining organisms. Decontamination is done with a gaseous mixture of ethylene oxide and Freon 12. This is applied at the subassembly level and to the entire spacecraft after it has been completely assembled. Thermal sterilization is performed by subjecting the completed spacecraft to 135° C for a period of 24 hours.

In order to insure that flight hardware will survive sterilization, it is necessary to test and evaluate identical hardware by subjecting it to a "type approval" treatment which is a little more severe than the actual flight sterilization treatments used. These type-approval requirements are stated in two Jet Propulsion Laboratory Specifications, GMO–50198–ETS, covering decontamination, and XSO–3075–TST–A, covering thermal sterilization. The former requires exposure to a mixture of 12 percent ethylene oxide and 88 percent Freon 12 at a relative humidity of 30 to 50 percent. Two 24-hour cycles are given, one at 24° C and the other at 40° C. The latter specification requires a three-cycle exposure at a temperature of 145° C in dry nitrogen, the duration of each cycle being 36 hours. Basically these specifications have been used in the evaluation of polymers in this test program.

The use of polymeric materials on spacecraft is extensive. A typical example is the Mariner IV electronic subassembly shown in figure 1. Some of the materials in this unit are epoxy adhesives, Teflon-insulated wire, epoxy and polyurethane encapsulants, Teflon and polyolefin sleeving, and polyurethane conformal coating. The largest use of polymers is in the area of electronic packaging; the next largest use is for electrical cabling and associated hardware. Other uses in addition to electrical are typically for thermal insulation, seals, bearings, structural adhesives, battery cases, and potting. The success of future space missions depends in large measure on reliable performance of these materials.

Test Program

The selection of polymeric materials (products) to be tested was given careful consideration. It was decided that we should evaluate the products already used for spacecraft applications first and not look for new materials that might be more amenable to sterilization. This approach has several advantages. For example, these polymers have been proven in service to meet the other demands of spacecraft operation. Additionally, some products have had a considerable effort expended on them over the years in developing processing techniques, specifications, and approved product sources. The program was started by testing the products used on the Ranger and the Mariner spacecraft. The materials presently

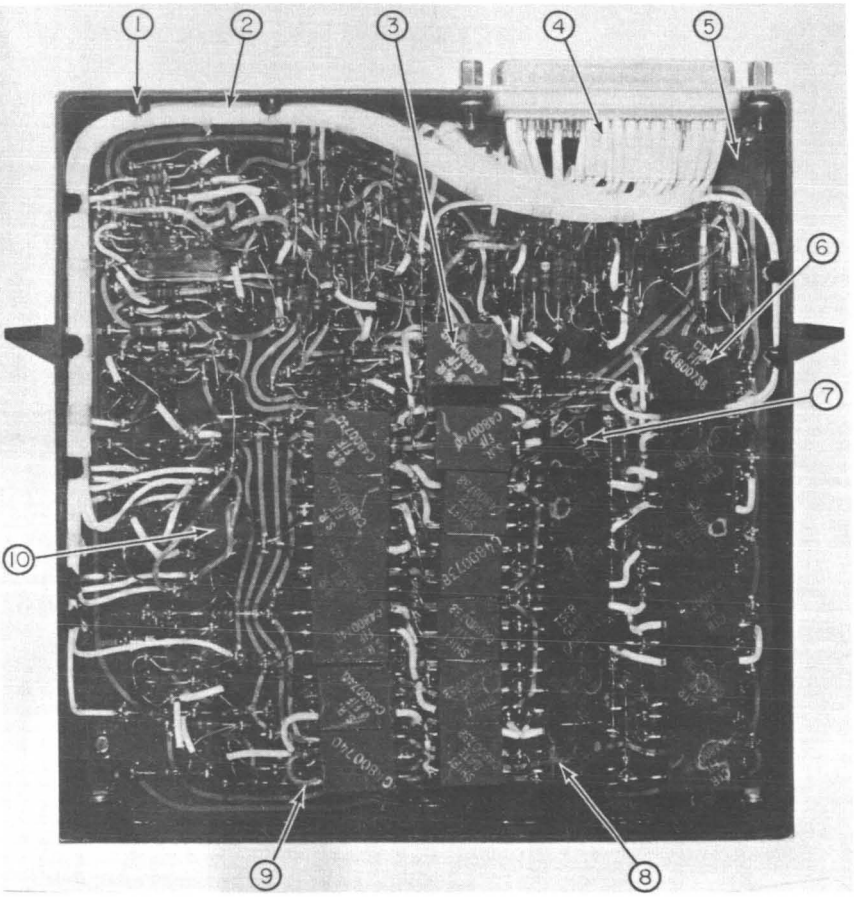

FIGURE 1.—MC–1 analog-digital television encoder subassembly used on Mariner IV. (1) Spot bonding (epoxy); (2) wire insulation (Teflon (TFE)); (3) module case (molded epoxy); (4) shrinkable tubing (polyolefin); (5) circuit board (glass-filled epoxy); (6) module case (molded epoxy); (7) module case (molded epoxy); (8) conformal coating (over entire electronics) (polyurethane); (9) sleeving (Teflon (TFE)); (10) terminal encapsulant (filled polyurethane).

under test, with their type and suppliers, are shown in table I.

The test plan used for evaluating materials is shown in figure 2. Material evaluation is accomplished in four steps: preliminary screening, thermal evaluation, ethylene oxide evaluation, and a combination of ethylene oxide and thermal. Materials that pass preliminary screening are continued on through the remaining steps of the program. Preliminary screening is done to eliminate the very poor materials early before much effort is expended on

TABLE I.—*Polymeric Products Presently Being Evaluated for Effects of Ethylene-Oxide Decontamination and Thermal Sterilization*

Trade name	Manufacturer	Material type
	Adhesives	
A–4000	Dow Corning Corp.	Silicone
R–823	Carl H. Biggs Co.	Epoxy
206 Cement	Caram Mfg. Co.	Polychloroprene
3022	Epoxy Products, Inc.	Epoxy
EC–1614 B/A	Minnesota Mining & Mfg. Co.	Epoxy polyamide
EC–2216 B/A	Minnesota Mining & Mfg. Co.	Epoxy
Eccobond 55/9	Emerson & Cuming, Inc.	Epoxy
Eccobond 55/11	Emerson & Cuming, Inc.	Epoxy
Eccobond 56C/9	Emerson & Cuming, Inc.	Epoxy
Eccobond 57C–A/B	Emerson & Cuming, Inc.	Epoxy
Epon 8/A	Shell Chemical Co.	Epoxy
Epon 422	Shell Chemical Co.	Epoxy phenolic
Epon 828/A	Shell Chemical Co.	Epoxy
Epon 828/2	Shell Chemical Co.	Epoxy
Epon 901/B–1	Shell Chemical Co.	Epoxy
Epon 901/B–3	Shell Chemical Co.	Epoxy
Epon 924A/B	Shell Chemical Co.	Epoxy
FM 96	Bloomingdale Rubber Co.	Epoxy
FM 1044	Bloomingdale Rubber Co.	Polyamide epoxy
GT 200	G. T. Schjeldahl Co.	Polyester
HT 424	American Cyanamid	Epoxy phenolic
A2/A	Armstrong Products Co., Inc.	Epoxy filled
PC 12–007A/B	Hysol Corp.	Epoxy
Pro-Seal 501	Coast Pro-Seal & Mfg. Co.	Polysulfide

RTV 102	General Electric Co.	Silicone
RTV 108	General Electric Co.	Silicone
RTV 140	Dow Corning Corp.	Silicone
Eccobond 26A/B	Emerson & Cuming, Inc.	Epoxy
EC 1103	Minnesota Mining & Mfg. Co.	Epoxy
RTV 891	Dow Corning Corp.	Silicone
E–3022	Epoxy Products, Inc.	Epoxy
Hysol 5150/3690	Hysol Corp.	Epoxy/modified amine

Encapsulants

Polycel 440R	Polytronics, Inc.	Polyurethane
PR1527A/B	Products Research Co.	Polyurethane
PR1930½	Products Research Co.	Silicone
Pro-Seal 777	Coast Pro-Seal & Mfg. Co.	Polyurethane
Scotchcast 3	Minnesota Mining & Mfg. Co.	Epoxy
Sylgard 182	Dow Corning Corp.	Silicone
Stycast 1090/9	Emerson & Cuming, Inc.	Epoxy
Stycast 1095/11	Emerson & Cuming, Inc.	Epoxy
RTV 60/T12	General Electric Co.	Silicone
Hapex 1200/1210	Hastings Plastics Co.	Epoxy
RTV 11/T12	General Electric Co.	Silicone
Stycast 2850 GT/9	Emerson & Cuming, Inc.	Epoxy
PR1930–2	Products Research Co.	Silicone
Scotchcast 241A/B	Minnesota Mining & Mfg. Co.	Epoxy
Stycast 3050/9	Emerson & Cuming, Inc.	Epoxy
Stycast 1095/9	Emerson & Cuming, Inc.	Epoxy
Stycast 1264 A/B	Emerson & Cuming, Inc.	Epoxy
Stycast 2741/15	Emerson & Cuming, Inc.	Epoxy
Eccocoat IC–2	Emerson & Cuming, Inc.	Polyurethane

TABLE I.—*Polymeric Products Presently Being Evaluated for Effects of Ethylene-Oxide Decontamination and Thermal Sterilization*—Continued

Trade name	Manufacturer	Material type
	Encapsulants—Continued	
Eccosil 5000	Emerson & Cuming, Inc.	Silicone
Stycast 2651/11	Emerson & Cuming, Inc.	Epoxy
Solithane 1001/1002	Thiokol Chemical Corp.	Polyurethane
Solithane 113/300	Thiokol Chemical Corp.	Polyurethane
Epocast 212/951	Furane Plastics, Inc.	Epoxy
Selectron 5119	Pittsburgh Plate Glass Co.	Polyester
Scotchcoat	Minnesota Mining & Mfg. Co.	Epoxy
Hysol 4251 (C7–4251)	Hysol Corp.	Epoxy polyester
Epocast 202/9615	Furane Plastics, Inc.	Epoxy
Eccofoam FP/12–6	Emerson & Cuming, Inc.	Polyurethane
Eccofoam S	Emerson & Cuming, Inc.	Polyurethane
Apco Foam 1414–1.5/EPY	Applied Plastics Co., Inc.	Polyurethane
	Elastomers	
AMS 3195	Rubatex Corp.	Silicone sponge
No. 805–70	Plastic & Rubber Products Co.	Butyl
Hadbar 1000/80	Hadbar, Inc.	Fluorosilicone
Hadbar 5000/50	Hadbar, Inc.	Fluorosilicone
L–308–8	Parker Seal Co.	Fluorosilicone
L–449–6/60	Parker Seal Co.	Fluorosilicone

N–195–7/70	Parker Seal Co.	Nitrile
PMP–6035	Pacific Molded Products Co.	Silicone
PMP–6100	Pacific Molded Products Co.	Silicone
PMP–42011AE	Pacific Molded Products Co.	Neoprene
RTV 501	Dow Corning Corp.	Silicone
RTV 615	General Electric Co.	Silicone
No. 1814	W. J. Voit	Butyl
S–417–7	Parker Seal Co.	Silicone
No. 1050–70	Plastic & Rubber Products Co.	Silicone
No. 391–5	Rubatex Corp.	Silicone
No. 391–7	Rubatex Corp.	Silicone
SR–349–70	Stillman Rubber Co.	Buna N
Viton 77–545	Parker Seal Co.	Fluorocarbon
Viton B–60	E. I. du Pont de Nemours & Co., Inc.	Fluorocarbon
Viton B–95	E. I. du Pont de Nemours & Co., Inc.	Fluorocarbon
B–318–7/70	Parker Seal Co.	Butyl
Hadbar 4000/80	Hadbar, Inc.	Silicone
Hadbar XB800–71	Hadbar, Inc.	Butyl
RC–5 No. 1852	Rubbercraft Corp.	Neoprene
RC–5	Rubbercraft Corp.	Silicone
Silastic 1410	Dow Corning Corp.	Silicone
SR 613–75	Stillman Rubber Co.	Butyl

Coatings

B–276	Westinghouse Electric Corp.	Epoxy
Cat-a-lac 443–1	Finch Paint & Chemical	Epoxy
Cat-a-lac 463–1	Finch Paint & Chemical	Epoxy
Corlar 585/586	E. I. du Pont de Nemours & Co., Inc.	Epoxy
Eccocoat VEA/B	Emerson & Cuming, Inc.	Epoxy

TABLE I.—*Polymeric Products Presently Being Evaluated for Effects of Ethylene-Oxide Decontamination and Thermal Sterilization*—Continued

Trade name	Manufacturer	Material type
	Coatings—Continued	
Eccosil 33	Emerson & Cuming, Inc.	Silicone
Varnish 220F	Westinghouse Electric Corp.	Alkyd
Hi-Heat Paint 171–A–28	Fuller Paint Co.	Silicone/aluminum
Insl–X–U86	Insl-X Products Corp.	Melamine
Interchemical 12412	Interchemical Corp.	Fluorosilicone
Laminar–X500 (white)	Magna Chemical Co.	Polyurethane
W 2374	Fuller Paint Co.	Alkyd/silicone
PR–1902	Products Research Co.	Silicone
TUF–ON 747–8	Brooklyn Paint & Varnish	Phenol aldehyde
UC–11659	Pittsburgh Plate Glass Co.	Silicone/aluminum
Uralane 241/973	Furane Plastics Co.	Polyurethane
Electrofilm 2396	Electrofilm Corp.	Sodium silicate/ MoS_2
Electrofilm 4306	Electrofilm Corp.	Phenolic/ MoS_2
Alkanex Varnish 9522	General Electric Co.	Alkyd polyester
B224–2 Varnish	Westinghouse Electric Corp.	Alkyd
Cat-a-lac 463–1–8	Finch Paint & Chemical	Epoxy
Eccocoat EC200	Emerson & Cuming, Inc.	Epoxy
No. 445 Silicone	Sinclair Paint	Silicone
Pyre-ML Varnish (RK–692)	E. I. du Pont de Nemours & Co., Inc.	Polyimide
SR–290	General Electric Co.	Silicone

Reinforced and unreinforced plastics		
Diall 52–20–30	Mesa Products	Diallyl phthalate
Diall FS–4	Mesa Products	Diallyl phthalate
Diall FS–10	Mesa Products	Diallyl phthalate
EG–758–T	Mica Corp.	Epoxy/glass
Fiberglass 91LD	American Reinforced Plastics	Phenolic/glass
Grade H–5834	Westinghouse Electric Corp.	Phenolic/glass
Laminate 500J	Budd Co.	Epoxy/glass/Cu
EG 752	Mica Corp.	Epoxy/glass
Laminate NS	Plastic Center	Phenolic nylon/glass
Lexan 103–112	General Electric Co.	Polycarbonate
Lexan 133–122	General Electric Co.	Polycarbonate
Micarta 8457G–10	Westinghouse Electric Corp.	Epoxy/glass
Micarta Grade 238	Westinghouse Electric Corp.	Phenol formaldehyde/glass-linen fabric
Micarta GX(H17480)	Westinghouse Electric Corp.	Epoxy/glass
Micarta H–2497(G–11)	Westinghouse Electric Corp.	Phenol formaldehyde/glass
Micarta HY180(G–10)	Westinghouse Electric Corp.	Phenol formaldehyde/glass
Micarta LE–221	Westinghouse Electric Corp.	Phenolic/linen
XP–206	Minnesota Mining & Mfg. Co.	Epoxy/glass
Films		
H-Film (Kapton)	E. I. du Pont de Nemours & Co., Inc.	Polyimide
Mylar Type C (1 mil)	E. I. du Pont de Nemours & Co., Inc.	Polyester
Mylar Type D (3 mil)	E. I. du Pont de Nemours & Co., Inc.	Polyester
Mylar Type M22 (1 mil)	E. I. du Pont de Nemours & Co., Inc.	Polyester
Tedlar 200 AM 30 wH	E. I. du Pont de Nemours & Co., Inc.	Polyvinyl fluoride

TABLE I.—*Polymeric Products Presently Being Evaluated for Effects of Ethylene-Oxide Decontamination and Thermal Sterilization*—Continued

Trade name	Manufacturer	Material type
	Films—Continued	
Mylar Type A (10 mil)	E. I. du Pont de Nemours & Co., Inc.	Polyester
Mylar Type HS (0.65 mil)	E. I. du Pont de Nemours & Co., Inc.	
Mylar Type D (5 mil)	E. I. du Pont de Nemours & Co., Inc.	
	Oils and greases	
Aeroshell 7A	Shell Oil Co.	Polyester/lithium soap
Apiezon Grease T	Shell Oil Co.	Hydrocarbon
DC–5	Dow Corning Corp.	Phenylmethyl silicone
Diallylphthalate	Union Carbide Corp.	Phthalate ester
DC–11	Dow Corning Corp.	Silicone
DC–200–350CS	Dow Corning Corp.	Poly(dimethylsiloxane)
Versilube F50	General Electric Co.	Silicone
	Tapes	
Mystic 7000	Mystik Tape, Inc.	Glass fabric/silicone adhesive
Mystic 7351	Mystik Tape, Inc	Mylar/rubber adhesive
Scotch No. 67	Minnesota Mining & Mfg. Co.	Glass fabric/epoxy
Tape No. 27	Minnesota Mining & Mfg. Co.	Glass fabric/epoxy
Tape No. 7455	Mystik Tape, Inc.	Al-Glass/silicone adhesive

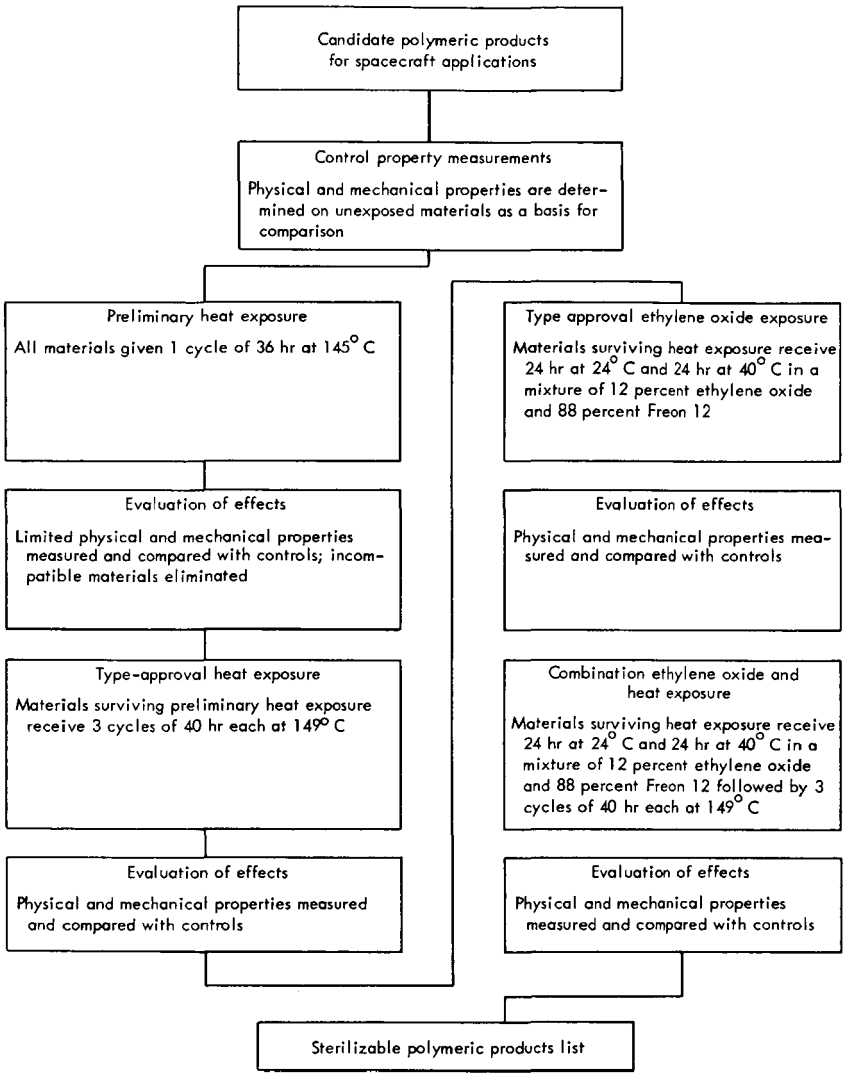

FIGURE 2.—Plan for evaluating polymeric products for effects of ethylene oxide decontamination and thermal sterilization.

them. This is accomplished by exposing each product to a single 36-hour cycle at 145° C and evaluating it for visual effects, volume change, weight change, and, in some cases, tensile strength. If the screening tests show any gross changes in properties, the material is rated incompatible with sterilization and is not tested further.

Products that pass preliminary screening are subjected to further exposures and testing. The next step for materials that

pass screening is exposure to the three-cycle heat environment. Again, the materials that fail this exposure are dropped from the program. The compatible materials are carefully considered, and a selected number from each category are continued on through ethylene oxide exposure and the combination ethylene oxide and heat exposure.

Exposure to the ethylene oxide and thermal environment is done in accordance with the type-approval treatments previously described, except the thermal treatment was increased in temperature to 149° C and the time for each cycle to 40 hours.

After a material is exposed to heat, ethylene oxide, or the combined environment, it is tested to determine if it has been degraded. To completely evaluate each polymer for its actual spacecraft application would require a very large, complex test program, however, because funds and time are not available for such a program, a simple approach is taken. Materials in the program are divided by function into eight categories: adhesives, coatings, encapsulants, films, oils and greases, reinforced and unreinforced plastics, elastomers, and tapes. For each category a group of standard test methods is selected for evaluating materials in that category. Table II shows the tests used for each category. This approach has the shortcoming that it only screens materials and does not actually qualify them for use on the spacecraft; but it does provide a good basis for selecting materials which will later be evaluated more fully in a specific application.

By reviewing the technical literature available for temperature and ethylene oxide effects on polymers, we hoped to find some help for making selections of materials compatible with sterilization. Considerable information on thermal effects was available for pure polymers, but it was surprising to find how little there was on compounded products. We did not expect to find much on compatibility with ethylene oxide, although a small amount of work was done in the early part of the Surveyor Project.

Manufacturers' bulletins and catalogs proved to be the best source of information. They usually contained some clue to the compatibility of specific products with heat. In most cases this information was given as a maximum useful temperature. In addition, a great deal of information was gained on both the methods for mixing and preparing materials properly and on the room-temperature properties of these materials.

Results

Each material evaluated in this program is rated into one of three classes: compatible, marginal, or incompatible. The criteria

TABLE II.—*Tests for Evaluating Compatibility of Polymers With Decontamination and Sterilization Treatments*

Quality evaluated	Category tested								Test standard
	Adhesives	Coatings	Elastomers	Encapsulants	Films	Oils and greases	Reinforced and unreinforced plastics	Tapes	
Tensile strength			X		X		X	X	ASTM D–638, D–412, D–882
Elongation			X		X				
Tensile shear strength	X								FTMS 175–1033, ASTM D–1002
Adhesion		X						X	ASTM D–2197, ASTM D–1000
Compression set			X[a]						ASTM D–395
Tear resistant					X				ASTM D–624
Hardness			X	X			X		ASTM D–676, ASTM D–1706
Viscosity/penetration						X			ASTM D–2196, ASTM D–273
Volume change			X	X					
Volume resistivity		X	X	X	X		X	X	ASTM D–257
Surface resistivity		X	X	X	X		X	X	ASTM D–257
Dielectric strength		X	X	X	X		X	X	ASTM D–257
Flexibility		X							FTMS 141–6223
Weight loss		X	X	X	X	X	X	X	

[a] Applied to heat-cycled materials only.

used are very simple; for a material to be compatible, its property data after exposure must show no degradation. A marginal rating is given when some loss in properties occurs, and only when a material is degraded so badly as to limit its usefulness is it rated incompatible. Where the line should be drawn between marginal and incompatible is established by judgment. Materials rated marginal or incompatible should be used only after careful consideration; however, these materials still can be used in special situations where compatible materials cannot meet the requirements if their losses in properties can be tolerated. It is important to study carefully the property changes with respect to the intended use. Changes in any property show a lack of stability in the material that could lead to unforeseen problems.

The results obtained in the program show the majority of materials are compatible with sterilization. Data obtained so far show about 20 to 25 percent of the materials tested to be marginal or incompatible. Most materials show considerable resistance to heat and ethylene oxide exposure, and in fact some materials are actually improved after heat cycling. It appears that substitution for the few materials found imcompatible or marginal would be easy. Although much testing remains to be done, we are confident that no insurmountable problems exist with the use of polymeric products on sterilized spacecraft.

Of the adhesives evaluated for thermal exposure, only six were degraded; all these suffered from a loss in shear strength. Several other adhesives tested had increased shear strength after exposure; this probably is due to postcuring brought on during thermal cycling.

Encapsulants appear to be the only category where a potential problem exists. Of seven products tested, three had excessive volume changes after either heat or ethylene oxide exposure; in one it was as high as 3.7 percent. Two others experienced a considerable reduction in hardness. A small volume change in these materials is critical to their successful use.

The data on products in most other categories show that only a few materials in each are affected. Reinforced and unreinforced plastics show very good resistance, with only two marginal ratings discovered to date. Four marginal materials have been found among the elastomers because of loss in strength and increased hardness. All the coatings have so far been found compatible, except for three temperature-control coatings that discolored. The only startling results obtained are on two very strength and elongation after exposure to ethylene oxide. Subse-

excellent high-temperature films that lost a significant amount of quent exposure to heat restored some of their strength.

The completed results on the list of materials now in the test program should be available in the near future. The testing should be completed in 1966 and the complete data will be published about that time.

Discussion

The resistance to thermal exposure is difficult to predict for polymeric products that are compounded with other ingredients. Most of the base polymers used in preparing products are thermally stable; typically they are silicones, epoxies, phenolics, fluorocarbons, and polyurethanes. However, in compounding products to do a specific job, ingredients are added to alter the natural properties of the base polymer, and they can also alter the products' heat resistance. These ingredients are classified as: plasticizers, curing agents, flexibilizers, accelerators, stabilizers, flow-control agents, pigments, and fillers. They are used to improve flexibility, resilience, impact resistance, and water-vapor transmission, to lower shrinkage, to raise or lower viscosity, to increase potential life, to lower exothermic temperature rise, to reduce cost, to lower density, to improve adhesion, and so forth. By the use of these modifiers the properties of the base polymers can be changed to fit a myriad of applications; this is what makes polymers so versatile.

If we were dealing only with the pure base polymers, our job would be easy. They have been studied by several investigators and have been found quite stable even after exposure to temperatures above the one with which we are concerned. Decomposition of the base polymer is not the problem we face; it is what happens to these other ingredients which causes trouble. Many are volatile, some are decomposed, and some react adversely with other ingredients or the base polymer. These are the factors that change the properties of material that has been degraded by thermal sterilization.

Unfortunately, the formulations of polymeric products are almost always proprietary. Without information on the specific ingredients in a material, it is impossible to understand its behavior when heated. Because of this we may never be able to predict the thermal stability of most products until they are tested.

Although ethylene oxide and Freon 12 are not considered to be very reactive with materials, there is some concern for their compatibility with polymers. Chemically, it is possible for ethylene oxide to interact with a polymer by physical absorption or by chemical reaction, both of which could affect the properties of the

material. The requirement for materials to be compatible with the combined effect of exposure to ethylene oxide and Freon followed by heat exposure can cause additional problems. It is apparent that any chemical reaction taking place between the ethylene oxide and the polymer (or any of its modifying ingredients) would be accelerated by the 145° C temperature of the heat exposure. Freon 12 used as a diluent in the decontamination gas mixture does not react chemically with most materials; however, it does have solvent properties that can cause swelling of some polymers. There is information reported in the literature on swelling of elastomers after prolonged contact with liquid Freon 12. Some evidence has been found in this program to show that Freon 12 has a similar effect on both elastomers and encapsulants.

From test data so far obtained, it appears that exposure to the mixture of ethylene oxide and Freon 12 is not very damaging to most polymeric products. Significant effects have been found in only two materials, Tedlar 200 and H-film, as previously stated. Physical absorption is believed to be responsible for this behavior, as subsequent thermal exposure of the H-film restores some of its lost strength. However, some chemical reaction could have taken place. A careful investigation is being conducted to understand these effects. It is possible that thin films may be severly degraded by reactions that would be superficial in a more massive product and therefore could go undetected.

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Sterilizable Electronic Packaging, Connectors, Wires, and Cabling Accessories

The determination that spacecraft equipment may require both ethylene oxide (ETO) decontamination and thermal treatments for sterilization has resulted in environment specifications covering these treatments. The effect of the decontamination and sterilization treatments on materials, processes, and components is being extensively investigated under the direction of the National Aeronautics and Space Administration (NASA).

This paper is concerned with sterilization effects on: (1) soldered and welded joints, (2) electronic equipment assembly processes and related materials, (3) electric connectors, and (4) wire and cabling accessories. Preliminary test results are presently available only on the soldered- and welded-joint program. The discussion of the other phases of work covered by this paper consists principally of a description of the test specimens and test methods. The sterilization treatment used was three cycles of 36 hours each at a temperature of 145° C in a dry nitrogen environment, while the ethylene oxide treatment was one cycle of 12 percent ethylene oxide and 88 percent Freon 12 by weight for 24 hours at 24° C and one cycle (same concentration) for 24 hours at 40° C.

Electric Soldered and Welded Joints

The electric connections tested are representative of the type of joint expected to be used on Voyager equipment. The soldered joints included 11 different material combinations representing electronic component leads soldered to terminals, electric wire soldered to terminals, and stranded conductors soldered to two types of connector cups (table I). All surfaces were tinned, prior to joining, with Sn-63 tin-lead solder, and a suitable flux determined by the composition of the material surface was used. All joints were hand soldered with the same type of solder, and a nonactivated rosin-base flux was used.

The welded joints included seven different material combina-

TABLE I.—*Material Combinations for Solder Joints*

[Solder-coated leads were baked at 150° C for 168 hours in argon; all other solid leads were baked at 200° C for 168 hours in N_2; gold-plating done per MIL–G–45204, Type I, Class 1]

Stranded conductor (#24 AWG, Teflon insulated) to connector cups:
Connector cup (JPL Spec. 20045/200–E)
Connector cup (JPL Spec. ZPH 2245–0300–B, DS317)
Stranded conductor (#24 AWG, Teflon insulated, per MIL–W–16878D, Type E) to solder-coated bifurcated terminal (JPL DS 167–7).
Solid conductors listed below to solder-coated bifurcated terminal (JPL DS 167–7):
Copper, gold-plated, OFHC, 0.020
Copper, solder-coated, OFHC, 0.020
Dumet, gold-plated, 0.020 per MIL–STD–1276, Type D
Kovar, gold-plated, 0.018 per MIL–STD–1276, Type K
Nickel, gold-plated, 0.025 per MIL–STD–1276, Type N–2
Nickel, bare, 0.025, per MIL–N–46026
Copper, gold-plated, OFHC, 0.020 to gold-plated bifurated terminal (JPL DS 99–7).
Components (¼-w axial lead resistors/solder coated) mounted between pairs of solder-coated bifurcated terminals (JPL DS 167–3) on glass-epoxy circuit board per MIL–P–13939C, Type FL–GE, 0.062 in. thick.

tions of component lead materials resistance-welded to nickel interconnect ribbon (table II). Weld schedules were determined for each material combination by the isoforce diagram method whereby weld energy (in W-sec) is plotted against weld-breaking strength for constant clamping forces.

Any effects of heat sterilization were expected to occur on the joint bond or interface rather than on the parent material or solder. The exception to this would be a load-bearing joint in which the elevated temperature strength of the solder is a major factor. Thus, the test plan consists of mechanical strength tests, electric resistance tests, electric tests during vibration, and metallographic examination of the various soldered and welded joints before and after the heat sterilization treatments (fig. 1). Stress rupture tests were also specified for the connection consisting of a conductor soldered to an electric connector cup, as this type of joint was considered most apt to be bearing a load during the sterilization treatment. An evaluation of the effects of heat sterilization on gold-plated surfaces that are soldered was accomplished by testing component lead materials with and without gold plating and by conducting electron microprobe analyses to determine the extent of gold diffusion in welded and soldered joints.

TABLE II.—*Material Combinations for Welded Joints*

Nickel (Inco 200) ribbon, 0.010 by 0.031 welded to the following:
Nickel,[1] bare, 0.016, per MIL–N–46026
Nickel,[1] gold-plated, 0.016, per MIL–STD–1276, Type N–2
Kovar,[1] gold-plated, 0.018, per MIL–STD–1276, Type K
Dumet,[1] gold-plated, 0.020, per MIL–STD–1276, Type D
Copper,[1] gold-plated, OFHC, 0.020
Copper, solder-coated,[2] OFHC, 0.020
Kovar,[1] gold-plated, 0.005 x 0.016, per MIL–STD–1276, Type K to itself.

[1]Leads baked at 200° C for 168 hours in inert atmosphere.
[2]Solder-coated leads baked at 150° C for 168 hours in N_2 or other inert gas.

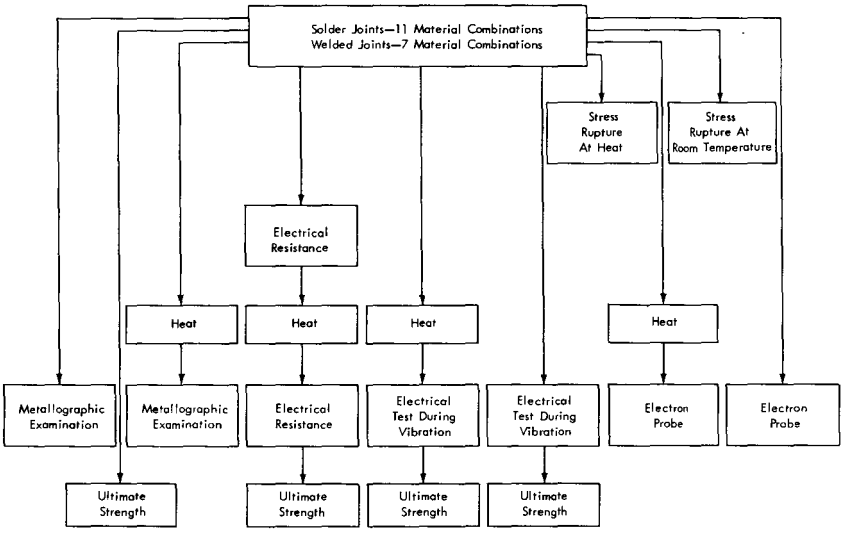

FIGURE 1.—Flow diagram for testing of soldered and welded joints.

Test work was also performed to determine the effect of ethylene oxide decontamination and heat sterilization treatments on the solderability of the various materials used in the fabrication of solder joints. Metallographic examinations and solderability determinations were made on the joint materials both before and after the ethylene oxide and heat sterilization treatments. This work was performed to determine possible problem areas in making equipment modifications and repairs involving soldering after preliminary decontamination and sterilization treatments.

An analysis of the test results showed, with one exception, that no appreciable changes occurred in the physical or electric proper-

ties of the soldered or welded joints because of heat sterilization. The most significant degradation observed in the tests on soldered joints occurred during the stress-rupture tests of the stranded conductor to connector cup joint. It was determined that the stress-rupture strength for 108 hours at room temperature was about 70 percent of the short-time ultimate strength, but that the stress-rupture strength for 108 hours at 145° C was only about 5 percent of the short-time ultimate strength at room temperature. This means that, if a joint of this type has a tensile strength of 15 pounds at room temperature, it may bear a load of only 0.75 pound through three cycles of the heat sterilization treatment.

Solderability tests of the materials specified for solder joints indicated no appreciable effect resulting from the ethylene oxide decontamination treatment. The heat sterilization treatment did result in poorer solderability of two materials tested. These were the solder-coated bifurcated terminals and the gold-plated Dumet leads with nickel undercoat.

An examination of the solder-coated terminals indicated possible errors in processing the terminals prior to the solderability test. Additional work is planned to determine the validity of the present data.

Metallographic examination of the Dumet leads, both before and after heat sterilization, revealed no differences which could account for the poorer solderability. Although not shown in table I, solderability determinations were also made on gold-plated copper leads with a nickel undercoat. Solderability of these leads was unaffected by the heat-sterilization treatment. Further investigation will be necessary to determine the reason for the poorer solderability of gold-plated Dumet (with nickel undercoat) after heat sterilization.

Electronic Equipment Processes

The investigation of sterilization effects on electronic equipment processes and related materials was divided into four subtasks: (1) embedment of welded modules, (2) conformal coating of printed wiring board assemblies, (3) adhesive bonding of metal and plastic surfaces, and (4) strain relief of mounted component leads.

Subtask (1) is related to a wire-connected modular packaging concept recommended for use on the Voyager project. This concept includes the use of a welded cordwood module (fig. 2) with leads welded to a standard header for interconnection.

The other three subtasks are applicable to a terminal-connected planar packaging concept, another of the packaging techniques

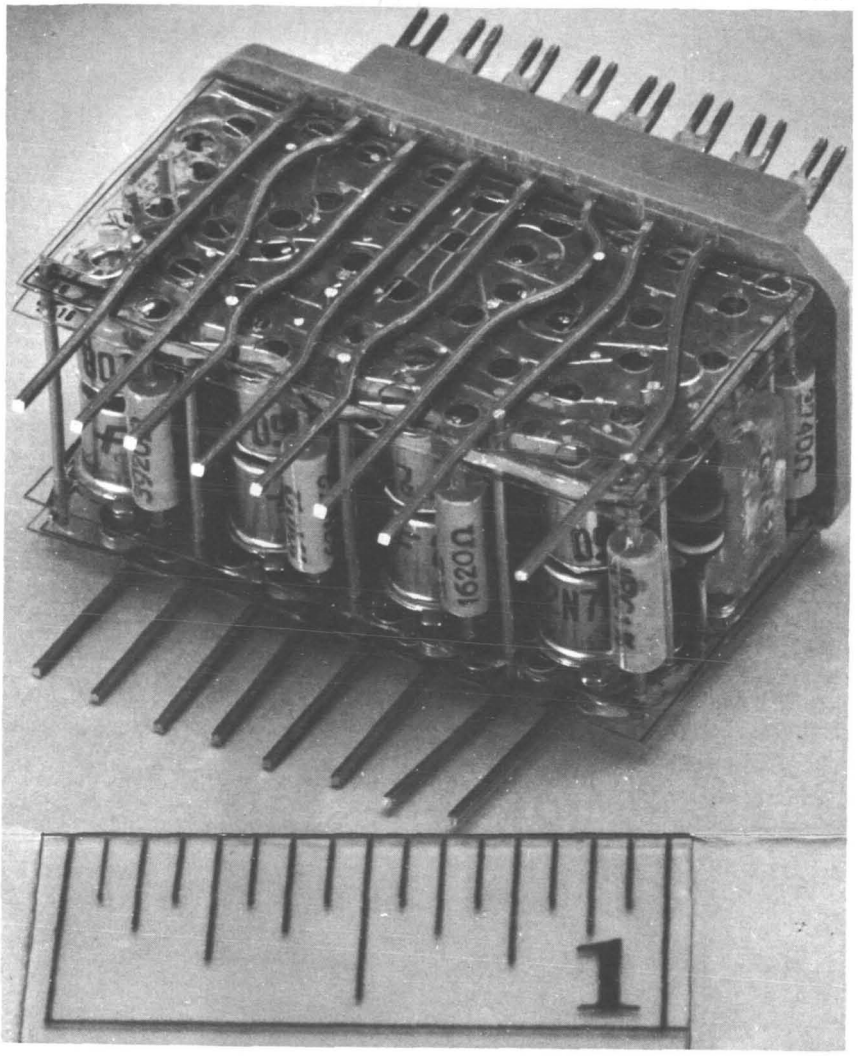

FIGURE 2.—Wirecon logic flip-flop, a welded cordwood module.

used in the Voyager equipment (fig. 3). This packaging concept mounts components between pairs of bifurcated terminals. The components are then covered with a conformal coating (subtask (2)). The adhesive bonding process (subtask (3)) is used to bond an insulation board to the terminal board. This subassembly is then bonded to a magnesium-alloy subchassis. Subtask (4) is designed to determine the stresses in mounted component leads during sterilization treatments and possible effects in solder joints. An evaluation of various methods of strain relief is also to be made, depending on preliminary test results.

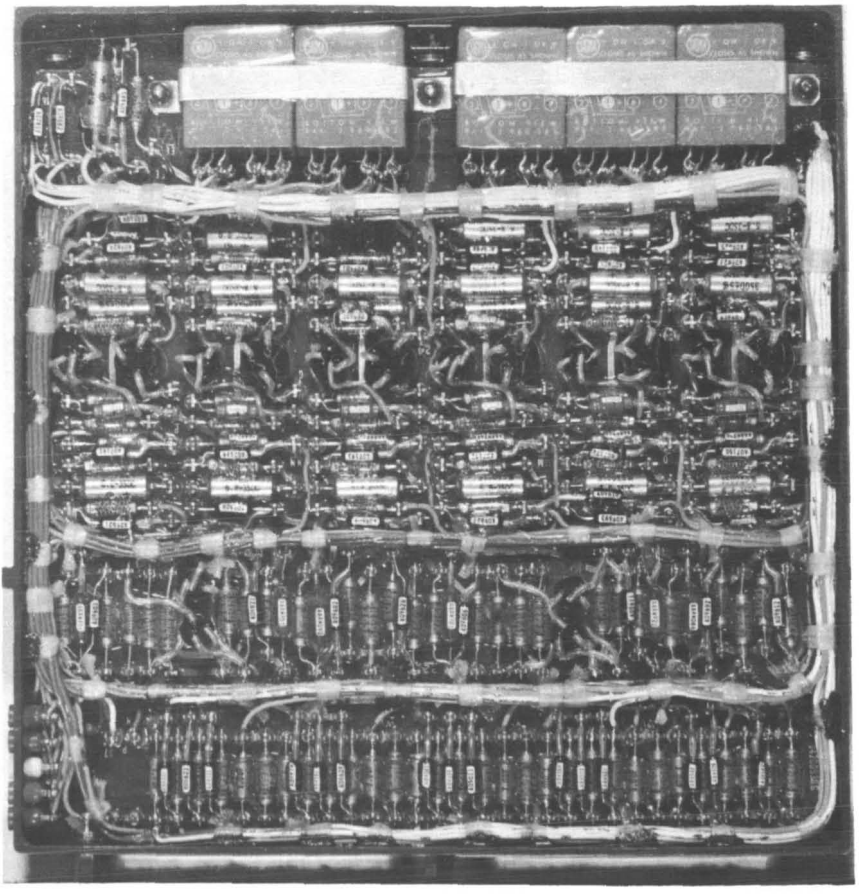

FIGURE 3.—Terminal-connected planar package.

The electronic components used in fabrication of the test modules for subtasks (1), (2), and (4) serve as sterilization treatment degradation indicators. All of these parts are burned-in for screening and stabilization purposes by using the heat sterilization environmental test specification requirement of 145° C for 108 hours. After module fabrication, the operating parameters of each component are determined.

The test specimens for subtask (1) (embedment of welded modules) consist of a series of modules each slightly less than 1 cubic inch in volume and containing 18 components with leads welded to pins of a wirecon header and to an interconnect ribbon. Five carbon resistors are included in the components within the module and are used to determine the module's internal stresses on embedded components. These groups of resistors have been cali-

brated by use of a deadweight hydraulic tester so as to determine changes in resistance versus external pressure. The remainder of the components are used to determine any degradation in operating parameters as a result of either the embedment or of the ethylene oxide decontamination and heat sterilization treatments.

Parallel tests are being conducted on embedment systems to measure embedment stresses by means of a thermometer increment method developed by R. E. Isleifson and F. D. Swanson of Minneapolis-Honeywell. This method, which was tested at JPL, shows that a thermometer calibrated for mercury rise versus pressure applied to the bulb can be used directly for embedment pressure measurements.

Test specimens for subtask (2) (conformal coating of printed wiring board assemblies) consist of a number of board assemblies, each containing 18 components mounted between pairs of bifurcated terminals. The glass epoxy terminal board is bonded to an insulated board and then to a magnesium base plate. Assemblies with and without conformal coating are subjected to the ethylene oxide and heat sterilization treatments. Four of the mounted resistors are used as pressure-indicating devices as in subtask (1), and the remainder of the components are tested for any degradation in operating parameters as a result of either the conformal coating or the ethylene oxide decontamination and heat sterilization processes.

The test specimens for subtask (3) (adhesive bonding of metal and plastic surfaces) consist of a series of 1-inch-wide, epoxy glass, laminate strips bonded to surface-treated magnesium alloy. Lap shear tests of the bonded specimens are conducted (1) as bonded, (2) after each cycle of the heat sterilization treatment, (3) after each cycle of the ethylene oxide decontamination treatment, and (4) after the combined ethylene oxide and heat sterilization treatments.

The test specimens for subtask (4) (strain relief of component leads) are the same as those for subtask (2), with components mounted between bifurcated terminals on a glass epoxy laminate bonded to an insulation board and then to magnesium alloy. Strain gages mounted on the component leads measure the dimensional change caused by the soldering process and then by the heat sterilization treatment. Operating parameter measurements are also taken on the various components to determine the effects of these strains on solder joints or part performance. These results will determine whether the method of mounting components with straight leads between pairs of terminals is acceptable or if methods of lead strain relief are to be tested and employed.

The test program outlined above has not been completed at this

time. It is believed that the test results will provide data on sterilization effects occurring within complex modules and will provide a suitable instrumentation approach for evaluation of additional, sterilizable polymers.

Electric Connectors

The determination of sterilizable electric multipin connectors is proceeding at the Jet Propulsion Laboratory (JPL) under a single work unit of the SRT Sterilization Program. At the present time a program of sterilization-effects testing is scheduled to begin under contract with the Hughes Aircraft Co. The plan of test is described in this report.

Selection of Sterilizable Connector Candidates

When candidates were selected for the sterilization test, the following designs were considered:

(1) A circular miniature design similar to the JPL Specification ZPH–2245–300, an improved version of MIL–C–26482, is still applicable and desirable for future spacecraft assemblies. An earlier version of this connector, called the "JC," has been performing for over 10,000 hours on the Mariner IV. It was also used on Ranger flights, and demonstrated reliable electric and mechanical performances.

(2) A rectangular subminiature design developed to JPL Specification ZPH–2245–302, an improved version of MIL–C–8384, is applicable and desirable for rack-and-panel assemblies. A subminiature D-connector was produced under JPL Specification 20045/200E for Ranger and Mariner IV assemblies. However, in order to assure reliable performances, a JPL modification was attached to each contact which exhibited a specified marginal contact retention force measurement prior to the last mating of any connector before flight. Although the subminiature D-connector is a favorable rack-and-panel configuration, basic changes to the contact design are mandatory if it is to be used in future spacecraft assemblies. Two promising design improvements are being explored for this possibility.

(3) A substitute circular miniature design similar to JPL Specification ZPH–2245–300 should be considered with the applicability of connectors designed to other military specifications. In the cases where a crimped attachment may be employed, the designs of NAS 1599 and MIL–C–38300 are promising sterilizable candidates. JPL electric evaluations of certain MIL–C–38300 connectors preliminary to sterilization have been favorable.

In addition to the above definitions of preferred connector

configurations, certain elemental features of selection which are necessary for the overall spacecraft application are:

(1) Nonmagnetic properties
(2) No petroleum additives or other lubricants
(3) Burrs less than 0.001 inch
(4) Attachment polarization
(5) Insert materials of silicone rubber, diallyl, and epiall are acceptable
(6) No outgassing metals (cadmium, zinc, tin)
(7) Interfacial seal
(8) Iridite or gold finishes
(9) Final plating on contacts: hard gold, Type II, Class II
(10) Locked coupling

More specific descriptions of the foregoing elements are included in the JPL detail design specifications and design standards drawings. In preparation for the sterilization-effects study, connector specimens of the above descriptions were procured and wired for test.

Test Plan

Figure 4 outlines three procedures of sequential testing which will be used to subject the candidate specimens to the sterilants. In this case, the sterilization method to be used is governed by JPL heat sterilization Specification XSO–30275–TST–A. It is basically identical to the proposed method of terminal heat sterilization which is intended for planetary spacecraft capsules. The decon-

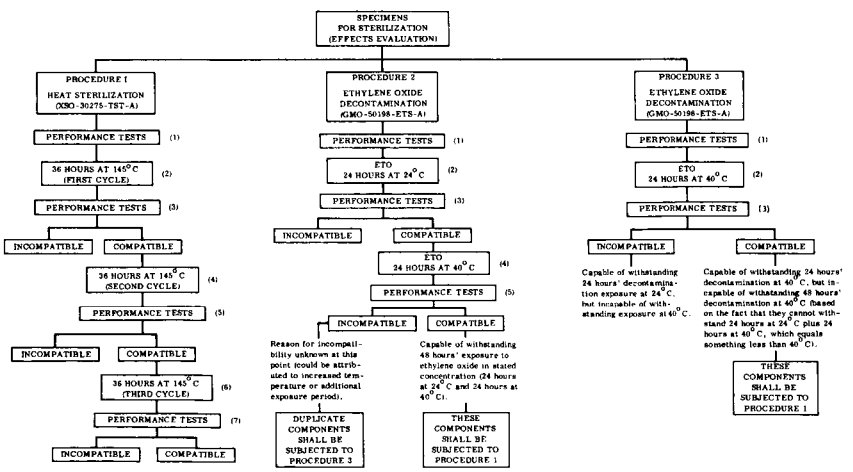

FIGURE 4.—Three procedures of sequential testing. "Performance tests" are specified visual, physical, and electric tests.

tamination method employs ethylene oxide and procedures of application which are governed by JPL Specification GMO–5019–8–ETS–A. It is also a proposed method for decontamination of parts which will be used in the assembly of sterilizable modules.

Connector specimens of each type of candidate in mated pairs will be arranged in four identical lots. During the test, one lot will remain unsterilized, but all lots will be subjected to the same performance test measurements as a basis of comparison. A second lot will be subjected to the heat sterilization cycles and measured for electric and mechanical characteristics following each heat cycle. As is noted by the steps of the block diagram in figure 4, specimens which have failed significantly will be set aside as being incompatible as a result of the heat cycles. In certain cases, it is expected that degradation of one or more elements of a connector might not prevent it from being tested to the next significant heat cycle for further evaluation of one of its other parts.

Procedure 2 is expressly designed to determine the specimen compatibility at 24° and 40° C in separate exposures with ETO. Should any connector specimens of the third lot not survive the last step of procedure 2, then an identical lot is subjected to the ETO sterilant of procedure 3 for a determination of whether incompatibility is a result of exposure at 40° C for 24 hours. The survivors of this procedure would be subjected to the heat sterilization cycles and tested for performance, thereby simulating a proposed method of parts decontamination and sterilization. Performance measurements of the interim steps would determine degrees of degradation.

Effects of Sterilants and Possible Problem Areas

A connector is basically composed of material, parts, and assembly elements which can be affected by the actions of the sterilants. More specifically, we consider the possibilities of degradations in the polymer of the insert and sealing gaskets, the plating and finishes, the joining adhesives and the base metal structure. Failures and defects may show up in any of the following ways.

Swelling or Decomposition of the Polymer From the Effects of ETO

In a recent examination of a diallyl insert for a rectangular connector, it was suspected that the insert was not completely cured. Under this condition, contact positions would change and possibilities existed for distortion of the insert under mating. The cognizant engineer employed a simple chloroform test to detect the presence of an undercured condition. The method is based on the ability of chloroform to dissolve incompletely crosslinked resins.

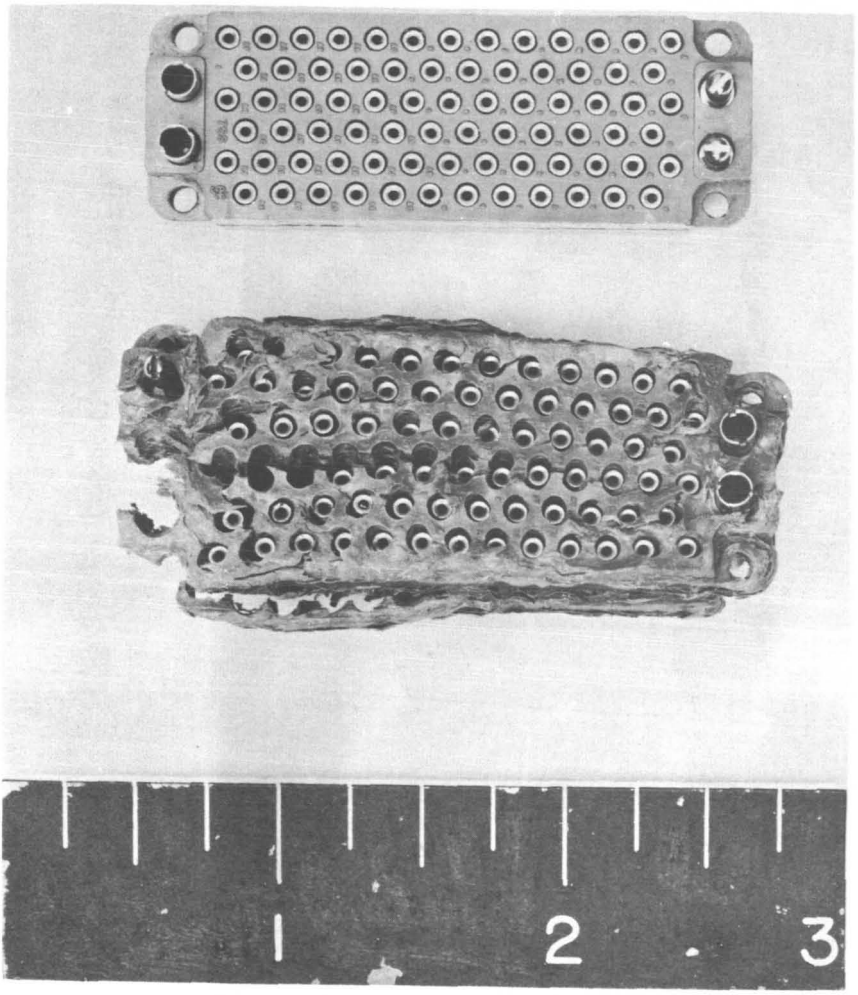

FIGURE 5.—Connector insert of diallyl material before and after being subjected to test consisting of boiling connector in chloroform for a period of 3 hours. Results of test show insert has not been fully cured.

Half of a specimen is boiled in chloroform for 3 hours. Subsequently it is removed and examined for defects which may be anything from roughness of the insert surface to outright disintegration of the insert. The latter case is illustrated in figure 5.

A similar specimen was divided and one part was just partially treated and compared with its other half which was postcured. A significant change in shape of the uncured portion was apparent, as is shown in figure 6, which is conclusive evidence that the manufacturer had not fully cured the diallyl.

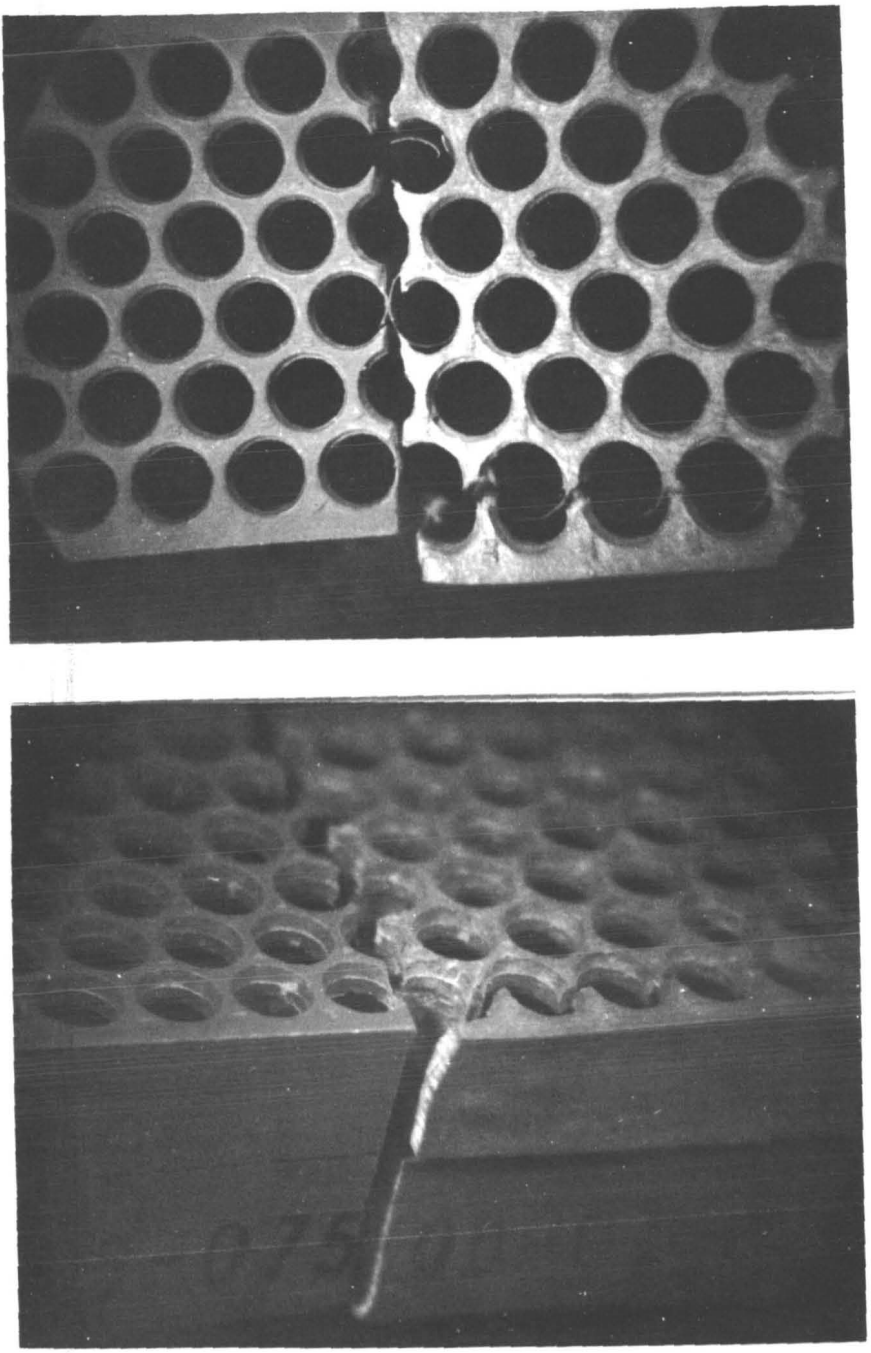

FIGURE 6.—Insert was sawed in two. Halves in left views were given additional cure of 2 hours at 300° F (149° C); half in right view was left "as cured" by manufacturer. Both halves were then boiled in chloroform for 3 hours.

Splaying of Pins Occurring From Heat and From ETO Exposures

It is not likely that heat will affect the selected candidates and cause splaying of pins. Actually both the subminiature D-connectors and the JPL JC's were designed for the sterilization requirement at the time when it was planned to sterilize the early Rangers, Mariners, and the Surveyor. These connectors, however, were never qualified as sterilizable parts. Additionally, it is anticipated that the connectors fabricated to MIL–C–38300 and NAS 1599 specifications might not be significantly degraded by the proposed sterilization heats to cause splaying of pins.

Ethylene oxide, on the other hand, is absorbed and might be suspected to cause displacement of pin positions.

Separation of Insert From Shell and Contacts Which Are Joined by Adhesives

Separation of the insert from the shell and contacts joined by adhesives has occurred on the JC design. It was subsequently corrected by the development of an improved insert mold and substitution of Dow Corning Sylgard 1377 adhesive. Twenty-five different adhesives were used in the development test before Sylgard 1377 was selected. The JPL DS 310 series connectors being produced under JPL ZPH–2245–300 detail design specification utilize a silicone rubber insert with Sylgard 1377 adhesives for attachment of contacts and the insert to the shell body.

Loss of Insert Material and Other Volatiles

Figure 7 illustrates the results of a standard volatile condensible materials (VCM) test which was performed on JB and JC circular miniature connectors. The JB connectors which contained neoprene inserts similar to the MIL–C–26482 designs were used on Rangers. The JC connectors which contained silicone rubber inserts were used on the Mariner IV spacecraft.

The subject test is performed in a vacuum of 1×10^{-6} torr (nominal pressure). The condenser plates were at 75° F. The "haze" which has deposited from the JC connector is apparent. Petroleum, which is used as a lubricant in molding neoprene inserts, has formed as droplets on the plate from the JB connectors. The acceptance criteria for condensibles are specified in JPL connector detail specification as 1.0×10^{-4} g/sq in.

Although Sylgard 1377 has been shown to be a suitable adhesive under normal atmospheric conditions, it is a suspected adhesive under heat sterilization and high vacuum pressures. At 300° F and a vacuum pressure of 1×10^{-5} torr there is an approximate 10 percent weight loss.

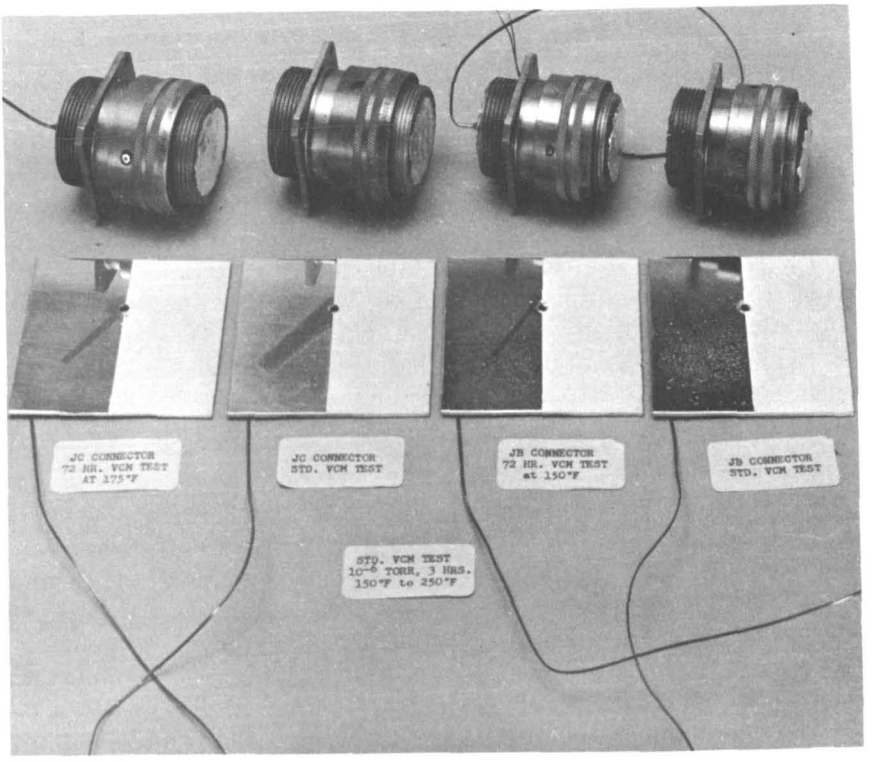

FIGURE 7.—Results of VCM test performed on JB and JC circular connectors.

Condensibles are detrimental to the spacecraft. They can be deposited on optics and thermally controlled surfaces. They can also coat electrical contacts and thereby increase the resistance.

Deterioration of Plating Deposits Under ETO Exposure

Sterilization investigations in the Surveyor program have observed plating outgassing after subjection to ETO exposures. Silver, for example, outgasses at rates less than 2 mils per year at 75° F; copper outgasses at rates greater than 50 mils and gold, at rates less than 2 mils per year. Both silver and copper can catalyze polymerization of ETO if moisture is present and might cause explosive reaction if acetylene contaminants are present in the ETO. The possibility of this can be seen because silver and gold deposits are sometimes absent in parts of connector contacts. Copper strikes and even silver can be exposed and missed during inspections.

It is not known to what extent one might suspect oxidation or other corrosive actions on silver and copper. Another possibility is peeling or flaking due to actions of heat and the ETO.

Decrease of Insulation Resistance and Increase of Dielectric Constant With ETO Exposures

Surveyor tests have shown that each ETO treatment progressively decreases the insulation resistance nonlinearly.

Wire and Cabling Accessories

As a subtask of the SRT work unit for sterilizable connector wire and cabling accessories, an RFP has been released requesting quotations for the study of sterilant effects. The plan is to fabricate cabling assemblies of a number of wires having different insulations which are tied and clamped according to recent cabling specifications. Hookup wire specimens have been procured on the basis of designs from JPL hi-rel Specification ZPH–2239–0940 using TFE and FEP insulations. These designs were specifically developed as promising sterilizable wires.

The list of wire insulations to be tested include polyalkene, H-film polyimides, ceramics, polyurethane, nylon-polyurethane, vinyl-acetate, polyester, irradiated modified polyolefin, polyvinylidene fluoride, and polyethylene-terephthalate.

Lacing cords specimens have been selected with materials of siliconized Dacron, Teflon, Teflon/glass, Dacron, nylon, and silicone glass. The specimens of cable clamps to be tested are made of Teflon, nylon, aluminum/Teflon, and aluminum/Silastic.

Test Plan

The test plan is similar to the approach to be used for connector compatibility evaluations. The procedures will test each part of the wire and cable accessories as assembled cables in order to determine the effects of interface.

Effects of Sterilants on Wires and Cabling Accessories

Some tests have been conducted in earlier studies of wire insulations and cable clamps. It was found that Teflon impregnated with glass increased in weight about 20 percent when exposed to ETO. It was previously mentioned that ETO has a tendency to decrease the insulation resistance of polymers. Teflon will also yield under pressures of 66 psi at 121° C. In more recent tests of materials by JPL, H-film, a polyimide insulation specimen, had sublimed after exposure to ETO.

Figure 8 is an illustration of entrapped air bubbles and other contaminants which have been found in a hookup wire wrapped with 2-mil H-film over ½-mil FEP. Under heat, the air bubbles expand and cause a separation of the insulation wrap. Figure 9 is an illustration of FEP over H-film insulation which contains many pinholes and stress lines radiating at right angles to the edge of the

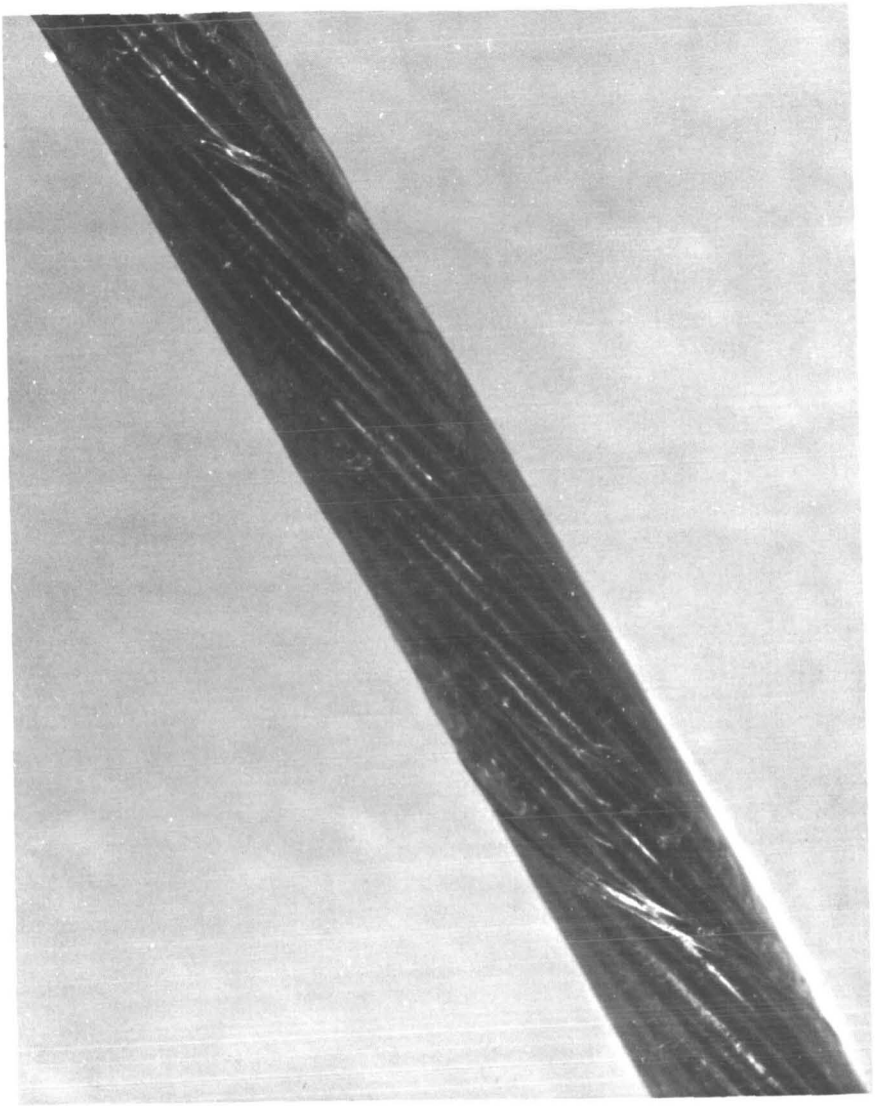

FIGURE 2.—Entrapped air bubbles and other contaminants in hookup wire wrapped with 2-mil H-film over ½-mil FEP. Color, amber; conductor No. 20.

insulation lap. Under heat, these fractures can propagate still further. The defects are susceptible to ETO absorption.

Qualification of Sterilizable Connectors, Wires, and Cabling Accessories

After the studies of sterilant effects are completed, it is planned to conduct a series of qualification tests of the final designs in much the same manner as that outlined in the military QPL require-

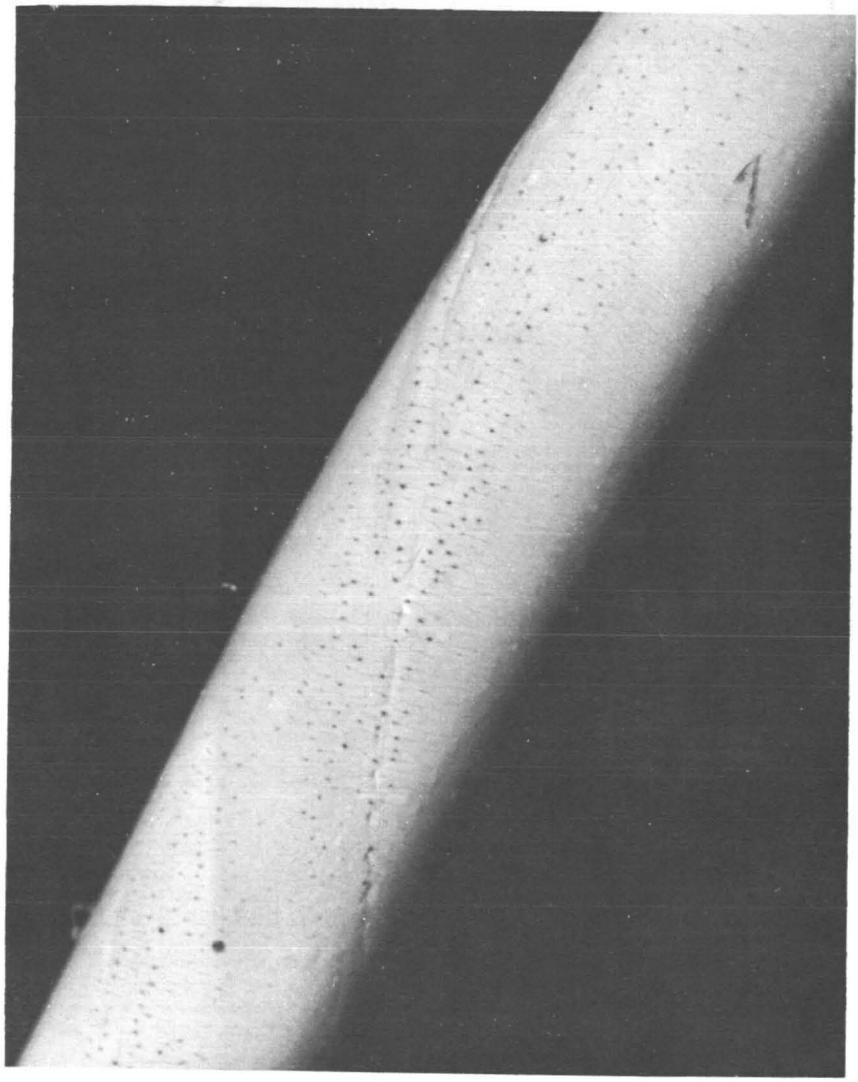

FIGURE 9.—Pinholes and stress lines radiating at right angles to length of wire in hookup wire with FEP over H-film. Color, white; conductor No. 16.

ments. In these cases, the JPL Qualification Test Specifications will be used. They are:

For connectors: ZPQ–224–5—General
For wire:

TFE, FEP precision hookup wire	ZPQ–2439–0940
H-film precision hookup wire	ZPQ–2439–0941
Form and winding wire	ZPQ–2439–0942
Cable accessories	ZPQ–2439–0943

The satisfactory completion of the qualification test entitles the design to be listed in Preferred Parts List ZPP–2061–PPL. This list will also state the qualified producing sources. All parts which have been qualified as sterilizable will be listed in JPL specification ZPP–2010–SPL, entitled "Electronic Part Sterilization Candidates for Spacecraft Applications."

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Batteries and Space Power Systems

The Jet Propulsion Laboratory (JPL) has a program to develop energy storage devices capable of performing satisfactorily after heat sterilization. The general objectives of the program are: (1) to obtain basic and new information regarding batteries and battery components subjected to heat-sterilization procedures, (2) to develop technology to assist in the proper designing and fabrication of heat sterilizable batteries, (3) to test and to evaluate components and units of heat sterilizable batteries, and (4) to produce a battery which will satisfactorily meet flight program requirements. It is intended that the knowledge and technology developed in this program be such that the application can be made to a variety of modes of heat sterilization.

A specific task is to develop the battery for the present planetary lander capsule project which must conform to these specifications: 150 to 600 watt-hour capacity, 1-year shelf life, specific energy greater than 25 W-hr/lb, 8- to 28-Vdc output voltage under rated load, 300-watt load capability at rated voltage, capability of four cycles of 100 percent discharge, rechargeable in less than 72 hours, operating temperature range of 10° to 65° C, capability of withstanding a heat sterilization of three cycles of 145° C for 36 hours each, and capability of withstanding severe shock and vibration loads (e.g., a 10 000-g peak symmetrical triangular pulse with 0.1-msec rise time followed by a 5000-g peak square pulse of 3-msec duration). The requirements that the electrical energy be delivered after heat sterilization and after the battery has sustained the cited shock and vibration loads are so rigorous that no available battery is adequate; consequently, a complete research and development program was undertaken.

Background

The research and development of a heat sterilizable power supply system began in April 1962 with a survey to determine the problem area in sealed silver-zinc (Ag-Zn) secondary batteries; attention was centered on this battery as a result of the energy density requirement. The investigation was performed by the

Delco-Remy Division, General Motors Corp., under contract to the Jet Propulsion Laboratory. By using a statement of work which required ascertainment of the problem areas in sealed Ag-Zn secondary batteries resulting from sterilization at 125° C for 36 hours and the study of components in the 100° to 130° C range, the scope of the work was expanded to include sterilization at 145° C for 36 hours and the study of components in the 100° to 145° C range. The range of cell testing was expanded from 36 hours at 125° C with the electrodes in any state of charge to include 145° C in the unformed state. Among the conclusions obtained in this cursory program were these for sterilization at 125° C: (1) the sterilized cells lose 35 percent of capacity after a 1-month activated stand; (2) the performances of sterilized unformed silver and zinc electrodes were satisfactory; (3) the separator combination of Dynel and fibrous sausage casing (FSC) was the best of those tested (an unformed cell using Dynel and FSC operated for 10 to 30 cycles at full discharge after sterilization); and (4) the unsupported Delco-Remy cell-case design could not withstand the temperature soak at 125° C. In addition, these observations for sterilization at 145° C were made: (1) there is a gas pressure buildup during sterilization and during the formation charge; (2) the electrolyte solution changes color; (3) the Dynel separator disintegrates; (4) the silver plates shed material; (5) there is a 50-percent decrease in capacity; (6) the open circuit voltage decreases rapidly; and (7) the cells develop leaks. This brief exploratory work provided the basis for a subsequent contract with Delco-Remy.

The second contract, begun in June 1963, was for an extensive program to investigate and develop materials, processes, and designs for a sealed Ag-Zn secondary battery capable of being sterilized at 145° C for 36 hours, the studies to be expanded to sterilization at 125° C and 135° C in some areas. Whereas the initial contract was a survey of the effects of heat sterilization on extant batteries, in this contract the purpose was to determine which components cause poor cell performance, the items to be studied being defined as the electrodes, the separator, the cell and battery cases, the seals, and the battery design. Among the conclusions obtained were these: (1) poor cell performance after sterilization at 145° C for 36 hours was due to degradation products from the separators (these products attacked the silver plate which resulted in a loss of capacity); (2) polyvinyl alcohol used as a binder in the negative plate produced high pressures during sterilization. (A satisfactory negative plate can be produced without this binder); (3) uncharged positive and negative

plates are not degraded by sterilization; (4) sterilization reaction products from nylon (E. I. du Pont de Nemours & Co.), Penton (Hercules Powder Co.), and Celcon (Celanese Chemical Co.) have no effects on cell performance; (5) sterilization of primary dry charged cells resulted in about a 40-percent loss of cell capacity due to decomposition of divalent silver oxide; (6) nylon is made brittle by sterilization and fails by cracking after sterilization; (7) the case-to-cover seal leakage problem was not solved; (8) none of the terminal-to-cover seals sustained the pressure tests; and (9) no available separator material was capable of functioning satisfactorily after sterilization at 145° C. Since Delco-Remy had surveyed all available separator materials and had concluded that superior separator materials must be developed to make possible the sterilizable battery, the major part of the program was then assigned to the development of this crucial item.

One of the conclusions from the Delco-Remy contracts was that separators fabricated from polyethylene base film by grafting with acrylic acid seemed to have potentialities for being modified further to yield separators with the necessary properties. On the basis of this, a development contract was given to Radiation Applications, Inc. (RAI), to explore these materials. Later, a contract was given for a parallel effort to the Narmco Division, Whittaker Corp., for developing new separator materials from eight new thermostable polymers, which were to be synthesized. A third separator development is part of the contract on the heat sterilizable battery being performed by the Electric Storage Battery Co. The progress of these contracts is described below.

Separator Development by RAI

The RAI program, begun in September 1964, covered the fabrication and testing of 51 different materials using polyethylene as the base film. The fabrication section of the program was as follows: (1) three densities of polyethylene were used in each process, except when noted otherwise; (2) in each procedure the polyethylene was grafted with acrylic acid (AA) using a Co^{60} source; and (3) three crosslinking procedures were used: (*a*) precrosslinking by irradiation with a 1.5-Mev electron beam to four radiation levels, (*b*) precrosslinking with three concentration levels of divinylbenzene (DVB) using a Co^{60} source, (*c*) postcrosslinking one base polymer with three concentration levels of DVB using a Co^{60} source, and (*d*) simultaneous crosslinking one base polymer with DVB and grafting with AA using a Co^{60} source; and (4) the effects of molecular weight and relative humidity were explored. The preparations are outlined in table I.

Each film was subjected to a test program designed to examine properties which were believed to be necessary for an adequate separator.

TABLE I. — *Preparation Procedures (RAI)*

1. Each of 3 densities precrosslinked using linear accelerator to 4 irradiation levels and then grafted with 1 concentration level of acrylic acid (AA) using Co^{60} at 1 irradiation level	→ 12 (2*)
2. Each of 3 densities postcrosslinked using linear acceleration to 1 irradiation level at 3 relative humidities after grafting with AA at 1 concentration level using Co^{60} at 1 irradiation level	→ 9
3. Each of 3 densities precrosslinked with 3 concentration levels of divinylbenzene (DVB) using Co^{60} at 1 radiation level and then grafted with AA at 1 concentration level using Co^{60} at 1 irradiation level	→ 9 (2*)
4. 0.917 density grafted with AA at 3 concentration levels using Co^{60} and then crosslinked with 3 concentration levels of DVB at 1 irradiation level using Co^{60}	→ 9 (2*)
5. 0.917 density simultaneously grafted and crosslinked using 9 ratios DVB/AA at 1 irradiation level Co^{60}	→ 9 (1*)
6. Each of 3 densities fractionated, low molecular weight fractions discarded, crosslinked using linear accelerator to 1 irradiation level, and grafted with AA using Co^{60} at 1 irradiation level	→ 3

In the first phase of the test program, the properties of the films after heat sterilization at 137° and 145° C were determined. Samples were taken at the end of each cycle of 137° and 145° C, and measurements were made of tensile strength, acid equivalence, dimensional changes, and resistivity. The resistance measurement was the governing criterion and in some cases repeated grafting procedures were used in efforts to lower the resistance into the acceptable range. In a few cases, acceptable values were never obtained, and these materials were not tested further.

The resistance measurement for 42 materials of the 51 prepared was low enough to allow complete testing in which the final test phase was for electrical capacity retention after three sterilization cycles at 145° C. The retention was greater than the arbitrary acceptance level of 90 percent after five deep cycles of charge and discharge for seven materials, the best capacity retention data being obtained for: (1) a material fabricated by precrosslinking 0.917-density polyethylene with divinylbenzene and then grafting with acrylic acid and (2) a material fabricated from precrosslinking 0.917-density polyethylene with the electron beam source to a total radiation dose of 70 Mrads and then grafting with acrylic acid. The detailed preparations for the seven materials are given in table II and the test data for the cell capacities are given in table III. Further testing will be done on the two best materials.

TABLE II.—*Procedures for Preparation of Acceptable Separator Materials (RAI)*

	1–1		1–2	
Linear accelerator crosslinking:				
Density	0.917		0.917	
Crosslinking dose, Mrad	50		70	
First graft:				
Solution (benzene-AA-CCl_4)	75–20–5		70–25–5	
Dose rate, Mrad/hr	0.014		0.015	
Total dose, Mrad	1.34		1.04	
Second graft:				
Solution (benzene-AA-CCl_4)	70–25–5		70–25–5	
Dose rate, Mrad/hr	0.014		0.014	
Total dose, Mrad	1.34		1.34	
	Precrosslink		Postcrosslink	
	3–1	3–2	4–1	4–2
Divinylbenzene crosslinking:				
Density	0.917	0.917	0.917	0.917
Crosslinking:				
Solution (DVB-benzene-MeOH)	1–1–98	1.5–1.5–97	1–1–98	1.5–1.5–97
Dose rate, Mrad/hr	0.025	0.025	0.025	0.025
Total dose, Mrad	0.55	0.55	0.58	0.58
Graft:				
Solution (AA-benzene-CCl_4)	25–70–5	25–70–5	25–70–5	25–70–5
Dose rate, Mrad/hr	0.021	0.021	0.014	0.014
Total dose, Mrad	1.43	1.43	1.68	1.68
	5–1			
Simultaneous crosslinking and grafting:				
Density	0.917			
Solution (DVB-AA-CCl_4-benzene)	0.6–24.4–5.0–70.0			
Dose rate, Mrad/hr	0.018			
Total dose, Mrad	2.16			

The conclusions derived from the RAI contract are: (1) no differences of separator degradation at 137° and 145° C were detected; (2) no differences of separator properties were detected for the conditions of sterilization in the absence of and in the presence of the silver electrode; (3) no further changes in properties occurred after the first cycle at either 137° or 145° C;

TABLE III.—*Capacities of Sterilized and Control Cells Constructed From Acceptable Separator Materials (RAI)*

Sample	Cell capacity, AH						Average relative capacity retention, percent
	Controls			Sterilized cells			
	C_1	C_2	C_3	S_1	S_2	S_3	
1–1:							
1	1.08	1.04	1.09	1.03	1.05	1.08	98.5
2	1.08	1.04	1.12	1.08	1.06	1.05	98.6
3	1.07	1.04	1.13	1.08	1.06	1.05	98.6
4	1.07	1.06	1.12	1.09	1.06	1.05	98.5
5	1.07	1.06	1.13	1.09	1.06	1.06	98.5
1–2:							
1	1.13	1.12	1.08	1.12	1.07	1.11	99.0
2	1.13	1.12	1.08	1.07	1.07	1.08	96.8
3	1.11	1.09	1.09	1.08	1.08	1.05	97.9
4	1.11	1.09	1.13	1.08	1.09	1.05	96.5
5	1.13	1.13	1.11	1.07	1.08	1.09	96.5
3–1:							
1	1.13	1.17	1.14	1.14	1.14	1.11	98.6
2	1.11	1.17	1.17	1.17	1.14	1.12	99.4
3	1.12	1.14	1.13	1.17	1.13	1.13	100+
4	1.13	1.14	1.14	1.09	1.11	1.12	97.4
5	1.11	1.11	1.13	1.08	1.10	1.11	98.3
3–2:							
1	1.14	1.13	1.16	1.11	1.14	1.17	99.6
2	1.11	1.17	1.15	1.09	1.13	1.12	97.4
3	1.13	1.14	1.13	1.10	1.13	1.12	98.5
4	1.11	1.14	1.17	1.09	1.11	1.12	97.1
5	1.13	1.16	1.14	1.07	1.11	1.11	96.0
4–1:							
1	1.13	1.15	1.16	1.16	1.19	1.17	100.
2	1.11	1.14	1.16	1.06	1.17	1.13	98.5
3	1.11	1.12	1.15	1.09	1.13	1.16	100.
4	1.12	1.13	1.14	1.05	1.09	1.10	95.6
5	1.11	1.12	1.14	1.08	1.08	1.09	96.5
4–2:							
1	1.12	1.14	1.15	1.04	1.04	1.07	92.4
2	1.12	1.10	1.14	1.01	1.04	1.01	91.0
3	1.11	1.11	1.14	1.02	1.03	1.04	92.0
4	1.10	1.14	1.11	1.05	1.03	1.05	93.3
5	1.09	1.12	1.10	1.03	1.02	1.05	93.4
5–1:							
1	1.10	1.08	1.11	1.03	1.08	1.03	95.4
2	1.05	1.07	1.11	1.04	1.13	1.08	100.
3	1.07	1.10	1.08	1.04	1.11	1.04	95.2
4	1.17	1.08	1.07	1.04	1.11	1.05	96.2
5	1.13	1.08	1.10	1.05	1.09	1.04	96.0

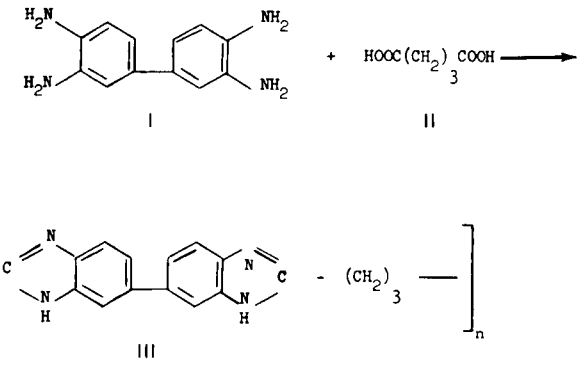
(4) all the seven separator materials from which the cells having greater than 90 percent capacity retention were constructed were made from 0.917-density polyethylene; and (5) satisfactory materials can be prepared using either irradiation or divinylbenzene crosslinking procedures.

Separator Development by Narmco

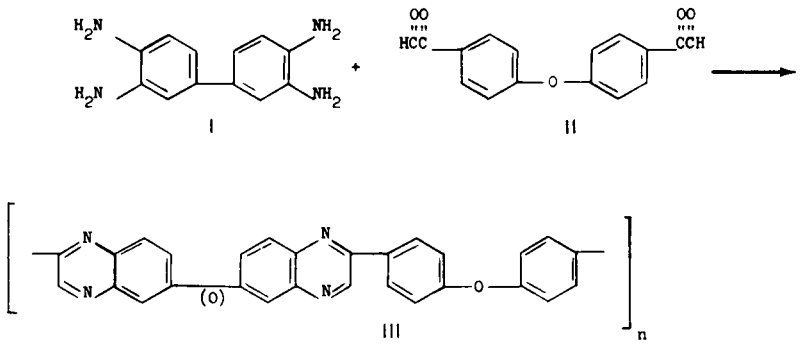
In the contract with the Narmco Division of Whittaker Corp., begun in June 1965, the field of the new thermostable polymers is to be investigated as a source for separator materials. In phase 1 of this program, Narmco is to: (1) synthesize eight distinct thermostable polymers to the highest possible molecular weight and/or to varying degrees of condensation or modification; (2) test the synthesized polymeric compounds; (3) fabricate films from the promising polymeric compounds; and (4) test the films for resistance to sterilization at 137° and 145° C. In phase 2, a 500-square-foot sheet of one candidate is to be produced and fully tested as a battery separator.

The polymers to be synthesized comprise polymeric aliphatic analogs of benzimidazole, benzoxazole, benzothiazole, and two quinoxalines. The aliphatic moieties derive from glutaric and sebacic acids by condensation with 3,3′-diaminobenzidine, 3,3′-dimercaptobenzidine, and 3,3′-dihydroxybenzidine. The quinoxalines are prepared from 4,4′-diphenyl ether bis glyoxal and 3,3′-diaminobenzidine and 3,3′, 4,4′-tetraaminodiphenyl ether. The products are: (1) poly-2,2′-(trimethylene)-5,5′-bibenzimidazole; (2) poly-2,2′-(octamethylene)-5,5′-bibenzimidazole; (3) poly-2,2′-(trimethylene)-5,5′-bibenzothiazole; (4) poly-2,2′-(octamethylene)-5,5′-bibenzothiazole; (5) poly-2,2′-(trimethylene)-5,5′-bibenzoxazole; (6) poly-2,2′-(octamethylene)-5,5′-bibenzoxazole; (7) poly-2,2′-[p,p′-oxybis(phenylene)]-7,7′biquinoxaline; and (8) poly-2,2′-[p,p′-oxybis(phenylene)]-7,7′-oxybisquinoxaline. (See fig. 1.)

As a prerequisite to the film-preparation stage, each of the synthesized polymeric compounds is subjected to preliminary screening tests to determine whether it has the necessary chemical properties for temperature and potassium hydroxide (KOH) resistance. These tests are: (1) general analysis using infrared and ultraviolet spectrophotometric methods; (2) determination of hydrolytic stability at 145° C in aqueous 40 percent KOH using inherent viscosity methods; (3) determination of oxidative stability in air at 250° C using the method of isothermal weight loss; (4) determination of oxidative stability in aqueous 40 percent KOH solutions containing silver ion; (5) solubility tests in appropriate solvents; and (6) determination of the glass transition tempera-



I	II	III
3,3'-diaminobenzidene	+ glutaric acid	→ poly-2,2'-(trimethylene)-5,5'-bibenzimidazole
"	+ sebacic acid	→ poly-2,2'-(octamethylene)-5,5'-bibenzimidazole
3,3'-dimercaptobenzidine	+ glutaric acid	→ poly-2,2'-(trimethylene)-5,5'-bibenzothiazole
"	+ sebacic acid	→ poly-2,2'-(octamethylene)-5,5'-bibenzothiazole
3,3'-dihydroxybenzidine	+ glutaric acid	→ poly-2,2'-(trimethylene)-5,5'-bibenzoxazole
"	+ sebacic acid	→ poly-2,2'-(octamethylene)-5,5'-bibenzoxazole



I	II	III
3,3'-diaminobenzidine	+ 4,4'-diphenylether bis glyoxal	→ poly-2,2'- [p,p'-oxybis (phenylene)] -7,7'-biquinoxaline
3,3', 4,4'-tetraamino-diphenylether	+ 4,4'-diphenylether bis glyoxal	→ poly-2,2'- [p,p'-oxybis (phenylene)] -7,7'-oxybisquinoxaline

FIGURE 1.—Polymer syntheses by Narmco Division of Whittaker Corp.

ture. On the basis of the results of these preliminary tests, promising polymeric materials are selected for further study and evaluation.

Polymeric materials, which possess the required temperature

and KOH resistances, are then evaluated in the film formation phase, at which point the processabilities of the polymers as films are studied and the properties of the resultant films are measured. The film formation phase includes studies of solution casting and hotmelt techniques and studies of the curing of films prepared from prepolymers. Measurements of hydrolytic and oxidative stabilities are made to determine the resistance to heat sterilization. These are prerequisites to the full test program of measurements of electric resistivity, electrolyte solution absorption, and rate of diffusion of the silver ion.

Although each of the polymers which has been synthesized has the requisite chemical resistance properties, the effort has now concentrated on the development of films from poly-2,2′-(octamethylene)-5,5′-bibenzimidazole. This emphasis is due to the superior film processability characteristic of this polymer. The work is now being directed to attempting chemical and physical modifications in efforts to lower the initial high resistivity value of 1.6×10^{6} ohm-cm. A procedure in which lithium chloride was incorporated into the film and then leached out, yielding a microporous structure, has been explored. However, the resistivity value was lowered only to 2.0×10^{5} ohm-cm by this process. Currently, chemical procedures are being used in which hydroxyethyl or carboxyl groups are introduced into the polymer via substitution at the —NH site in the imidazole ring and by sulfonation. These procedures are intended to introduce increased hydrophyllic character into the polymer and decreases in resistivity should result.

Separator Development by the Electric Storage Battery Co.

The Electric Storage Battery Co. (ESB) will develop a copolymer of divinylbenzene and methacrylic acid for a separator material as part of a research and development contract on the heat sterilizable battery. The separator development includes fabrication and testing phases. Preliminary data indicate that this copolymer is not degraded by the heat sterilization conditions.

Research and Development Program for the Ag-Zn Battery by ESB

A research and development contract was awarded to ESB in September 1965 to study, develop, fabricate, and test sealed Ag-Zn and silver-cadmium (Ag-Cd) battery cells capable of functioning satisfactorily following heat sterilization, high impact, and vibration procedures. (These procedures, which have already been described, are subject to changes resulting from varying space flight requirements.) The contract provides for electrochemical studies on electrodes, electrolyte solutions, and separators; for

chemical and physical studies of case materials; and for the design, fabrication, and testing of cells of both the Ag-Zn and Ag-Cd systems. The testing of the most promising plastics for cells, polyphenylene oxide and polyphenylsulfone, has begun. Preliminary cell designs are being considered, and calculations of stresses associated with certain cell designs have been made. This contract and the inhouse program at JPL are the major comprehensive efforts underway at this time.

Nickel-Cadmium (Ni-Cd) Testing Program

Ni-Cd cells (Sonotone, D size, 3.5 amp-hr) are being tested and evaluated in a program by TRW Space Technology Laboratories under contract to JPL. The program comprises testing the float, stand, and cycle characteristics of sterilized cells which are uncharged, charged partially to various degrees, and fully charged. Preliminary results show that the loss of capacity increases with increasing degrees of charge. The data are not sufficient to indicate the effects on cycling. The tests are continuing.

Inhouse Program at JPL

The inhouse sterilizable-battery program at JPL has been intended to be an advance development effort as well as to serve as a complementary effort to contractual work. This program comprises cell testing and evaluation, resistivity measurements, case material testing and evaluation, and impact testing. This pattern will continue with increasing emphasis on developing a particular battery to meet specific space-flight requirements.

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Alternate Approaches to Sterilizable Power Sources

The heat sterilization of spacecraft required for lander missions has created the problem that some components are unable to withstand the temperature environment. This is especially true of secondary batteries, which are expected to function normally and be capable of hundreds of charge-discharge cycles after sterilization. These batteries, however, either experience a significant decrease in capacity or will not operate after undergoing the sterilization cycle of three 36-hour periods at 145° C. This failure or decrease in capacity can be attributed mainly to degradation of the separator material, and possibly, the electrodes.

Because of this fact, considerable time and effort are being expended to obtain batteries capable of being sterilized without degradation. Some progress has been made, and laboratory batteries have been built that can operate for a few cycles after sterilization although with some capacity decrease. These batteries could perform satisfactorily where limited cycling is required, but a flight-ready unit that could operate for hundreds of cycles after sterilization is not presently available. No existing cell can be sterilized without experiencing some degradation.

What possibilities are there that a secondary unit capable of hundreds of cycles can be produced? Beside the approach being followed by the Jet Propulsion Laboratory, a few companies have investigated the possibility of producing a sterilizable primary or secondary unit. This paper is concerned with the approaches being used by these laboratories and the current status of their work. The Astropower Laboratory of Douglas Aircraft Co., Inc., Missile and Space Systems Div., and The Eagle-Picher Co. are working with the silver-zinc system. They base their hopes to achieve a sterilization capability on novel separators. Electro-Optical Systems, Inc., a subsidiary of the Xerox Corp., is developing an electrically regenerative fuel cell that should be capable of sterilization, and Gulton Industries, Inc., has tested sterilizable components for a battery with an inorganic separator.

In some cases, the work being carried on in this area is the result

of efforts in other directions. This is the case with two programs which the Lewis Research Center of NASA is funding. The production of a sterilizable battery is not the primary objective of these programs; however, the batteries from both programs show that they can be sterilized and operated for useful periods of time after high-temperature storage. The following résumés present in more detail the aforementioned approaches.

Douglas Aircraft Co., Inc., Astropower Laboratory

The NASA contract with the Astropower Laboratory of Douglas Aircraft Co., Inc., is intended to produce an improved secondary silver-zinc battery utilizing a sterilizable inorganic separator that can operate efficiently over an extended temperature range (50° to 210° F) for at least 1500 cycles at a 25-percent depth of discharge. Standard silver-zinc cells are limited in that they operate best over a narrow temperature range (50° to 120° F) and have very limited short-cycle lives (100 to 200 cycles) at shallow depths of discharge. Any variation in temperature or depth of discharge reduces the performance significantly.

The silver-zinc battery is a desirable power source in that it is capable of providing twice the power per unit weight of the next best system, silver-cadmium. Astropower Laboratory has developed a separator that may allow the silver-zinc battery to compete with the other secondary batteries in terms of life and at the same time exceed them in energy density and operating-temperature range. The inorganic separator has this capability because it withstands high temperatures, resists oxidation, and inhibits dendritic growth and the migration of the soluble electrode species.

Two-plate cells have operated for more than 2700 cycles at 25° C and 2200 cycles at 100° C. At higher temperatures, however, the number of cycles declines to a point where only 30 cycles are obtained at 150° C. All these tests were carried out at 20-percent depth of discharge. Charge-discharge characteristics of two typical cells are shown in figures 1 and 2. Calculations based on these data indicate that amphere-hour efficiencies of these cells are comparable to those of commercial cells. In addition to the attributes already mentioned, it has been found that the battery can be sterilized and subsequently cycled at room temperature after the sterilization. Figure 3 shows the performance of a cell that has been sterilized and then tested. This cell ran for over 2000 cycles after sterilization. It must be pointed out that these cells are not sealed and that during the test electrolyte was added. No attempt has been made to seal the cell, since the preliminary objective was to determine the performance capability of the inorganic separa-

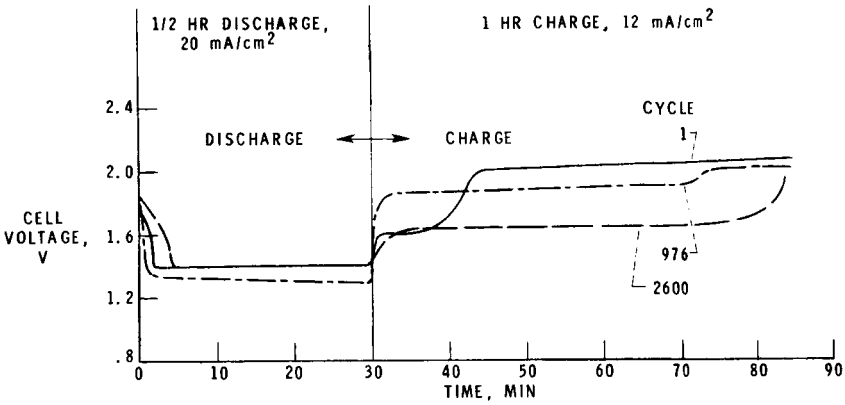

FIGURE 1.—Charge-discharge cycle test of Douglas silver-zinc secondary cell at 25° C. Depth of discharge based on low rate capacity (16-hour discharge), 17 percent.

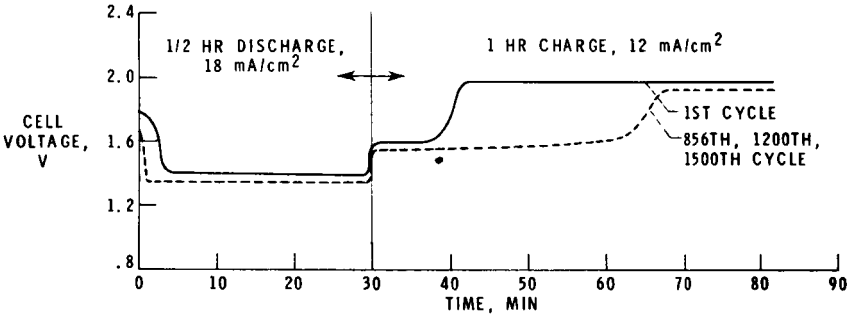

FIGURE 2.—Charge-discharge cycle test of Douglas silver-zinc secondary cell at 100° C. Depth of discharge based on low rate capacity (16-hour discharge), 17 percent.

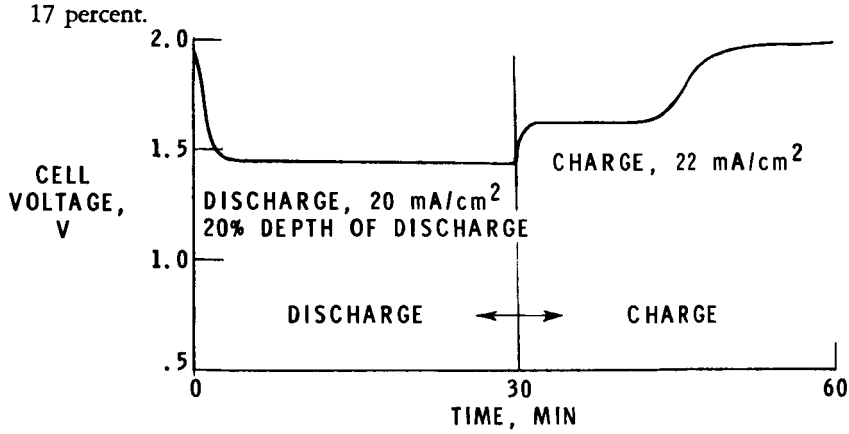

FIGURE 3.—Discharge-charge curve of Douglas silver-zinc secondary cell at 25° C following heat sterilization.

tor. Results of the testing of two-plate cells have indicated that there is much to be gained from further development of this unit.

The next step in development will be to design, build, and test a multiplate cell. Initially, a 5-ampere-hour multiplate cell will be developed. The assembly techniques and manufacturing processes will be selected so that an easy translation from this cell to one of any size or capacity can be made. A preliminary look at the multiplate cell indicates that a design capable of passing environmental tests and performing for at least 1500 cycles over a wide temperature range (25° to 100° C) is feasible. A preliminary design for the multiple cell has been completed, and work is proceeding toward tailoring the components to the design. The target date for completion of the evaluation of the 5-ampere-hour cell is late 1966.

The Eagle-Picher Co.

The Eagle-Picher Co. has reported that it has a silver-zinc battery capable of withstanding heat sterilization. An 8-ampere-hour unit has withstood three 36-hour cycles at 145° C with no apparent degradation. Similar designs, unsterilized, have shown an activated-stand capability of over a year. Cells built in April 1965 were sterilized and have undergone a charge-stand-discharge cycle 12 times since then. Eagle-Picher has sold similar cells to a number of companies for testing; they feel a more thorough testing of these units is desirable. The NASA Langley Research Center and Avco are among those performing tests, but the results are not available as yet.

Electro-Optical Systems, Inc.

Another program, which is in a more advanced state of development, is underway at Electro-Optical Systems, Inc. (EOS). The device being developed is an electrically regenerative hydrogen-oxygen fuel cell. The battery consists of a cell stack that is utilized as a fuel cell during the discharge period and a water electrolyzer during the charge period. Integral gas-storage tanks contain the hydrogen and oxygen generated during charge and the electrolyte holds the water generated on discharge.

Such a device offers a number of advantages for space application which existing secondary batteries do not, including the potential for sterilization. Cells have been operated over a range of temperatures from 70° to 150° C. No extensive testing has been done at the higher temperatures, the majority of tests having been run at 70° to 100° C. Single cells, 6-cell batteries, and 34-cell batteries have been tested in the aforementioned ranges. Figure 4

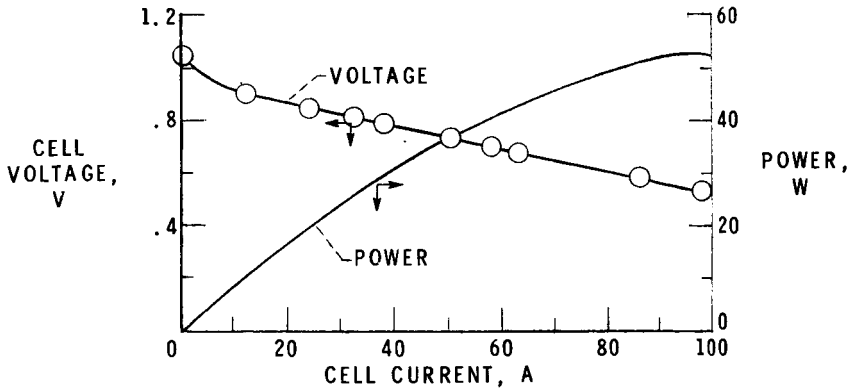

FIGURE 4.—Performance at 125° C of cell built by Electro-Optical Systems, Inc. Electrode area, 180 sq cm.

shows the discharge characteristics of a single cell at 125° C. At this temperature, the cell performs very efficiently in short-time tests. However, life data have not been obtained. Figure 5 shows a pressure curve and a charge-discharge curve of a cell operating at 150° C. Charge voltage is somewhat lower and discharge voltage is higher than that at lower operating temperatures. Figure 6 shows cell performance at 75° C after a 67-hour soak at 150° C; for comparison, performance at 75° C prior to the heat soak is also

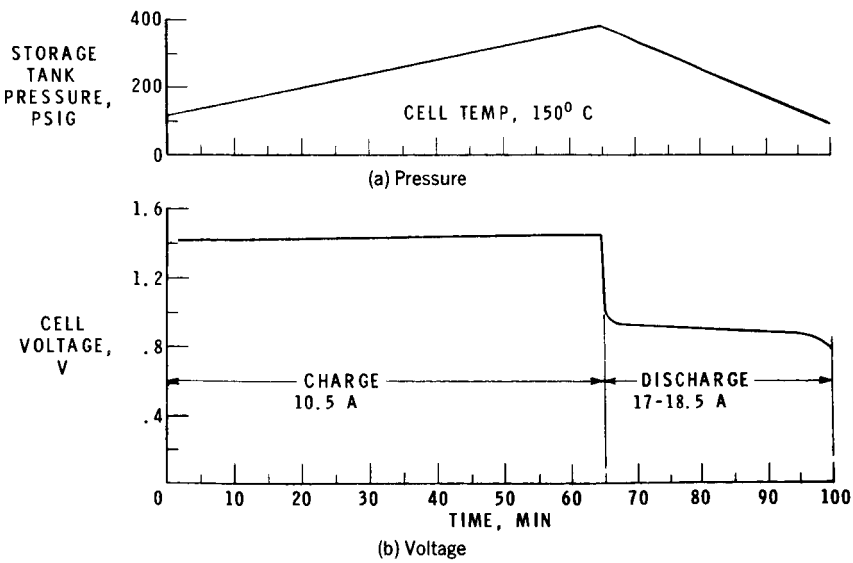

FIGURE 5.—High-temperature test of single-cell regenerative hydrogen-oxygen fuel cell at 150° C.

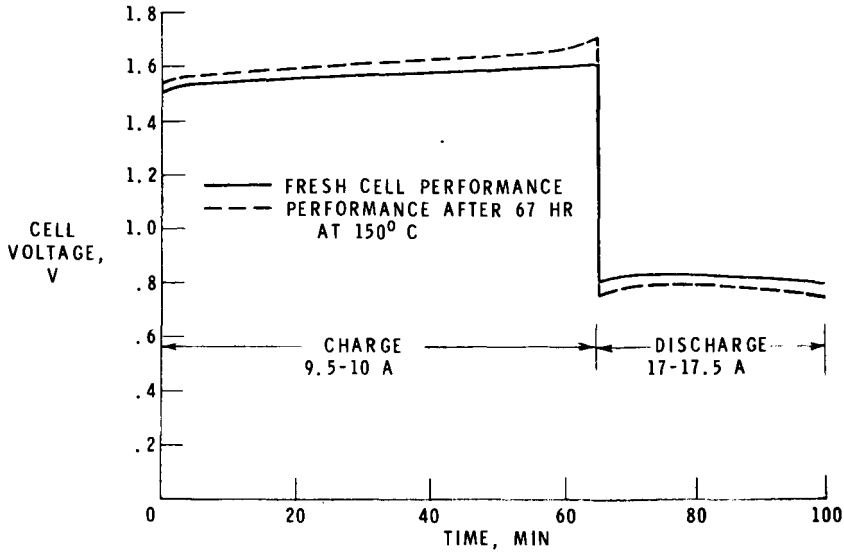

FIGURE 6.—Effect of high-temperature storage on cell performance at 75° C.

shown. A slightly higher charge voltage and a lower discharge voltage were observed after the 150° C exposure. Work is now underway at EOS to eliminate this loss, which appears to be associated with oxidation of the oxygen electrode.

The present performance capability of the six-cell fuel cell is as follows:

Watt-hours per pound:	
35-min discharge	10–12
60-min discharge	17–20
Operation temperature, °C	Up to 150
Power level, W	90
Thermal sterilization capability	Potentially sterilizable
Cycle life at full depth (90° C)	>300
Operating current density, discharge, mA/cm^2	100
Overload capability	2:1

The basic design, the materials of construction, and the operating characteristics all indicate that this secondary fuel cell should be capable of efficient operation after sterilization.

Gulton Industries, Inc.

Gulton Industries, Inc., has indicated that it has performed sterilization tests on nickel-cadmium cells and some work on another sterilizable system. It was found that the nickel electrode degraded during sterilization. Gulton Industries has, as of this date, two electrodes and an inorganic separator which can be

sterilized, although these components have not been tested together in a cell.

Conclusions

It appears that two or three of the approaches that have been proposed for obtaining sterilizable secondary batteries have a chance of meeting the requirements for a lander mission. Of the two approaches discussed in detail in this paper, the Douglas silver-zinc cell must still be developed into a finished sealed cell capable of withstanding the environmental conditions that would be imposed by such a mission. There seems to be little question, however, that this cell can withstand the rigors of thermal sterilization and perform satisfactorily. The major problems to be solved lie in the conversion of a laboratory device into a reliable piece of flight hardware.

The electrically regenerative fuel cell has operated both as a single cell and as a multicell unit with a cycle life greater than 300 at 70° to 90° C. It has also operated at 150° and at 75° C after a 150° C storage of 67 hours. Work now underway on the electrodes and membrane should enhance reliability, improve cycle life considerably at all temperatures, and enhance sterilization capability.

The approaches offered by Gulton Industries, Inc., and The Eagle-Picher Co. appear to offer some promise on the basis of the information available to the author at this time. As more test data become available, a more accurate appraisal of their merits relative to those of the other methods discussed will be possible.

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Guidance-and-Control System Sterilization

The sterilization of guidance-and-control systems is treated from the standpoint of both the components and systems. In the components effort, the status of development work in optical sensors, inertial devices, and actuators is discussed. The work in optical sensors is directed toward the development and investigation of the problems associated with photoconductive detectors and an image dissector. In each case the difficulty is primarily in the selection of materials which will simultaneously meet thermal sterilization and performance requirements. A gas-bearing gyro, an accelerometer, and an electrostatic gyro are included in the present inertial sensor development areas. Attempts to sterilize inertial components of the early Ranger spacecraft are also discussed. The actuator investigations concern servomotors, a direct-current torquing device, and a high-ratio small-volume gear train. Differential expansion and dimensional stability are the major study areas for these devices. The systems effort is directed toward establishing the need for the specific components required and the problems anticipated in the sterilization of entire systems for future missions. The assumption is made that the sterilization problems of standard electronic components are being investigated elsewhere; therefore, this subject is discussed only as it relates to a specific device. In some cases new system mechanizations are proposed as a solution to the sterilization problem. Finally, the major problem areas foreseen are mentioned.

A capsule which is to land on the surface of Mars may require several different subsystems that fall in the category of guidance-and-control equipment. In a typical mission, after the capsule is separated from the spacecraft bus, it may require an attitude-control system for orientation prior to a deflection maneuver. During this deflection maneuver, which puts the capsule on an impact trajectory, an autopilot may be required to achieve a sufficiently accurate trajectory change. A coasting period follows in which the capsule may or may not be attitude controlled. In order to obtain an acceptably soft landing, an active control system utilizing a retro engine may be needed during the final descent. Finally, landed operations may require guidance-and-control functions. Determining the location of the capsule on the surface of the

planet is in this category, as well as pointing antennas at the Earth or solar-radiation collectors at the Sun.

This paper is concerned with the sterilization of guidance-and-control equipment from both the components and systems viewpoints. Systems considerations are necessary to provide the component programs with guidelines on the selection of devices to be investigated, on the type of mechanizations that seem best suited for a particular application, and on the presterilization testing that may be necessary. The components are divided into three classes: optical sensors, inertial sensors, and actuators. Sterilization of specific electronic components is mentioned only when those components are peculiar to guidance-and-control circuits. Thus, even though sequencers and computers are part of guidance-and-control equipment, their sterilization problems are assumed to be associated with standard electronic equipment and are not covered here.

Throughout this paper the greatest emphasis is placed on heat, or thermal, sterilization. Where a materials compatibility problem may arise because of the use of some other sterilant, such as ethylene oxide, this situation is noted. Much of the emphasis in the component development work is placed on the component's ability to survive a high-temperature short-time sterilization cycle because high temperatures usually present the most severe conditions to the component. For design verification, a temperature of 145° C for 36 hours has been used. In some cases, however, even at lower temperatures, the duration of exposure to heat is the critical factor; this then becomes the major emphasis in the investigation and development programs.

This paper is divided into four areas in which sterilization problems of guidance-and-control equipment are currently being studied: systems, optical sensors, inertial sensors, and actuators. A status report of the results achieved and a brief discussion of planned work in each of these areas are given. Finally, in order to present a more complete picture, additional work that should be done is mentioned.

Systems

The objectives of a systems effort in sterilization investigations are: (1) to define the components required for guidance-and-control subsystems on landing capsules, (2) to establish new subsystem designs when current mechanizations cannot be sterilized, (3) to study the compatibility of component sterilization methods with systems-level sterilization techniques, and (4) to establish testing philosophies and procedures for final systems tests. Since the

component investigations are dependent upon objective (1), this task must be undertaken first. The remaining objectives have strong interrelations with the component programs and can be studied simultaneously.

Required Components

The determination of necessary components for capsule control systems is made by studying previous capsule investigations and tabulating the types of devices required. Where this information is unavailable, projections are made as to the types of subsystems that may fulfill mission requirements. In each case, since a specific set of mission requirements is unavailable, it is impossible to specify the components that will be required completely. One can be somewhat more general, however, and say that high-quality gyroscopes and accelerometers, Sun sensors, possibly a star or planet sensor, and actuators for attitude-control, autopilot, and articulating devices will be required.

Subsystem Redesign

In present techniques of mechanizing some control systems, the circuits use large-value capacitors (some in excess of 4000 μF). Since this requires the use of wet-slug-type or wet-foil-type capacitors, it is unlikely that these capacitors will survive thermal sterilization. Anticipating this result, other circuit mechanizations not requiring these components have been considered. Among the mechanizations for timing circuits is a digital technique utilizing a counter and pulse generator. This mechanization decreases the size of the required capacitor by the number of counter stages. Because of the high reliability of silicon semiconductors, the reliability of this type of circuit may be considerably greater than that of conventional mechanization. Other special circuits have been studied which will eliminate the use of large-value capacitors in attitude-control and autopilot systems. It is planned to breadboard the circuits considered and subject them to thermal sterilization followed by functional testing.

Compatibility of Sterilization Methods

Part of the systems investigation is directed toward insuring that the systems sterilization requirements are compatible with the component tolerance. As an example, consider two devices: A star tracker is most critical with the high-heat, short-time sterilization cycle, and Sun sensors are most critical with the low-heat long-time cycle. The systems investigators must be aware of this type of component incompatibility and must make appropriate adjustments in the subsystem mechanization. The solution may be either

to increase component tolerance or to modify the system design concept. This same problem arises in the overall sterilization of the capsule and should be handled in a similar manner.

Testing Philosophy

In addition to the components problems that have to be solved, an overall systems testing philosophy must be established. It is necessary to explore the factors involved in making a decision of whether to employ subsystem presterilization tests coupled with a high confidence level or poststerilization systems-level tests of critical functions. It is probably possible to obtain the necessarily high confidence level in guidance-and-control subsystems by thorough testing of several engineering evaluation models of flight hardware both before and after sterilization. There still remains a basic flaw in eliminating a final poststerilization systems test. There has been no check of the interface connections between the individual subassemblies and between the control systems and other systems. To preserve overall capsule reliability, verification of the interface integrity is imperative; therefore, functions which must be poststerilization tested are those which maximize the verification of the interfaces. For example, a function which would not have to be tested is one where the input and output are monitored in the same subassembly, such as the continuity between an input to an attitude-control amplifier and its output. A function, such as generating a signal in a star sensor and monitoring its effect elsewhere in the system, would be a necessary poststerilization test, since it would verify many interface connections.

Much more consideration must be given not only to subsystem testing but also to capsule-system testing. This is so because final sterilization will probably be done on a capsule-system basis and all final testing would then necessarily be at a capsule-system level. At this stage of the investigation it seems that the best approach would lie somewhere between the two extremes. Establishing a high confidence level in subsystem performance after sterilization would be desirable. In addition, certain system-level, interface-verification tests would probably be necessary. Further work on this subject will hopefully indicate the best choice.

Optical Sensors

In the area of optical-sensor sterilization, attention is focused on two areas: (1) sensors requiring moderate-to-low sensitivity, such as Sun sensors and short-range planet sensors working in the visible spectrum; and (2) sensors requiring high sensitivity, such as star sensors and long-range planet sensors working toward the blue end of the spectrum. The primary effort in both of these areas

has been on the development of detectors which can be sterilized. It is anticipated that sterilizable detectors will be integrated into subsystems to demonstrate detector-system compatibility.

Cadmium sulfide photoconductors have proven to be highly satisfactory for sensors requiring low-to-moderate sensitivity such as Sun sensors and planet sensors. Detectors of this type have previously been used in Ranger and Mariner Sun sensors, and on Mariner for the Canopus-tracker Sun shutter, Earth detector, narrow-angle Mars gate, and Sun gate. Because of this experience, cadmium sulfide photoconductors look promising for use on future missions. To meet sterilization specifications imposed by planetary soft landers, a program was initiated to obtain cadmium sulfide photoconductors able to withstand thermal sterilization requirements. The following three basic characteristics are necessary:

(1) The overall light-to-dark resistance ratio must not be degraded with temperature.
(2) The resistance change with temperature must be both minimized and predictable.
(3) Temperature characteristics must be matched from photocell to photocell.

The cadmium sulfide cells used to date are manufactured from a sintered form of cadmium sulfide; in their production, they "see" temperatures of 300° C or greater. After this high-temperature process, indium electrodes are deposited on the cells. The melting point of indium is 156° C, very close to the high temperature required for sterilization. Present experience indicates that cadmium sulfide cells fabricated with indium electrodes will not stand the high temperature of sterilization. The low-temperature long-time sterilization cycle will probably degrade the cadmium sulfide itself more than the high-temperature short-time cycle. Therefore, present thinking indicates that electrodes of nickel or an equivalent metal must be used and that the high-temperature short-time cycle must be used for sterilization.

Presently available cadmium sulfide photoconductors exhibit large resistance changes with increased temperature. A portion of the change is permanent, but random in magnitude and hard to predict. It is felt that cadmium sulfide cells produced by vacuum deposition might exhibit superior temperature — resistance characteristics. With this in mind, cells have been produced by vacuum deposition and are currently undergoing evaluation. Very early test results are favorable.

Landing vehicles may require star sensors, antenna-pointing Earth sensors, and possibly approach-guidance planet sensors. For all of these applications the image dissector looks attractive. The

electrostatic image dissector has been shown to be a superior detector where high sensitivity, long life, and ruggedness are important parameters. An electrostatic image dissector is shown in figure 1. A program to develop a flightworthy electrostatic image dissector capable of being heat and gas sterilized was undertaken in June 1964.

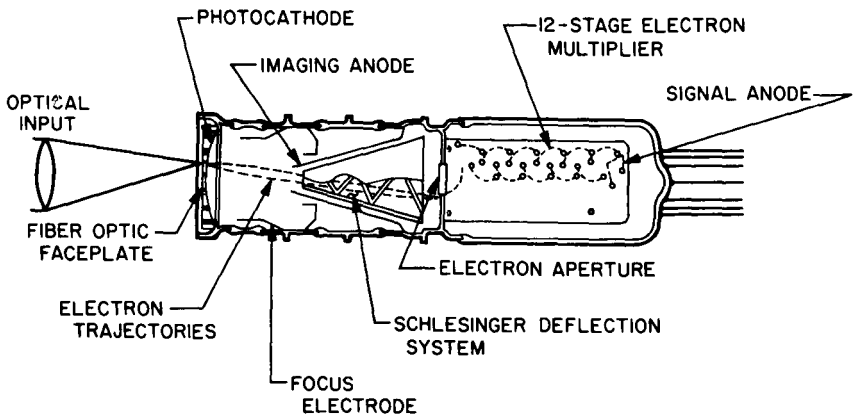

FIGURE 1.—Electrostatic image dissector.

Gas sterilization (12 percent ethylene oxide and 88 percent Freon 12, by weight) is not considered to be a problem. Thermal sterilization has adverse effects on the most sensitive photocathodes because they generally employ cesium, which is quite volatile at the high sterilization temperatures. Because of this inherent weakness in phototubes using cesium, the bialkali photocathode has been given primary attention. The bialkali surface is expected to withstand elevated temperatures because the alkali metals sodium and potassium have lower vapor pressures than cesium and therefore are less likely to migrate.

In this development, emphasis is placed on obtaining the highest possible sensitivity. Special attention is being paid to the multiplier gain which can be obtained with these tubes, since in the absence of cesium the secondary-emission ratio of conventional silver-magnesium dynodes is somewhat reduced. The approach to the problem of achieving useful cathode sensitivity and gain has involved the fabrication of over thirty 2-inch photomultiplier tubes, each incorporating a processing experiment. Each tube was processed under close control so that after repeated sterilization the various effects of the activation schedule on sensitivity, again, and stability could be studied. Heating and cooling rates, temperature, activation bake times, pressure, photoresponse, gain, and

leakage were monitored throughout the processing of each tube. After it was activated under close control, each tube was subjected to a high-temperature aging cycle. Although this aging cycle reduces the photoresponse of the tube, it has a stabilizing effect on the photoresponse and gain for subsequent sterilization cycles. General improvements in photoresponse and stability have resulted from the processing of each successive group of tubes. The results of this work are reported in detail on pages 91 to 94 of reference 1.

The activation process developed in the 2-inch photomultiplier is currently being applied to the image-dissector geometry. Preliminary results indicate that the performance in the image dissector will be similar to that in the photomultipliers.

Ultimately, the detectors being investigated will have to be integrated into a complete sensor. This will involve bringing together the detector optics, processing electronics, and mounting hardware. Special attention will have to be given to the optics if any two or more of the elements of dissimilar materials are cemented together. The additional components may or may not be critical in the sterilization procedure.

Inertial Sensors

As part of the Ranger Block II program, an effort was initiated to provide sterilization capability for the inertial sensors. Thermal sterilization at that time consisted of one 24-hour soak at 125° C.

Both gyros and accelerometers were used in the Ranger spacecraft. Initially the gyro underwent only minor modifications of sealants and adhesives to make it capable of the 125° C soak; however, a series of catastrophic failures was encountered. These failures were traced to a breakdown of insulation on the wire used in the gyro signal generator and torquer. After an evaluation of improved insulations, the most qualified was subsequently used in all Ranger gyros. No further catastrophic failures occurred after this change. Typical drift performance changes brought on by thermal sterilization showed an average spin-axis mass-unbalance change of 1.03°/hr/g and an average input-axis mass-unbalance change of 0.41°/hr/g. Although no catastrophic accelerometer failures were induced by the exposure of the instruments to the heat sterilization cycle, bias (null offset) shifts were much larger than could be tolerated without some form of recalibration prior to flight. It was assumed that the large bias shifts encountered were largely due to warping of the seismic-proof mass assembly under the high sterilization temperatures. The assembly was originally fabricated in several parts and cemented together with special

epoxy adhesives. Since it was felt that a monolithic structure would be more stable at elevated temperatures, an improvement program was directed toward the development of a method for fabrication and assembly of a one-piece pendulum structure. The newly designed instrument exhibited approximately 40 percent of the bias shift of the unimproved accelerometer.

As an alternative technique, radiation sterilization of gyros was attempted using 10^7 R/hr of beta particles from a cobalt source for a period of 36 hours. Testing showed no significant performance change due to radiation, and one unit was subsequently successfully life tested for 1000 hours. Further attempts to utilize radiation sterilization were not made because of the limited pile size and operational problems of sterilization of complete gyro module assemblies.

No further efforts to improve either Ranger gyro or accelerometer performance were undertaken, since the sterilization requirements were abandoned on this program.

The gyro on which sterilization development is presently being conducted has a hydrodynamic gas-bearing spin motor and a hydrostatic fluid-supported gimbal (fig. 2). Such a gyro may find use on spacecraft or capsule missions requiring precision gyro performance up to 10 000 hours. The sterilization development plan was organized into three phases. The first phase was a review

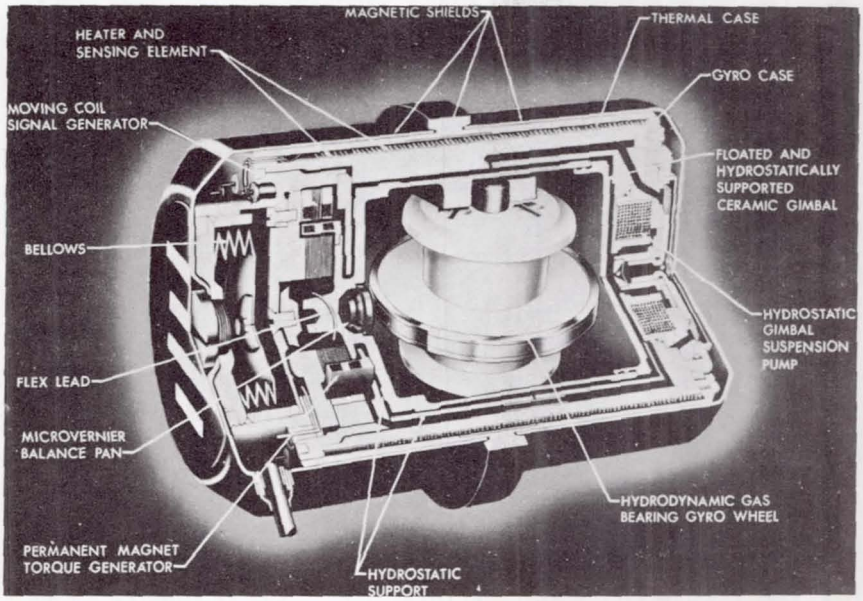

FIGURE 2.—Gas-bearing gyro.

of the existing gas-bearing gyro design to uncover weak areas, to conduct material studies, and to make necessary component redesigns to achieve a sterilizable gyro. In the second phase, a dummy gyro test vehicle without a spin motor was to be built and evaluated to guide the final gyro design. Finally, in the third phase, a complete sterilizable gyro was designed and built; it is currently being evaluated.

In the first phase, an evaluation program was conducted to determine structural epoxy strengths before and after sterilization. The test results pointed to the best epoxies for particular applications, and, in general, the epoxies were stronger after sterilization than before. The gyro case material was changed to improve dimensional stability. Thermal-expansion difference between dissimilar materials was investigated to uncover possible areas of overstressing during sterilization. The results of this investigation indicated that the spin-motor hysteresis ring and the hydrostatic-pump stator assembly presented thermal mismatch problems, and these were redesigned.

Also as part of the first phase, a flotation-fluid evaluation was conducted. Before the sterilization development program began, it was known that the existing flotation fluid could not withstand sterilization temperatures without chemical breakdown. Another flotation fluid was selected for evaluation with the component parts. Test samples representing actual gyro materials were immersed in the second fluid and soaked at 145° C for 1000 hours. Periodic tests were made to evaluate the chemical activity by measuring the specimen weight changes and observing surface appearance changes. In most cases, slight material changes did occur but were not of importance. In other cases, significant changes took place and appropriate alterations were made. The second fluid has a lower density than the original, and therefore the gimbal density was reduced to maintain flotation. This was accomplished by reducing the gimbal cross section and increasing its length. The hydrostatic pump and pickoff and torquer assembly had to be moved and modified to allow for the gimbal changes.

Upon completion of the subcomponent and material studies, the second phase was initiated, in which a dummy-gyro test vehicle was constructed without a spin motor, but with all other design changes included. This test vehicle was evaluated for gimbal torques before and after each of five sterilization cycles. The torque test results are tabulated in table 1 as an equivalent drift rate in degrees per hour. Stability from cycle to cycle is the most important consideration rather than absolute drift rate.

As indicated in table I, fluid torques are reasonably stable. This

TABLE I.—*Effect of Sterilization Cycles on Gimbal Torques*

Run	300° F soak time, hr	Flex lead torque, deg/hr	Flex lead torque change, deg/hr	Fluid torque, deg/hr	Fluid torque change, deg/hr
0	0	−5.03	.....	−0.51	+0.13
1	20	−3.54	+1.49	−.38	+.13
2	39	−.99	+4.04	−.48	+.03
3	21	−.28	+4.75	−.50	+.01
4	23	+.47	+5.50	−.43	+.08
5	21	+.13	+5.16	−.47	+.04

is an important result, as it indicates relative stability of the case and gimbal near the fluid flow gaps. However, flex lead torque was very unstable and showed large changes from one cycle to the next. This was corrected by forming subsequent flex leads from a more stable material and cycling at a temperature well above sterilization temperature before installation into the gyro. Additional evaluation of the improved flex leads indicated drift-rate shifts from one sterilization cycle to the next on the order of 0.04° per hour.

Following the dummy-gyro evaluation, final gyro design and fabrication were completed incorporating all improvements resulting from the investigation program. During the testing of this gyro, it was necessary to make design changes in the spin-motor stator, in the stator winding potting, and in the gimbal suspension pump. The gyro was rebuilt incorporating these changes and went through a complete test sequence of five sterilization cycles, with performance checks after each cycle.

The test results are summarized in figure 3. One can see that the mass unbalance and reaction torques have a definite trend with each sterilization cycle. The mass unbalance trend may be caused from flotation fluid absorption and the reaction torque shifts are likely from flex lead null shifts. Fluid torque is reasonably stable; this indicates the case stability. The overall performance shift with sterilization cycles did not meet performance goals; however, passing sterilization without catastrophic failure is considered a significant accomplishment in itself.

Subsequent testing indicates that g-sensitive drift terms of this gyro are continuing to change while the g-insensitive drift rate appears to have stabilized. The problems encountered during the proof sterilization cycles and in continuing testing will be further studied on a second sterilization model presently being fabricated.

There has been a small effort underway for approximately a

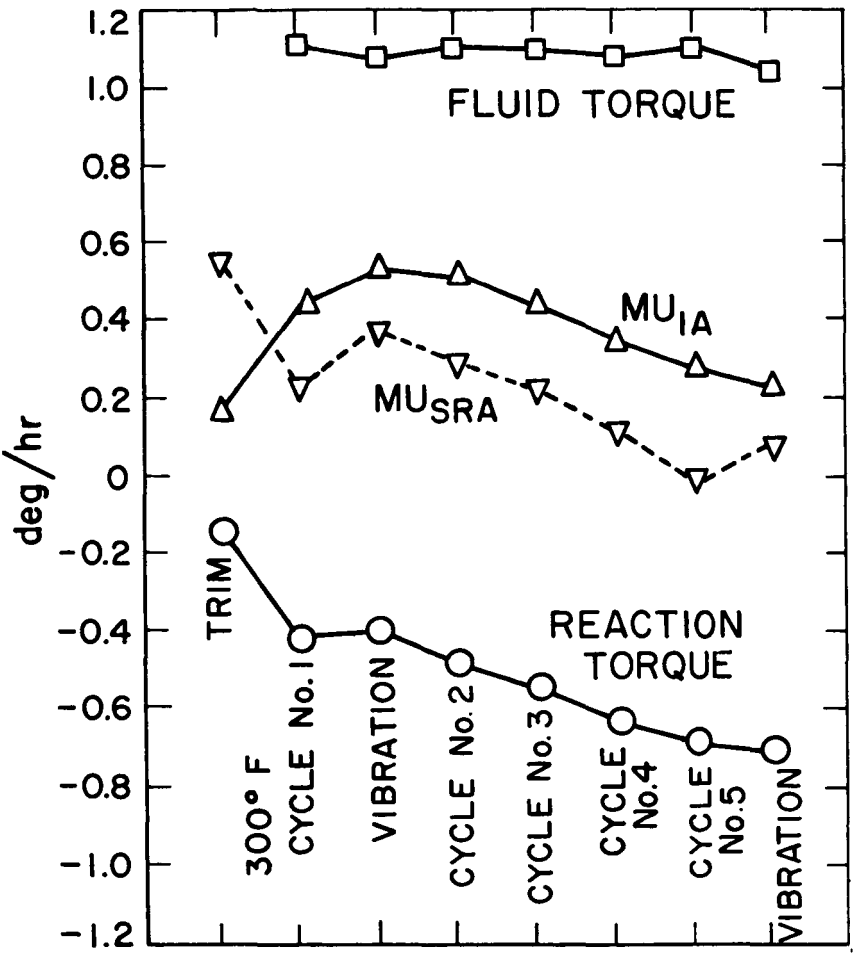

FIGURE 3.—Gyro balance torque history.

year to investigate the problems associated with sterilizing an accelerometer. The primary effect of heat sterilization on the accelerometer under test has been unacceptably large bias shifts. At the present time, a unit that seems to have a better chance of surviving the sterilization environment is being sought.

The final work in the inertial area is being done on an advanced device called an electrostatic gyro (ESG). The principle of this gyro is very different from that of conventional gyros. In an ESG a spherical rotor is freely suspended in an electrostatic field set up by six diametrically opposed electrodes forming a spherical cavity around the rotor. Optical pickoffs view a pattern on the rotor and provide information to a computer to determine the relative

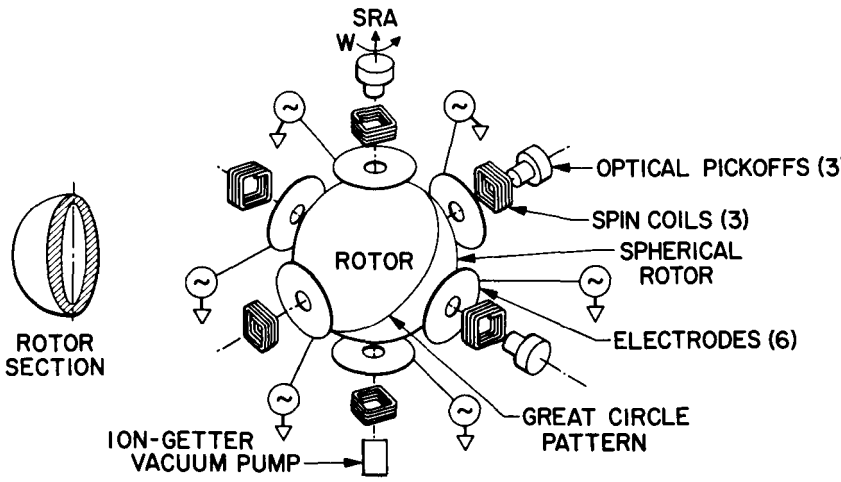

FIGURE 4.—Electrostatic gyro, exploded view.

orientation of rotor spin axis and gyro housing. Figure 4 is an exploded diagram of an ESG.

In conjunction with the basic ESG development program, principal components were subjected to thermal sterilization to determine what additional development would be necessary to insure that the gyro will ultimately have thermal sterilization capability. Problems encountered were blistering of rotor plating; failure of electronic components, a photo diode, and a preamplifier; and an inductance change in the suspension-system output transformers. Test results indicate that nonplated rotors and electroless nickel-plated envelopes can survive sterilization. The optical pickoff assembly suffered no problems from sterilization. The problems with the electronic components, the photo diode, and the preamplifier resulted from the use of solid-state components which were not designed to pass thermal sterilization. It is expected that these problems will be solved by proper selection of electronic components. The inductance change in the transformers requires further investigation to determine its implications.

Sterilizable, inertial-quality gyros are now state-of-the-art. The work in accelerometers has not progressed so far; however, usable devices can probably be developed. An advanced gyro design, the ESG, seems to offer no unsolvable sterilization problems. For a more detailed discussion of recent work in this area, see pages 95 to 97 of reference 1.

Actuators

The types of actuators included in this discussion are gas systems for attitude control, jet vane actuators for autopilots, and

any actuator for a spacecraft appendage such as an antenna, a science platform, or a guidance sensor. Depending upon the complexity of the landing capsule, any or all of these devices may be necessary. Since a great deal of work has been done toward providing the designer with materials capable of withstanding high temperatures, the problem of material compatibility has largely been eliminated. Probably the two greatest problems associated with heat sterilization of actuators are differential expansion and dimensional stability. With a reasonable degree of caution, however, they should not offer any insolvable problems.

The approach of this investigation is to fabricate and test certain devices or components which are similar to those anticipated for use on a landing capsule. As problems arise, design changes are made and retesting determines the sterilizability of the unit.

As an example, a new type of detenting servomotor has been under development. This motor could find use as the prime mover in an appendage actuator. In this work, which has essentially been successfully completed, the motor was designed, fabricated, and tested. Careful attention was given to the selection of the materials to be used, and the resulting unit showed no problems with expansion or dimensional stability when subjected to the thermal sterilization environment.

Another device presently under development is a jet vane actuator for use in autopilot systems. Since the output shaft must have a total travel of only $\pm 30°$, ball bearings have been replaced by flexure supports. Even though ball bearings have been successfully used in high-temperature applications, it is felt that the use of a flexure support will result in fewer problems. In addition, this actuator will be more compatible with a high-vacuum environment.

It is also planned to test a potentiometer and a high-ratio, small volume gear train for thermal sterilization survival. The potentiometer will contain a conductive plastic element, which is thought to be the critical element. In the gear train the primary concern is the extent of differential expansion. Even though a wide temperature range can be taken into account in the gear-train design, during thermal sterilization it seems likely that temperature gradients will exist which may cause some problems.

Conclusions

The work being done in the guidance-and-control area toward the development of sterilizable spacecraft equipment indicates that, in spite of the difficulties encountered, there are no insur-

mountable problems. In the cases where nonsterilizable electronic components have been used in previous systems, the mechanizations can be changed to eliminate those parts. The problems encountered in thermal sterilization are primarily material selection in optical sensors; material selection, differential expansion, and dimensional stability in inertial sensors; and differential expansion and dimensional stability in actuators.

In all areas, work must continue so that we can gain further knowledge of the effects of thermal sterilization on performance characteristics of these components. As this information becomes available, it can be used to establish a testing philosophy for not only the guidance-and-control subsystems but also the entire landing capsule.

Reference

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Sterilizable Communications and Data-Handling Systems

This paper is concerned with that part of the sterilization program at the Jet Propulsion Laboratory (JPL) associated with the spacecraft telecommunications system. Thus far, work has been undertaken on and significant results have been accomplished with: (1) pressure transducers, (2) magnetic-tape recorders, and (3) magnetic-core memories.

The pressure-transducer program has consisted of the following phases:

(1) Investigating available pressure transducers and selecting those potentially capable of withstanding sterilization
(2) Subjecting the selected transducers to sterilization testing
(3) Evaluating and analyzing the test results to define any problems resulting from the sterilization environment

A program was undertaken to define and solve all the problems associated with the sterilization of a typical spacecraft magnetic-tape recorder. The program was outlined so that each component to be included in the final transport subsystem was individually designed or developed for sterilization compatibility and exposed to sterilization tests. After qualification of the individual transport components, a complete transport was assembled, subjected to sterilization testing, and subsequently life tested.

A typical magnetic-core memory subsystem was defined and a program was undertaken to solve all the problems associated with its sterilization. All the individual components within the memory were evaluated and qualified for sterilization compatibility. The complete magnetic-core memory is presently being assembled and will be exposed to sterilization testing.

As has been indicated, specific spacecraft telecommunications hardware has been developed or procured and exposed to sterilization testing. Results have been analyzed and the subsequent development and testing undertaken where deemed necessary. A detailed presentation of the individual task descriptions, work accomplished, and future development plans for each of the aforementioned telecommuncations subsystems and devices is the subject of this paper.

For centuries, man has turned to the heavens and pondered the question: Is he alone in the enormity of the universe he perceives? The very nature of man, always curious and searching for the why of his existence, makes it inevitable that he will actively seek the answer to this question. If the answer cannot be found within the

restricted boundaries of his immediate home on Earth, it is only natural that eventually he will find a means to escape his confinement to journey into the unknown regions of outer space. There he will visit other worlds, continually searching for a clue that might indicate that extraterrestrial life does in fact exist and he truly is not alone.

In order to prepare for his actual journeys to other planets, man is presently sending unmanned probes to these worlds to gather information that will reduce the magnitude of the unknown that presently confronts him. Such unmanned missions will become increasingly more complex as techniques are mastered, and prior to man's actual visit manmade devices will actually land upon unexplored planetary surfaces to gather, process, and send information back to Earth. Such information will include in its content experimental data designed to indicate the presence or absence of living organisms.

At this point, one should reflect upon a basic fact: this information should not be the result of monitoring the existence of life forms from Earth that had been transported to the unexplored planet via the space vehicle. Consequently, it becomes apparent and mandatory that all living organisms originating from Earth must be destroyed prior to a spacecraft's impacting other planets so that such contamination cannot occur. This, in summary, is the present purpose of the NASA Sterilization Program.

The present NASA Sterilization Program requires that all spacecraft flight subsystems be required to withstand $137 \pm 2°$ C in an inert atmosphere for 24 hours for flight acceptance and that all prototypes survive three 36-hour exposures to a $145 \pm 2°$ C temperature for type approval. In addition to the aforementioned dry-heat requirement, there is also a requirement that the equipment survive decontamination exposure to an ethylene oxide (ETO) atmosphere at 35 percent relative humidity and 40° C for 24 hours.

Certainly of significant concern is that part of the sterilization program at JPL associated with the spacecraft telecommunications system. The spacecraft telecommunications system consists of equipment required for receiving, gathering, processing, and transmitting telemetry and command information.

Certain telecommunication subsystems consist of components and materials common to other systems and subsystems on the spacecraft. For instance, the sterilization compatibility of digital and linear circuits consisting of electronic components would most naturally fall under the cognizance of the JPL Component Parts Group. However, certain other subsystems and devices are unique to the spacecraft telecommunications system. It is in these areas

that the nature and extent of any and all problems resulting from exposure to JPL sterilization testing must be defined and adequate solutions effected by the JPL Telecommunications Division. Thus far, investigation in the following three critical areas has been undertaken and significant results have been accomplished:

(1) Pressure transducers
(2) Magnetic-tape recorders
(3) Magnetic-core memories

A presentation of the task description, work accomplished, and future plans for each of the above telecommunications subsystems and/or devices is the subject of this paper.

Sterilization of Pressure Transducers

Pressure transducers are fundamental to a spacecraft mission in that they monitor pressures associated with both engineering measurements (measurements concerned with the performance of the spacecraft) and scientific measurements (measurements concerned with the environment external to the spacecraft). The Telecommunications Division of JPL, being responsible for the transducers required for the engineering measurements, initiated an in-house program to define and solve all the problems associated with the sterilization of engineering pressure transducers. This program was organized to consist of the following five phases:

(1) Investigate various commercially available pressure transducers and select those potentially suitable for spacecraft requirements
(2) Study the mechanisms and constituent materials of the selected transducers to determine their potential capability of withstanding the sterilization environment
(3) Subject transducers selected as potentially sterilizable to JPL TA and heat-sterilization testing
(4) Evaluate and analyze the test results in order to define any problems resulting from exposure to the heat-sterilization environment
(5) Solve any major problems defined from the evaluation and analysis of the test results for each transducer if deemed feasible and/or necessary

Selection of Transducers

Previous experience at JPL with pressure transducers indicated that significant temperature problems may occur as a result of the heat-sterilization environment. However, this experience was primarily based upon work with transducers from only two manufacturers. Consequently, an investigation was initiated to contact

other manufacturers and determine the present transducer state of the art. A transducer compendium published by the Instrument Society of America was used to generate a list of potentially suitable companies possessing capability in the pressure-transducer area. From this list, numerous companies were contacted and, of these, three were selected as potentially most suitable. These three companies were visited and individual transducer mechanism and manufacturing processes observed and evaluated. A general conclusion from these visits was that present transducers manufactured by these companies might, with additional emphasis placed on selection of materials, withstand the JPL sterilization environment with no degradation. One transducer each was obtained from two of these companies for the purpose of subjecting them to JPL sterilization testing and evaluating the subsequent results. These transducers are shown in figures 1 and 2.

The transducer shown in figure 1 is a commercially available unit (Microsystems Type PT8–S/N 703) capable of measuring pressures in the range of 0 to 50 through 0 to 5000 psi. It is specifically designed for applications wherein an unamplified voltage output of high value is desirable. It is a semiconductor strain gage constructed of corrosion-resistant materials. The mechanism consists of an integrally machined diaphragm to which four active semiconductors are bonded. The pressure cavity is fully welded and possesses no sealed or threaded joints.

The pressure transducer shown in figure 2 (Giannini Type 461 322–A1V6–80–75) is a commercially available unit capable of pressure measurements from a minimum range of 0 to 300 psia to a maximum range of 0 to 3500 psia. It is a hermetically sealed Bourdon-tube-type instrument having a highly accurate potentiometric output.

The aforementioned transducers were received from their manufacturers and calibrated with the equipment illustrated in figure 3.

Testing of Transducers

Following calibration, each transducer was subjected to a complete dry-heat sterilization test. During this test, the transducers were exposed three times to a temperature of $145 \pm 2^{\circ}$ C for 36 hours with stabilization to ambient conditions between each exposure. Between each such temperature cycle the transducers were pressurized and checked against the original calibration points, as plotted in figures 4 and 5.

Following sterilization testing, each transducer was subjected to JPL TA vibration testing. This consisted of subjection to a combined 10-g rms noise plus 4-g rms sinusoidal exposure for 2 min-

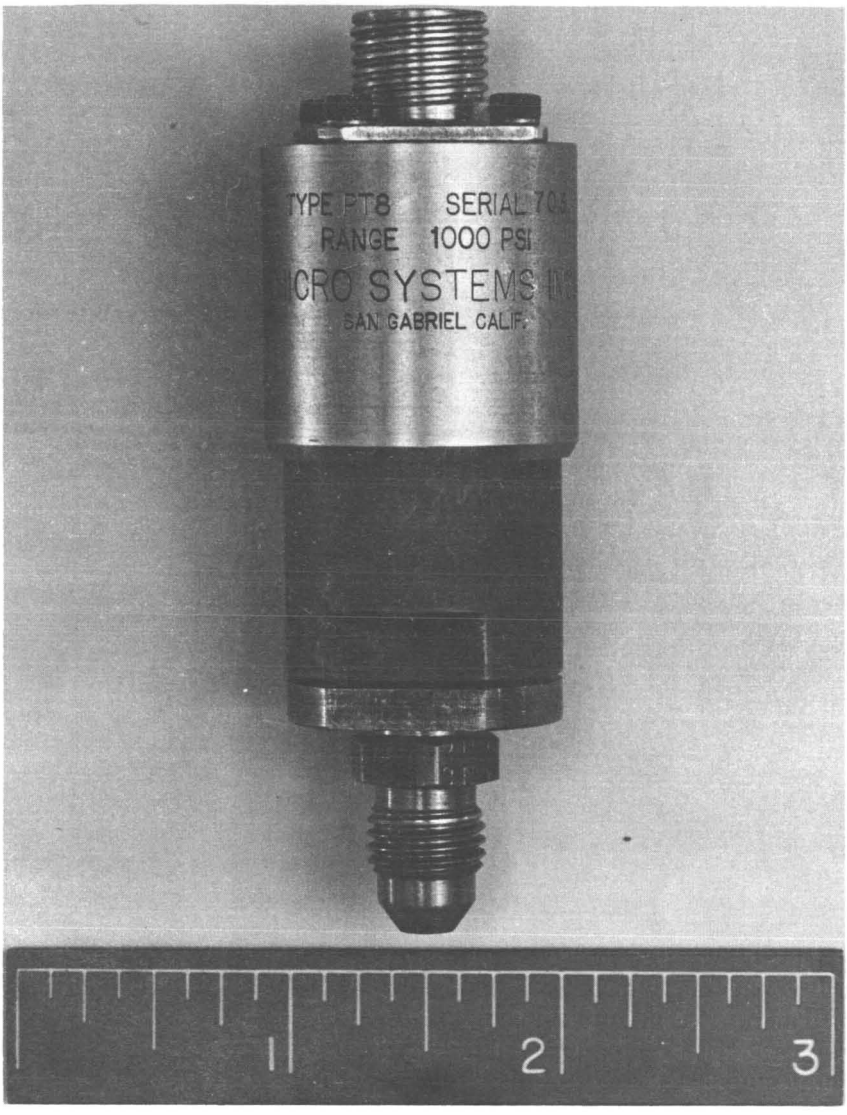


FIGURE 1.—Microsystems pressure transducer.

utes and a 5-g rms noise plus 4-g rms sinusoidal environment for 6 minutes. This test was conducted in all three planes; after the test the original calibration points were again checked.

A shock test was then performed on each transducer. This test consisted of five 200-g shocks, each of 0.7-msec duration in each of three axes. Following this test, the transducers were again checked against their original calibration.

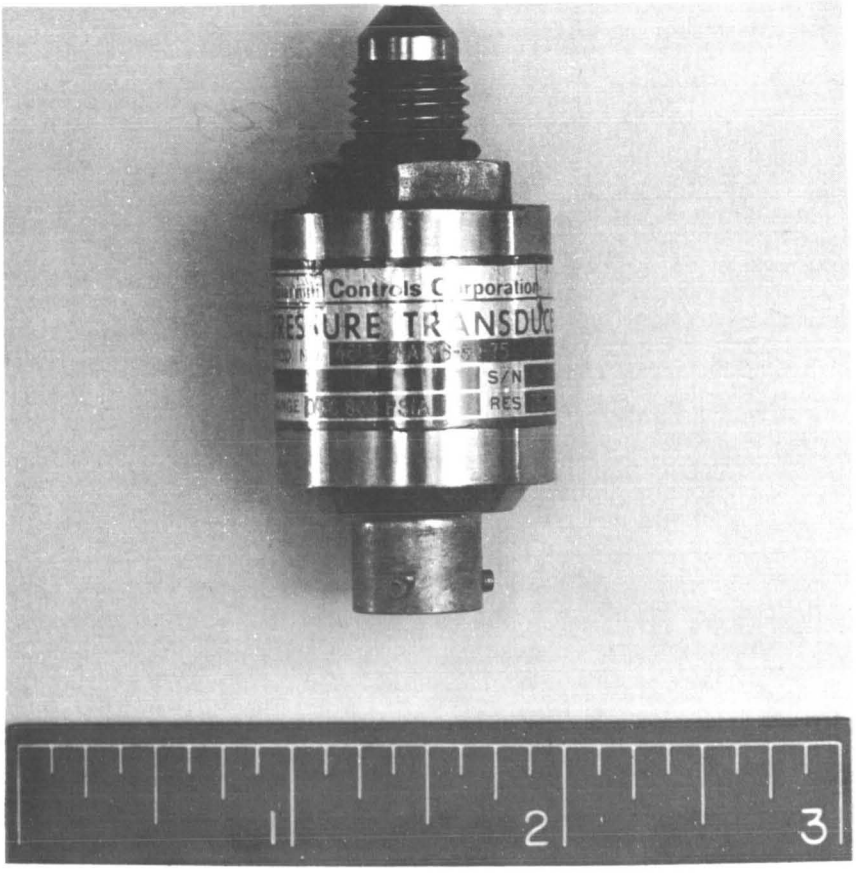


FIGURE 2.—Giannini pressure transducer.

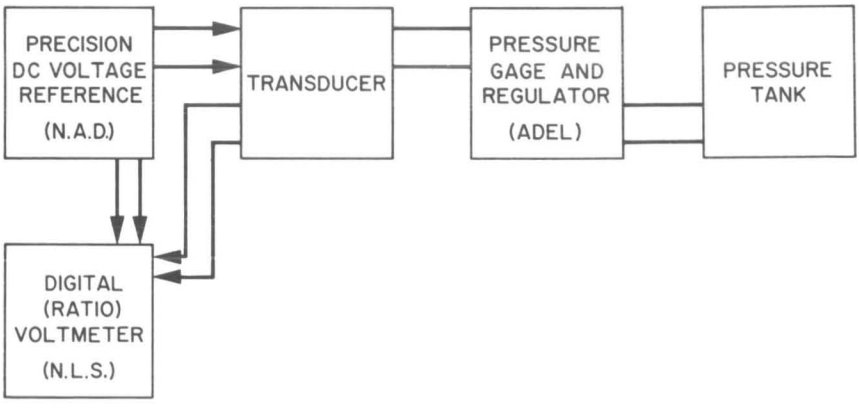


FIGURE 3.—Setup of equipment for calibration of pressure transducer.

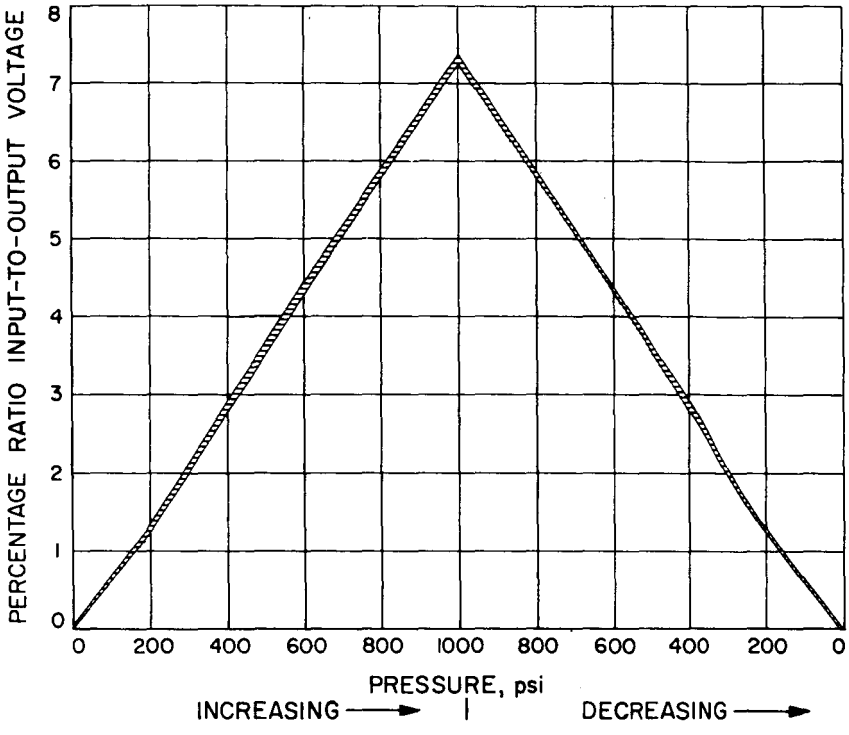


FIGURE 4.—Microsystems Type PT8 pressure transducer, serial no. 703.

Graphs indicating the maximum error band resulting from the points of extreme variation are plotted for each transducer in figures 4 and 5, respectively. These plots were derived from maximum variations from the original calibration monitored throughout the entire testing program previously outlined. As can be seen from figures 4 and 5, variations from the original calibration are within ± 2 percent for both transducers.

Results of Tests

Post-testing examination of the transducer from Microsystems, Inc., revealed no detectable degradation. However, a silicone potting material was used on some connections in the Microsystems unit. From testing and analysis on another program (tape-recorder sterilization), this material has been considered questionable for use in a space environment. Consequently, if this or similar transducers are considered for spacecraft applications, a suitable replacement for the silicone potting should be investigated.

Post-testing examination of the transducer from Giannini Controls Corp. disclosed no major degradation either. However, some

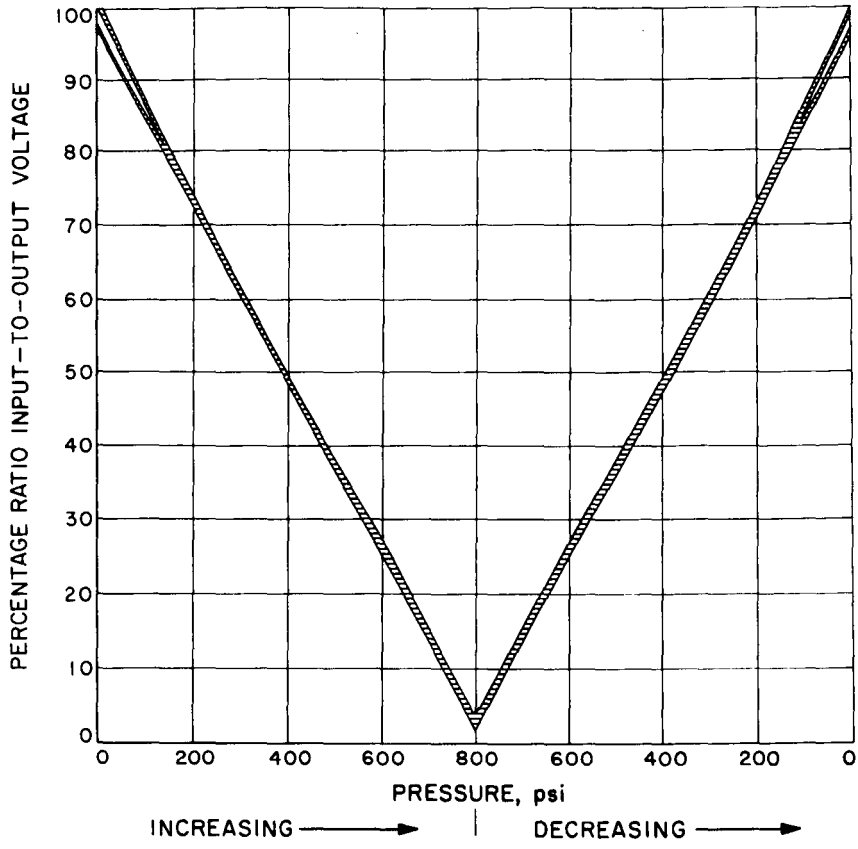


FIGURE 5.—Giannini Controls Type 461 322–AIV6–80–75 pressure transducer, serial no. 2–1.

evidence of discoloration and creep of the potentiometer insulation material was detected. Although materials used in this transducer for mounting and insulating the potentiometer resistance wire were affected by the sterilization environment, better materials have been developed recently and are available. For instance, an organo-silicon varnish, which combines long thermal life with high bond strength, has been developed by the Dow Corning Corp. (GP77) and should be potentially suitable for a sterilization application.

Sterilization of Magnetic-Tape Recorder

Data storage is a necessary requirement for a deep-space telecommunications system because of the bandwidth limitations of the communication link. Consequently, it is necessary in numerous cases to accept data inputs at comparatively high rates and to store

and read out at a rate commensurate with these limitations. For video applications, input data rates are high, and the quantity of data needed to produce useful information is very large. Therefore, use of data-storage systems with magnetic-tape recorders as the primary storage medium appears to be the most feasible approach for such high-capacity applications considering the present status of data-storage technology. In addition, for future data-handling systems in which data-compression techniques are employed to use the available bandwidth more efficiently, large storage capacity will also be required. In summary, there is little question that magnetic-tape recorders will continue to be increasingly important subsystems for future deep-space telecommunications systems.

The need for magnetic-tape recorder systems in spacecraft has been apparent for some time, so the problem of sterilization compatibility was initially considered several years ago. Previous experience with tape recorders had indicated that the heat-sterilization requirement could represent a serious problem for numerous components of the transport subsystem. Although the magnetic-tape and Mylar belts were of chief concern, heads, motors, bearings, lubricants, seals, and the transport plate were also considered potentially susceptible to damage when exposed to sterilization temperatures.

Definition of Program

A program was undertaken in 1963 to define and solve all the problems associated with the complete sterilization (by both dry-heat and ethylene oxide) of a typical spacecraft magnetic-tape recorder. The endless-loop Mariner IV type magnetic-tape recorder was chosen as the basis for the transport design because it was more like actual flight hardware than any other transport under development at JPL. The program was outlined so that each component to be included in the final transport subsystem would be individually designed and developed for sterilization compatibility and then exposed to complete dry-heat sterilization tests. From these tests, a complete experimental and analytical analysis was to be performed to define adequately any degradation affecting the performance or life of the components. If problems resulted, an attempt was to be made to solve them so that a reliable sterilizable component would be available for inclusion into a sterilizable transport subsystem. Since these components were to be assembled into a sealed case, the only component requiring ethylene oxide decontamination survival would be the pressure seals. Therefore, the program was defined so that only the seal material was to be exposed to ethylene oxide testing prior to transport assembly.

When sterilizable components were available, they were to be assembled into a complete flightworthy tape-transport subsystem which would be exposed to complete sterilization testing with its performance before and after sterilization monitored and analyzed. Any detection of degradation in performance reliability would be determined from these results and an attempt would be made to solve it. Assuming that a sterilizable transport subsystem was achieved, the final phase of the program was to be a life test to determine the effects, if any, of sterilization on life and reliability.

Component Evaluation

A contract was awarded to Raymond Engineering Laboratory, Inc. (REL), of Middletown, Conn., in 1963 to accomplish the aforementioned program. The initial phase of this contract was concerned with the attainment of sterilizable components for a Mariner IV type of transport. The components individually so considered were: (1) magnetic tape, (2) pressure and drive belts, (3) record-playback motors, (4) rotating modules and lubricants, (5) record-reproduce heads, (6) transport plate, (7) preamplifier electronic modules, (8) end-of-tape sensors, and (9) covers and seals.

Magnetic Tape

Initially in the program, an industrywide search was conducted to determine the availability of a high-temperature magnetic tape capable of surviving the dry-heat sterilization environment with minimum deterioration of performance and reliability characteristics. Several tape manufacturers were initially contacted and their tapes were selected and evaluated. A summary of results and conclusions follows.

(1) *Minnesota Mining & Manufacturing Co. (3M).* — Two 3M magnetic tapes, LR1220 and LR1353, were selected for evaluation. Both of these tapes have a Mylar backing and are considered to be the best lubricated tapes available from 3M. Tests revealed that the LR1353 tape, recommended for use at temperatures up to 225° F, was by far the most capable of surviving high temperatures. Following sterilization, samples of LR1353 tape showed no significant deterioration in electrical output. However, mechanical deterioration was apparent in that excessive shrinkage (approximately 2 percent) and reduced tensile strength were evident following sterilization exposure.

(2) *Memorex Corp.* — Only standard Mylar-backed tapes rated for temperatures far below the sterilization temperature were available from Memorex. Sample tapes from Memorex were unusable after exposure to the 300° F sterilization testing because of

the excessive deterioration of electrical and mechanical characteristics that resulted.

(3) *Remington Rand Office Systems Division of Sperry Rand Corp. (Univac).* — The only potentially high-temperature tape available from Univac was a Mylar-backed tape called Univac Electroless. A sample of this tape was obtained and exposed to sterilization testing. However, the oxide film deteriorated so excessively that the tape was entirely unusable following sterilization.

(4) *Reeves Soundcraft Corp.* — Samples of several Mylar-backed tapes were obtained from Reeves Soundcraft. Following numerous temperature tests, one of these tapes appeared to retain its stability much better than the others. This tape was lubricated with graphite on one side and had an oxide coating on both sides of the Mylar base. Reeves produced some samples of this double-oxide tape with a high-temperature polyimide backing developed by Du Pont known as H-film to improve temperature-resisting capability. Several 6-foot samples were obtained for backing in place of Mylar and exposed to sterilization testing. The sterilized samples of H-film tape showed no significant degradation in performance or mechanical characteristics following sterilization tests.

Samples of metal tape were also obtained from Reeves. However, the electrical performance of the metal tape was extremely poor compared with that of Mylar- or H-film-backed tapes. In addition, the tape had a tendency to curl after passage over the small radius of the capstans.

(5) *Thin Films, Inc.* — Several samples of tape with H-film backing were obtained from Thin Films, Inc. This tape exhibited a very low output as compared with other tapes tested and indicated poor temperature stability of the oxide coating following sterilization.

(6) *The Arnold Engineering Co.* — Several samples of metal-backed tape were obtained from Arnold Engineering. However, as with the Reeves metal tape, electrical and mechanical performances were extremely poor.

From the foregoing evaluation program, it was concluded that H-film represented the most promising backing material for a sterilizable magnetic tape. In addition, the Reeves H-film-backed tape previously discussed was the only tape evaluated that showed promise of satisfactory survival of the sterilization environment. Therefore, a contract was awarded to Reeves for the production of a usable reel of H-film-backed tape. Reeves was instructed to use their proprietary oxide binder, which employs oxide coating on both sides of the tape, because of its potential high-temperature

stability. From this contract, 6000 feet of H-film tape were produced and sent to REL for evaluation. Preliminary tests indicated an electrical output comparable to good Mylar instrumentation tape.

A 300-foot pack was initially made and exposed to sterilization. This test resulted in a failure because of inadequate curing of the tape. Therefore, a curing process was incorporated which consisted of a 24-hour exposure to 150° C with a dry nitrogen wash over a loose tape pack. An ensuing sterilization test of a 300-foot pack of cured tape exposed a new problem: excessive shedding of the graphite lubricant. A burnishing machine was therefore constructed for the removal of excessive lubricant; this machine removed the excess lubricant by passing the tape over an abrasive surface.

A 300-foot tape pack of cured and burnished H-film tape was then mounted in a transport case and exposed to complete sterilization testing with very encouraging results. The pack started normally with no evidence of discontinuities or voids. A total of 310 passes was accumulated on the tape before the amount of lubricant debris collecting about the head and rotating parts was considered to be of serious proportions. This is a normal or expected capability of lubricated tape in an endless-loop pack.

Although the Reeves H-film tape survived the sterilization environment with no significant degradation in performance or life detected, several unique characteristics of the tape were determined as a result of the curing and sterilization process. One of these is that the binder system undergoes a modification during high-temperature exposure wherein the oxide becomes so affixed to the H-film tape that it cannot be removed by normal means. Prior to curing of the tape the oxide surfaces are easily removed by means of ordinary solvents.

Another characteristic is a definite increase in stiffness of the H-film tape following curing at 150° C. This apparently was the cause for an increase in AM from 10 to 17 percent following curing. This increase in stiffness is probably a byproduct of the aforementioned binder modification.

A third characteristic is a definite outgassing of the H-film tape during the curing process. Analysis of several tape samples at JPL revealed gas byproducts consisting of small traces of polyimide (H-film) and acrylate materials (binders and adhesives). It was necessary to incorporate a nitrogen flowthrough during the curing process to allow the removal of these byproducts.

In addition, splicing of cured H-film tape requires special consideration. The normal splicing operation requires removal of oxide from the two ends of tape to be spliced. The ends are then

bonded together with a separate piece of H-film. However, as was mentioned previously, the oxide is extremely difficult to remove from H-film tape. Therefore, the splice must be made over the oxide. Tests of such splices indicate that the splice is adequate. In fact, no failure of oxide interface has ever occurred because the H-film lap will break before this can happen.

In summary, H-film-backed tape was developed and has survived JPL sterilization testing with no significant degradation in performance or life of the tape. Since no other known tape demonstrated this capability, this tape was selected as the magnetic recording tape to be used in the fabrication of the sterilizable tape transport.

Pressure and Drive Belts

Initial tests of Mylar belts at sterilization temperatures indicated that Mylar would probably be unsatisfactory. Therefore, several belts were fabricated from H-film in the hope that this would provide significant improvement at high temperatures. These first H-film belts were unsatisfactory because they became wrinkled. It was found that the wrinkles could be prevented by rotating the belts during the curing process. Following the development of a satisfactory curing process, several H-film belts were fabricated and exposed to sterilization testing with extremely encouraging results.

Comparative sterilization tests of Mylar and H-film belts were conducted with the belts mounted in a skeleton transport. Test results showed that the tension of the capstan-to-capstan Mylar pressure belt was reduced over 60 percent by the sterilization environment; this made the belt completely unusable. On the other hand, an H-film playback motor-to-first-idler belt and an H-film pressure belt lost 16.5 and 24.5 percent of their initial tension, respectively. These are usable figures in that the belts can be adjusted initially to approximately 20 percent increased tension to compensate for the decrease in tension during sterilization.

Sterilization testing of unmounted belts also indicated a definite superiority of H-film over Mylar. H-film belts experienced no significant dimensional change, whereas Mylar belts normally suffered a typical reduction in length exceeding 5 percent with evidence of actual damage. In addition, Mylar stress characteristics were affected to a much greater extent. Mylar typically showed a change in tensile modulus of more than 18 percent, whereas the corresponding modulus change of H-film belts was normally 2.5 percent as a result of the sterilization process.

In summary, H-film belts were found to be far superior to Mylar

belts in the sterilization environment. With the initial tension of the H-film belts set approximately 20 percent above the nominal figure, the belts indicated a capability of surviving the sterilization environment with no significant degradation. Therefore, H-film belts were selected for use in the sterilizable tape transport.

Record and Playback Motors

The record and playback motors required by the transport were designed by H. C. Rotors, Kew Gardens, N.Y., and fabricated by REL. These motors were both highly stable as hysteresis synchronous motors having an efficiency of 25 to 30 percent. Prior to sterilization of the motors, the laminations were spray varnished, assembled, bored, and fluidized with an epoxy compound. This process included exposure to several temperature cycles, the most extreme being 115° C for 4 hours. This was necessary to drive off any volatiles from the Hysol or varnish used for cementing the laminations together.

The finished record and playback motors were exposed to complete sterilization testing, with very little deterioration resulting. There was a measured reduction in stall torque of 10 percent in the playback motor at −10° C and a comparable increase in both synchronization and stall torque of the record motor at room temperature. Small changes were similarly noted in bearing-running torque, but these changes were considered insignificant. Experience has shown that bearing-torque tests are generally difficult to repeat within ±10 percent under ideal conditions. Therefore, any effect upon the motor bearings brought about by the sterilization process appears dependent upon life testing to be truly indicative.

In summary, the motors developed for the sterilizable transport indicated a capability of surviving the sterilization environment with no indicated deterioration in performance. Therefore, these motors were deemed acceptable for use in the sterilizable tape transport.

Rotating Modules and Lubricants

An investigation of available lubricants was initiated, with indications that the Anderson L 245X oil used in the Mariner IV transport bearings would probably survive the 145° C sterilization temperature. This conclusion was primarily based upon discussions with the manufacturer. In addition, a preliminary design study of the rotating modules required by the transport indicated no evident problems which would require a significant redesign of the Mariner IV transport units. On the basis of these results, the following rotating modules were assembled and exposed to sterili-

zation testing: (1) two capstan units, (2) one reel assembly, (3) two pressure-belt modules, and (4) one idler assembly. Of the 6 assemblies tested, 5 had insignificant changes, whereas one of the capstan assemblies experienced an increase in runout of almost 12 times the initial value. In addition, the coastdown time for this module increased by a factor of 2. An investigation of the cause of failure was conducted wherein the module axial preload was found to have decreased. A reduction in lubrication was eliminated as a cause because it could account for the increase in coastdown time but not runout. Although a detailed autopsy of the module was conducted, no definitive cause for the failure was determined.

According to one supposition, the excess runout was attributed to the relaxation in preload combined with an adding together of the tolerances of certain mechanical dimensions such as surface flatness, parallelism, and length of separators in a negative fashion. A factor contributing to this effect could be associated with the possible use of material lots having different heat-treatment histories for the manufacture of the two capstan assemblies. To substantiate this possibility, the failed capstan assembly was reworked and resterilized, and it demonstrated no apparent effects as a result of exposure to the sterilization environment. Consequently, a procedure of exposing a mechanical item to an environment slightly in excess of the required sterilization environment and then reworking to account for shifts may be a practical approach in obtaining a good temperature history.

Since all six rotating assemblies had survived sterilization (although one required rework and a second sterilization test), they were designated for use in the assembly of the sterilizable transport.

Record and Reproduce Heads

Preliminary testing indicated that magnetic heads capable of surviving the sterilization environment would require some development. Applied Magnetics Corp. (AMC), Goleta, Calif., was selected to provide the heads for this program. The initial heads produced by AMC had high curing-temperature (400° F) bonding materials such as Union Carbide R–61 silicone varnish. Three such heads were exposed to and survived sterilization at AMC. However, they had not been mounted nor had the pressure of tape been applied to them. Subsequent testing with the heads mounted in a transport resulted in the failure of all three. The primary failure modes were typified by: (1) core shifting, (2) changes in gap width, and (3) changes in inductance. Detailed consideration of these failure mechanisms resulted in the conclusion that a head

design stressing a matching of temperature coefficients was necessary. In addition, the possibility of a unique head design incorporating a retaining spring to return the pole pieces to their original position following sterilization was considered worthy of investigation.

To obtain thermal expansion coefficient compatibility, molds of RTV rubber were fabricated, a strip of metal was placed in the bottom, and epoxy was poured over it. Following cure, the end of the metal and epoxy strip was clamped and relative cure versus temperature characteristics were measured by means of a set of rulings at the end of the strip.

The aforementioned spring arrangement was investigated but rejected because it aggravated the problem of pole-piece "stepping." Although the primary problem in heat sterilizing a head is concerned with gap opening, slight anomalies in head construction can cause significant misalinement of the pole pieces in the plane of the tape. The most successful approach to this problem consisted of a process in which a "poor-match" epoxy was used to fill the back volume of the head. With expansion due to temperature, the back gap tends to open; this, in turn, causes the front gap to have a tendency to close and results in an effective fulcrum in the area of the assembly screws. Since the coefficient of expansion and the position of the screws were extremely critical, the study resulted in the selection of one screw per head so that one core half is effectively floating (the original head assembly technique used a separate screw for each core half). In addition, a relatively resilient rubber was selected for the fill between the core and shell rather than all epoxy.

Four sets of heads using such design techniques were produced in which each set used different shell materials. The four materials used were aluminum, aluminum-bronze, KR Monel, and brass. All four sets were exposed to sterilization testing and remained within the required design tolerances. According to the test results, the aluminum head was superior, so it was selected for inclusion in the sterilizable transport.

Transport Plate

The effect of sterilization temperature upon the transport mounting plate is one of expansion. This does not initially appear to be a serious problem when one considers the fact that the plate should return to its original condition following exposure to heat. However, because of this expansion, motor, idler, and capstan shafts will vary in spacing during sterilization. Therefore, when combined with the thermal coefficient of expansion of the asso-

ciated belts, the expansion causes a net change in belt tension to occur. For the sterilizable tape transport being developed, the plate was made of AZ31B magnesium, while the drive and reduction belts were to be made of H-film. Since the coefficient of expansion of magnesium is greater than that of H-film, and since the drive belt lengths were equally greater than the pulley centerline distance, the thermal changes very nearly canceled. This could not be accomplished with the reduction belts, however, so there was a loss of tension of 15 to 25 percent. Therefore, the transport-plate design was considered satisfactory for use in the sterilizable transport with the provision that the reduction belt tensions be initially preset to the high side of their original tolerances to compensate for this loss in tension during sterilization.

Preamplifier Electronics

Since it is desirable to mount the tape-recorder playback preamplifiers close to the playback head for minimum noise pickup, the preamplifier electronics package was one of the sterilizable components to be included in the sterilizable transport being developed. The differential preamplifier circuits were packaged in modules consisting of discrete components which were assembled by a cordwood-construction method. Three such preamplifier modules were assembled into a shielded can identical to that used on the Mariner IV flight program. The only change from the Mariner IV packaging was replacement of phenolic circuit boards with epoxy glass boards because phenolic deteriorated as the result of exposure to a temperature of 145° C.

Subjection of the differential preamplifier assembly to complete sterilization testing resulted in no detectable problems that might affect performance or reliability. Therefore, the preamplifier assembly was deemed acceptable for use in the sterilizable tape transport.

End-of-Tape Sensor

The approach used in the Mariner IV transport for end-of-tape sensing consisted of metallic fingers contacting a conductive area of the tape. The gold-plated Mylar-aluminum laminate used for Mariner IV was found to be unsuitable because of a breakdown of the gold edges at sterilization temperatures. An initial approach consisted of bonding a 0.3-mil gold leaf to the oxide side of the H-film tape. This was unsuccessful because the gold leaf cracked and did not adhere to the oxide. Another approach consisted of depositing silver by the Brashear (mirror-silvering) technique. However, this met with failure in that the Brashear-deposited silver volatilized at sterilization temperature. A third approach,

which proved to be more successful, consisted of a spraying process using a silver lacquer. Following sterilization, the silver lacquer remained intact with a resistivity of approximately 200 milliohms per inch.

Although selected for use in the fabrication of the sterilizable transport, the silver-lacquer technique is too noisy at low tape speeds. Therefore, further work is necessary in this area. An optical end-of-tape sensing technique appears most attractive for future applications at this time.

Covers and Seals

The transport was designed to be contained within a case which is hermetically sealed with Parker Viton rubber Gask-O-Seals. Considerable work had been accomplished on the Mariner IV program to achieve a design producing a reliable seal. Therefore, the Mariner IV transport case and covers were sealed using the same identical design and exposed to complete dry-heat sterilization testing. Although the leak rate approximately doubled at a case temperature of 145° C, it returned to normal after cooling to ambient. Although this added leak rate contributed to a gas-pressure loss of 0.8 pound per square inch during the 108-hour sterilization period, this was considered insignificant. In addition, the covers bulged approximately 3/32 inch, indicating a need for a stronger cover. However, no degradation of the seal or sealing area was detected.

The seals were also subjected to ethylene oxide (ETO) decontamination testing. The case and covers were assembled and internally pressurized with 1 atmosphere of a 90/10 nitrogen-helium gas mixture. Leak rates were monitored before and after ETO decontamination cycles. These tests showed no adverse effect on the seal integrity as a result of the ETO exposure. In addition, no evidence of surface corrosion or discoloration was detectable as a result of the ETO tests.

In summary, the basic sealing technique used for Mariner IV was considered satisfactory and, therefore, selected for use on the sterilizable tape transport.

System Evaluation

Test Procedure

Following qualification of the individual components, the complete transport was assembled and subjected to complete dry-heat sterilization testing (three 36-hour periods at 145° C). After sterilization, the transport started normally with no indications of sticking or binding. The head outputs, flutter, and AM were

normal. The transport was operated for approximately 25 passes of the tape before the case was opened. Opening of the case revealed the following:

(1) A black stain was on the Dow 17 finish adjacent to the Viton seal rubber.
(2) Whitish crystals were present adjacent to the seal toward the inside of the case.
(3) A soft gray deposit that soon volatilized was present on the reel housing.
(4) A surface scale was present on the beryllium copper clutch spring and capstan "True-Arc" (C-Clamp) that gave the appearance of fine diamond dust.
(5) Slight creepage and a crack were apparent in the epoxy of the AMC magnetic head (not located in the tape path); however, the gap width had remained constant with no shifting or stepping.
(6) The brass flywheel, the clear anodized aluminum pulleys, and all solder connections had an etched or dulled appearance.

Photographs of the opened transport following sterilization are shown in figures 6 and 7.

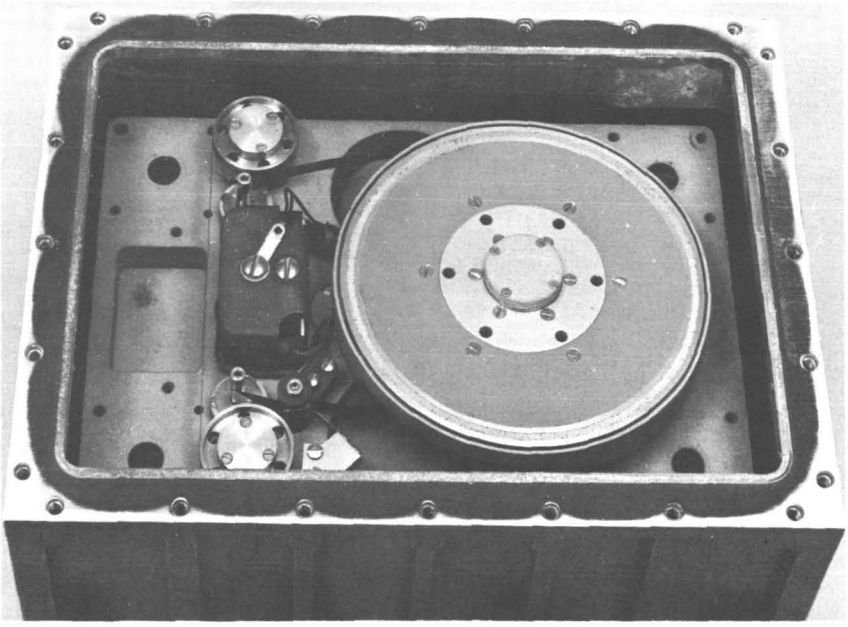


FIGURE 6.—Sterilized transport; top view.

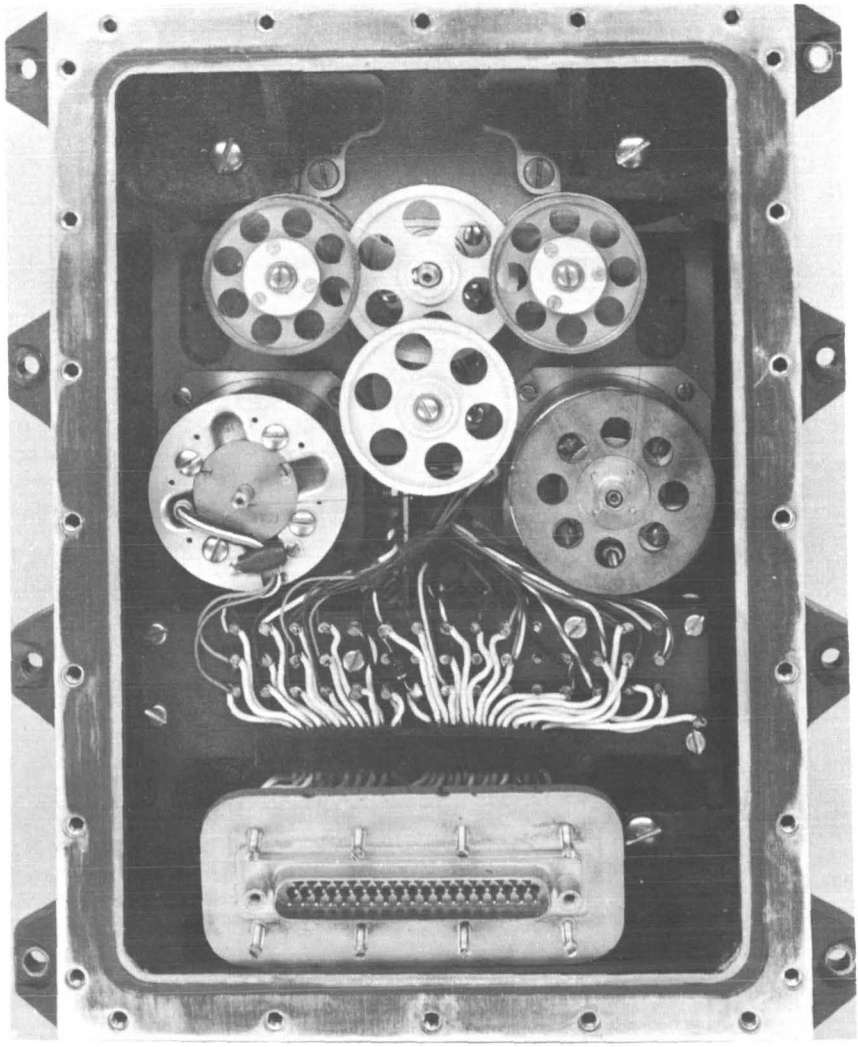


FIGURE 7.—Sterilized transport; bottom view.

An evaluation and analysis of the foregoing results of the sterilization testing were undertaken. Some preliminary results of this evaluation are summarized as follows: a sample of Viton rubber, cured to magnesium, was tested at JPL to investigate the reactions described in items (1) and (2) above and for sulfur and chlorine presence as the effect of sulfur or beryllium copper is similar to that observed on the spring and True-Arc. The Viton is a fluor-hydrocarbon and did not contain sulfur or chlorine either in formula or as contaminants. In pursuit of another possibility, Dow-Corning No. 55 silicone grease was used as an aid to

hermetically sealing the transport case. In all previous testing on the case before, after, and during heat sterilization and chemical decontamination, this grease had not been used. Since these tests were successful, the grease is probably not necessary. However, it was applied to the sealing area prior to sterilization and appeared to be the cause for the whitish crystals (analysis shows these to be mainly silicon and magnesium). As a further indication, the No. 55 pneumatic grease was tested for sulfur and chlorine and was found to contain chlorinate aromatic silicon with lithium oleate, plus some diphatic hydrocarbon additives. In addition, the total volatile material was found to be over 16 percent when the grease was heated to 100° C for 23 hours.

Following the aforementioned examination of the assembled transport, a life-test program was undertaken. A program of slow and fast tape velocities (0.01 and 12 in./sec) was established. After 5 days of life testing, a capstan bearing appeared to fail (it became noisy and caused increased flutter). After 10 days, the flutter again increased, indicating additional bearing failures. At that time, the life testing was interrupted and preparations for a complete disassembly and analysis of the transport begun. Clean-room facilities were made available at JPL wherein the transport was disassembled.

Transport Disassembly and Analysis

Following the removal of the covers, the first step in the disassembly of the transport consisted of the removal of the head shield. A collection of material thought to be either oxide or tape lubrication was noted inside of the shield. It was also noted, following examination of the heads, that the tape was depositing two piles of lubrication or oxide at the head contact area (fig. 8).

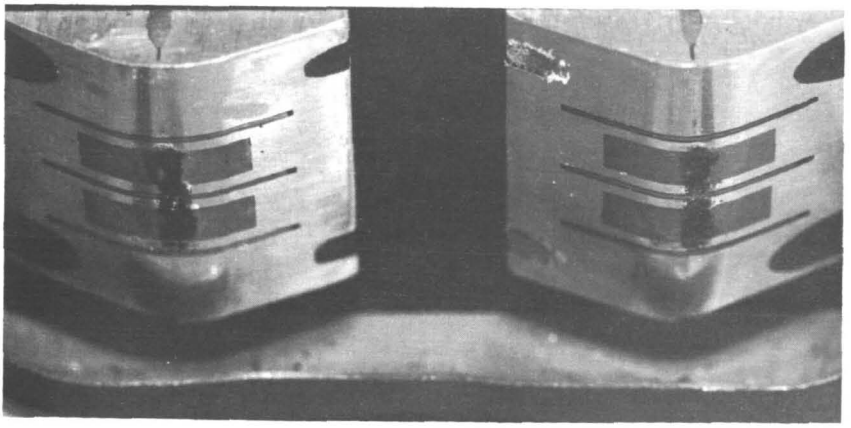


FIGURE 8.—Head deposits.

The next step in disassembly consisted of the removal of the tape pack, drive belt, pressure-pad assembly, and reel collar and shim. The collar showed corrosion, while the drive belt was definitely dirty with vertical scratches. The wiper pad shown in figure 9 was loaded with debris. The reel assembly showed a gray-white growth or deposit on the edge of the reel gaps near the tape (figs. 10 and

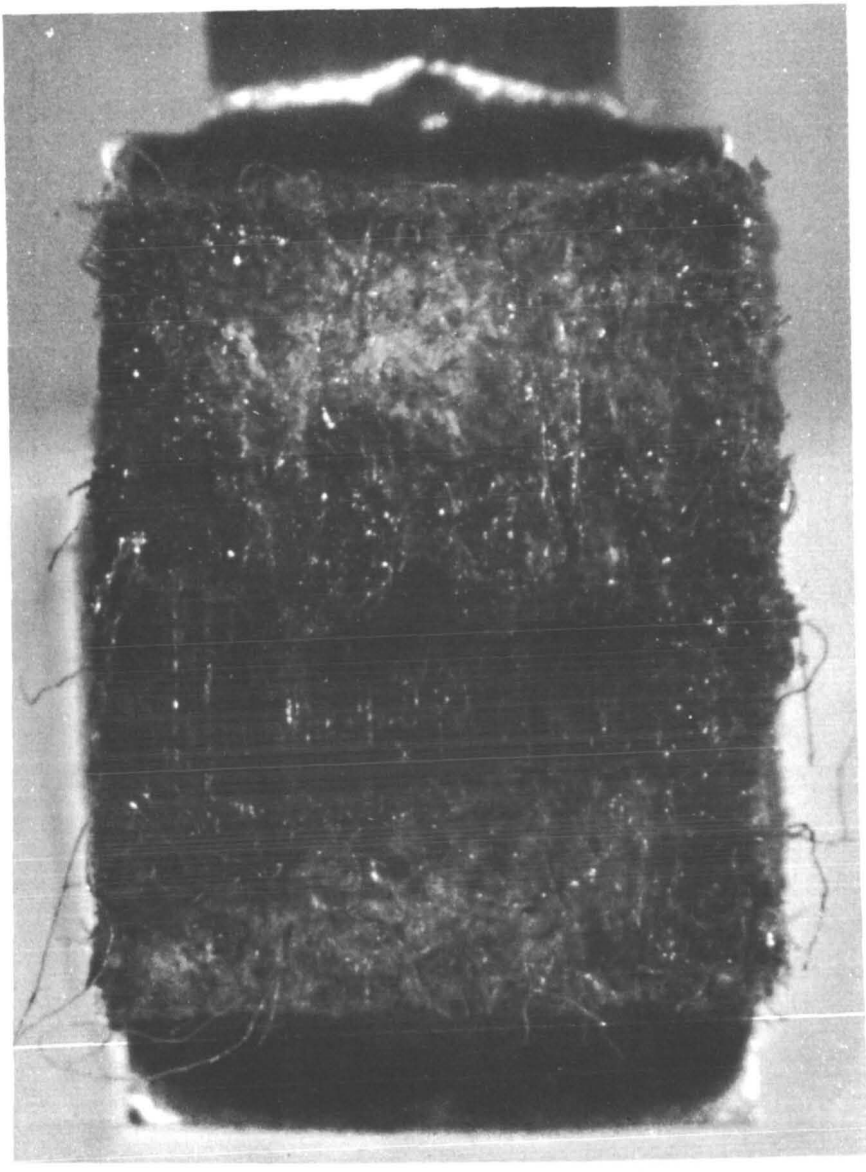


FIGURE 9.—Pressure pad.

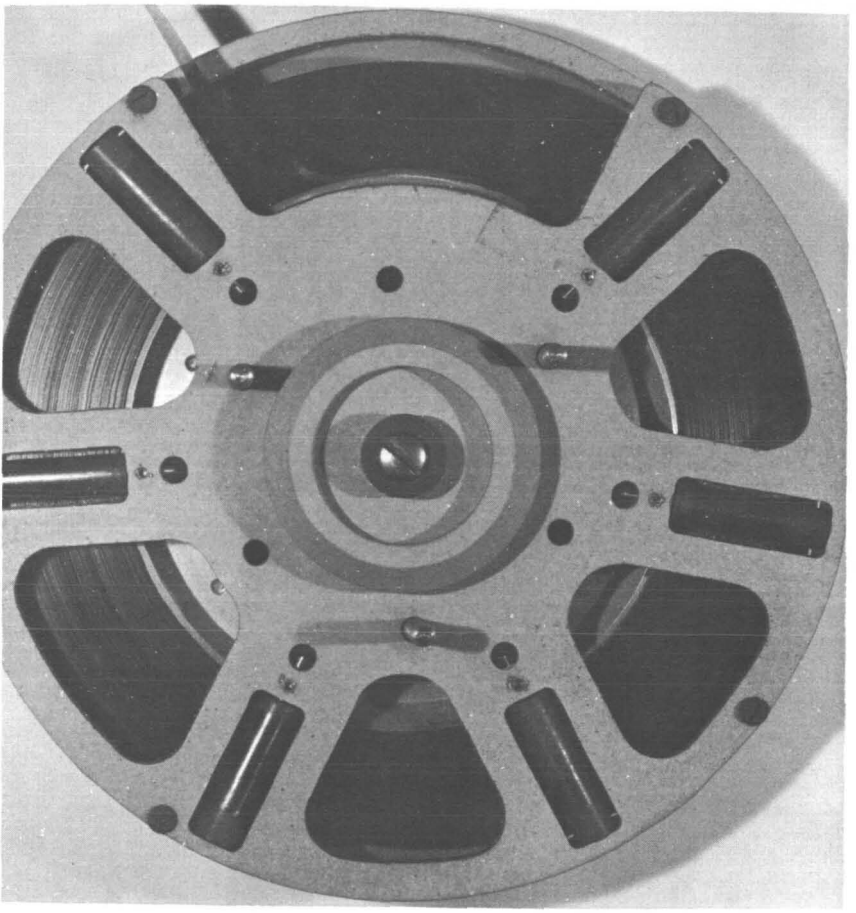


FIGURE 10.—Reel assembly.

11). The tape roller shown in figure 11 indicated a large collection of lubricant or oxide deposit. However, it is interesting to note in figure 10 that the degree of this contaminant varied from roller to roller. In fact, as can be noted from figure 11, one tape roller is virtually free of this material. Figure 11 also shows the tape pack to be stacked unevenly.

At this point, examination of the other side of the transport plate showed a capstan belt to be "riding" approximately one-sixteenth inch off the downstream capstan pulley. Further examination showed that the downstream capstan appeared to have radial play. The aforementioned belt started to center when pressure was applied to aline the capstan and the unit was rotated. It was also noted that all visible solder joints on this side of the transport plate were very dull. In addition, the four circuit-board tiedown

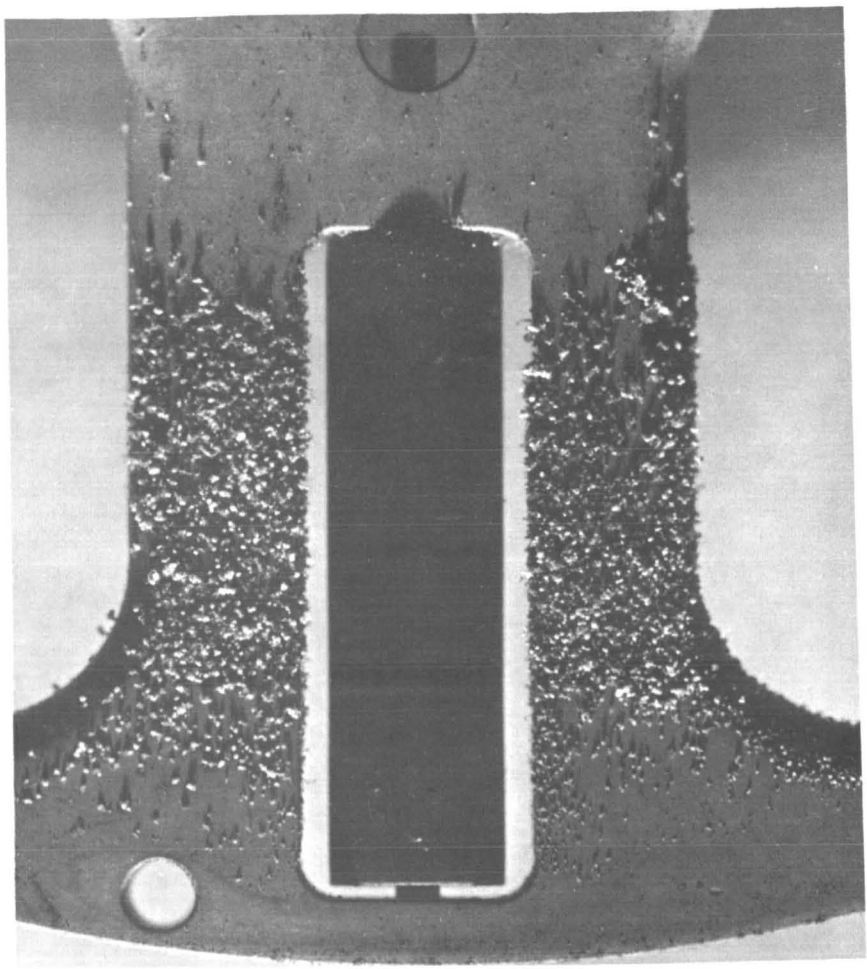


FIGURE 11.—Tape-pack deposits.

screws and one from the playback motor cleat were found to be loose.

The disassembly continued with the removal of all belts, the motor assemblies, and the first idler. The first-idler assembly bearings were found to have failed. Although the assembly was not locked up, it was very close to it. There were two extremely rusty screws at the bearing retainer.

All wires from the transport to the circuit board were clipped and labeled, and the board and connector assembly were removed. The transport plate was then removed from the case. Close examination of the removed transport plate revealed that most of the tiedown screws associated with the capstan and the pressure-

belt pulley assemblies were very rusty. Corrosion was also found under all subassembly tiedown cleats. A nut, a washer, and the main tiedown screws of the sense guide assembly were loose. The outer bearing of the right pressure-belt pulley assembly fit the shaft much too tightly, whereas bearings failed in both the downstream capstan and the second idler.

Disassembly of the downstream capstan was then begun (fig. 12). The internal Eaton ring was very corroded (figs. 13 and 14).

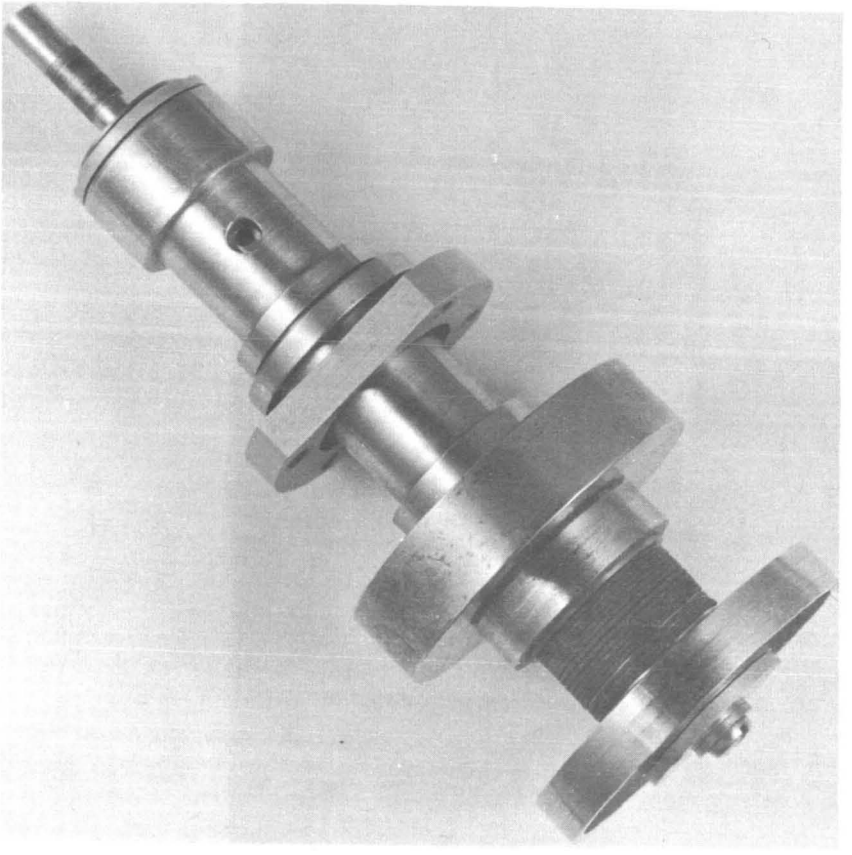


FIGURE 12.—Capstan assembly.

This corrosion had transferred as powder to bearings in the clutch assembly. With preload removed from the downstream capstan shaft bearings, the shaft turned in the inside diameter of both bearings. The dust covers were then removed from both downstream capstan body bearings. Final-stage-type failure reflected in spalling and metal failure with many rust-powder deposits was noted in the bearings (fig. 15). The bearings of the downstream

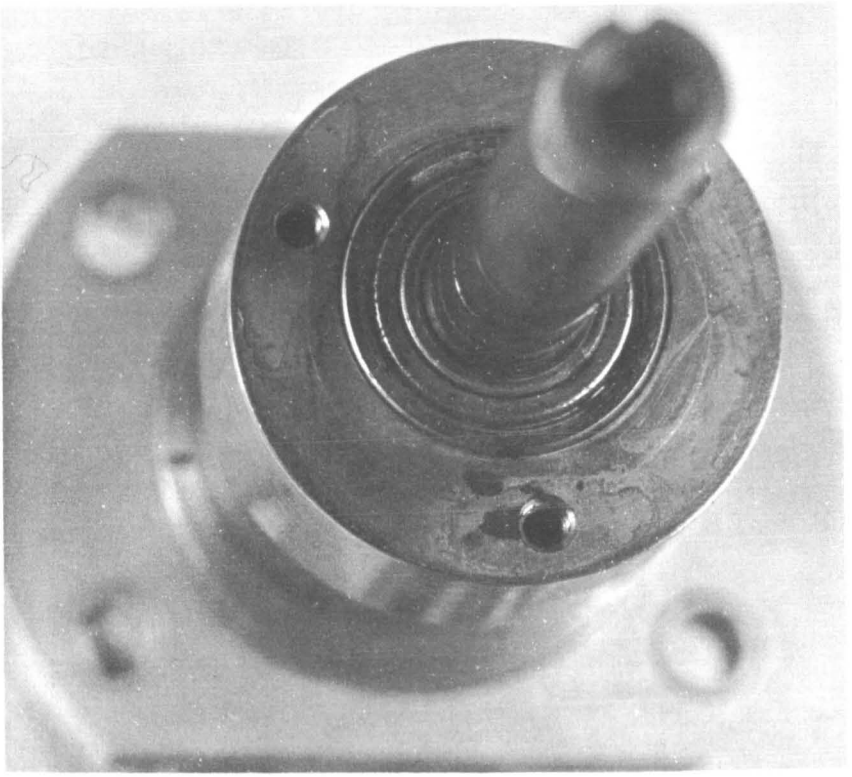


FIGURE 13.—Capstan bearing failure.

clutch were found to be in the early stages of rust-type failure. Close examination showed all lubrication to be gone, and there were rust deposits on the contact edges of the ball separator.

The final stage of disassembly involved the tape-pack assembly. The screws were very rusty, and the reel plate showed heavy deposits of material in the area of the rollers where the edge of the tape works. The reel bearings and retainers showed various types of corrosion, some of which were not found elsewhere in the transport. In addition, lubrication was present, but in some cases it had developed a tan, thick, and grainlike appearance.

Results of Analysis

Following the aforementioned disassembly and examination of the sterilized transport, an extensive chemical analysis program has been undertaken and is presently in progress at JPL to determine the nature of and cause for the various degradatory contaminants and depositions. Since these chemical interactions

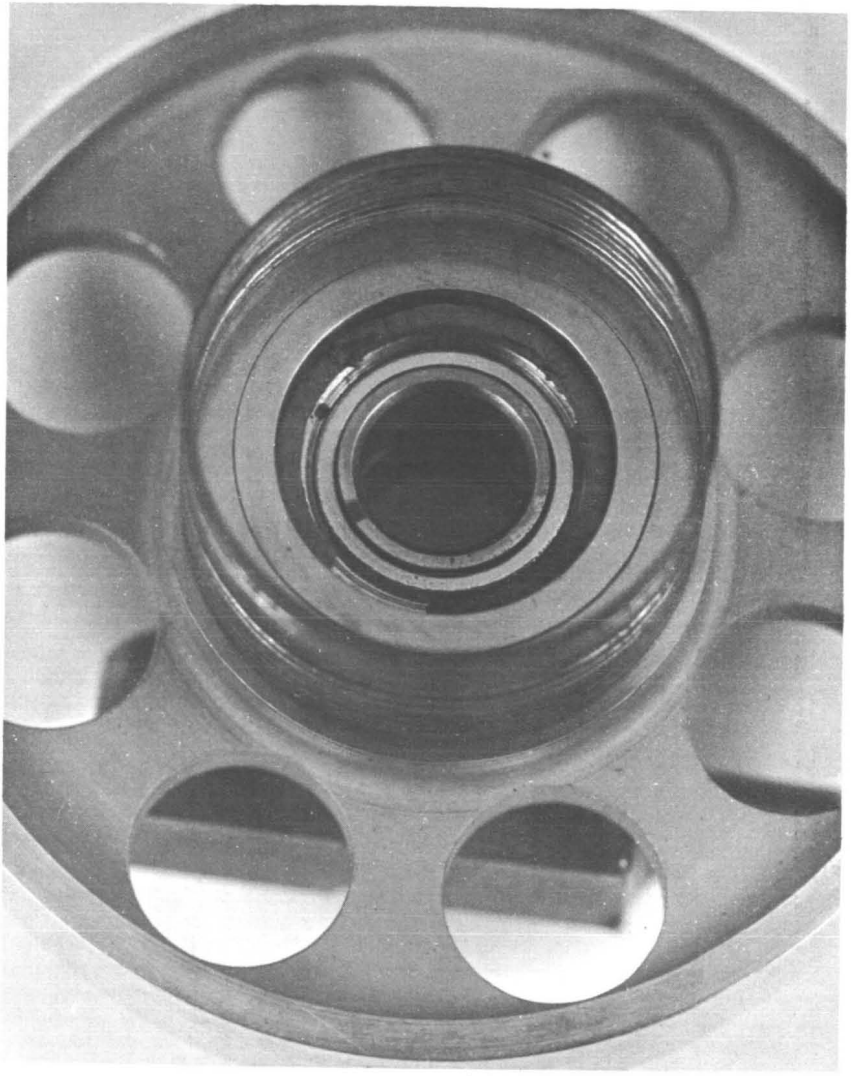


FIGURE 14.—Capstan pulley corrosion.

appear to be the cause for the life-test failure of the transport, it will be necessary to isolate and minimize them so that adequate reliability and life-performance characteristics can be realized.

Sterilization of Magnetic-Core Memory

Spacecraft data-handling systems will require medium-capacity storage for numerous applications, including programing, buffering, and data processing. Presently, the most proven storage

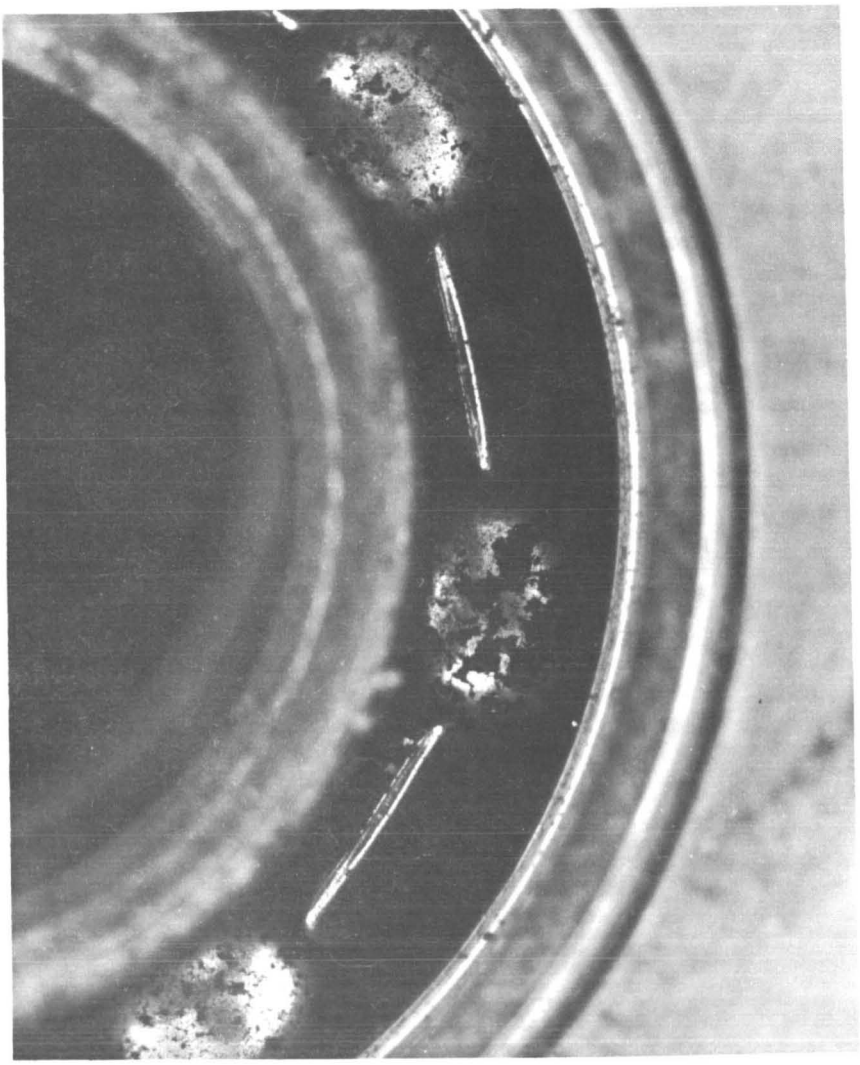


FIGURE 15.—Ball-bearing corrosion.

technique for capacities from 10^3 to 10^5 bits is the magnetic-core memory.

Since forthcoming planetary orbiter and capsule systems will require sterilization compatibility, an effort was undertaken in 1964 to define and solve all the problems associated with the complete sterilization (both dry heat and ethylene oxide) of a typical spacecraft magnetic-core memory. An 8000-bit random-access core memory was defined for development into a sterilizable

subsystem. The program was outlined so that each component and material to be included in the final memory would be individually selected or developed for sterilization compatibility and then exposed to complete dry heat and ethylene oxide sterilization tests. Following these tests, a complete experimental and analytical analysis was to be performed to define adequately any deterioration affecting performance or life. If problems resulted, an attempt was to be made to solve these problems so that qualified components would be available for inclusion into a sterilizable core-memory subsystem. Such a sterilization subsystem was to be fabricated and exposed to complete sterilization testing and its performance before and after sterilization monitored and analyzed. Any detection of degradation in performance or reliability would be determined from these results and an attempt would be made to solve them. Assuming that development of a sterilizable core-memory subsystem was achieved, the final phase of the program was to be a life test to determine further the effects, if any, of sterilization on life and reliability.

A contract was awarded to Electronic Memories, Inc., Hawthorne, Calif., in 1964 to carry out such a program. The initial phase of this contract has been concerned with the attainment of sterilizable components and materials for the defined memory subsystem. Presently, all components and materials proposed for use in the memory have been designed, tested, and qualified for surviving both the dry heat and ethylene oxide environments.

A description of all components and materials required for the core memory prior to ethylene oxide testing is included in table I. The complete test results of ethylene oxide sterilization cycles 1 and 2 are itemized in tables II through IV.

The pretest preparation and measurements of all core-memory components and materials prior to heat sterilization are described in table V. The effects of heat sterilization on these same components and materials are given in table VI.

As noted from the test results, no significant component or material deterioration resulted from either the ethylene oxide or heat-sterilization exposures. Therefore, the next phase of this program, consisting of the fabrication of a complete core memory using sterilization-qualified components and materials, is presently in progress.

Summary of Results

Significant work in the development of sterilizable spacecraft telecommunications components and subsystems has been accomplished by JPL. The three areas treated in this paper are: (1)

TABLE I.—*Description of Materials and Components Before Ethylene Oxide Exposure*

Description	Remarks
SS 4090	Material is clear and tacky; when subjected to fingerprint impression, it will flow back to a smooth, glossy surface with time
RTV 108	Has slight milky color when material builds up, is dry to touch, has rubbery base; indication of air bubbles when material is completely cured in air
RTV 140	Physical characteristics of this material: semi-clear in color, slightly tacky, dry-to-firm rubbery base with air bubbles when cured in air
Plane no. 1 with SR 585 (for magnetic data, see table IV)	Potting is clear in color, silghtly tacky, has good flowing characteristic around cores; a number of air bubbles trapped around wires and cores; indication of flakes of insulation from magnet wires clinging to wires and indication of crazed area on wires wound through cores
Plane no. 2 with Sylgard 184	Potting compound is clear in color, dry to touch, has good flowing characteristics around cores, no indication of any bubbles; magnet wire insulation chafed or crazed; cannot be ascertained whether crazing or chafing is actually on wire insulation or potting compound
Plane no. 3 with Q20046	Potting compound is silver-gray in color, has a rubbery base, is dry to touch, indicates irregular surface with some cores exposed
Plane no. 4 with RTV 881	Creamy or tan in color, opaque, dry to touch, has rubbery base
Formvar 35 brown or tan color magnet wire; STD, tight 5 percent stretch	Insulation looks all right; some dust and dirt particles are stuck to wires
Specimen of Cat-L-Link	Very hard, bonded well to glass board with glossy black and slightly discolored
Specimen of iridite aluminum	Pinky-gold in color
Specimen of black anodize	Fairly even dull-black finish
Multilayer circuit board with Cannon Type "D" connector attached	No separation noted; good appearance
Electronic components: resistors, capacitors, etc.	All components look all right. (For values, see table II)
Welded modules	Potted — good appearance; no separation of header board. Unpotted — looks all right

TABLE II.—*Test Evaluation of Electrical Components for Ethylene Oxide Exposure*

Description	Part no.	Value	May 27, before ETO, cycle 1	June 25, after ETO, cycle 1	July 2, after ETO, cycle 2
R_1	RN 60 B 332, T.I., 1 percent	332 Ω	333.4	333.8	333.4
R_2	¼ W, Ohmite 5 percent	68 Ω	68.2	65.0	68.0
R_3	RN 15 x 32 R7F, T.I., 1 percent	23.7 Ω	23.64	23.0	23.5
R_4	1/10 w, 5 percent	100 Ω	99.89	100	110.2
R_5	10 percent T.I.	180 Ω	181.4	179.6	182
R_6	2 percent	620 Ω	620	617	622
T_1	2XRHA, Technitrol, Pin 4 to 5		0.827 Ω	0.810	0.824
	2XRHA, Technitrol, Pin 1 to 2		0.828 Ω	0.821	0.889
	2XRHA, Technitrol. Pin 2 to 3		0.820 Ω	0.823	0.959
	2XRHA, Technitrol, Pin 1 to 3		1.580 Ω	1.524	1.550
C_1	35 Vdc±20 percent Sprague	1 μF	0.98 μF	0.99 μF	0.99 μF
C_2	200 V 10 percent Vitramon	150 PF	150 PF	146 PF	150 PF
C_3	CY 13C12 IF 500 Vdc	121 PF	126 PF	124 PF	125 PF
CR_1	1N 753A		20 mA at 6.2 V	20 mA at 6.2 V	20 mA at 6.2 V
CR_2	40100 Fairchild		10 mA at 0.9 V	10 mA at 0.88 V	10 mA at 0.9 V
			10 μA at 100 V	10 μA at 100 V	10 μA at 100 V
CR_3	40100 GE		100 μA at 75 V	100 μA at 78 V	100 μA at 78 V
CR_4	40100 GE		100 μA at 85 V	100 μA at 85 V	100 μA at 85 V

TABLE III.—*Test Evaluation of Core Plates for Ethylene Oxide Exposure*

Serial no.	Date	25° C			95° C			Description
		Min DV1	Max DV1	Max DV2	Min DV1	Max DV1	Max DV2	
1—SR 585	6–6	32	51	4	35	54	4	Before encapsulation
	6–12	34	52	4	39	56	4	After encapsulation; before ETO cycle 1
	6–25	36	50	2	32	54	4	After ETO cycle 1
	7–2	35	52	3	40	56	2	After ETO cycle 2
2—SYL 184	6–6	4	51	4	40	54	4	Before encapsulation
	6–12	36	52	4	39	54	4	After encapsulation; before ETO cycle 1
	6–25	40	51	2	34	50	3	After ETO cycle 1
	7–2	43	54	2	42	52	2	After ETO cycle 2
3—Q 20046	6–6	40	50	4	40	53	4	Before encapsulation
	6–12	40	51	4	42	58	4	After encapsulation; before ETO cycle 1
	6–25	42	52	2	40	56	3	After ETO cycle 1
	7–2	43	55	3	45	60	2	After ETO cycle 2
4—RTV 881	6–6	38	52	4	42	52	4	Before encapsulation
	6–12	36	49	4	42	58	4	After encapsulation; before ETO cycle 1
	6–25	42	54	2	34	48	4	After ETO cycle 1
	7–2	42	54	3	42	58	2	After ETO cycle 2
5—No potting	6–6	39	50	4	38	54	4	Before ETO test
	6–25	40	50	2	36	49	4	After ETO cycle 1
	7–2	40	53	3	44	56	2	After ETO cycle 2

pressure transducers, (2) magnetic-tape recorders, and (3) magnetic-core memories.

The results of the pressure-transducer effort indicated that designs capable of surviving the deep-space environment are currently available. However, prior to the commitment of any pressure transducer to a specific program, the following four-phase program should be undertaken:

(1) A list of all materials used in the transducer should be obtained from the manufacturer and submitted to critical evaluation and analysis. Any questionable material should be replaced with suitable materials having better known characteristics in the particular environment.

(2) Following presterilization calibration and testing, a representative sample of the selected transducer should be subjected to complete sterilization testing.

TABLE IV.—*Ethylene Oxide Sterilization Test Results*

Description	Test results	
	After ethylene oxide test cycle no. 1	After ethylene oxide test cycle no. 2
RTV–108	Same as before ETO cycle 1	Unaffected throughout test
SS–4090	Do.	Do.
RTV–140	Do.	Do.
Plane no. 1 with SR 585	Do.	Do.
Plane no. 2 with SYL 184	Do.	Do.
Plane no. 3 with Q 20046	Do.	Do.
Plane no. 4 with RTV 881	Do.	Do.
Heavy thermaleze wire	Do.	Do.
Formvar (all wires)	Do.	Do.
Triple nyleze no. 38 std (red color)	Looks OK; 5% stretch; looks OK	Do.
Specimen of Cat-A-Link	No noticeable change	Do.
Specimen of iridite aluminum	Do.	Do.
Specimen of black anodize	Do.	Do.
Multilayer board	Do.	Do.
Electronic components: resistors, capacitors, etc.	All check out electrically (see table II); visually OK	Do.
Welded modules	Check electronically as previously; visually OK	Do.
Polythermaleze no. 40 magnetic-wire:		
Specimen no. 1	Lump of varnish at one spot	Same as cycle 1
Specimen no. 2	Gelatinlike material	Do.
Specimen no. 3	No noticeable change	Do.
Specimen no. 4	Same as specimen no. 3	Do.
Specimen no. 5	No noticeable change	Do.
Specimen no. 6	Looks OK	Do.
Specimen no. 7	Insulation cracks but no separation of insulation	Do.
Specimen no. 8	Same as specimen no. 7	Do.
Specimen no. 9	Do.	Do.
Specimen no. 10	Looks OK	Do.
Specimen no. 11	Do.	Do.
Specimen no. 12	Do.	Do.

(3) Following poststerilization testing and evaluation, the transducers should be exposed to complete type-approval testing as required by the particular mission program.

(4) Following type-approval testing, the transducers should be adequately life tested to insure that latent unreliability was not introduced by the sterilization environment.

The magnetic-tape-recorder program has resulted in the development of a magnetic-tape-recorder transport similar to that flown in the Mariner IV spacecraft for the purpose of determining and solving all the problems associated with its sterilization. Each component developed for and used in the transport was individually exposed to and survived the required sterilization environment prior to transport assembly. Consequently, the feasibility of developing individual transport components capable of surviving the sterilization environment was demonstrated. However, the assembled transport, when exposed to the sterilization environment, was affected significantly by the creation and collection of depositions and debris resulting from apparently numerous chemical interactions. Consequently, several transport bearings failed after only a few days of poststerilization operation. The cause of these chemical interactions and resultant contaminants is presently being investigated and will continue to be investigated until fully

TABLE V.—*Preparation of Core Mats for Heat Sterilization*

(a) Specimens 1–8

Specimen no.[a]	Adhesive material	Curing temperature and time
1	SR–585	Air dry at room temperature for 1 to 2 hr
2	SR–585	Heat cure at 300° F for 5 min
3	DC–280	Air dry at room temperature for 1 to 2 hr
4	DC–280	Heat cure at 300° F for 30 min; temperature is then increased to 350° F for 8 min
5	SR–585	Air dry at room temperature for 1 to 2 hr
6	SR–585	Heat cure at 200° F for 30 min
7	DC–280	Air dry at room temperature for 1 to 2 hr
8	DC–280	Heat cure at 200° F for 30 min; temperature is then increased to 480° F for 8 min

[a] Specimens nos. 1 through 4 were positioned in oven vertically during curing operation; specimens nos. 5 through 8 were positioned in oven with cores facing downward during curing operation.

TABLE V.—*Preparation of Core Mats for Heat Sterilization*—Continued

(b) Specimens 9 and 10

Temperature, °C	Core mat specimen no.[a]	Minimum "1"	Maximum "1"	Maximum "0"	Testing current
25	9	36	52	2	Drive = 355 mA
	10	38	54	?	Inhibit = 310 mA
					$t_r = 0.2$ μsec
					$t_w = 1.0$ μsec
95	9	40	54	2	Drive = 330 mA
	10	42	55	2	Inhibit = 310 mA
					$t_r = 0.2$ μsec
					$t_w = 1.0$ μsec
−20	9	38	58	2	Drive = 370 mA
	10	40	54	2	Inhibit = 310 mA
					$t_r = 0.2$ μsec
					$t_w = 1.0$ μsec
25	9	38	50	2	Drive = 355 mA
	10	38	52	2	Inhibit = 310 mA
					$t_r = 0.2$ μsec
					$t_w = 1.0$ μsec

[a] Specimens nos. 9 and 10 were prepared with 8 × 2 arrays of EMI 36101 cores and embedded on board with DC–280 (adhesive); specimen no. 9 was cured at room temperature; specimen no. 10 was cured at 200° F for 30 min; temperature is then increased to 480° F for 5 min.

TABLE V.—*Preparation of Core Mats for Heat Sterilization*—Continued

(c) Specimens 11–20

[Strobe time = 0.4 μsec; sense system = 200 Ω into a 10-turn loop — no capacitance readout with current probe; tester No. 3 and Bemco oven]

Specimen no.	25° C				95° C			
	Min DV1	Max DV1	Max DV2	Testing current	Min DV1	Max DV1	Max DV2	Testing current
11	[a]34	[a]52	4		[a]39	[a]56	4	
	32	−51	4		−35	−54	4	
12	[a]36	[a]52	4		[a]39	[a]54	4	
	±40	−51	4		+40	−54	4	
13	[a]40	[a]51	4		[a]42	[a]58	4	
	±40	−50	4		−40	−53	4	
14	[a]36	[a]49	4	$I_{pw} = 355$ mA $I_{pr} = 355$ $I_z = 310$ mA $t_r = 0.2$ μsec $t_w = 1.0$ μsec	[a]42	[a]58	4	$I_{pw} = 330$ mA $I_{pr} = 355$ $I_z = 310$ mA $t_r = 0.2$ μsec $t_w = 1.0$ μsec
	−36	−52	4		±42	±52	4	
15	[a]38	[a]52	4		[a]40	[a]56	4	
	−37	+51	4		−38	−54	4	
16	[a]38	[a]52	4		[a]40	[a]52	4	
	+38	±48	4		±42	−54	4	
17	[a]40	[a]54	4		[a]40	[a]56	4	
	±40	+53	4		+42	±56	4	
18	[a]36	[a]52	4		[a]42	[a]53	4	
	+40	−50	4		+42	+53	4	
19	−39	+50	4		+38	+54	4	
20	+40	−52	4		+43	−57	4	

[a] Value after encapsulation.

TABLE V.—*Preparation of Core Mats for Heat Sterilization*—Concluded

(d) Component Measurements

Description	Remarks
Q–1, –50020	At 40 mA, 4 mA step reads 0.16 volt
(V_{ce} sat., H_{fe})	At 30 mA, 1 mA step reads 0.21 volt
	V_{ceo} = 29 V min = 15 V at 0.1 mA
	V_{cbo} = 65 V min = 35 V at 0.1 mA
Q–2, –50012	V_{ceo} = 55 min = 40 at 1 mA
	V_{ce} sat. = At 300 mA step reads 0.34 V
	At 300 mA step reads 0.38 V
	V_{bc} sat. = At 300 mA, 30 mA step reads 1.1 V
Q–3, –2N2801	H_{fe} = At 200 mA, 30 mA step reads 1 V
EM 40100	Diode breakdown voltage
	At 100 μA, trace reads 120 V
	Forward voltage at 10 mA reads 0.76 V
	Forward voltage at 400 mA reads 1.49 V
C_1	3.2 μF
C_2	12 pF
C_3	179 pF
R_1	100 Ω
R_2	91.5 Ω
R_3	22.1 Ω
R_4	689 Ω
R_5	178 Ω
R_6	615 Ω
R_7	99.2 Ω
Emerson & Cuming 1090	Good appearance
TI Module (drive switch no. 1); part no. 901139	Check electrically
TI Module (drive switch no. 2); part no. 901136	Check electrically
Welded module	Check electrically

TABLE VI.—*Effects of Heat Sterilization on Core Mats*

(a) Specimens 1–8

Specimen no.	Remarks
1	Considerable sag, cores toppled
2	Considerable sag, cores toppled
3	Sag slightly, core positions indicated good
4	No noticeable sag, core positions are very good
5	Core and adhesive shifted from square pattern
6	Similar to specimen no. 5
7	Cores shifted slightly; adhesive is tight, no sagging; core pattern indicated good
8	Adhesive looks firm, no sagging, good appearance, and excellent core pattern

TABLE VI.—*Effects of Heat Sterilization on Core Mats*—Continued

(b) Specimens 9 and 10

Temperature, °C	Specimen no.	Minimum "1"	Maximum "1"	Maximum "0"	Testing current
25	9	42	59	6	Drive = 375 mA Inhibit = 270 mA $t_r = 0.2$ μsec $t_w = 0.2$ μsec
	10	46	61	8	
95	9	48	62	6	Drive = 350 mA Inhibit = 270 mA $t_r = 0.2$ μsec $t_w = 0.2$ μsec
	10	48	64	8	
−20	9	48	64	7	Drive = 300 mA Inhibit = 270 mA $t_r = 1.0$ μsec $t_w = 1.0$ μsec
	10	48	68	9	
25	9	46	60	8	Drive = 375 mA Inhibit = 270 mA $t_r = 1.0$ μsec $t_w = 1.0$ μsec
	10	44	64	9	

(c) Specimens 11–20

[Readout at leaking time; sense system = 200 Ω into a 10-turn loop; tester no. 31

Specimen No.	25° C				95° C			
	DV1 Min.	DV1 Max.	DVZ Max.	Testing current	Min. DV1	Max. DV1	Max. DVZ	Testing current
11	40	54	2		41	56	2	
12	40	54	2		41	56	2	
13	40	54	2	X&Y Read & write 355	41	56	2	X&Y Read & write 330
14	40	54	2		41	56	2	
15	38	54	2		40	58	2	
16	36	52	2	$t_r = 0.2$ μsec	40	54	2	$t_r = 0.2$ μsec
17	36	56	2	$t_w = 1.0$ μsec	46	58	2	$t_w = 1.0$ μsec
18	40	54	2	$I_z = 310$ mA	44	56	2	$I_z = 310$ mA
19	40	54	2		44	56	2	
20	40	54	2		44	56	2	

analyzed and until corrective measures can be taken so that adequate reliability and life performance characteristics can be realized.

TABLE VI.—*Effects of Heat Sterilization on Core Mats*—Concluded

(d) Component Measurements

Description	Remarks
Q–1, –50020	At 40 mA, 4 mA step reads 0.25 volt
(V_{ce} sat., H_{fe})	At 30 mA, 1 mA step reads 0.34 volt
	V_{ceo} = 29 V, min = 15 V at 0.1 mA
	V_{cbo} = 65 V, min = 35 V at 0.1 mA
Q–2, –50012	V_{ceo} = 55 V, min = 40 V at 1 mA
	V_{ce} sat. = At 300 mA, 30 mA step reads 0.34 V
	At 300 mA, 10 mA step reads 0.38 V
	V_{be} = At 300 mA, 30 mA step reads 1.1 V
Q–3–2N2801	H_{fe} = At 200 mA, 30 mA step reads 1 V
EM 40100	Diode breakdown voltage
	At 100 μA, Trace reads 119 V
	Forward voltage at 10 mA reads 0.76 V
	Forward voltage at 400 mA reads 1.49 V
C_1	3.2 μF
C_2	12 pF
C_3	179 pF
R_1	100 Ω
R_2	91.5 Ω
R_3	22.1 Ω
R_4	689 Ω
R_5	178 Ω
R_6	615 Ω
R_7	99.2 Ω
Emerson & Cuming 1090	No noticeable change
TI Module (drive switch no. 1); part no. 901189	Unaffected throughout test
TI Module (drive switch no. 2); part no. 901136	Unaffected throughout test
Welded module	Unaffected throughout test

The magnetic-core-memory sterilization program will also result in the development of a complete subsystem for the purpose of determining and solving all the problems associated with its sterilization. At this time, the complete memory subsystem has been defined and all components tested and qualified to pass both dry-heat sterilization and ethylene oxide decontamination requirements. Presently, the complete memory subsystem is being fabricated, after which it will be exposed to complete sterilization testing and evaluation.

WINSTON GIN
Jet Propulsion Laboratory

Heat Sterilization of Pyrotechnics and Onboard Propulsion Subsystems

Chemical-propellant rockets, both liquid and solid, for primary propulsion and possibly for auxiliary power supply, and electroexplosive subsystems are being investigated at the Jet Propulsion Laboratory (JPL) in order to arrive at engineering design criteria and constraints applicable to these components when they are incorporated into heat-sterilized spacecraft. General problems, presently applied developmental solutions, and future work are described.

Progress is reported on the development of a general-purpose, hot-bridge-wire squib initiator which, in addition to meeting the heat-sterilization requirements, will have other desirable reliability and safety characteristics and will meet certain mission-peculiar constraints. Exploratory tests have been performed on the power-switching and pyrotechnic control circuitry used in the Mariner Mars and Ranger Block III spacecraft. Test results show no evidence of serious degradation of the electrical components and functions.

The technology required for a sealed, liquid-propellant, supply system which withstands sterilization temperatures is being developed. The principal conclusions of an analysis of the general relationships among the thermodynamic and spatial variables of a closed system consisting of the liquid propellant and the propellant tank are presented. Preliminary testing shows that, with 6Al4Va titanium tankage which is adequately prepared, hydrazine can be successfully subjected to sterilization temperature cycling in a completely closed system.

Progress has been made in the determination of the ability of current solid propellants to withstand the sterilization temperature cycling and in the design of a motor incorporating qualified propellants with associated rocket hardware. Possible methods of achieving substantial decontamination prior to terminal heat sterilization are described. Design considerations for configurations that may yield minimum-stress propellant grains are discussed and exploratory test results are shown.

The conclusion is reached that the requirement for dry-heat sterilization at temperatures up to 145° C for three 36-hour cycles imposes no fundamental obstacles for pyrotechnic, liquid-propellant, or solid-propellant subsystems beyond the normal development and optimization problems.

The supporting research and advanced development at the Jet Propulsion Laboratory directed at the problems of subjecting spacecraft onboard chemical-propulsion and pyrotechnic subsystems to sterilization by dry heat and of assuring reliability for

subsequent mission performance are reported. Chemical-propellant rockets, both liquid and solid, for primary propulsion and possibly for auxiliary power supply, and pyrotechnic subsystems are being investigated in order to arrive at engineering design criteria and constraints and, where necessary, to develop those technologies applicable to these devices.

Applications on Board Spacecraft

On board a planetary entry capsule or lander which must be terminally sterilized by dry heat there are, typically, a pyrotechnic subsystem and a chemical-propulsion subsystem. The pyrotechnic subsystem comprises electrically initiated explosives (electroexplosives) and their associated devices and the electronic firing-control (power-switching) equipment. Pyrotechnic functions performed may include the firing control of the capsule-deflection rocket motor or deorbit rocket motor, ejection and control of the retardation subsystem, separation of the payload from the entry package, and operations during the landed phase. Related items may include mechanically initiated explosive primers with delay trains and linear-shaped charges for specialized functions such as parachute deployment and sterilization-canister removal.

The chemical-propulsion subsystem may be of liquid type, with the attendant feed and thrust-chamber assemblies, or it may be of solid type, with its component parts. Typically, a solid-propellant rocket motor deflects a capsule into an entry mode, whereas a throttleable liquid-propellant motor functions as the retrorocket to brake the landing onto the planet's surface. Related items may include a liquid-propellant gas generator to operate a turboalternator for postlanded, limited-life electrical power and a small solid-propellant rocket motor for spin stabilization during entry.

General Problems

It might appear that there would be overwhelming difficulties with the dry-heat sterilization of chemical propellants and pyrotechnic materials, since these are themselves heat-producing, energetic, or explosive substances. However, the autoignition temperatures of these substances (at about 250° to 300° C) are two or three times higher than the presently accepted sterilization temperatures, and therefore, in general, self-deflagration does not constitute a threat except possibly in the case of large solid-propellant charges as discussed later. The major difficulties stem from the side effects of high-temperature soaking: phenomena such as chemical decomposition, degradation of mechanical or ballistic properties, high vapor-pressure buildup, and stresses due to differences in thermal coefficients of expansion.

Although the only currently prescribed policy for attaining sterility is a terminal dry-heat soak, other methods of sterilizing chemical propulsion and pyrotechnic equipment have been considered. These other methods are irradiation, incorporation of sporicidal agents, and special fabrication and assembly procedures. However, these methods have been relegated to being employed to reduce the initial population of viable organisms prior to the terminal sterilization by dry heat.

The general requirement that propulsion and pyrotechnic equipment withstand surface exposure to ethylene oxide (12 percent) and Freon 12 (88 percent) appears to present no problems Liquid propellants would be contained in sealed vessels and solid propellants would be protected by some type of seal at the nozzle. There is experimental evidence that even if a leak in the seal permitted the mixture of ethylene oxide and Freon 12 to encounter the solid propellant, the effect would not be detrimental. However, long-term exposure of propellant may result in detrimental effects; experimental evidence for this condition is incomplete. Explosive chemicals in pyrotechnic devices would be hermetically sealed. Pyrotechnic electronics and other inert propulsion hardware would not be detrimentally affected.

Pyrotechnic Subsystem

Power-Switching Unit

The effects of sterilization temperature cycling on the pyrotechnic power-switching unit can be determined by examining the effects on electronic piece parts and components, cabling, electronic packaging, and encapsulation techniques, since the unit, in some respects, is a typical electronic subassembly. The unit seems to present no serious problems. As an initial gross evaluation of the sterilization effects, the unit for the Mariner Mars spacecraft was subjected to temperature cycling for three cycles from ambient to 135° C and was checked for accumulative degradation of the major components or functions. This unit was not designed to meet sterilization heat requirements; rather, it was selected for initial evaluation because it probably represents the basic technical design approach to be adopted for Voyager spacecraft.

The pyrotechnic power-switching unit for the Mariner Mars initiates squibs when commanded by switches in other subsystems. The input consists of 2.4-kilocycle, 50-volt power and switch closures from the central computer and sequencer. The outputs consist of exponentially varying current pulses of 11 amperes (peak) to each of 14 squib-initiating channels and a 20-millisecond switch closure to each of two telemetry output channels. These

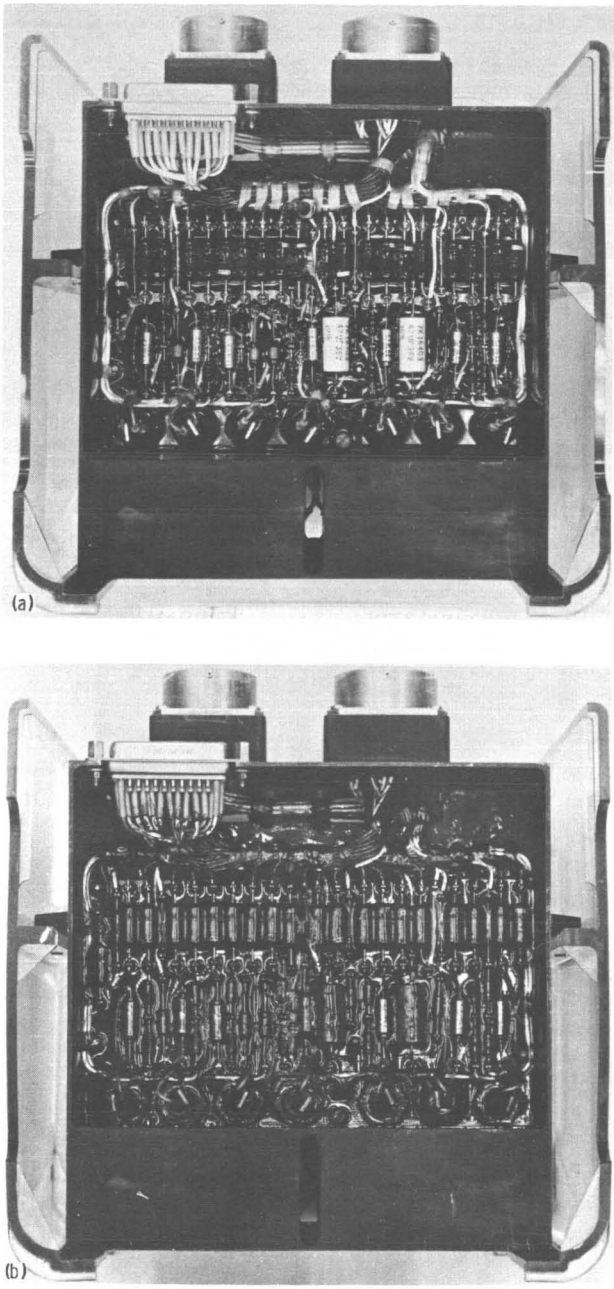
high-output currents with limited input current are achieved by the use of capacitor banks discharged by means of solid-state switching (silicon-controlled rectifiers).

Operations to command firing of squibs and to provide telemetry output signals were done before heat cycling and after each heat cycle. Measurements were also made of the energy-storage capacitance (GE, tantalum, wet-foil type) and leakage current and of the forward leakage current of the silicon controlled rectifier (a Minuteman, Hi-Rel, type C35). Figure 1(a) shows the appearance of the unit before any heating; figure 1(b) shows it after two cycles of heating. No visible damage occurred externally beyond the increased darkness of the paint on the case and of the conformal coating. Measured results indicated that somewhat less than the specified minimum peak current was delivered to several squib functions and that, generally, the total capacitance remained constant, whereas capacitance leakage current increased and the leakage current of the silicon-controlled rectifier decreased at the completion of each heat cycle. The unit survived the three heat cycles and operated without failure; however, since the capacitor-bank leakage current increased severalfold after the final cycle, one concludes that capacitor degradation probably occurred.

A similar series of tests was conducted with the pyrotechnic power-switching unit for the Ranger Block III spacecraft. (The earliest version of the Ranger Block II spacecraft was designed for sterilization by heat at 125° C for one 36-hour cycle in type-approval testing; however, the sterilization requirement was later waived for lunar missions.) Unlike the Mariner Mars unit, the Ranger unit operates by means of battery power rather than storage-capacitor discharge and utilizes mechanical relays rather than solid-state switches. After type-approval-level sterilization heating, one significant subsystem parameter, the net resistance, increased by about 2 percent; this indicates the possibility of continuing degradation. The responsible components or elements could not be identified. The effect may be current dependent. One inertial switch also failed after heat cycling. The failure mode was mechanical in that the moving parts of the switch seized.

No life testing on either the heat-sterilized Mariner Mars or the Ranger Block III unit had been performed, since these were gross, exploratory tests done to reveal general problem areas by using available assemblies and since the discrete failures were not positively identified.

Present efforts are centered mainly around the capacitor, which will be characterized for effects of sterilization heating when used in typical pyrotechnic power-switching circuits. Much information



(a) Before sterilization heating.
(b) After sterilization heating.

FIGURE 1.—Mariner Mars pyrotechnic control unit.

pertinent to the design of such circuits will evolve from general qualification and screening of electronic components, development of reliable electrical connections, and development of electronic packaging techniques for the sterilization requirement.

Electroexplosive Devices

Electrically initiated squibs and the mechanical devices which they actuate (e.g., actuators such as pinpullers and valves) typically comprise the remainder of the pyrotechnic subsystem. It appears that the problem of designing the devices is straightforward and involves routine attention to details, such as mechanical interferences resulting from mismatches in temperature coefficients of expansion and the use of organic materials (e.g., for O-ring seals) which do not have adequately high temperature resistance. Valves and pinpullers, for instance, are presently available which will accept the type-approval levels of sterilization heating. In general, mechanical devices which do not have functional chemical or electrical interfaces do not pose design problems, especially if they do not have to operate at the temperature of sterilization heating.

Qualification of an initiating squib has been identified as a difficult engineering problem. The difficulty arises from the variety of requirements imposed upon the squib in addition to that of sterilization temperature cycling. These requirements are:

(1) Heat sterilization in a flight-approved environment, 135° C for 24 hours

(2) Safety, including the ability to withstand 1 watt and 1 ampere for 5 minutes without firing and the ability to withstand electrostatic discharges of approximately 25 000 volts from a 500-picofarad capacitor applied between pins and case or between pins, at any pressure or altitude

(3) Reliability through dual bridges and freedom from critical dependence upon uninspectable qualities

(4) Performance and special requirements, including small size and weight, incorporation of an integral connector, exclusion of magnetic materials, capability of withstanding severe temperature shock, capability of withstanding firing pressures of at least 30 000 psi without significant rupture or venting, and capability of withstanding high-impact forces

Recognizing that the squib requirements are stringent, JPL surveyed the squibs available in 1964 and found that not one completely met all of the indicated requirements. The single

candidate which most nearly met the requirements was the Apollo Standard Initiator (ASI), a small, hot-bridge-wire pressure cartridge that, as an independent gas-producing or heat-producing module, will initiate other devices (ref. 1). The ASI appears to meet the sterilization heating requirement and is presently being evaluated against other requirements peculiar to Voyager.

Since no available squibs could meet all the requirements, the development of a new squib was initiated on the basis of a concept which incorporates dual bridges, connector-type 3/8-inch by 24 threads, and 1-watt and 1-ampere no-fire capability. This is the same basic concept of the ASI, so that technological improvements of either squib can automatically apply to the other. Work was started on the new squib in mid-1964; the effort was directed by JPL and done partly inhouse and partly by several small subcontractors. At the present, all but one of the important problems have been solved by a combination of judicious selection of materials and design solutions using the selected materials. A satisfactory body material has been found (Inconel 718, which is effectively nonmagnetic), and thread seals have been proven which will meet the 30 000-psi requirements. A new pin seal has been demonstrated, and a satisfactory connector design has been evolved. Bridges produced by film deposition of nichrome, an advanced technique, have been developed at JPL; these bridges bypass the old problem of obtaining reliable joints between the pins and the bridge wires and also provide an order-of-magnitude improvement (about 10 watts compared with a normal value of 2 watts) in no-fire capability. Resistance to damage from severe temperature shock (−185° to 150° C) has been demonstrated by the film bridge. An acceptable matchhead formulation has been mixed and loaded at JPL by using zirconium, potassium chlorate, and barium nitrate. The remaining task concerns development of a satisfactory antistatic discharge shunt to provide against accidental initiation via electrostatic paths. A sketch showing the new squib as presently conceived is presented in figure 2.

Percussion-initiated (as opposed to electrically initiated) devices, which may be used, for instance, in parachute deployment, appeared originally to display anomalous behavior after exposure to sterilization temperature cycling. It has now been shown that the sterilization temperature cycling as such is not the cause of the problem. The problem seems to arise from improper mechanical handling during the installation; i.e., the crimping of the percussion cap into the cartridge or explosive device.

Detonating explosives normally would be avoided in spacecraft

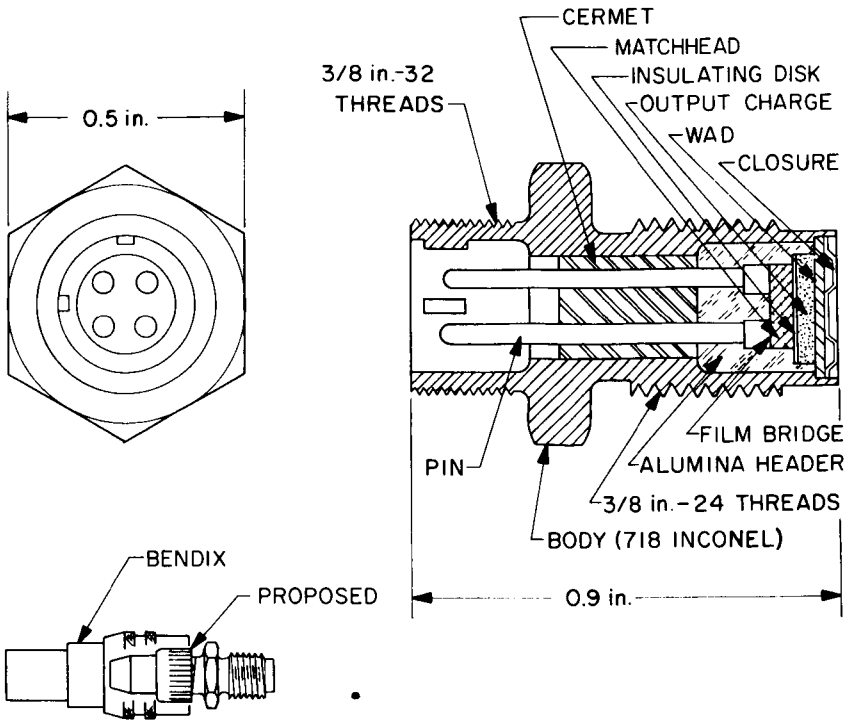


FIGURE 2.—New squib concept developed by JPL.

applications, but might be required for flexible, linear-shaped charges in operations such as the opening of the sterilization canister containing the sterilized capsule. Various available detonating explosives which will meet the sterilization requirement are being evaluated and selected.

Liquid-Propellant Subsystem

General Problems

From the aspect of propulsion, whether of liquid-propellant or solid-propellant type, terminal sterilization by heat soak has the combined disadvantages of forcing the adoption of lower performance systems and introducing an extra element of hazard during the sterilization of the assembled capsule or lander system. Alternatives which would provide greater payload weights would be separate sterilization of the propulsion hardware and of the propellant prior to loading, possibly using heat in both cases, and assembly under aseptic conditions. It is recognized that aseptic assembly is a major undertaking, but in some conditions the

performance level may be critical. Separate sterilization can still be performed to reduce the initial biological contamination, of course.

The principal concerns associated with soak at elevated temperature of a sealed liquid-propellant propulsion system are: (1) the vapor pressure of propellants. (2) the compatibility of propellants with wetted components, and (3) the stability of the propellants. For example, N_2O_4 has a vapor pressure of about 750 psi at 135° C. Substitution of inhibited red fuming nitric acid (IRFNA) results in a propellant oxidizer system with a lower vapor pressure of about 110 psi at the same temperature but costs a decrease in specific impulse from 319 to 305 lb_f-sec/lb_m in a representative application. The high-performance cryogenic liquid propellants are automatically ruled out for two reasons: (1) the required insulation would work against the heat-sterilization process, and (2) if the sterilizing heat passed through the insulation, intolerable pressures would be generated. (It may be noted that the vapor-pressure function of temperature of many propellants is very steep and that the acceptance or rejection of a propellant depends critically on the specified sterilization temperature.) The stress corrosion of N_2O_4 in contact with 6Al4Va titanium, which otherwise would be an excellent pressure-vessel material for spacecraft, is severely aggravated at high temperature and may force the use of less efficient aluminum tankage. As for the stability of propellants, some of the amine fuels (hydrazine and hydrazine mixtures) are known to undergo catalytic decomposition at a rate which is highly temperature dependent.

Special design attention must be paid to the avoidance of propellant leaks at sealing points due to differences in thermal expansion. For example, leakage through the propellant valve could be prevented by appropriate blowout disks which could be opened after sterilization. The tankage must be overdesigned either in pressure or volume to accommodate the increase in temperature, or else vent valves could be added to relieve tank-pressure buildup during the heating.

Whereas it appears that oxidizers such as N_2O_4 and red fuming nitric acid have a devastating effect on spores, it has been demonstrated that certain hydrocarbon fuels and N_2H_4 do not exhibit such an effect. Mr. E. G. McNall of the Dynamic Science Corp. reported recently, in a letter, that N_2H_4 permits a half-lifetime to the *B. subtilis* spores of about 1 week at room temperature. Aircraft jet fuels in certain environments have been plagued with the problem of microbial contamination, which leads to clogged filters and sludge formation. Although the source of the contami-

nation is rather controversial, the viability of many species of bacteria and fungi in the jet fuels has been well established (ref. 2).

Optimization of Liquid-Propellant Propulsion-System Operating Parameters

Liquid-propellant engines for spacecraft are generally designed to use pressurized rather than pumped feed systems, since relatively low chamber pressures can be used and since comparatively small velocity increments are required. Heat sterilization of cold-gas pressurizing systems does not pose any problems because personnel safety requires that the pressure vessels for such systems be designed with a safety factor of 2.2 (ratio of burst pressure to maximum working pressure). If the heat sterilization is carried out remotely, the safety factor of 2.2 is probably adequate. The increased pressure due to vapor-pressure increase or thermal expansion would probably not exceed the allowable burst pressure; therefore, the tanks would not have to be strengthened.

The most affected part of the engine is the propellant tankage and supply subsystem because of the strong dependence of vapor pressure of many propellants upon temperature. The pressurization gas tank pressure increases by 40 percent as the temperature is raised from 20° to 145° C, but the propellant vapor pressure for some propellants increases by an order of magnitude.

An analysis (ref. 3) was performed to optimize theoretically the liquid-propellant rocket-engine operating parameters with the purpose of minimizing the penalties which arise from the increased pressure. First the regulated-gas, pressure-fed, propellant tankage system was treated. The analytic problem to be solved was the determination of the mass and wall thickness of a spherical propellant tank as parametric functions of the thermodynamic and spatial variables of the propellant-tank system when the following variables are known:

(1) Operating pressure (typically, 220 psi).
(2) Tank material (typically 2014–T6 aluminum alloy).
(3) Weld factor (typically 2.0) and safety factors.
(4) Propellant mass and properties; propellants examined are N_2H_4, IRFNA, N_2O_4, unsymmetrical dimethyl hydrazine (UDMH), kerosene, and H_2O_2.
(5) Ullage space above the propellant is prepressurized to some fraction of the nominal operating pressure with an inert gas in equilibrium with the propellant vapor.
(6) The system is heated from temperature T_1 to T_2 without venting (typically from 20° to 145° C).

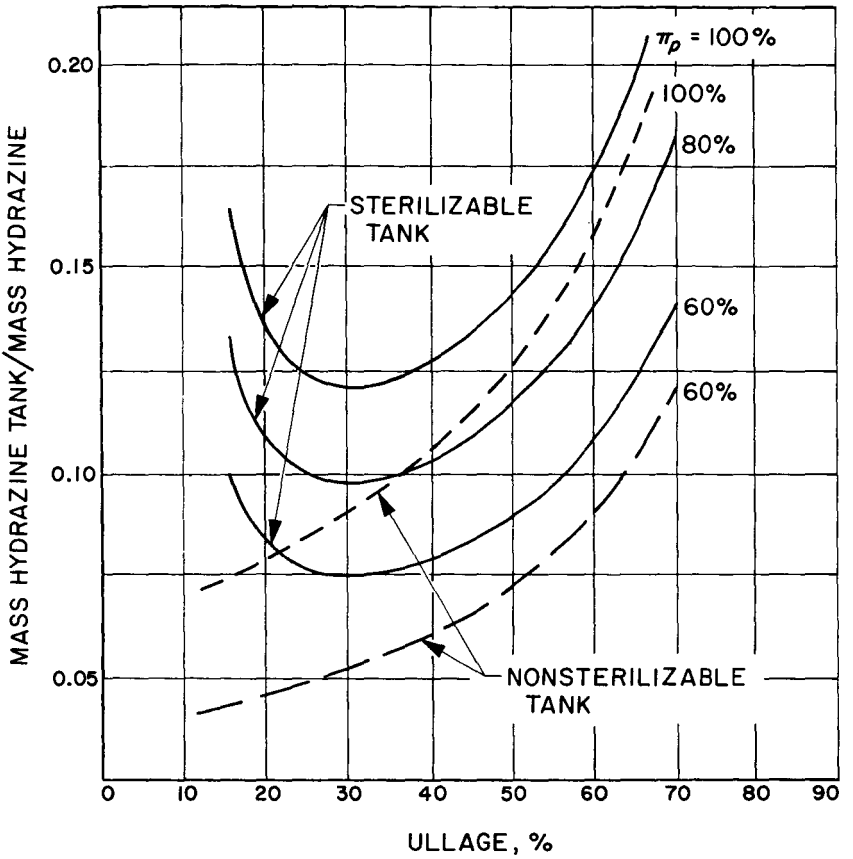


FIGURE 3.—Ratio of tank mass to propellant mass in a hydrazine system at different prepressurization levels. (π_p prepressurization fraction; tank material, 2014–T6 aluminum alloy; operating pressure, 220 psia; allowable stress of tank at 293° F, 43 600 psi; sterilization temperature, 293° F; weld factor, 2.)

A typical result from reference 3 is shown in figure 3. The calculation was also performed for the comparable case where heat sterilization is not required of the propellant tankage system. It can be seen that, for a given prepressurization fraction, an optimum ullage exists. Optimum ullage fractions for other propellants are shown in table I, and the ratio of system mass for sterilizable to that for nonsterilizable systems is shown in table II.

Next the internally pressurized or "blowdown" tankage situation was treated. This system contains both a monopropellant and the pressurizing gas (helium) required for propellant expulsion.

TABLE I.—*Optimum Ullage Fractions*

Propellant	Optimum ullage, percent
Hydrazine	30 to 31
IRFNA	35.5 to 37.5
N_2O_4	44 to 48
UDMH	36 to 40
Kerosene	27
H_2O_2	30

TABLE II.—*Ratio of System Mass for Sterilizable to That for Nonsterilizable Systems*

Propellant	Ratio of system mass for prepressurization level, percent, of—	
	60	100
Hydrazine	1.8	1.7
IRFNA	2.7	2.3
N_2O_4	9.4	6.7
UDMH	2.7	2.3
Kerosene	1.4	1.4
H_2O_2	1.6	1.6

The propellant and gas are separated by a thin, flexible membrane of negligible mass. During operation the pressure within the tank is allowed to decay from its initial pressure to a minimum pressure attained at completion of propellant expulsion. For a given ullage fraction (that part of the total tank volume occupied by gas), the above-mentioned minimum pressure determines the amount of pressurization gas which must be loaded. After loading the tank, the system is heat sterilized without venting. The problem is to find the dependence of the tank mass and wall thickness upon minimum tank pressure.

Typical results show that the ullage fractions which yield the optimum mass ratio are near 60 percent for these blowdown systems and that the inert mass of sterilizable systems is approximately twice as large as that of nonsterilizable systems operating at comparable conditions.

Preliminary Experimental Results

Hydrazine, N_2H_4, is a state-of-the-art liquid monopropellant often used for space propulsion. Although its vapor pressure at 145° C is only 35 psig, it is known that under certain conditions

hydrazine will decompose and build up pressure in an unvented container. Apparently there is a catalytic effect on the kinetics of decomposition between metal and the monopropellant at elevated temperature. Since 6Al4Va titanium is a very likely selection for tank material, it was used in a combined feasibility test with hydrazine (ref. 4). The test employed a flight-weight, spherical tank of 6.5-inch inside diameter, 0.096-inch wall thickness, 3600-psig design working pressure, and 7920-psig minimum burst pressure. The tank was cleaned and then passivated with 3½ pounds of hydrazine, leaving an ullage of 37 percent. The ullage space was purged with nitrogen and the tank was prepressurized to 7 psig.

The loaded tank was heated at 145° C for three cycles of 35½, 37, and 46½ hours, respectively. After the third cycle and cooldown to room temperature, an increase of 16 psi over the original tank pressure (7 psig) was recorded. Chemical analysis of the propellant before the test showed the hydrazine to be 99.3 percent pure and that after the test, 98.9 percent pure. Even if this difference were identified as real, such a small change would not noticeably affect performance, except that gas in solution, if excessive, would be a problem. The maximum pressure generated during the third cycle was 93 psig.

This early test showed that, with adequately cleaned, compatible tank material, hydrazine can be subjected to sterilization temperature cycling in a closed system without deleterious effects.

Future Work

Plans are now being formulated to explore the next major type of heat-sterilizable, liquid-propellant engine: an Earth-storable, bipropellant system which would deliver more performance than monopropellant hydrazine. The oxidizers of the higher performing bipropellants have the disadvantage of generally having higher vapor pressures than do those of the monopropellants and, being more reactive, may present compatibility problems such as stress corrosion. The objective of the next effort is to apply the type-approval level of sterilization temperature cycling to a complete flight-weight, bipropellant rocket system with qualified components and then subsequently to test-fire the engine. The engine size has been selected at a nominal 100-pound thrust with a burn time of about 300 seconds. Probably an existing thrust chamber such as that developed for the Apollo or Surveyor spacecraft will be used. Components which will be studied include propellant valves, a gas regulator, possibly throttle valves, and propellants with positive expulsion devices such as a metallic diaphragm.

Solid-Propellant Subsystem

General Problems

As with the liquid-propellant engines, the critical problems with solid-propellant motors arise because provisions which can be adopted to permit sterilization by heat engender some loss of overall propulsion-system performance. A solid-propellant motor characteristically consists of a propellant grain which is a viscoelastic, composite material with a certain shape designed to achieve the desired ballistic performance, an elastomeric insulation which protects the case containing the grain and the combustion gases during operation, an igniter, and a nozzle. The problems arising from high-temperature soaking of such equipment are: (1) differences in thermal expansion and contraction, which stress the areas of case-insulation-propellant bond and compressively stress the grain itself; (2) degradation of the propellant's mechanical, physical, or ballistic properties; (3) the characteristically low thermal diffusivity of the solid propellant which, if the grain is very large, will require very long times to reach the equilibrium value of sterilization temperature at every part; and (4) thermal gradients caused by unsymmetrical heating or extremely thick propellant webs which can lead to stresses severe enough to crack the grain.

Again the solutions to these problems lie in minimizing the inert-weight penalties by optimum design using carefully selected materials. The inert-weight penalty varies with the size of the motor. For a small unit such as a spin motor, a low value of mass ratio is of little importance. For a large motor, used typically for the deflection or deorbit of a large capsule containing about 100 pounds of propellant, the decrease in mass ratio from that of a nonsterilized, state-of-the-art design may be of the order of 10 percent. The compressive stresses due to differences in thermal contraction are aggravated if the motor is designed to have very high volumetric loading, a further consideration which may lead to decreased mass ratio.

Heat sterilization, as such, appears to result in no degradation to the specific impulse of present-day solid propellants. However, an unrelated, mission-peculiar requirement exists which specifies that the solid-propellant exhaust shall contain no condensable products such as aluminum oxide, which could deposit on spacecraft components, create unbalancing moments, or cause communications blackout. This requirement rules out the use of propellants with energetic metallic fuel additives and causes a decrease in deliverable specific impulse by about 8 to 10 percent.

Decontamination Prior to Terminal Heat Sterilization

At first examination, it might appear that certain ingredients commonly utilized in solid-propellant formulation are already bactericidal. Typical ingredients are the nitroglycerin in double-base propellants, the epoxy and imine curing agents in many composites, and the toluene diisocyanate in polyurethane formulations. However, the evidence at present is that solid propellants as a class are not self-sterilizing. Furthermore, the indications are that the conventional processes of manufacturing these ingredients are probably responsible for the introduction of the larger portion of the contamination, which, while perhaps low or of the same level as contamination in the processing of other biologically clean materials such as food, is still high relative to planetary quarantine standards set for spacecraft.

The interior and inaccessible interfaces of the inert components of the solid-propellant rocket, such as the nozzle, the nozzle-case interface, and the insulation-case interface, can be separately heat sterilized before loading the propellant. The propellant itself might be decontaminated to reduce the biological load by a process developed at JPL (ref. 5) for use with a propellant made of polyurethane, ammonium perchlorate, and aluminum. This process consists of purging the mixer and casting system before the introduction of propellant ingredients with a mixture of 12 percent ethylene oxide and 88 percent Freon 12, followed by an addition of pure ethylene oxide amounting to 6 percent of the total liquid-fuel ingredients. The ethylene oxide creates a boiling, purging action during the mixing at low pressure; in fact it causes lower viscosity mixture and thus provides a better mixing action. Two flight-weight motors, each with 60 pounds of this propellant, were processed in this manner and test fired with no detrimental effect to their mechanical properties or ballistic performance.

Bacterial assay of propellant inoculated with 10^6 *B. subtilis* var. *niger* spores per cubic centimeter and subsequently treated with 6 percent ethylene oxide during the mixing process originally showed that sterility was achieved. However, refinements to the techniques of inoculum recovery from solids have raised a doubt about the effectiveness of the process in achieving sterility, although there is reason to believe that the level of biological contamination was greatly reduced. Furthermore, the addition of ethylene oxide to the propellant was accomplished with the polyurethane formulation which cannot survive sterilization heat cycling. This special processing remains to be checked again with the heat-sterilizable propellants, using improved bioassay techniques.

The broad problem of reducing the initial biological count prior to heat sterilization by performing biologically clean manufacture and assembly of chemical rockets and pyrotechnics remains to be examined with care. There are implications of complicated procedures which could drastically affect the acceptability of present manufacturing and assembly techniques. It is possible to consider steps such as the following: (1) decontamination by heat or other means of the propellant ingredients prior to mixing, or (2) imposing a heat sterilization cycle during the curing phase of the propellant manufacture.

Design Considerations

Two general classes of solid-propellant rocket designs can be considered: the cartridge-loaded and the case-bonded classes. The cartridge-loaded grains are typically separately cured, trimmed, and loaded into the case by using some appropriate support. The decoupling of the grain from the motor case that is thus accomplished allows for greater differences in thermal contraction and expansion. The significant disadvantage is the decrease in mass ratio resulting from the inclusion of a grain support structure, case-wall insulation, and a large end closure. The case-bonded motors typically contain propellant that is cured in the case and bonds to a liner-insulation material which in turn is bonded to the case. This design results in a high mass ratio because, in many areas of the case, the yet-unburned propellant itself acts as insulation for the case and because high loading density can be achieved. As an illustration of the comparative performance of the two types of designs, a motor with a total weight of 100 pounds might carry only 50 pounds of propellant in a pessimistic estimate for a cartridge-loaded design, but it might carry 84 pounds of propellant in an optimistic estimate for a case-bonded design. However, the disadvantage of the case-bonded design is that the stresses from differential contraction must be relieved or minimized.

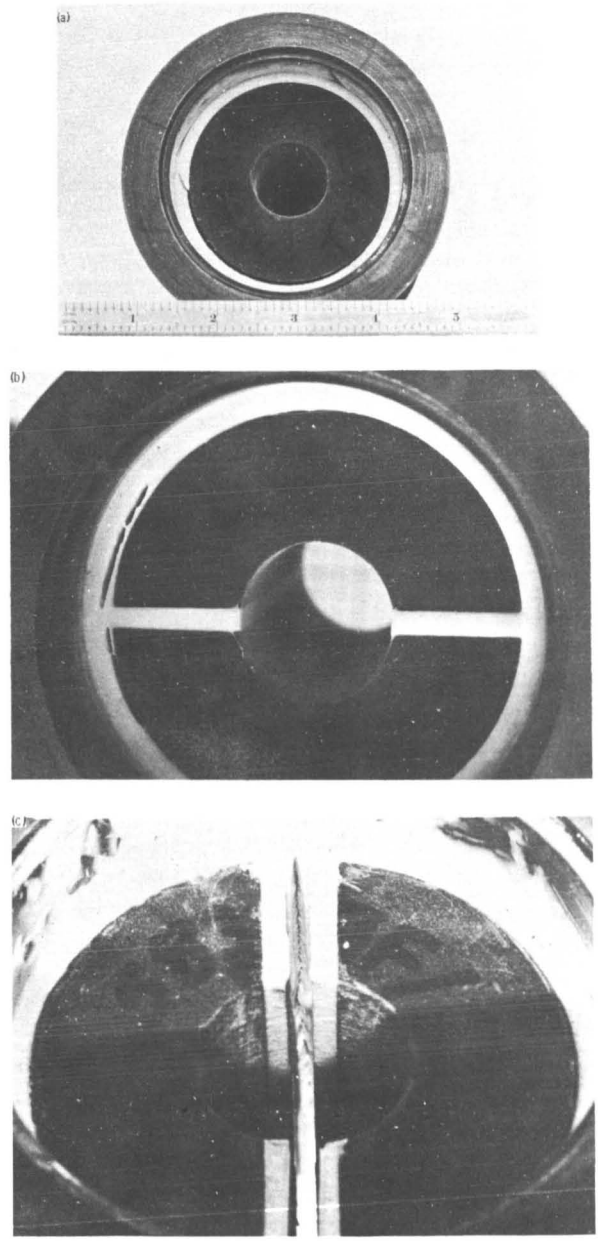
Currently available propellants which meet the condensation-free exhaust requirement and which appear to retain adequate mechanical and ballistic properties after sterilization temperature heat soak are formulations of a polyester styrene and ammonium perchlorate and a polybutadiene acrylic acid, imine-cured, ammonium perchlorate (ref. 5). An example of a composition which will not survive heat sterilization is the JPL polyurethane ammonium perchlorate propellant which otherwise has superior mechanical properties well qualified for the normal space applications. The

polyurethane binder apparently undergoes a reversal of the crosslinking cure process and effectively returns to its initial liquid state upon the application to the sterilization heat cycle. Most contemporary, composite, double-based propellants are not normally stable at high temperatures and are thus also ruled out for heat sterilization. Reference 5 further demonstrates that the test configuration, like the final motor grain configuration, strongly determines the mechanical integrity and survivability of the propellant. Either hardening, which might be caused by continued polymerization, or softening, which might be caused by the breakage of crosslink bonds, could occur in the propellant undergoing heat sterilization. Even if not catastrophic, adverse effects might appear with respect to ignitability. The presence of oxygen and moisture during heat sterilization could also have a detrimental effect.

The objective of the heat-sterilizable, solid-propellant, rocket-motor design is the achievement of the highest mass ratio with the configuration which has the least internal stress and still maintains adequate mechanical properties for both the propellant and the inert hardware at the sterilization temperature. Areas of stress concentration, such as star points with sharp radii of curvature, must be avoided. The slotted grain configuration is a possible solution to the design problem of minimum-stress grains; however, it is still not without special problems. The design problem is illustrated in an exploratory sterilization heat cycle test performed at JPL, and typical results are shown in figure 4. Figure 4(a) shows how a tubular charge (a polyester styrene, ammonium perchlorate propellant) potted into the case with RTV silicon elastomer failed: The liner separated from the charge at part of the periphery after the first heat cycle. Figure 4(b) shows how a slotted grain whose slot is filled caused a separation within the liner itself, but had less stress to relieve at the periphery. Figure 4(c) shows how a slotted grain with a filled slot which is itself slotted now transfers the stress relief to the well of the slot. In case-bonded motors, design compromises such as stress relief boots in the insulation may be required. Inert-material selection must be judicious; for example, an epoxy-glass filament-wound case provides a high mass ratio but has a lower coefficient of thermal expansion than has stainless steel.

Future Work

A combined effort by JPL and industry for conducting heat-sterilizable motor design studies and test demonstrations is underway. General design problems will be further analyzed, and promising motor designs will be studied in detail, including structural



(a) For tubular charge.
(b) For slotted grain with filled slot.
(c) For slotted grain with filled slot, which is slotted.

FIGURE 4.—Results of grain configuration tests.

analyses for the thermal loads. Several 50-pound, flight-weight motors with suitable modifications, such as slotted grains or special grain supports, will be loaded, heat sterilized, and test fired to evaluate presently known design solutions and to focus on critical problem areas.

More fundamentally oriented work is in progress to advance the technology of grain support with the development of a heat-sterilizable foamed material which will be lightweight, will provide grain support, and will absorb the expansion and contraction strains. Additional work has been planned for the control of biological contamination of heat-sterilizable propellant (one method would be the incorporation of ethylene oxide during the processing) ; the success of such work depends on the continued improvement of the micro-organism recovery and assay techniques from solid materials. Failure mechanisms, both stress and chemical degradation, need to be investigated.

The effect of scale on the stress field in a motor will also be investigated. For a typical motor with 100 pounds of propellant, dimensional changes as large as 0.1 inch might occur at the sterilization temperature. The method of supporting such a large or a larger grain may require techniques radically different from those usable for smaller grains.

Another basic problem, which is aggravated by scaling to larger motor sizes, arises from the theory of thermal initiation of explosives. It is known that if the surface of a solid explosive is subjected to a high temperature, the explosive will self-heat from internal chemical decomposition. If the surrounding temperature is above a certain critical temperature, after a period of induction time which depends on the specific chemical and physical constants, geometry, and size, the explosive will deflagrate from the runaway reactions. Methods have been developed (ref. 6) for the prediction of the critical temperature for a given propellant based on differential thermal analyses. The higher the ambient temperature is above the critical temperature, the shorter the induction time to self-deflagration. Theory predicts, for example, that the critical temperature for a propellant based on polybutadiene acrylic acid, with a 12-inch diameter in a solid cylindrical shape, will be 124° C. Experiment demonstrated that such a configuration with the specified propellant deflagrated after 167 hours in a 149° C oven and after 45.3 hours in a 162° C oven. The critical temperature for a 30-inch-diameter cylinder is 110° C, and experiment demonstrated that deflagration occurred after 270 hours at 138° C.

Concluding Remarks

It appears reasonable to conclude that the requirement for dry-heat sterilization at temperatures up to 145° C for three 36-hour cycles imposes no fundamental obstacle for pyrotechnic, liquid-propellant, or solid-propellant subsystems. The gross engineering problems have been recognized, and technical feasibility for these equipments has been demonstrated in a variety of tests and investigations. The course of action throughout has been to identify the failure modes and to discover the optimum solutions which minimize the penalties on performance and reliability.

Demonstration of the ability of the subsystems to withstand the launch and space environments, especially vacuum, remains to be done when the detailed subsystem designs are determined. Material and propellant selection and design studies have taken such factors into account, and no serious poststerilization environmental problems are presently anticipated.

There are several operational problems which will bear additional study and which may be solved by the development of specialized procedures. These problems fall into two areas: (1) minimization of the preterminal sterilization biological load to some level dictated by project policy, which would imply clean manufacture and assembly; and (2) launch-pad operational problems. The latter area includes: (1) the inability to conduct inspection of the pyrotechnic or propulsion equipments after heat sterilization (perhaps some remote inspection technique such as radiography must be developed); (2) the increased hazard, specifically to the spacecraft, during heat sterilization owing to the presence of explosive components; and (3) the possibility that the propulsion subsystem may be a large part and also a low-thermal-diffusivity part of the heat-sterilized spacecraft, which would require a long heat-up time and consequently would impose a longer exposure of all other spacecraft elements. One possible operational solution for this last problem is separate, internal heat sterilization of the propulsion module followed by assembly into the spacecraft for terminal sterilization, at which time only the external part of the propulsion module needs to be brought up to sterilization temperature.

Finally it must be remarked that the engineering analyses and tests herein reported have been conducted with reference to a specific sterilization heat cycle; significant changes in temperature, duration, and cycles can exert a drastic effect on a pyrotechnic and propulsion subsystem capability to survive a different heat cycle.

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Session V—Discussion

Luckey, *McDonnell Aircraft Corp.*: No bacteriological data were presented in this session. Does this mean that none were taken in these studies?

Goddard: I think that the answer to this is that all the work was directed toward the problem of settling the heat-cycle requirements as a result of the biologists' determinations. How can you design and fabricate components, pieces, and equipment so as to survive the particular heat cycle described and be reliably operable thereafter?

Luckey: I assumed that this was true and it seems to me that we are building a relative vacuum for the biologists. You have a good opportunity to explore bacteriologically these components as they are being tested mechanically. I think it would be most helpful to have bacteriological work done on these components. For example, early in this session it was suggested that such and such a temperature will not kill bacteria. You remember the tailing effect with radiation? I suggest that there is a tailing effect with heat and you might indeed end up with organisms which were more resistant than you expected. I think that it is very important to run a survey as you proceed in this testing.

Miles: I tried to touch on this; it was originally decided among the departments that the engineers would turn over the components for assay to the biologists. We have done this and there are quite a few samples in the hands of the biologists to be assayed. As to the tailing off, we have had much discussion about this new set of requirements. In a paper given by Davies and Horowitz at COSPAR in Argentina, much was made of the fact that there was curvature rather than a straight-line descent in that soil curve and we think they may have something.

Luckey: It seems to me that many of these liquid propellants are indeed highly germicidal compounds; ethylenimine, for example. I think this might be a very fine equivalent to ethylene oxide. Ammonium perchlorate and hydrazine sound like fine bactericidal compounds. Have you considered the possibility of not having to heat sterilize any of these?

Gin: Yes; we have looked at this problem to see whether these propellants, liquid or solid, are indeed self-sterilizing. I have a specific piece of information in mind. At Dynamic Sciences, *B. subtilis* was exposed to hydrazine at room temperature; the half-life was 1 week. So there are bacteria that will survive even in

hydrazine. The stronger oxidizers, like nitric acid, are probably also self-sterilizing. The solid propellants that we have examined, such as the polyurethane system, are not self-sterilizing. It contains TDI which we thought would be an effective sterilizing agent, but this has not been found to be the case.

Beuf, *General Electric* Co.: Mr. Miles, do you think it would be more appropriate to check each and every component and material for outgassing of substances which might be deposited on optics or do you think it would be better to do this on a subsystem basis as Mr. Arens did with his recorder–

Miles: Well, I would hate to design a fairly complicated device, any one component of which could be fatal to the project. I would say we took the right approach in starting with the components. We do have some doubters in our hardware line who say that we do not need any real systems built up, that we know all the components are good. We have other people who say that even if we built up something from this and checked it out, we don't need anything comparable to the 10 000-hour life test for the system as Lockyear needed for his parts. Now I am sure that I have overstated their position, but their inactivity at times makes me feel that they have understated the seriousness of the problem. I really think that both are required and I would not want to trust parts work alone nor would I want to start on systems work without having parts work behind me.

Foster, *Hughes Aircraft* Co.: Mr. Miles, did I understand you to say that one of the new requirements is a class 100 laminar downflow room for facilities? If so, at what level? Black box, subsystems, or spacecraft?

Miles: I will paraphrase a memorandum from the Bioscience Office: "You can't get into the final sterilization chamber unless you have a sufficiently low load, namely 10^8. You can arrive there by several paths. If you choose to follow the clean-room path you will have to perform these operations in a class 100 clean room." They have specified a laminar downflow class 100 clean room.

Hall: The present consensus is that this will have to be applied only at the subassembly level if it can be shown that the microbial load is greater than 10^8 without such care in subassembly.

Miles: Some of the parts can, indeed, be assayed, and they will be. Some, however, will not fall into a representative category and, accordingly, they in turn may require special procedures. Granted that we could assay all the parts, it is my understanding that we could then assemble with parts that are assayable and heat decontaminated. We would then stay in the clean room for the manufacture of the subassembles. Alternately, we could use 10

D-values based on the soil cycle to arrive at the subassembly level. From there, there is no latitude; you must stay in the clean room for final capsule assembly.

Orhon, *Aerojet General Corp.*: Mr. Kohorst, in your presentation you showed a table of tests which you performed with your polymeric materials. I was wondering why you do not have a test showing loss or gain of weight in polymeric materials after sterilization (especially grams-per-square-centimeter loss or gain) ? On the other hand, I was wondering why in the adhesion test you use only tensile shear and not peel tests?

Kohorst: As far as weight gain and loss, we did make measurements. They were not included in that table. As to the peel tests, we tried to develop a program that would give us the most information on the most materials in the shortest time at the least cost. I am sure you all recognize that we must make compromises.

Orhon: Did you find loss in certain cases and gain in others?

Kohorst: Yes.

Connelly, *Decontamination, Inc.*: I would like to touch on a potential problem area, which includes the microbiological disciplines, the electronic field, materials engineering, and process engineering. It concerns the problem of soldering. Soldering is usually accompanied by a fluxing operation. The types of flux most widely used are resin types. Other work is done with material such as glutamic acid. Speaking as a chemist, not a biologist, it occurs to me that either of these materials could provide some nutrients. Since you are trying to get a low level of biological contamination before reaching the final stage, it would be desirable to eliminate these nutrients. The process engineer is faced with a problem when using solder flux, since the solvent used to remove the flux often removes some of the ink and paint identification on the parts. The other alternative has been glutamic acid, but it is a water-base system and the space agency has opposed water-base cleaning of circuit boards. Other processes available could eliminate these problems completely by using sonic activation of the solder pot. Is this an area of concern for spacecraft work?

Holtze: Our work concerned the effect of heat sterilization on the solder joint itself. In these tests the component leads were pretinned using activated flux and then cleaned in joints with a neutral resin flux. I am no microbiologist, but I can see how the flux could serve as a nutrient. We have not gotten into that part of the program. In normal practice we remove the flux by washing in a suitable solvent and we have not damaged the markings. Incidentally, we have an investigation underway of markings which resist fading during heat sterilization. If we could avoid

using a flux it would be helpful, since washing procedures may cause entrapments that could act as nutrients.

Koesterer, *General Electric* Co.: One of the design criteria should be to have nonnutrient flux involved in spacecraft components. There are some other hydrocarbon greases which I believe certain microbes can metabolize. Thus, the selection of materials is best handled at the design criteria stage. If the problem still exists, then invoke decontamination techniques.

Session VI

Capsule Structures and Payloads, Procedures, and Facilities

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Effects of Sterilization on Separation, Entry, Descent, and Landing Phases of a Capsule Mission from an Engineering Mechanics Perspective

This paper is limited to a discussion of certain problems, and of the status of the approaches to the solution of these problems, associated with the capsule separation, descent, and landing phases of a Mars mission. A distinction will be made between problems associated with the ability of a particular subsystem to withstand the sterilization environment and operate properly and those problems associated with possible recontamination of the capsule at some time after the capsule has undergone terminal heat sterilization. The recontamination problems arising during the capsule-spacecraft separation phase in the vicinity of Mars will be stressed. The discussion of recontamination of the capsule by the spacecraft assumes a nonsterile spacecraft.

Flight Capsule-Spacecraft Separation Phase

The requirement for sterility of all subsystems entering the Martian atmosphere within the next 50 years significantly complicates the flight capsule-spacecraft separation phase. In order to assure capsule sterility, a sterilization canister must completely enclose the entry capsule from final assembly until such a time that nonrecontamination of the entry capsule can be assured. The sterilization canister must provide an effective biological barrier and at the same time must be capable of reliable separation from the capsule and/or spacecraft.

In order to minimize the possibility of recontamination of the capsule during flight, it is desirable to retain the sterilization canister until planetary encounter. Maximum protection from biological recontamination of the separated capsule results if the sterilization canister and capsule are separated integrally from the flight spacecraft (type A in fig. 1). In the type A separation sequence, the capsule, enclosed within the sterilization canister, is separated from the spacecraft. Following a separated cruise sufficiently long to allow the capsule to go beyond the influence of

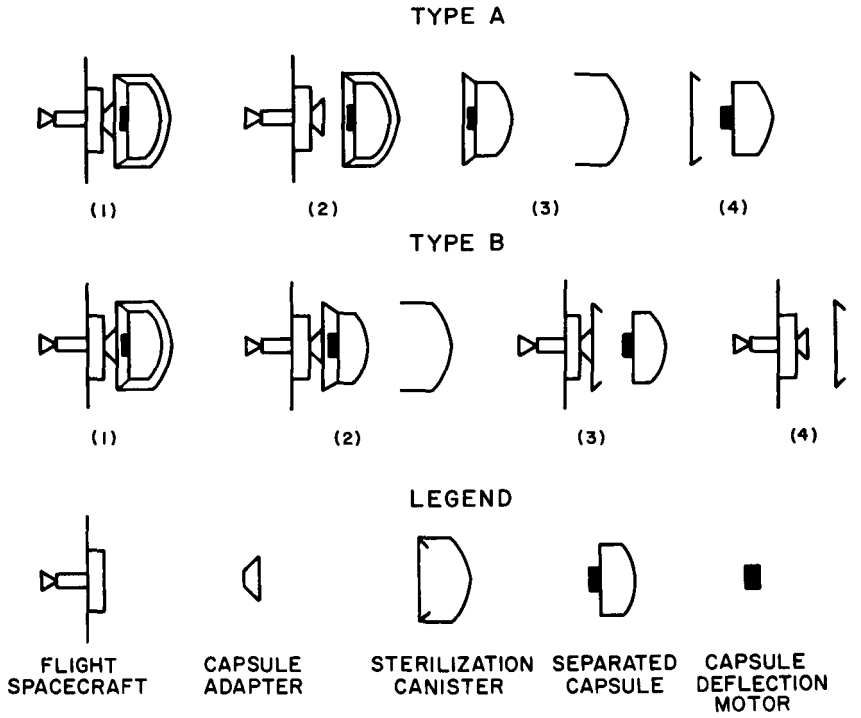

FIGURE 1.—Capsule-spacecraft separation sequences.

possible contamination from the spacecraft attitude-control gas jets or other contamination sources, the sterilization canister joint is broken; then the forebody section of the canister is separated from the separated capsule and aft section of the sterilization canister.

This entire discussion assumes a capsule-deflection delivery mode either from a direct-approach trajectory or "out of orbit." In all capsule-deflection modes the capsule must perform a propulsive maneuver after separation from the spacecraft in order to place the capsule on a Mars-impact trajectory. If the capsule does not perform a set of turns prior to performing its deflection maneuver, the forebody portion of the canister must be given a lateral velocity prior to deflection motor firing so that the capsule will not be driven into the canister foresection. The capsule is then separated from the aft section of the sterilization canister and is prepared to perform its deflection maneuver. If the capsule is required to perform a set of turns following separation from the spacecraft, these turns can be performed following release of the

foresection of the sterilization canister; thus the potential collision problem during capsule-deflection motor firing is eliminated.

In the type B separation sequence (fig. 1), the forebody section of the sterilization canister is separated prior to capsule separation from the spacecraft. In this mode, the surface of the capsule forebody is potentially exposed to the plume of the spacecraft attitude-control gas jets. Another potential source of capsule recontamination may be very small dust particles which settled on the spacecraft during assembly. The presence of these very small dust particles on the leeward side (away from the Sun) of the solar panels on Mariner IV are believed to have been responsible for brightness increases seen by the Mariner IV Canopus sensor. These particles can be dislodged from the spacecraft during canister or capsule separation or by micrometeoroid impacts and deposited on the capsule once the foresection of the canister has been released. Another potential source of contamination is slow outgassing of various materials on the spacecraft. This gas could potentially condense on the exposed capsule surfaces following forebody canister jettison.

In the case of the "out of orbit" mode wherein the capsule and spacecraft are both carried into orbit, the sterilization canister separation problem is further complicated. If the sterilization canister foresection is separated prior to orbit insertion, the capsule forebody is exposed to a portion of the exhaust gases from the orbit-insertion engine; also, the orbit-insertion maneuver produces a large disturbing force which may dislodge significant numbers of dust particles on the spacecraft. In either case, the possibility of contamination of the foresection of the capsule is increased over that of the direct-approach case.

If the sterilization canister foresection is retained through the orbit-insertion maneuver and released at some later time in Mars orbit, the acceptable orbit altitude may be determined by the requirement of a 50-year lifetime of the sterilization canister in orbit about Mars. Since the canister will have a very low ballistic coefficient (mass per unit drag area), its lifetime in orbit will be much less than that of the spacecraft. In addition, the presence of a large body floating in nearly the same orbit as that of the spacecraft could produce problems for the spacecraft Canopus sensor should the canister come into view at some later time. One potential solution is not to release the canister but to hinge the foresection so that the capsule can be separated; then the canister would be reclosed and retained on the spacecraft. This approach is undesirable since it limits view angles for spacecraft experiments and increases the attitude-control requirements of the spacecraft

in orbit about Mars. A good clean solution to this problem has not been identified to date.

From the preceding discussion it appears that, generally, separation of the capsule within the sterilization canister is most desirable to prevent recontamination of the capsule. However, from an operational and dynamics of separation standpoint, the type A approach is least desirable. First, there is no redundant capability for removing the foresection of the sterilization canister if approach A is used. In approach B, if the initiation of separation of the forebody section of the canister from the capsule system is unsuccessful, an independent, redundant initiation activated by the spacecraft may be employed. Secondly, the masses and inertia of the canister relative to the capsule are approximately 1 part in 10, whereas, the masses and inertia of the canister relative to the capsule and spacecraft (including orbit-insertion motor) are approximately 1 part in 100. Therefore, separation perturbations to the capsule system are much less when the canister is separated while the capsule is attached to the spacecraft. These separation perturbations will affect the pointing accuracy for the capsule-deflection maneuver and will affect clearance requirements for the capsule-sterilization canister.

As discussed previously, there is a definite collision problem associated with forebody canister release in the capsule-deflection attitude. If the forebody section of the canister is released from the cruise attitude of the spacecraft and capsule and then the capsule and spacecraft perform a set of turns to place the capsule in the proper attitude for the deflection maneuver, the problem is eliminated. In approach A, capsule turns following canister forebody separation would be necessary; thus, there would be added complexity in the capsule sequence and increased electronic logic requiring sterilization. This latter point is significant, since it is desirable to minimize the number of electronic subsystems and/or components which must be heat sterilized in order to maximize capsule system reliability.

Although they will not be discussed here, the time at which the forebody section of the canister is released is also affected by capsule temperature-control considerations and capsule meteoroid-protection requirements. In total, the sterilization canister-capsule combination produces a whole set of systems interactions which would not be present if the constraint of noncontamination of Mars did not exist.

If the type B capsule-separation sequence is to be adopted, we must ascertain how the nonrecontamination constraint can be satisfied. As discussed previously, all potential sources of con-

tamination on the spacecraft will, at most, contaminate the capsule external surfaces. This fact is extremely important, since the capsule surfaces can be exposed to a biologically lethal environment prior to entry. Horowitz, in a paper to be published in *Science,* has shown that the unfiltered solar ultraviolet flux at Mars provides sufficient energy to kill the most resistant bacteria in a matter of minutes. The total integrated ultraviolet flux between 2400 angstroms and 2800 angstroms will annihilate a typical bacterial cell in one-half minute. Even the most resistive cells are annihilated in 10 minutes. Therefore, if the capsule were programed to perform a slow-roll turn during its approach to the planet, thus exposing all external surfaces to the Sun for at least 10 minutes, the external surface could be sterilized. Although not the subject for this paper, it would appear that the solar ultraviolet would sterilize the orbit-insertion exhaust plume or attitude-control gas prior to encounter with Mars, thus eliminating these sources of contamination. This slow-roll turn is also desirable from a capsule temperature-control standpoint and, therefore, should be examined to determine its operational problems.

If the foregoing approach is unacceptable from a planetary quarantine standpoint, it may be necessary to sterilize portions of the spacecraft system, such as the attitude-control gas and lines. An overall probability of capsule recontamination must be evaluated prior to taking these steps.

In summary, the constraint of noncontamination of Mars significantly affects capsule system spacecraft separation, capsule preentry operational sequence, and certain capsule system mechanizations; and perhaps this constraint affects even spacecraft system mechanizations.

One of the most important capsule system mechanizations affected by the noncontamination constraint is the sterilization-canister separation joint. The requirements of this joint are to provide an acceptable biological barrier and to provide reliable, uniform separation of the forebody and aftbody sections of the sterilization canister. The performance must be obtained with minimum timing dispersion in order to assure a clean separation of the two portions and to limit interference with the capsule aeroshell by means of a lightweight system.

The joint must be effectively continuous in order to provide an acceptable biological barrier. Two types of joints have been considered; the first is a continuous joint which employs a shaped charge for breaking the joint, and the second is a semicontinuous joint which utilizes the change in shape of a gas-filled tube to break the joint. The shaped charge offers the prospect of a good

continuous joint but produces debris, a portion of which will impact the capsule aeroshell and may produce damage. The shaped charge certainly will complicate ground assembly operations and safety requirements in the vicinity of the capsule following shaped-charge installation; also it will make capsule disassembly difficult, should this be required following ground systems tests. The utilization of the shaped charge also requires a separation and an assembly joint, as shown schematically in figure 2.

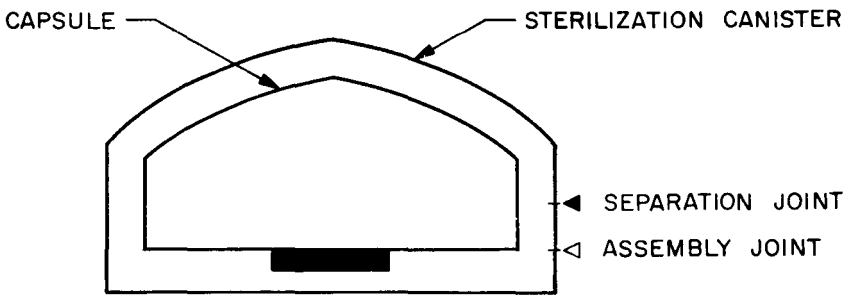

FIGURE 2.—Schematic of sterilization canister joints.

The second type of joint is much more desirable and has been adopted as a nominal approach in our present design studies. This mechanism was designed by Bamford of JPL and is shown in figure 3. In this approach the assembly (field) joint and separation joint are integral. The separation joint consists of a circumferential ring with a male and female portion. A small elliptical cross-section tube is housed in the female section. As the tube is inflated from an external gas supply, it changes shape to a circular cross section so that sufficient force is provided to shear the pinned joint. If necessary from a noncontamination standpoint, the joint can be locally filled with epoxy in order to provide a continuous seal. As shown in figure 3, the male and female portions of the joint are designed to slip over the canister shell and are bolted to the shell; therefore, an integral assembly and separation joint is obtained. One of the most desirable features of this approach is that the inflated tube does not rupture; therefore, all gases are contained. Although this may not be an optimum joint, it does have the necessary characteristics of the canister separation joint.

The breaking of the canister separation joint is the most critical phase of the canister release. However, in order to separate the forebody section of the canister, a mechanism for imparting a velocity increment to this portion of the canister is required. A cocked-spring separation mechanism utilizing pin-puller initiation

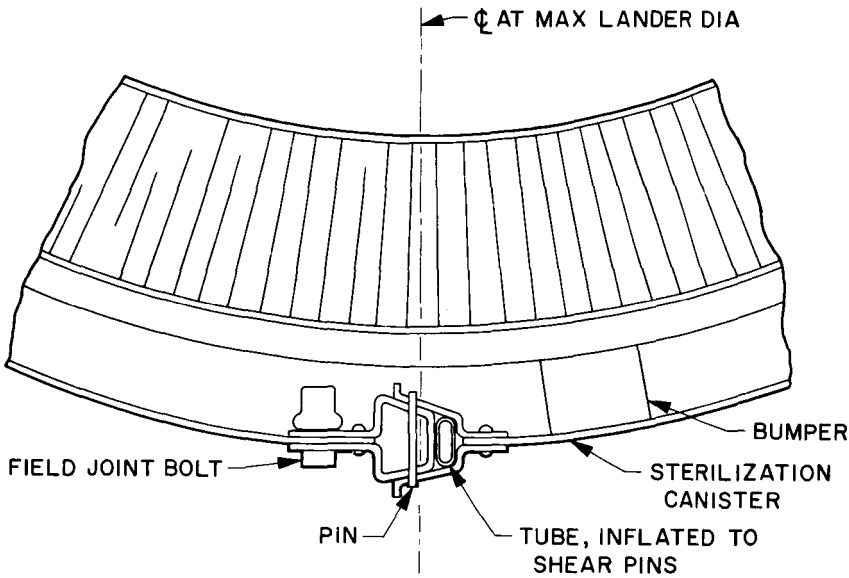

FIGURE 3.—Sterilization canister joint.

is presently envisaged; this arrangement does not appear to produce any particular problem other than those commonly encountered in over-the-nose shroud releases. In summary, potential mechanizations for canister release and separation have been devised. It is now necessary to demonstrate experimentally the characteristics and performance of these mechanisms.

Capsule Entry Phase

From an engineering mechanics standpoint, the two entry subsystems affected by the sterilization requirement are the aeroshell structure and the heat shield. It is believed that both systems are capable of withstanding the type-approval and flight-acceptance sterilization requirements; however, a brief review of certain effects which must be considered in their development is warranted.

The present approach to the capsule aeroshell structure is to utilize epoxy or phenolic reinforced fiber-glass sandwich construction. The only problem may be to qualify certain materials for this application; however, this appears to be directly soluble. Materials such as 91–LD presently appear acceptable from a sterilization standpoint. Although incomplete data exist at present, it appears that adhesives such as Epon 924 can be considered for bonding the face sheets (skin) to the honeycomb core. Even with the lightweight skins and low core densities required for the Mars

entry capsule, it does not appear that material selection for the capsule shell produces any particular problem.

There appear to be a large number of potential heat-shield materials which can survive the sterilization environment, since some of these materials are cured at temperatures in excess of 400° F. Some of the heat-shield materials which are being evaluated are phenolic Nomex, epoxy-phenolic-silica-fiber, and methyl phenyl silicones. The methyl phenyl silicones are of particular interest; since they appear to be resistant to the interplanetary environment, they have desirable, very low, brittle transition temperatures (−150° to −200° F), and they exhibit acceptable radiofrequency transparency under certain entry conditions.

Specimens of a silicone-elastomer material (ESM 1004AP, a methyl phenyl silicone elastomer) were obtained and the response of these specimens to the thermal-vacuum environment following heat sterilization was evaluated. An evaluation of the performance of this material is being carried out jointly by JPL and the Ames Research Center. The effort at JPL included the exposure of an 11- by 6- by 1-inch specimen to dry nitrogen at 300° F for 5 days in order to simulate the dry-heat sterilization environment. The material was then placed in a vacuum system held at 2 to 5×10^{-7} torr for 14 days at −100° F. The sample of ESM 1004AP showed no significant weight loss (<0.5 percent) as a result of thermal or vacuum tests. The material took a slight permanent set when deflected by its own weight in the vacuum test, but was not in any way brittle at −100° F upon removal from the vacuum test system. In any case, it does not appear that this heat-shield material would cause a problem in any of the predicted Mars preentry and entry environments.

The foregoing detailed evaluation has been performed on only one heat-shield material. A major effort to evaluate various heat-shield materials for Mars entry application is being undertaken by JPL in concert with Langley Research Center and Ames Research Center. The encouraging data from this first set of experiments indicates that a choice from a large variety of heat-shield materials should be possible.

Payload Terminal Descent Phase

Present Voyager mission approaches anticipate the use of a parachute during the terminal descent phase of certain types of capsule missions. The effect of the sterilization environment on parachute materials was evaluated in 1964 by the Cook Electric Co. (JPL Contract No. BE4–229753). The final report of the study is given in reference 1 and the salient results have been

presented by Nagler, reference 2. Silk, nylon 66, Dacron, and Nomex, along with representative pyrotechnic materials required for parachute initiation, were exposed to thermal and ethylene oxide (ETO) testing followed by vacuum tests ranging from 5 to 30 days. Thermal testing employed three cycles at 294±3° F for a 36-hour cycle and ETO testing consisted of exposure to a mixture of 12 percent ETO and 88 percent Freon-12 for two temperature cycles of 75±5° F and 104±5° F for 24 hours each. The vacuum exposures were performed under 5×10^{-6} torr at a temperature of approximately 160° F.

Of the materials tested (tests included sudden shock tests simulating opening loads), both Dacron and Nomex appear to have acceptable material performance, with material variations being less in Nomex. Folded or twisted and compacted specimens of all the materials showed no measurable variation from the flat specimens. Sewed specimens, on the other hand, lost 10 percent of their strength, probably because of fiber damage.

Ten suppliers of pyrotechnic devices and materials were contacted; five supplied pressure-generating devices and two supplied reefing cutters. All devices operated after exposure to all standard environments. Certain seals on the pressure-generating devices were purposely punctured and some of these samples failed. The failures indicated that vacuum exposure of sealed devices for periods of 7 to 9 months (Mars interplanetary transfer) may be detrimental. Subsequently, verification of the ability of reefing cutters to withstand sterilization has been obtained by JPL and Frankford Arsenal.

Landing Phase

During the impact and landing phase, some type of energy-absorbing material will be required to reduce the impact shock to acceptable design levels. In the case of parachute terminal descent and with the combined low Martian surface pressure of 5 millibars and expected high continuous winds (approximately 200 ft/sec), an efficient impact energy absorber is vital to the landing mission. Three types of energy-absorbing system to accommodate impact speeds of approximately 200 to 300 feet per second are being evaluated at JPL. These impact limiters employ balsa wood, plastic honeycomb, or a pneumatic gas bag for energy dissipation. Sterilization acceptance programs are presently being performed on only the plastic honeycomb and balsa wood impact-limiter materials, with the emphasis on the latter.

The plastic honeycomb material is being developed by the General Electric Co. One task within the contract calls for expo-

sure of flat honeycomb specimens to three cycles of heat at 300° F for a duration of 36 hours in dry nitrogen. A minimum of 48 specimens will be tested as one part of the impact-limiter development program. A minimum of 9 static and 12 dynamic crushing tests on vented specimens will be performed at temperatures of −100° F, ambient, and 200° F after the sterilization cycle exposure. These tests have been scheduled for the spring of 1966.

A major JPL inhouse program is underway to evaluate the effect of the sterilization environment on balsa-wood impact limiters. The approach being taken is to evaluate the balsa-wood crushing stress and specific energy following exposure to various vacuum and thermal environments. Figure 4 shows a visual comparison of balsa-wood specimens following heating in a sealed capsule. The unheated sample in the center is shown for comparison purposes. Specimen 7.1–6 (left in fig. 4) was heated for 58.1 hours at 300° F

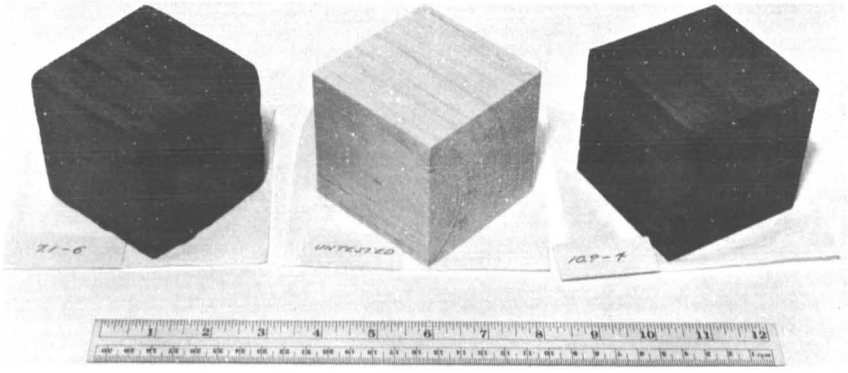

FIGURE 4.—Comparison of balsa-wood specimens following heating in sealed capsule.

in a sealed capsule. The capsule experienced a pressure rise of 127 psi; this indicated that decomposition of the balsa wood resulted. Specimen 10.9–4 (at right in fig. 4) was heated at 300° F for 121.2 hours in a sealed capsule purged with dry nitrogen prior to test. In this case the specimen exhibited a pressure rise of 141 psi; this also indicated decomposition.

Other work included a series of balsa-wood compression tests which were performed on a different set of samples. Typical examples from these tests are summarized in table I. All the crushing stress data are effective for static crushing. Two significant results can be seen from table I. First, the absence of moisture from the impact-limiter material increases its specific energy, and thus its energy-absorbing capability for the same weight. Second,

TABLE I.—*Balsa-Wood Compression Tests*

[Crushing speed, 1 in./min.; ram area, 1 in.^{2}]

Specimen	Pretest treatment	Moisture content, percent weight	Density, lb/ft^3	Crushing stress, psi	Specific energy, ft-lb/lb
8–23–1	As-received material	8	8.89	1535	19 300
8–75–3	Conditioned at 10^{-5} torr for 17.1 hr	0	6.47	1410	26 500
8–46–2	Dried at 145° C for 108 hr (not encapsulated)	0	8.57	2220	30 800
8–38–1	Heated at 145° C for 108 hr in stainless steel capsule	.....	6.64	583	10 600

the specimens which are exposed to the sterilization environment but not encapsulated show increased performance over the as-received material, whereas the encapsulated specimens demonstrate large reduction in performance. This program is being continued and exposures up to 500 hours are presently being performed.

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Design Criteria for Typical Planetary Spacecraft To Be Sterilized by Heating

This paper describes work performed by the General Electric Co. for the George C. Marshall Space Flight Center of the National Aeronautics and Space Administration (NASA) under Contract NAS 8–11107 entitled "A Study of Design Guidelines for Sterilization of Spacecraft Structures" and Contract NAS 8–11372, Part I, entitled "Design Criteria for Typical Planetary Spacecraft To Be Sterilized by Heating." The purpose of this work was to establish guidelines for the design of planetary spacecraft subject to heat sterilization.

In performing this work, the concept of heat sterilization within a sealed sterilization barrier was rigidly followed. Because of uncertainties regarding actual requirements, a conservative range of environments was specified for this work; they extended from a maximum of 150° C for 60 hours to a minimum of 135° C for 24 hours. These may be contrasted with the latest approved range which extends from a maximum temperature of 160° C for 3 hours to a minimum temperature of 105° C for 336 hours. Analytical and test phases were designed to present solutions to sterilization problems where possible and to define problems better where solutions are not readily available. Primary emphasis was placed on the response of structural and mechanical components to elevated temperatures.

Design guidelines and related sterilization data from this work and other sources have been collected in a volume entitled "Manual of Procedures for Spacecraft To Be Sterilized by Heating, Volume I—Design Guidelines" (ref. 1). This manual presents in a single volume a body of information dealing with design problems which arise from the requirements to sterilize a planetary lander by heat in accordance with current NASA policy.

Typical Lander

Description

At the present time, the first planetary lander has not been defined, and the vehicles under consideration comprise a wide design range. The availability of a range of launch vehicles that can be used for Mars missions and the uncertainty over specific experiments to be conducted on the first mission have resulted in the spectrum of lander sizes and weights shown in figure 1. The

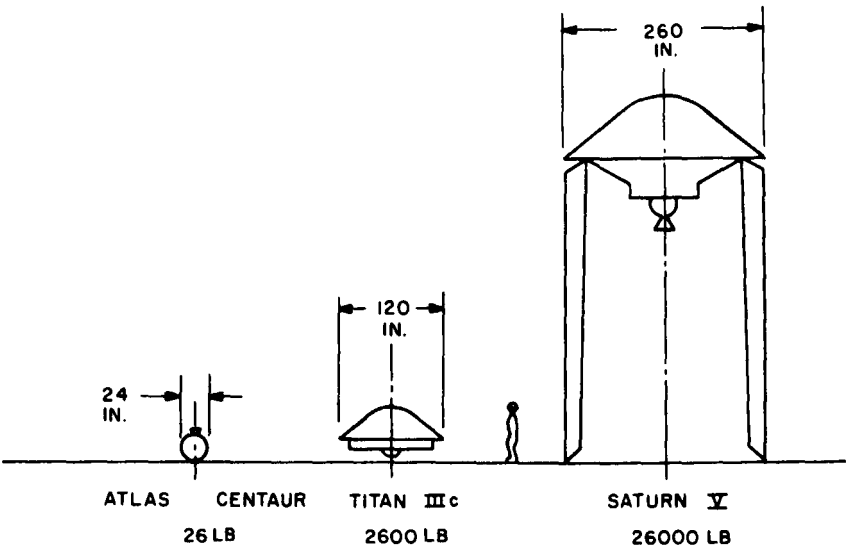


FIGURE 1.—Size spectrum of Mars landers.

smallest vehicles proposed are nonsurvivable landers which employ no retardation devices and are destroyed on impact; these vehicles would be designed to obtain data on the Martian atmosphere (refs. 2 to 4). The largest vehicles would contain numerous biological experiments (refs. 5 and 6). For the largest vehicle shown in figure 1, approximately 5000 pounds, or 22 percent of the 26,000 pounds of total lander weight, would be available for the scientific payload. It is interesting to note that, with a lander this size, the weight limit is set by considerations of fairing envelope volume rather than capability of the launch vehicle.

The vehicles shown at the two extremes in figure 1 are shown in greater detail in figures 2 and 3. Both vehicles have considerable merit but, since the first landers have not been defined, it is necessary to deal with the more general problems common to all types of landers. When very large landers are considered, the sterilization

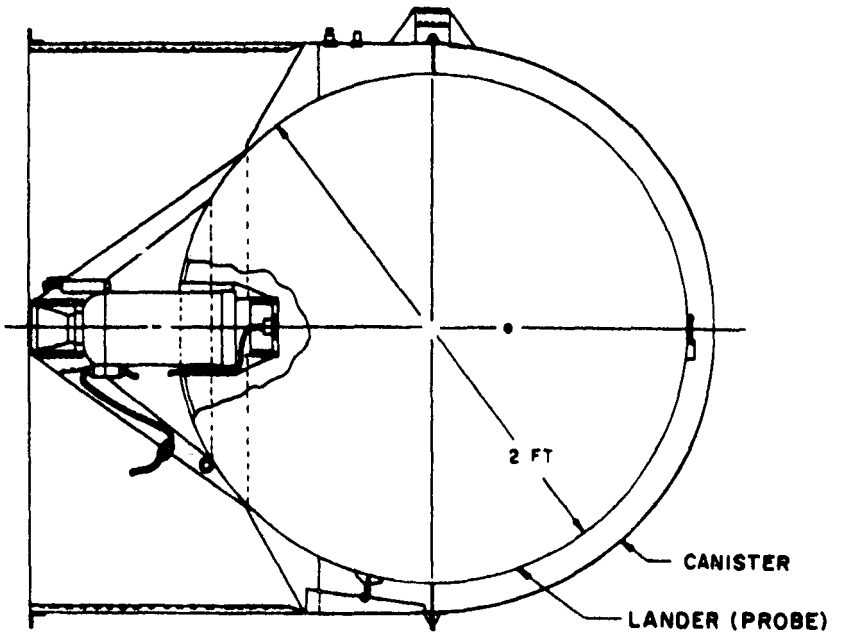


FIGURE 2.—Typical Mars atmosphere probe.

problems are magnified and, in particular, the need for major new facilities becomes apparent.

Another factor that must be considered in describing a typical lander is the aerodynamic shape selected for a Martian mission. At the present time it appears that no less than five basic shapes are worthy of consideration; these shapes are summarized in figure 4. Although final selection of a lander shape for the first mission has not yet been made, the sphere-cone shape was used as a model for the work described in this paper.

The design of each item of equipment on the lander will be affected to some degree by the sterilization requirement. As a minimum requirement, materials must be selected which can withstand sterilization temperatures. Considerable literature exists on time-temperature properties of the more commonly used metals; however, there is a definite lack of such information for non-metalics. Testing has been done on many questionable items such as balsa-wood impact limiters (ref. 7), parachute materials (ref. 8), solid propellant rockets (ref. 9), and explosive devices (ref. 10), and it appears that, with proper material selection, good design practice, and an adequate test program, these and related items may be designed to withstand sterilization.

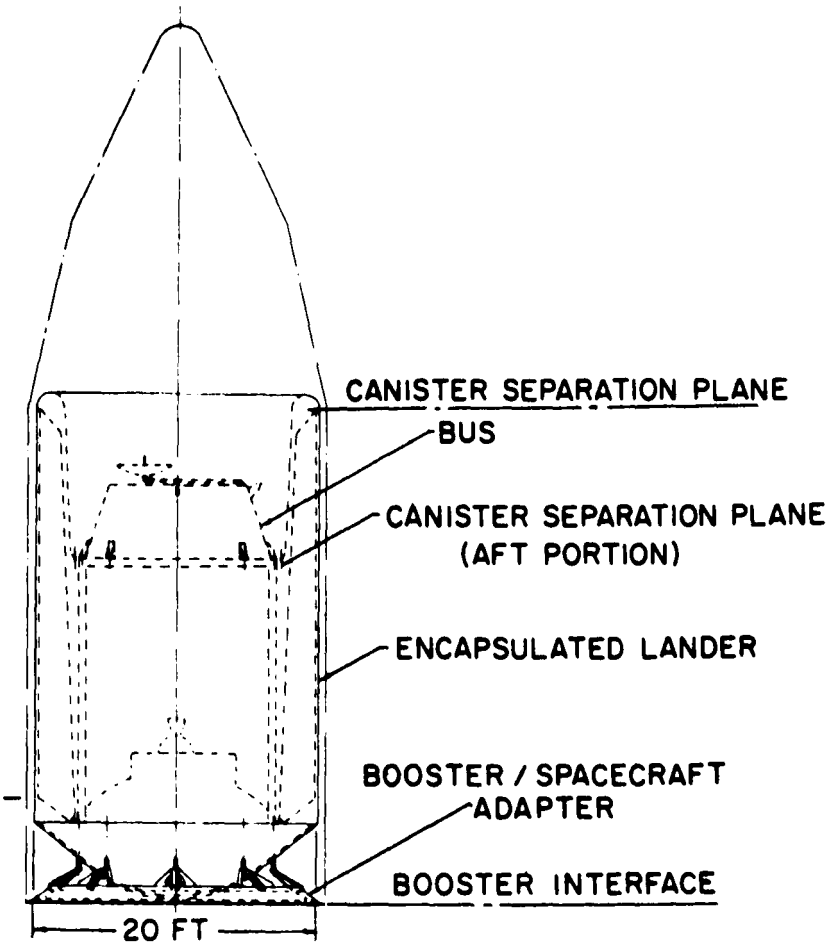


FIGURE 3.—Mars vehicle, soft lander.

There is, of course, a possibility that some components included in the lander may not be capable of terminal heat sterilization. In order to circumvent this problem perhaps these components might be sterilized prior to installation in the vehicle, then thermally protected during the terminal heat cycle, or sterilized and installed in the vehicle after the vehicle has undergone terminal sterilization. Since it is highly undesirable to penetrate the biobarrier after sterilization, the first method appears to be the better approach, although both methods would require biological qualification. However, even the first method introduces many system penalties and design problems. A thorough tradeoff study

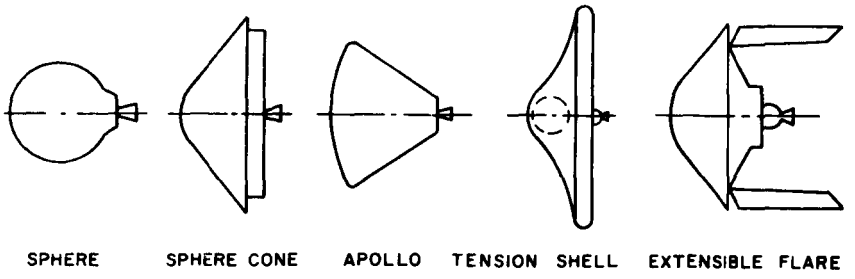


FIGURE 4.—Typical entry capsule shapes.

of these problems must be made if heat-sensitive components are included in the lander; it is too early for any recommendations in this area at this time.

Study of Joints

Concern with sterilization effects on structural and mechanical joints led to a general study of the problems that are attributable to the sterilization environment. The study evaluated the joints and joining techniques required for a typical lander from the standpoint of design, stress analysis, thermal analysis, manufacturing, reliability, biophysics, and weight. The general conclusion that can be drawn from all work to date is that the selection, design, and fabrication of joints to withstand the sterilization environment do not present any unique or limiting problems which could interfere with a successful program. The major points which lead to this conclusion may be briefly summarized as follows:

(1) It is not practical to make all joints of a typical spacecraft under biologically controlled conditions
(2) No types of joints or joining processes need be eliminated solely on the basis of incompatibility with sterilization requirements
(3) Structural design of joints which require sterilization does not require new techniques or methods
(4) With very few exceptions, design of joints for maximum thermal conductivity is not required for sterilization compatibility
(5) A number of specific types of fasteners must be eliminated because they are not compatible with the sterilization cycle
(6) Joining processes for use within a clean room require special development and special monitoring
(7) Reliability may be improved by application of clean-room techniques and procedures

(8) Biologists must specify what load reduction steps or procedures are appropriate for various spacecraft joints

Analyses

The response of a spacecraft to thermal sterilization is its response to the time at elevated temperature. Sterilization as such has no effect on the vehicle. The heating cycles applied to achieve sterility simply constitute a time-temperature design condition to be considered with others such as ground handling, powered flight, deep space vacuum, entry heating, and impact. Sterilization-induced loads and stresses can be analyzed and designed for by techniques of thermostructural analysis and design previously developed which are part of the regular professional equipment of structural designers and analysts. No gaps in analytical methods are apparent which would delay or interfere with the successful design of a planetary lander subject to the sterilization requirement.

Sterilization by heat is unique in at least one respect, however. For many analytical conditions where heat is applied to the vehicle (e.g., solar heating), insulation can be used to prevent thermal stresses and distortions, but the sterilization condition requires that all structure, in fact the entire vehicle, be heated uniformly and for specified lengths of time. Thus the structure must be designed for the condition and not around it.

During the course of this study a number of basic structural elements and conditions were analyzed to determine their response to the sterilization environment. These elements and their associated thermostructural problems were considered representative of the types which will be inherent to a typical lander design. They included:

(1) Entry shield
(2) Beams
(3) Compression members
(4) Shells
(5) Plates and panels
(6) Clamping rings
(7) Bearings
(8) Interrivet buckling
(9) Tanks and pressure vessels

Analyses of these items showed that design techniques and principles which represent good practice do not, in general, conflict with the requirement for sterilization. Particular attention should be paid, however, to thermal compatibility and to deflections. It does not appear that sterilization heating constitutes a critical

design condition for the structural components designed to accommodate static and dynamic loads of powered flight. For components which experience their maximum design loads on the ground, thermal stresses due to sterilization are additive and govern the design; this condition occurs primarily for such components as springs, band clamps, and other preloaded members.

Tanks and pressure vessels are special cases of major structural elements which experience their critical loading condition under the sterilization environment unless acceptable techniques for charging after terminal heating are identified. An example of this may be shown in the design of a typical pressure vessel. It was found that a design sterilization temperature requirement of 150° C when imposed on a pressurized tank increased the wall thickness and tank weight by 80 percent. This increase was the result of the pressure increase and a 20-percent reduction of material properties at 150° C.

Two of the major problems due to sterilization of the lander are the effect of thermal gradients across sections of thick material and the effect of long-term elevated temperature exposure on the material properties of the structure, electronics, and other components. The severity of the thermal gradients and the subsequent thermal stresses are governed in part by the rate of heating and cooling, the method of heat application, and the flow paths inside the vehicle.

Heating Problems

The ideal solution to the thermal sterilization of a spacecraft would be to heat the canister and vehicle throughout to the desired temperature instantaneously and to obtain equally instantaneous cooling at the conclusion of the sterilization cycle. Such a procedure, by whatever means it could be accomplished, would introduce a minimum of thermal degradation and minimum thermal stresses. The total time at elevated temperature for components would also be reduced, and the least impairment of reliability would result. In an actual spacecraft such instantaneous heating is not feasible; therefore, the solution requires a tradeoff between thermal gradients and transient times.

If we consider a typical vehicle which is enclosed in a sterilization canister, the first problem becomes the application of heat to the canister. This can be handled by the use of heater blankets, radiant heating, or a heating oven. The heating within the canister presents two possible choices. The first is to heat the vehicle in an evacuated canister; the second is to heat with a gas within the canister at or near atmospheric pressure.

In order to compare the effects of vacuum heating with heating within an atmosphere, an analog analysis was performed for a typical 1200-pound lander. In the examples studied, gaseous sterilization reduced the time to reach sterilization temperature (150° C) almost 50 percent from the vacuum sterilization case. On the basis of a 60-hour soak time, this results in a 10-percent reduction in the total time at elevated temperatures. Another advantage of gaseous heating was that it reduced the most extreme temperature gradients by as much as 60 percent. Gaseous heating has the added advantage of increasing joint conductances and increasing the apparent thermal conductivity of multilayer insulations.

The most important means of heat transmission between canister and vehicle is by gaseous conduction and convection. Free convection heat transfer is somewhat limited because of the particular geometry and orientation of the canister and vehicle. Values of free convection coefficients would range between 0.5 and 1.0 Btu/hr ft^2 °F (3 to 6 W/m^2 °C). However, the use of gas circulation devices can easily result in forced convection coefficients of 2 to 4 Btu/hr ft^2 °F (11 to 22 W/m^2 °C), with readily attainable gas velocities. When one considers the thermal resistance effects of any entry heat shield system and related external thermal protection systems, it is seen that there is no clear advantage in excessive convection coefficients on the outside of this heat shield because heating in an area of minimum thermal protection may be more beneficial.

If the heat transfer between the heat shield and the payload capsule is considered, there exist more and better thermal conduction paths in the electronics, experiments, and other scientific payload compartments than in the canister-to-vehicle heat-flow region. However, here again the use of forced convection heat transfer will result in more rapid and, what is even more important, more uniform heating.

In order to obtain maximum efficiency from forced convection, special attention must be given to the arrangement of components within the vehicle. Equipment, wire harnesses, and tubing should be spaced to permit adequate gas-flow paths. This is particularly important for components of large thermal mass which, because of their slow response times, will be the limiting items in the heating and cooling portions of the sterilization cycle. Table I shows some of the typical advantages and disadvantages of forced convection heating sterilization cycles.

If forced convection heating is to be accepted, a method must be devised for obtaining such forced convection. The most desirable means would be by the use of separate fans for the region between

TABLE I.—*Advantages and Disadvantages of Forced Convection Heating*

Typical advantages	Typical disadvantages
Shortened heating cycles Reduced thermal gradients Elimination of most joint resistances Permits thermal short circuits Can reach most complex components buried inside black boxes Can partially defeat thermal insulations	Requires fans or other circulators May transport dust or other particles to sensitive instruments such as optics Contamination problems may arise The presence of a fan motor may introduce a hot spot

the canister and capsule and for the internal capsule and its components. An alternate method would be the use of externally supplied heated gases. This, of course, requires penetrations through the canister and results in the likelihood of introduction of organisms (i.e., contamination). This, therefore, is not a desirable approach.

An important problem is that of knowing when the coldest component has reached the desired sterilization temperature so that the time-temperature exposure cycle can start. Analytic methods can be and have been used. However, they are primarily beneficial in a general sense rather than for specific component temperatures. Analytic predictions of the "coldest" component are not an easy task because of the thermal remoteness of items such as transistors and similar components.

From the standpoint of added weight and systems reliability, it may be difficult to install a large number of thermocouples or other monitoring devices throughout the flight vehicle to assure that the coldest point is monitored during sterilization. Because of the no-access requirement, it would be impossible to remove such devices after the sterilization cycle. The recommended approach is to install a limited number of thermocouples in selected locations in the flight vehicle and monitor these during the final sterilization cycle. Preceding this, however, it is necessary to perform an identical thermal sterilization cycle on a thermally similar vehicle which has been extensively instrumented. This sterilization test vehicle (STV) can be a prototype or other test vehicle or model, provided it is thermally similar. It should have instrumentation in the same location as that of the flight vehicle monitoring instrumentation. In addition, there should be extensive instrumentation in the STV for the purpose of assuring that every last part is up to sterilization temperature. Comparison between the monitoring and

STV instrumentation should also indicate thermal problem regions which can thus be handled reliably without access to the flight vehicle. The following thermostructural test program is representative of the approach that can be taken on an STV.

Thermostructural Tests

In order to determine the effect of a thermal sterilizing environment on a typical planetary lander, a series of thermostructural tests was performed. Selection of a test vehicle was based on specific requirements to —

(1) Determine the length of the heating and cooling transient times of a realistic entry vehicle

(2) Compare predicted thermal and structural analyses with test results

In order to fulfill these requirements, two test vehicles were assembled from major elements of a General Electric Co. Earth reentry vehicle. The differences between the two vehicles were mainly in the degree of complexity and assembly control. Vehicle A was a complex vehicle used to study the effects of the different heating methods and cycles on the thermal transients and gradients in a realistic entry package. Vehicle B contained a less complex entry package that could be readily analyzed, both thermally and structurally. Vehicle A weighed approximately 200 pounds (90 kilograms) when fully assembled. Vehicle B weighed approximately 180 pounds (80 kilograms). Externally, the configurations of the vehicles were identical. A photograph of the vehicle installed in the test oven is shown in figure 5. Both vehicles had the same thermal shield, thrust cone, aft cover, and base plate. The heat shield was made from General Electric ESM material with a fiber-glass liner. This material is excellently suited for thermal protection of a Mars landing vehicle and has been proposed for that purpose previously.

Vehicle A was instrumented with approximately 100 thermocouples installed on prime structure, on components, and inside the components. No analysis was done prior to testing. Vehicle B contained 120 thermocouples and 42 active strain gages. Transient heating times, temperature gradients, and stresses were predicted by analysis prior to testing. A photograph of the instrumented B vehicle is shown in figure 6.

A summary of the test conditions for the seven tests performed on the two vehicles is given in table II. Tests A1 and A2 were performed with the vehicle thermal shield and thrust cone structure exposed directly to the oven air. Tests A3 and A4 and the three B tests were performed with the vehicle enclosed in a

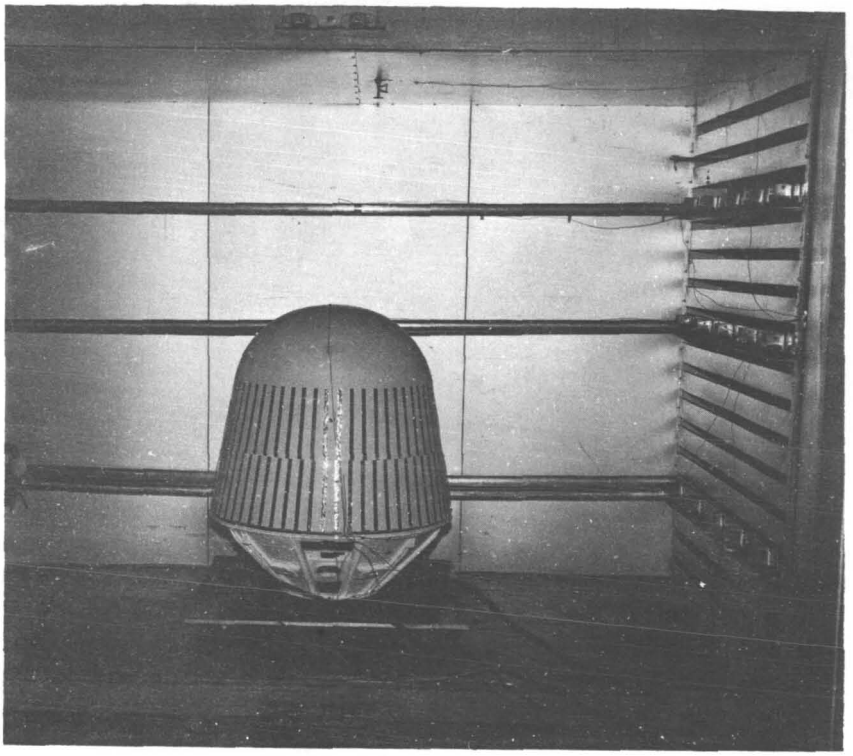


FIGURE 5.—Vehicle A installed in oven.

canister. The canister, constructed from 0.063-inch-thick aluminum, was used as a thermal barrier only in order to protect the vehicle from the air circulation caused by the oven heating fans. A photograph of the canister is shown in figure 7.

For all tests, the oven air temperature was increased from room

TABLE II.—*Thermostructural Test Conditions*

Test	Canister	Fan in vehicle	Fan in canister	Soak, hr
A1	No	Yes	Yes (oven)	0
A2	No	No	Yes (oven)	9
A3	Yes	No	No	64
A4[a]	Yes	No	No	0
B1	Yes	Yes	Yes	0
B2	Yes	No	Yes	0
B3	Yes	No	No	0

[a] 42° F oven overheat; 25° F canister overheat.

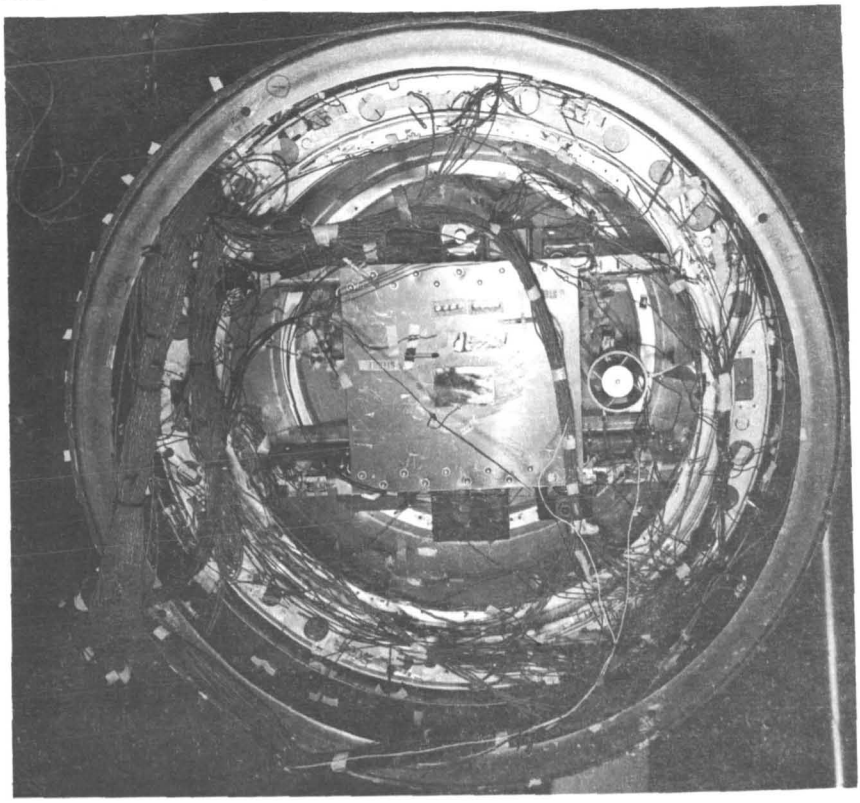


FIGURE 6.—Instrumented vehicle B; aft view.

temperature at the rate of 2° F per minute. Except for test A4, the upper temperature limit was 300° F (149° C) ; for test A4, the oven air was deliberately overheated to 342° F until the canister reached 325° F. The oven temperature was then gradually lowered at a variable rate so that the vehicle temperature never exceeded 300° F. A 9-hour temperature soak was included in the A2 test, and a 64-hour soak, in the A3 test. The soak period was defined as beginning when the coldest thermocouple reached 300° F.

The important test results are summarized in tables III and IV. The reduction in the time to reach a sterilization temperature of 300° F (149° C) is quite significant as the number of fans is increased. The optimum number and locations of fans, however, have not been studied in this program; a "best guess" location, which was dictated in part by attachment locations, was picked for the fans. These tables also show the time required to reach 300° F (149° C) and 290° F (143° C) from an initial value of 70° F (21° C). As one would expect, the time to reach 290° F (143° C), which

TABLE III.—*Vehicle A; Summary Test Results*

Test conditions	Test	Elements	Max. temp. difference, ΔT, °F	Time to reach max. ΔT, hr	Time to reach 290° F, hr	Time to reach 300° F, hr
Oven fans, vehicle fan	A1	Heat shield and thrust cone	18	2	2¾	
		Capsule structure	48	2	4½	
		Components			4½	7
Oven fans only, no vehicle fan	A2	Heat shield and thrust cone	50	2	5½	
		Capsule structure	86	2½	8	
		Components			8	12
Oven fans only, vehicle in canister	A3	Heat shield and thrust cone	58	2	8½	
		Capsule structure	61	3	14	
		Components			14	23
Oven fans only, vehicle in canister, 25° F canister overheat	A4	Heat shield and thrust cone	64	2¾	7½	
		Capsule structure	61	3	10	
		Components			10½	18

TABLE IV.—*Vehicle B; Summary of Transient Temperatures*

Test conditions	Test	Elements	Max. temp. difference, ΔT, °F	Time to reach max. ΔT, hr	Time to reach 290° F, hr	Time to reach 300° F, hr
Oven fan, canister fan, vehicle fan	B1	Heat shield and thrust cone	42	1¾	4½	
		Capsule structure	18	2	4¼	
		Components			5¼	6¼
Oven fan, canister fan, no vehicle fan	B2	Heat shield and thrust cone	47	2	3¾	
		Capsule structure	20	2	3¾	
		Components			5¼	6¼
Oven fan, no canister fan, no vehicle fan	B3	Heat shield and thrust cone	46	2¼	9	
		Capsule structure	20	2½	9¼	
		Components			10¼	12
Oven fan, no canister fan, no vehicle fan	B3 cool-down	Heat shield and thrust cone	57	3½		
		Capsule structure	26	2½		
		Components				

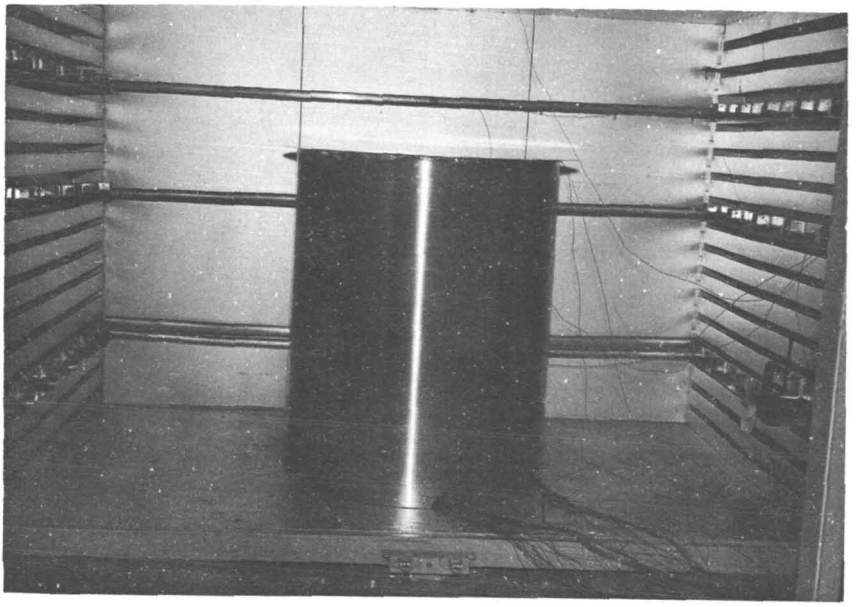


FIGURE 7.—Vehicle A enclosed in test canister.

is roughly equal to 95 percent of the final temperature rise, is approximately two-thirds of the time to reach maximum temperature. Theoretically, the maximum temperature, which is the imposed oven temperature, will be reached after an infinite time. In practice, when this temperature is reached within 1° or 2° C, one can assume the vehicle has reached steady-state conditions. The effect of overheat may be seen by comparing the transient times for tests A3 and A4. The A4 overheat condition showed a decrease in transient time for the payload components of 3½ hours to reach 290° F and 5 hours to reach 300° F.

A comparison of pretest analysis with the test data obtained from vehicle B tests showed that —

(1) Temperature gradients during the transient heating and cooling periods and the times for components to reach equilibrium temperature are difficult to predict. This is due mainly to the forced convective heat transfer mode which is introduced when fans are used. Thermal analyses for nonsterilized spacecraft are normally confined to radiation and conduction in a vacuum environment. Thus the thermal analysis for the sterilization condition is more difficult than that for the flight condition.

(2) Present methods of stress analysis are adequate for predicting maximum thermal stresses in the vehicle. Accurate predictions of stresses due to thermal gradients during the transient

period are somewhat limited because of the difficulty of accurate temperature predictions. However, it was shown during these tests that these stresses, which were on the order of 3000 psi, are small compared with the steady-state (300° F) stresses. These steady-state stresses were caused by the difference in expansivities of mating members and for some conditions reached 14 000 psi.

Alinement Test

The probability of the installation of alinement-sensitive components such as gyros, antennas, and attitude-control nozzles on the vehicle requires that an investigation be made to determine the distortion effects of heat sterilization. While some of the permanent distortions due to thermal sterilization are amenable to analysis, no analytical methods exist to predict distortions which result from such factors as —

(1) Raw material manufacture
(2) Residual stresses resulting from forming of detail parts
(3) Heat-treatment history of detail parts
(4) Stresses caused by assembly restraints such as welding fixtures and riveting jigs
(5) Joint slippage caused by differential thermal expansion of mating parts

In order to determine these distortion effects, an optical alinement test was performed on a typical vehicle which had not previously been heated.

Eleven surface mirrors were installed at various locations on the vehicle structure and on typical components mounted on the structure as shown in figure 8. The relative angular positions of these mirrors were measured both before and after a 60-hour heat soak at 302° F (150° C). The alinement test setup is shown in figure 9.

A comparison of the optical data showed that the maximum angle change between any two mirrors due to the heat cycle did not exceed ½° arc. Distortions of this order must be considered in lander design. However, studies of presently proposed lander systems indicate that they are within the anticipated tolerance for alinement sensitive equipment.

Surface Finish Experiment

The relationship between surface roughness and relative ease of sterilization is of interest to spacecraft engineers. It has been suggested that space vehicles would be easier to sterilize if all surfaces were smoothly machined and polished. The requirement for smooth, polished surfaces would greatly increase the cost and

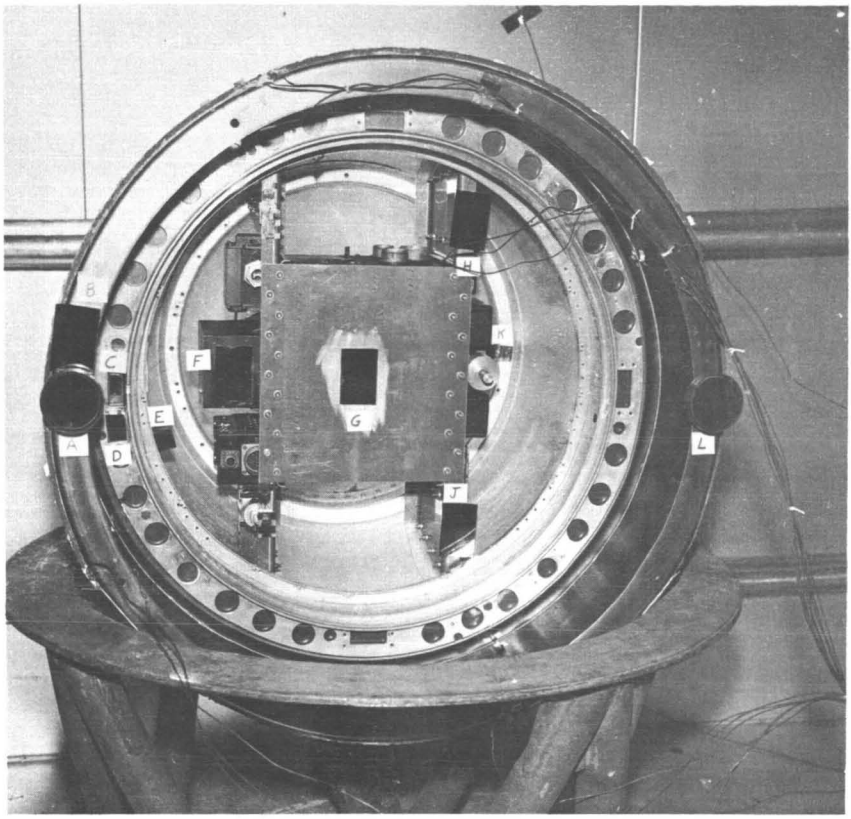


FIGURE 8.—Mirror installation for alinement test.

complexity of a program. Furthermore, the surface of many materials is not machined prior to use. For example, the surface roughness of typical sheet stock as purchased may approach, as a best case, 16 microinches rms.

Since the relationship between surface and time to sterilize is of interest to design engineers, a simple series of tests was performed using —

(1) An easily identifiable pure culture of an organism with known heat resistance (*Bacillus subtilis* var. *niger*)

(2) Samples of a single material (2024 aluminum) with varying degrees of known surface roughness (4 to 125 microinches rms)

The results of these tests led to the following conclusions:

(1) Relatively short thermal treatment at a temperature of 135° C will significantly reduce the level of microbial spores on a typical spacecraft material, 2024 aluminum,

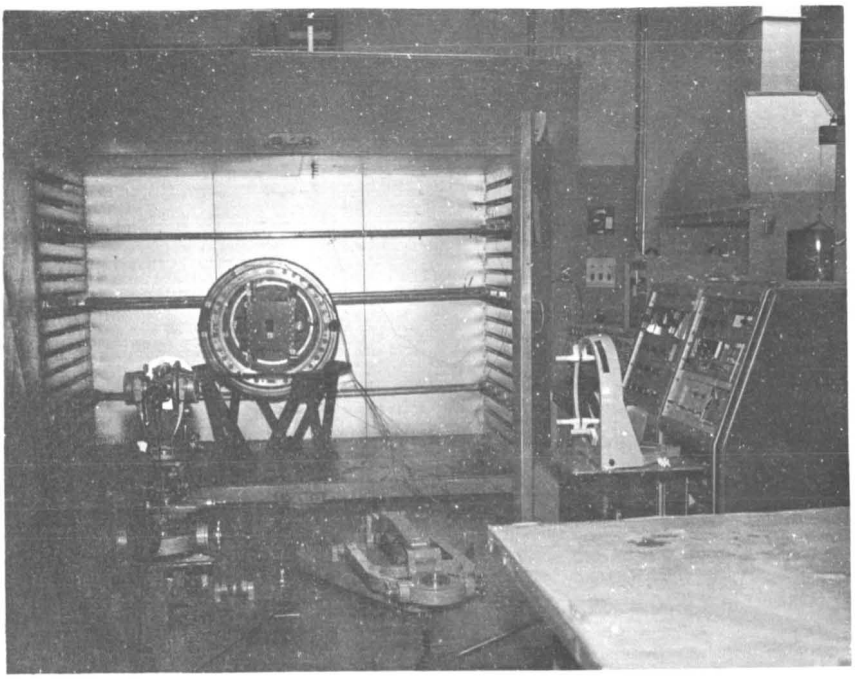


FIGURE 9.—Alinement test setup.

with surface finishes ranging between 4 and 125 microinches rms.

(2) It appears that finishes for machined aluminum surfaces better than 125 microinches rms are not required for sterilization purposes in light of the 135° C, 24-hour sterilization treatment.

Typical Canister (Biobarrier)

Design Requirements

One of the basic requirements of the sterilization policy prescribed by NASA for planetary landing spacecraft is that the landing assembly will be enclosed in a bacteriological barrier (canister) to maintain cleanliness and sterility. After decontamination, the canister will not be opened within any portion of the Earth's atmosphere which might contaminate the landing assembly (ref. 10). This barrier, or canister, must maintain a germ-free environment from the final sterilization cycle until separation of the lander from the spacecraft. Although this functional requirement sounds deceptively easy, on close examination it becomes a significant design obstacle to be overcome.

Part of the difficulty in designing a sterilization canister stems

from the requirement to maintain an absolutely germ-free environment under conditions where monitoring is neither practical nor possible. Secondly, aside from its function as a biological barrier, the canister will be required to perform other functions such as thermal control, bus-lander interface, and handling protection.

The configuration of the canister is dependent on the size and shape of the lander, the shape of the fairing, and the relative positions of booster, spacecraft, and lander. Numerous design requirements can be identified for each of these variables, but those which are basic to most lander configurations can be summarized as follows:

(1) Provide a barrier against contamination from dirt, dust, micro-organisms, spores, or other viable contaminants
(2) Withstand all service and flight environments, but specifically be compatible with heat sterilization and supplemental biological load reduction by any approved techniques
(3) Provide separation and jettison mechanisms for opening in space and deflecting the lid or cover from a planetary entry trajectory
(4) Provide the separation interface between the lander and the remainder of the spacecraft
(5) Transmit structural loads from the lander to the spacecraft or from the booster to the lander, depending upon relative position
(6) Provide structural support for other equipment as dictated by the design of the system (e.g., support for heat rejection radiators to cool the sealed lander). This requirement will occur in cases where the lander power is supplied by a radioisotope thermoelectric generator (RTG) (see fig. 10).

Materials

Several considerations must be evaluated in enclosing the lander in a canister. As an example, the choice of materials is considered. Three basically different aproaches to canister design can be identified:

(1) Rigid materials
(2) Flexible materials
(3) Combination of rigid and flexible materials

The Ranger sterilization canister was completely rigid, and although decontamination was accomplished using gaseous ethylene oxide, a similar canister could be used for heat sterilization. A typical configuration for a rigid canister is shown in figure 11.

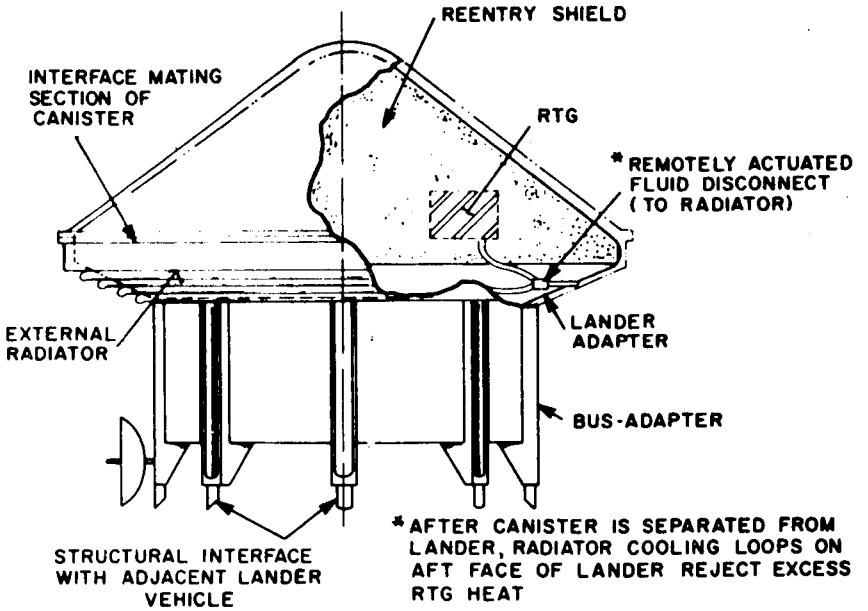


FIGURE 10.—Typical 6200-pound lander showing integration of external radiator with sterilization canister.

Several design problems for this type of device can be identified. Internal pressure will build up during terminal sterilization, and unless properly vented, could result in a structure failure of the canister. Upon cooling, the canister would draw a vacuum and be subjected to collapsing pressures unless sterile gas is added. Following terminal sterilization, no access of any kind can be made without resterilization. The rigid canister will be heavier than the flexible film canister; however, it will be less susceptible to handling damage.

The use of film isolators for spacecraft applications has been considered previously, but to date there has been little detailed configurational work along these lines. One of the most objectionable characteristics involves the jettison and separation of a thin film from an object whose shape is relatively complete. A flexible film canister for a typical spacecraft envelope is shown in figure 12. A summary of the design characteristics of flexible film sterilization canisters is provided in table V. Several of the film materials which appear promising for canister design are listed in table VI.

Weight

Weight considerations play an important role in the design of the sterilization canisters. For recent studies of small landers, the

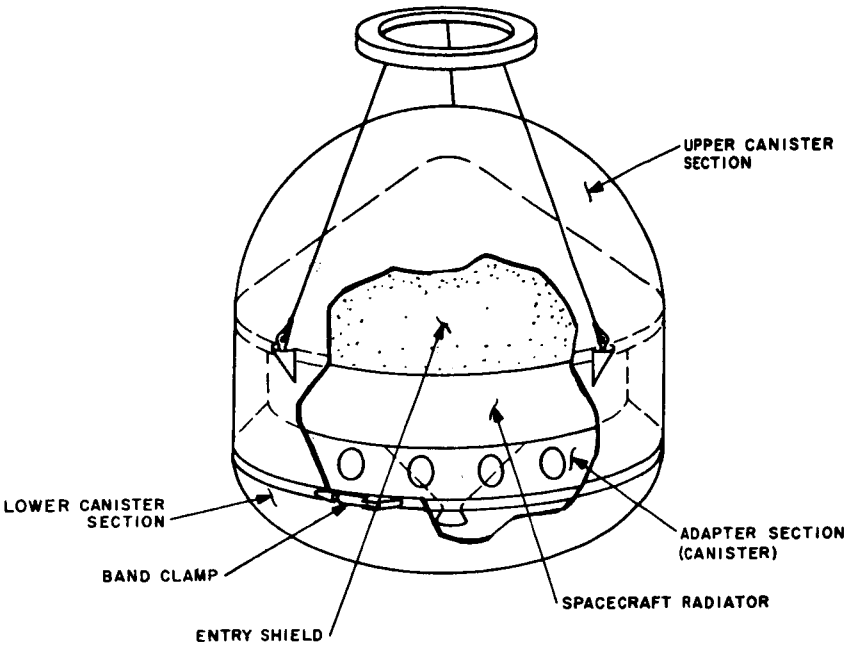


FIGURE 11.—Sterilization canister with rigid container.

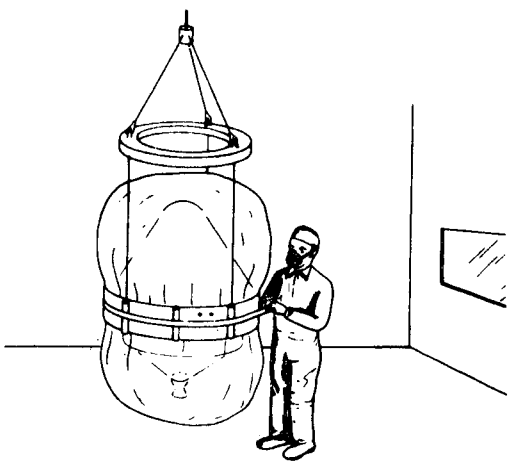


FIGURE 12.—Flexible film sterilization container.

ratio of canister weight to lander weight — without meteoroid protection — is in the range of 9 to 16 percent, as shown in table VII. It is not likely that the percentage can be reduced significantly as lander size increases. Techniques for minimum weight design must be balanced against possible risks of penetration or handling damage. Weight fractions required for the

TABLE V.—*Film Materials*

Material	Thickness range, in.	Temperature range, °F	Specific gravity
Cellulose acetate	0.0008 –.010	300–400	1.28–1.31
FEP fluorocarbon	.0005 –.020	400–525	2.15
Polyamide (nylon)	.0005 –.030	180–375	1.129
Polytrifluoro-chloroethylene	.005 –.030	300–390	2.1 –2.15
Polyester-peterphthalate (Mylar)	.00015–.014	300	1.38–1.395
Polytetrafluoro-ethylene (Teflon)	–.010	500	2.1 –2.2
Polyimide (H film)	.001 –.004	+500	1.2

TABLE VI.—*Design Characteristics of Flexible Film Sterilization Containers*

Evaluation	Characteristic	Discussion
Advantages	Visibility	Good, transparent film permits interior to be seen
	Ease of manipulation	Certain operations could be performed through the film; gloves can also be attached
Disadvantages	Weight	Lighter than metallic cans
	Ease of repair	Repairs can be made with adhesives or hot-wire sealing techniques
	Separation problems	Difficult to jettison a thin film bag
	Susceptibility to penetration or other damage	Handling damage is possible
	Difficulties with efficient reinforcement	Inserts require additional strength; sewing or stitching not practical; bonding causes abrupt section changes
	Sterile passthrough required	Locks can be bonded in; hot-wire techniques also used; source of handling damage

TABLE VII.—*Weight Relationships Between Landers and Sterilization Canisters*

Total probe weight, lb	Canister weight, lb	System weight, lb	Ratio of canister to system weight, percent
46.8	9.0	55.8	16.1
1950	350	2300	15.2
6060	600	6650	9.0

TABLE VIII.—*Takeoff Weight of Various Gases in Ranger Fairing*

Gas	Takeoff weight, lb
88 percent Freon 12 12 percent ethylene oxide	42.4
Nitrogen	11.9
Helium	1.7

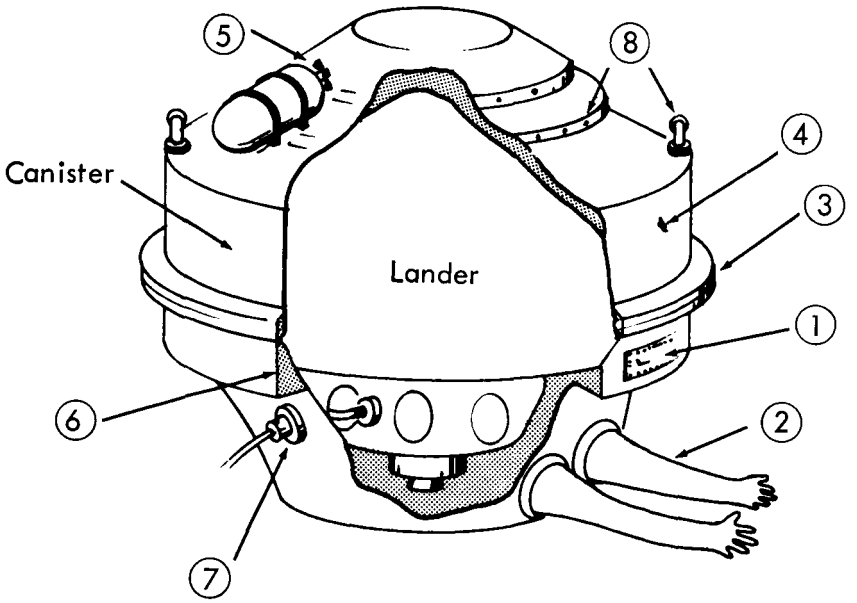
canister will vary with vehicle size and shape as well as with materials and construction methods selected for the barrier.

In reducing takeoff weight of the system, one area that should be considered is the weight of gas in the canister. The replacement of filling gas by lighter purge gases should be investigated. On the Ranger program, ethylene oxide used as a sterilizing gas was replaced with dry inert gas to reduce weight (ref. 12). The weights of various gases on takeoff in the 164-cubic-foot Ranger fairing are shown in table VIII. The weight differences assume greater significance when one considers that the volume of the canister for the largest lander studies for the Mars mission could exceed 10 000 cubic feet. Various combinations of gases might be desirable to combine ease of leak detection (Freon, helium, krypton 85), chemical inertness (helium, nitrogen, argon), or biocidal properties (ethylene oxide, hydrogen chloride, sulfur dioxide, chloroform, or carbon tetrachloride). When purge gases are used, weight allowances for entrance and exit valves should be made. If venting during ascent is required, preliminary weight estimates should include a check valve.

Pressure Design

The design of the canister as a pressure vessel should be considered as one alternative. If the canister is not vented during the sterilization cycle, the internal pressure will increase slightly from 14.7 to 21 psi. At this increased pressure it is probable that leakage from the canister will increase. It is generally assumed that the internal pressure within the canister will be maintained slightly above ambient in order to maintain a slight positive outflow. The most probable leak sources for a typical canister are shown in figure 13. Each specific canister configuration will have its own leak characteristics.

Techniques for repairing leaks in a biologically acceptable manner have yet to be identified. The major problems in this area arise from the definition of biologically acceptable leaks and the manner of controlling or repairing them. These problems are summarized as follows:



1. Leaks at seals, gaskets or diaphragms for access ports, viewing windows or glove ports. 2. Leaks due to punctures, tears, permeability of gloves (integral with canister). 3. Leaks through large diameter O-rings or seals at mating flanges of canister segments. 4. Leaks from punctures or penetrations of the metallic canister segments. 5. Leaks at bulkhead fittings for pressurizing gas (sterile conducting medium). 6. Leaks at fittings for tiedown and separation of lander. 7. Leaks at potting or mechanical bulkhead fittings for electrical wire harness. 8. Leaks at bolts or rivets through canister walls.

FIGURE 13.—Leak sources. Typical canister.

(1) Types and sizes of leaks likely to occur on the canister
(2) Methods of leak detection and location appropriate for use under realistic service conditions
(3) Biologically acceptable repair methods and procedures
(4) Test techniques for verification of leak repairs

The possibilities for contamination have not been removed after the sterilized spacecraft has been launched. Numerous sources of contamination during transit exist. They include contamination from particle impingement from the orbiter and canister and, in particular, from —

(1) Squib-actuated devices
(2) Solid propellant engines
(3) Cold gas attitude-control systems
(4) Outgassing from coatings, greases, paints, etc.
(5) Puncture by micrometeorites

These potential hazards may constitute the weak link in an otherwise well-constructed chain unless provisions are made early in the design cycle to eliminate them. The exterior surface of the canister is contaminated by definition. Prior to separation, it may be desirable to increase its internal pressure slightly, perhaps with a biocidal gas, in order to prevent contamination during separation.

Separation

For this discussion it is assumed that the bus is decontaminated, not sterilized, and is directed in such manner that it will not contact the planet. Contamination of the planet could then occur only through contamination of the lander or from nonsterile portions of the spacecraft bus which are inadvertently placed on an impacting trajectory. Contamination in flight can occur whenever nonsterile particles from the bus or canister exterior impinge on the lander. These factors argue in favor of leaving the canister sealed until just prior to Mars entry.

The first step in the separation sequence is to open the canister and jettison the cover. Separation can occur by explosively severing a V-band clamp. The use of V-band clamps is not the only separation technique that can be used, however. When the capsule is sealed in its sterilization canister, the interface between mating sections of the canister must be leakproof. At some point the canister must be opened and jettisoned, and it is customary to speak of the interface for opening it as if it is the same interface used to seal it. In the design of an actual sterilization canister, two separate interfaces are required: (1) one for sealing it on Earth, and (2) one for opening it in space, and there is no reason to constrain the design such that these two always coincide.

The employment of the shaped charge as a separation technique for mating elements has been evaluated. This technique permits mating at one interface and separation at another. The use of shaped charges for cutting large-diameter shells is well established. In actual practice, cuts are often made without shaped charges but with high-order cutting explosives such as a mild detonating fuse (MDF). This scheme permits the use of bolted or flanged connections for mating elements of a sterilization canister. At the interface, permanent seals can be made by using adhesives, braze alloys, or other materials to insure freedom from leaks.

After the canister has been opened, its cover must be deflected from an impacting trajectory, as must all items of loose hardware generated upon opening. This event can probably be accomplished

by using springs although, for very large canisters, rocket motors may be lighter.

Lander separation from the opened canister can be seen in best perspective by remembering that "There is no interface between the lander and the orbiter." In place of this single interface there are two mechanical interfaces: (1) the lander-canister interface, and (2) the canister-orbiter interface. The requirements for each of these interfaces are significantly different. The lander-canister interfaces, both mechanical and electrical, must be made within the canister. They must also be capable of being broken on command when approaching Mars. This implies explosively actuated components, which are actuated by a signal initiating from either the lander or the orbiter, which in turn is triggered from Earth. The interfaces between the canister and the spacecraft (mechanical and electrical) do not require separation.

The significant aspect of the lander-canister interface is the fact that it is inaccessible after the sterilization cycle. All disconnects are made on the inside of the canister; this includes structural and electrical disconnects as a minimum. Other disconnects may be required depending on the size and complexity of the lander.

Design Manual

The conclusions of this work are being summarized in a design manual (ref. 1) for use by spacecraft designers and engineers faced with the unique requirements imposed by thermal sterilization. The purpose of this manual is to collect in a single volume a body of information dealing with design problems which arise from the requirement to sterilize a planetary lander by heat in accordance with current NASA specifications.

In the preparation of the design manual it was assumed that users (e.g., design engineers) are interested in all aspects of design, manufacture, test, and installation of the flight vehicle. Accordingly, those aspects of sterilization problems other than design, but which affect spacecraft design, were considered appropriate for inclusion.

Because of the unique nature of the sterilization requirement, it is not sufficient for a designer to be familiar with the thermal and structural effects of the heat cycle on his component or system. He should also have some familiarity with microbiology and with biological cleanliness. Since the spacecraft may have to be compatible with chemical load reduction techniques as well as with the thermal sterilization requirement, a designer should have a working knowledge of the choices at his disposal.

The manual contains information which will be useful to

engineers faced with the task of designing a heat sterilizable spacecraft. It does not eliminate the need for standard reference works; however, information from standard sources was included wherever it was particularly applicable to problems caused by thermal sterilization.

Where possible, general design guidelines have been formulated. Since planetary spacecraft have not yet become standardized, these guidelines are applicable to typical vehicles only and they require judicious modification in specific cases.

Conclusions

One of the major contributions of this study was the preparation of a design manual (ref. 1). Engineers will find this manual useful when faced with the task of designing a heat sterilizable spacecraft.

Significant design guidelines which were obtained during the course of this study may be summarized as follows:

(1) The introduction of a sterile atmosphere into the containing canister is preferable to sterilization in a vacuum. The advantages gained include: shorter thermal rise times, the elimination of a 14.7-psi overpressure design requirement on the containing canister, and the elimination of an atmospheric containment hazard caused by leaks at the interface between mating sections.

(2) The analysis of the transient thermal behavior of the spacecraft during the sterilization cycle is more complicated than the thermal analysis for the space-flight condition because of the convection effect of the canister filling gas.

(3) Present analytical techniques are adequate for the prediction of stresses after the local thermal environment has been defined.

(4) Two types of stresses are caused by thermal sterilization: stresses due to different thermal expansivities of adjoining structural members and transient stresses due to thermal gradients in the vehicle. The first stresses are a maximum and the second stresses disappear for the steady-state heat soak condition.

(5) For typical spacecraft the first stresses mentioned above are a multiple of the second stresses. The largest stresses during thermal sterilization occur at the interfaces of stiff mating members which have different thermal expansivities.

(6) Stresses due to the sterilization environment are not likely to be critical for structural members which carry high inertia loads during boost and maneuver conditions. They probably are critical for members which carry their full design load before boost (e.g., preloaded separation rings, pressure vessels, etc.).

(7) The maximum permanent distortions of a sphere-cone planetary entry vehicle tested were less than ½° of arc. This does not appear to be critical for Martian landers presently under consideration.

(8) The heat shield acts as a major thermal barrier which retards both heating and cooling. This is particularly true for spherical landers. The use of nonmetallic materials in structures and/or impact limiters aggravates this problem.

(9) Techniques for improved, forced convective heat transfer within the canister and the spacecraft are essential to the promotion of rapid uniform heating and cooling during terminal sterilization.

(10) No joints or joining techniques need be rejected solely on the basis of incompatibility with terminal sterilization. Every type of joining technique can be made acceptable from a sterility standpoint by careful planning and controls.

(11) Design for thermal sterilization is consistent with good present spacecraft design practices, if special attention is paid to details concerning heat transfer and relative deformations.

(12) Biological tests show that the use of highly polished or finely machined surfaces does not significantly improve the ease of sterilization of a spacecraft.

(13) Canister design presents a major task. It strongly affects the lander design and vice versa, particularly with respect to configuration, separation mechanism, and thermal control.

(14) Most structural materials can be used in sterilizable spacecraft provided their thermal properties and the degradation of their mechanical properties are accounted for.

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Sterilizability of Scientific Payloads for Planetary Exploration

A brief description is given of the methods employed by the National Aeronautics and Space Administration (NASA) in the selection of scientific payloads for planetary missions. An analysis has been made of the sterilization problems facing experimenters in connection with several instruments that could be included in a planetary-landing scientific package. The solutions to these problems have been discussed rather briefly, but enough detail has been presented so that others working on similar problems will know where to obtain the best available information.

Instrumented scientific payloads are the essence of our unmanned flight programs designed to acquire fundamental information about the planets; such information will include the physics and chemistry of the planetary surfaces and interiors and other environmental characteristics, including the possible existence and nature of extraterrestrial life. Because of the high cost of planetary exploration as well as the technological problems of instrument development, extreme care must be taken to insure that our scientific payloads are as systematic and methodologically sound as possible. There are many aspects of an experiment which must be taken into consideration in judging its scientific value as compared with that of alternative experiments. Several useful criteria for evaluating experiments to be landed on a planet are: the criticalness of the questions to be answered, the planning value of the data for future experiments, various factors of complementarity with other instrumentation, the reliability of the experiment and its instrumentation, and the sterilization problems associated with an experiment.

Current methods of space investigation employ multiple, independent, preprogramed experiments. It should be realized that there is a fundamental difference between the manner in which scientific research is conducted in terrestrial laboratories by means of ordered sequential investigations building on preceding results and the current methods of space investigation employing individual preprogramed experiments. It may be necessary in the future

to employ a completely new approach to the scientific exploration of the planets. Such an approach would be a recognition of the need for carrying out scientific investigations with computer-managed laboratories rather than with landing capsules comprising a collection of discrete experiments. One such laboratory under feasibility study is the automated biological laboratory (ABL). This point is made so that the focus for the problems of the sterilizability of scientific payloads can be properly oriented.

At the present time a selected experimenter for a flight mission is required to produce, in accordance with mission specifications, acceptable hardware for incorporation into the flight payload or to work with a NASA center in the production of flight instrumentation for his experiment. Ultimately, it will be necessary to have all instrumentation responsibilities centered in the group which produces the computer-managed laboratory. In this paper the approach to the sterilizability of scientific payloads is on the basis of individual experiments proposed either by NASA centers, by qualified academicians, or by members of the aerospace industry.

While a flight experiment is being developed it passes through several model stages about which there must be mutual understanding of terms. Table I defines these instrument models as they are used in the context of this paper.

The experiments that will be of interest in planetary exploration will involve the interplay of several scientific disciplines. Table II lists a few life-detection experiments as well as some environmental experiments that bioscientists suggest for consideration in scientific payloads for planetary landing missions. The inclusion of all these experiments would not necessarily be practicable in any given payload, for the makeup of any payload must be on the basis of the factors given previously, such as criticalness, reliability, and sterilizability.

When NASA has decided that a particular space exploration mission is to be undertaken, there is a definite procedure for the selection of the specific experiments for each of the mission flights. Table III lists briefly the sequential steps that are taken in choosing a flight mission payload. This procedure is culminated by the final selection of a mission payload by the Space Science Steering Committee and the approval of the payload by the Associate Administrator for the Office of Space Science and Applications of NASA Headquarters. This procedure allows a wide spectrum of experiments and experimenters to compete for space and weight in the payload. The experimenters for a payload are not restricted to those who have had previous NASA support in the develop-

TABLE I.—*Definitions of Instrument Development Stages*

Stage (status)	Definition
Functional breadboard model	Functional feasibility of experiment must be demonstrated with model, which need not meet flight weights and sizes
Flight-size breadboard model	This instrument must meet requirements of previous model and must meet flight restrictions for weight and size
Engineering prototype model	This model approximates flight hardware configuration and is designed to satisfy environmental and operational constraints
Type-approval model	A model built in final flight configuration and subject to modification during testing program
Temperature-control model	This model need not be a functional instrument; it has same size, shape, and thermal characteristics as flight instrument
Structural test model	A dummy model that has exactly the same mechanical characteristics as those of flight model
Proof test model	This unit is identical to flight model but is needed for final proof of design
Flight model	This hardware is launched as part of spacecraft payload

TABLE II.—*Experiments of Interest for a Program of Planetary Exploration for Detection of Extraterrestrial Life*

Environmental experiments	Exobiological experiments
Characterization of organic matter	Detection of macromolecules
Determination of surface H_2O	Detection of increase in macromolecules
Analysis of atmospheric components	Growth and metabolism; measured by CO_2 evolution
Ionic constituents and pH of surface matter	Detection of catalytic activity
Radiation (ultraviolet)	Detection of optical activity or measurement of optical isomers
Surface and subsurface temperatures	Detection of porphyrins
Atmospheric temperature and pressure	Soil respiration

TABLE III.—*Brief Outline of Present Method of Selecting Experiments for Flight Payloads by Office of Space Science and Applications (OSSA)*

Step	Function
1. Proposals: (a) Unsolicited (b) Solicited	Scientific community can propose experiments at any time or respond to AFO (Announcement of Flight Opportunity)
2. Subcommittees of Space Science Steering Committee (SSSC)	Review, evaluation, and recommendations
3. Chairman of SSSC and responsible program office director	Subcommittee chairman reports actions
4. Cognizant NASA Center for project management	Evaluation of technical feasibility and spacecraft integration problems
5. Director of Sciences, OSSA Space Science Steering Committee	Review and recommendations of proposed payload
6. Associate Administrator, OSSA	Approval of payload

ment of their experiments prior to the announcement of the flight mission. It is understandable that those with past experience in flight experiment development or those who have flown other flight missions will have more experience in both writing the necessary details of their proposed experiments and in conveying their thoughts and ideas to the various committees involved in the selection of the scientific payload.

The sterilization requirements for scientific instrumentation apply only to those items to be landed on a planet's surface, and it is on the forthcoming Martian exploration missions that our attention must immediately be focused. In June 1965, the Voyager Program Office made a preliminary announcement of flight opportunities (AFO) for the 1971 Voyager mission to Mars. This preliminary AFO contained a brief mention of the sterilization requirements that the landed Voyager experiments must experience: "All Voyager 1971 experiments must meet the planetary quarantine constraints. Landed capsule experiments must meet stringent sterilization requirements. Advice and assistance in meeting sterilization requirements will be rendered by NASA to prospective experimenters." The actual dry-heat sterilization cycle that the scientific instrumentation must undergo has not been defined at this time, but will be chosen from the table of spacecraft sterilization times described by Bruch in the paper in these proceedings entitled "Dry Heat Sterilization for Planetary-Impacting Spacecraft." The final selection of a sterilization cycle for the Voyager capsule will be made after the capsule and its

scientific payload have been better defined and the effect of the sterilization cycles on the equipment more fully assessed.

The sterilizability of scientific instruments for planetary landing missions has been a subject of concern at NASA Headquarters for the past 2 years. In July of 1964, I was given the responsibility of pushing the development of sterilizable bioscience experiments and accompanying instrumentations. Don Easter of Lunar and Planetary Programs was assigned the monitoring of the development of sterilizable instrumentation for physical science experiments.

A detailed memorandum about the sterilization requirements for experiments and approaches to solving sterilization problems was sent to nine experimenters funded by the Bioscience Programs Division. The letter to the experimenters asked for knowledge of the compatibility of chemical reagents, nutrient media, reagent (media) cells, sample (cell) holders, light (lamp) sources, mirrors, prisms, optical trains, beam-choppers, phototubes, electrodes, motors, wiring, plastic tubing, galvanometers, microammeters, photomultipliers, and so forth, that are used in their experiments and instrumentation with the dry heat and ethylene oxide cycles specified in the memorandum. The sterilization specifications were briefly summarized in the memorandum as follows:

(1) All planetary-impacting hardware will be subjected to terminal dry-heat sterilization in an inert atmosphere at a temperature of 135° C for 22 hours.

(2) All prototype hardware must pass qualification heat trials (heat compatibility tests) in an inert atmosphere at 145° C for 36 hours for each of three cycles (total time of 108 hours).

(3) All prototype hardware must be tested for its compatibility with ethylene oxide (ETO) as contained in gaseous mixtures of ethylene oxide and Freon 12. The parameters for the ETO compatibility tests are 500 ± 50 milligrams ETO per liter of space, temperatures of 24° C and 40° C, relative humidity in the range of 30 to 50 percent, and time periods of 24 hours for each temperature cycle.

The prospective planetary mission experimenters were given the choice of responding either by letter, by phone, or by request for an official visit. The response to the request for information was slow. Although the investigators had known that planetary-impacting hardware would be sterilized, a majority did not expect a solution to the sterilization problem to develop from their efforts. A general feeling prevailed that some group with NASA would solve the difficulties inherent in the sterilization of exobiological instrumentation. Many felt that sterilization should be a part of

the total environmental testing (heat, ethylene oxide, acceleration, vibration, thermal vacuum, and shock) for the specified flight missions.

The majority of these bioscience experimenters were unable to comment on the sterilizability of the electronics or other engineering items in their instrumentation. Their meager responses did uncover problem areas relative to sampling devices, plastic seals, lubricants, electrodes, dialysis membranes, chemical reagents, and biological media. Analysis of these items indicates that some of them are inherently heat labile and that sterile insertion must be used if they are to be included in the scientific payload. Because the adequacy of sterile insertion techniques is still undefined, this sterilization procedure remains to be accepted by NASA Headquarters. At this time, terminal dry heat is the only approved procedure for spacecraft sterilization.

The difficulty of obtaining a response from the bioscience experimenters required some review by our office. One can appreciate the feeling of the experimenters who do not want to become involved with hardware programs unless they are certain that their experiments will be selected for a defined flight mission. In order to avoid unnecessary expenditures of time and funds, it has been necessary that the sterilization requirement not be imposed too early in the experiment development program. This approach was satisfactory until about a year ago when it became obvious that some group within NASA must act as a knowledgeable source for the disbursement of information to experimenters requesting help. Consequently, the Materials Section at the Jet Propulsion Laboratory (JPL) was encouraged to act as a consulting group on sterilization matters to NASA experimenters. This arrangement was to continue until the Voyager project could take over the sterilization responsibility for its scientific payloads.

In February 1965, the Materials Section of JPL contacted several potential Voyager experimenters at JPL as well as several bioscience experimenters funded by NASA Headquarters. The letter to the experimenters requested the following information:

(1) A list of all the materials which are presently used or are projected for use in their life-detection experiments
(2) A list of components which were in common use in their experiments (these components were to be analyzed for their materials content)
(3) An up-to-date set of drawings for each instrument.

Also enclosed to the experimenters was a list of anticipated materials problems for each of the experiments as presently understood in terms of the instrumentation for that experiment.

The following materials problem areas were suspected to exist for the Wolf Trap life-detecting device under development by Vishniac and Weston at the University of Rochester (ref. 1) and subcontracted to Ball Brothers Research Corporation for prototype hardware fabrication:

(1) Circuitboards, potting compounds, conformal coatings, solders, contacts, and insulation for electronic components and modules
(2) Heater components
(3) Gimbal mount lubricants
(4) Container seals
(5) Seal for syringe hole for nutrient loading
(6) Tubing for high pressure
(7) Filter mesh for pickup sphere
(8) Lens seals
(9) Seals or gaskets for explosive actuators

The aforementioned life-detection experiment has been chosen to illustrate the development of sterilizable instrumentation. The contractual requirements for the Wolf Trap hardware call for a passive thermal soak of one cycle for 24 hours at 145° C. This cycle has been given to one breadboard model that was delivered in 1964, and it will be given to one engineering model to be delivered in December 1965. This engineering model is the first approach to flight quality hardware and will be capable of limited environmental testing. No requirement exists in the Wolf Trap development effort for ETO decontamination.

In response to the JPL request for materials information, the Ball Brothers Research Corp. furnished a list of materials used in the Wolf Trap instrumentation. A condensation of the JPL analysis of these materials is presented in table IV. It would behoove other potential experimenters to look at this inventory and to ascertain for themselves the type of information that can be furnished by the Materials Section at JPL.

Further information about the sterilization status of the Wolf Trap instrumentation is given in table V, which lists the passive heat sterilization history of the end items to be used in the engineering model from unpublished data by Buckendahl. It is estimated that 60 percent of the listed components are "off the shelf" items produced to manufacturers' specifications. The remaining 40 percent are Ball Brothers approved parts purchased in accordance with a specific source control drawing; these parts have been subjected to extensive testing, including power age. All the items that failed were "off the shelf." Of the 163 transistors listed under engineering model end items, 111 were devices that

TABLE IV.—*Comments by JPL Materials Section Regarding Materials Actually Used in Development of Sterilizable Wolf Trap Instrumentation*

Materials	Comment
Fluorocarbon (tubing)	Tendency to cold flow; undesirable quantities of volatile materials released by heating
Vinylidene fluoride (O-rings and seals)	Suspected incompatibility with Freon 12
Metals and ceramics	Not affected by sterilization
Epoxy resin	Recommended that only 2 specific catalysts be considered for use; volatile recondensables are a problem
Silicone elastomers	Recondensable material lost under vacuum exposure at high temperatures
Glass fabric-epoxy resin printed wiring board	Many epoxy-glass circuitboards do not pass a sterilization or volatile-condensable materials tests

had been used in the breadboard model. The causes of failure as listed in table V represent only the consensus between the design group and the reliability group. The indicated failures are catastrophic failures which would deviate instrument performance from stated specifications.

Another device which is undergoing intensive testing for sterilizability is the gas chromatograph (GC) being developed at JPL (from unpublished data by Bowman and ref. 2). Gas chromatography can be used to analyze anything that can be put into a stable gaseous state. It is ideally suited for atmospheric and surface analysis of a planet. The development of a flight-type GC has been directed toward perfecting individual components that will meet the sterilization requirements (dry heat and ETO) and also will survive three mutually orthogonal planes of impact at 10 000 g's.

The evaluation of the electronic components in the GC is being carried out primarily by the JPL component parts qualification group. The electronic components are selected from parts types evaluated in the JPL electronic parts sterilization program. Preliminary investigations indicate that tantalum and metalized Mylar capacitors will need more extensive evaluation, and possibly more development, to assure meeting the environmental requirements. Mylar capacitors are an acceptable electrical substitute for metalized Mylar capacitors. These substitute items are consider-

TABLE V.—*Wolf Trap Electronic Component Passive Heat-Sterilization History*

Component type	Number of engineering model end items, heated for 1 cycle at 160° C for 4 hr and 1 cycle at 160° C for 20 hr	Total failures
Resistors:		
Hot molded carbon composition	349	0
1% metal film	127	0
1% carbon film	4	0
Power wire wound	8	0
Capacitors:		
Silver-mica	20	0
Ceramic	25	0
Solid tantalum oxide	2	0
Diodes:		
Silicone general purpose and zener	60	0
Transistors:		
Silicone NPN and PNP	163	1
Field effect	10	1
Relays:		
Miniature TO–5 can	15	1

ably larger in size and are therefore less desirable for an instrument of high-impact tolerance. The other GC electronic components do not appear to pose unusual environmental problems (ref. 2).

All the GC mechanical components are being individually developed because commercial components do not exist or the existing ones will not pass either high-impact or sterilization tests. In general, Teflon, stainless steel, and aluminum are used in the design of the mechanical components for the gas chromatograph. Some of the problem areas and their solutions that have been encountered in the development of a sterilizable gas chromatograph are given in table VI.

A third device that is relatively advanced in development for sterilizability is the planetary atmospheric mass spectrometer that has been assembled at the Goddard Space Flight Center. This instrument weighs approximately 7 pounds and measures the discrete masses associated with argon, carbon dioxide, nitrogen, oxygen, and water. The temperature stability of the RF oscillator circuitry posed a serious sterilizability development problem. Temperature-stable coil core materials were necessary in order to prevent temperature drift following sterilization. Furthermore, field effects transistors had to be substituted for the electrometer tubes, which were degraded by the heat treatment. This mass

TABLE VI.—*Problem Areas in Design of Sterilizable Gas Chromatograph*

[Unpublished data of Bowman]

Problem area	Solution
Tantalum capacitors	Used foil tantalum capacitors voltage derated 30 percent in addition to normal-use design derating
H_3 detector ionization source	Investigated H_3 loss at elevated temperatures
Carrier gas pressure regulator	Care in mechanical design and selection of materials reduced differential expansion problems
Plumbing connections	All connections are solid encapsulated in a manifold system in chassis; Viton A O-rings seal individual components to manifold
Sample valve	Completely contained Teflon valve face
Carrier gas tank	Tank was designed for test pressure 2.5 times greater than nominal pressure at room temperature
Adhesives	Use of high-temperature epoxy adhesives
Low-temperature shielded cable insulation	Use of Teflon insulated cable
Nylon parts (possible ETO incompatibility)	Parts fabricated from Teflon or diallyl phthalate GDI–30

spectrometer, as well as several pressure gages for measurements of planetary atmospheric pressures in the range above 50 torr, have been heat treated at 145° C for 48 hours for several cycles by the Goddard group.

In August 1964, the Bioscience Programs Office of NASA Headquarters initiated a feasibility study with the Aeronutronic Division of the Philco Corp. for an automated biological laboratory (ABL) as a scientific payload for a planetary landing capsule. As part of that feasibility study, the effect of thermal treatment on typical reagents, growth media, and equipment used in life-detection experiments was examined (ref. 3). The objective of this task was to explore the degree to which sterilization incompatibility may be expected for materials and reagents typical of those that would be used on experiments for an ABL type of payload. Although all the details of this study cannot be presented in this paper, a brief analysis is given in the following paragraphs.

Tests were made on reagents and materials in glass containers with and without added coupons of Teflon, or Teflon plus tantalum. The coupons represented interfaces which would occur in packaging the various materials. For these assemblies of materials and interfaces, alternative approaches to achieve sterilization compatibility may exist, but these alternate designs were not tested in this particular program. Observations were made on the immediate effects of the thermal treatment, the effects which occurred during 5 months of storage after thermal treatment, and the influence of the packaging materials and added coupons on the materials immediately after thermal treatment and also after the 5-month storage period (ref. 3).

Table VII summarizes concisely a few of the data that have been obtained in these sterilization studies for an ABL. Very severe degradation was noted with the growth media and with the dialysis membranes. It is apparent that serious development work

TABLE VII.—*Dry-Heat Sterilization*[a] *Compatibility Testing of Materials and Reagents Typical of Those That Could Be Used on an Automated Biological Laboratory*

[From final report on Contract NASw–1065]

Materials	Results
Microbial growth media:	
Dry	Sugar- and/or protein-containing media were charred
Wet	All protein- and/or sugar-containing media were severely deteriorated
Phosphate assay reagents:	
Dry	Turned faintly yellow
Wet	Ammonium paramolybdate reagent formed crystals
Water	No effects
Ion-exchange resin—wet	Retained normal capacity
Dialysis membranes:	
Dry	Brown and crisp
Wet	Remained colorless, but lost all strength
Thiocarbocyanine dye:	
Dry	Crystals failed to dissolve completely; new absorption maxima obtained in protein reactions
Wet	Decolorized
Packaging materials—wet	No effects on tantalum or Teflon; Viton B compression disk was deformed

[a] Dry-heat sterilization was 24 hours at 135° C or 145° C.

is necessary if these materials are to meet our dry-heat sterilization cycles. If the adequacy of sterile insertion techniques can be proven, the use of alternate types of sterilization procedures could be considered in order to allow these items on the payload.

Other techniques that have been suggested for use in sterilization of scientific payloads include sterile assembly, ionizing radiation, filtration of fluids, and chemical sterilants for fluids. All these procedures must meet the same probability requirements for sterility as those specified for dry heat. It should be emphasized that development efforts are underway with some of these techniques to establish their adequacy in meeting planetary quarantine requirements.

Although the preceding paragraphs have described the development of sterilizable instrumentation with the supporting research and technology funds of several program offices, it is obvious that this type of effort must be undertaken by the Voyager project in the near future. Just how the Voyager project will support the development of sterilizable scientific instrumentation has not been specified. As was indicated in table III, the selection of the flight payload will be the first step before money can be expended for the development of Voyager mission experiments. Meanwhile, it is mandatory that efforts be continued to isolate various sterilization problem areas so that attempts at solutions can be undertaken even before the Voyager project becomes fully engrossed with this problem. The Bioscience Programs Office further requests that anybody who has problems in the development of sterilizable scientific instrumentation for planetary payloads should contact either NASA Headquarters or the knowledgeable groups at JPL. Although the extent of the service that can be given is rather limited at this time, the individuals will certainly be given the best information available and will be directed to the best sources of information regarding engineering developments useful in achieving solutions to their problems.

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Manufacturing and Handling Procedures for Planetary Spacecraft Which Are To Be Sterilized by Heating

The work described in the present paper was performed by the General Electric Co. for the George C. Marshall Space Flight Center of the National Aeronautics and Space Administration (NASA) under contract NAS 8–11372. This work was performed in advance of any particular mission or application, and it has not been submitted to, nor approved by, either the Voyager Project Office or the Office of Bioscience. The aim of this work is the preparation of procedures for the manufacture, assembly, and handling of typical planetary spacecraft which are to be sterilized by heating. These procedures are to cover the full cycle of the spacecraft from the receipt of raw material through vendor procurement, manufacture, assembly, test, transportation, sterilization, checkout, and prelaunch. Since the contract is less than one-half completed, this is only a progress report and no final conclusions have been drawn.

Bioclean Manufacturing, Assembly, Test, Checkout, and Launch

Manufacture and Assembly

Bioclean manufacture is required so that the number of organisms on the lander-canister assembly, prior to the terminal sterilization, is sufficiently small to assure reliable sterilization under the NASA-specified time-temperature regime outlined on July 21, 1965, in a letter from the Special Assistant for Planetary Quarantine to the Director of Lunar and Planetary Programs, NASA. However, not all piece parts and components have to be manufactured under bioclean conditions. Those which have been shown to be reliable after repeated exposure to thermal sterilization or which can be satisfactorily cleaned by chemical means may be manufactured by conventional methods and then sterilized or cleaned (biological load reduced or bioload reduced) before assem-

bly into the next higher echelon of the lander. The determination of when the above conditions are satisfied is an engineering function, but it is of vital importance to the manufacturing process. In fact, it has such far-reaching consequences that it should be carefully considered during the design of the lander in order to reduce the number of components requiring bioclean manufacture.

In the discussion which follows, components that cannot withstand repeated exposure to elevated temperatures are called "heat sensitive." It is these heat sensitive items toward which the efforts of contamination control must be primarily directed.

Each heat sensitive assembly may, in turn, be made up of components which are heat tolerant and those which are heat sensitive. Again, the heat tolerant components do not present too great a problem. They can be bioload reduced by heat. The heat sensitive components, however, must be handled in a bioclean manner at all times until they can be encased in a biobarrier. Similarly, heat sensitive components are made up of heat tolerant and heat sensitive parts. The former are manufactured in a conventional manner, and the bioload is reduced by heat before assembly with heat sensitive parts into a heat sensitive component. The latter are manufactured in a bioclean environment throughout.

The decision of whether to make or buy a part is based upon an evaluation of a number of factors such as vendor capability, capacity, bioclean manufacturing facilities, reliability, cost, and delivery. The decision in the case of a heat tolerant item remains essentially unchanged. However, the imposition of a bioclean requirement on a heat sensitive item is a significant deviation from usual vendor requirements and is treated in more detail in a later section of this paper.

Test

Bioclean manufacture requires that the degree of contamination be reliably established before manufacturing operations are performed. The operations themselves must be performed under controlled conditions, and evidence must be provided to this effect. The buildup of any assembly is interspersed with subsystem checkout tests. These tests must be performed with bioclean connections to the flight equipment so that the actual test equipment can be located outside the bioclean area. Bioassays will be required at key assembly points; they constitute additional tests to be performed within the assembly cycle. After completion of the lander assembly and prior to its enclosure in the canister, an initial

systems test must be performed. It includes electrical, pneumatic, and mechanical compatibility tests and system performance tests. Compatibility tests with the operating support equipment are also required.

All of these tests may either be performed in a bioclean room with the test equipment located in an adjacent area which need not be bioclean or be performed in a nonbioclean quality control and testing area, provided the lander is enclosed in a protective shroud during the tests and during transport to and from the test area. If the latter procedure is followed, the following novel steps are added to the assembly sequence: (1) enclose lander in shroud prior to transfer from the bioclean assembly area to the nonbioclean test area, (2) clean exterior of shroud (preferably in an airlock) before return to the bioclean area, and (3) remove shroud.

After checkout the lander will be inserted and sealed in the canister (biobarrier), and the integrity of the canister will be verified. The lander-canister assembly will then be tested under vibration followed by a performance verification. Thermal vacuum tests must then be performed followed by a complete systems test. It does not appear feasible to perform either the vibration test or the thermal vacuum test in a laminar flow clean room. Therefore, the previously outlined procedure for transport to and from the nonbioclean test areas must be employed. At the conclusion of thermal vacuum testing, provisions must be made to release sterile, or at least bioclean, gas into the canister at a controlled rate to avoid contamination, excessive pressure, or condensation.

The integrity of the canister must be checked repeatedly at various stages of the assembly and test cycle. At the very least such checks must occur after initial assembly, after enclosure of the lander, after vibration testing, and after thermal vacuum testing.

Checkout and Launch

Sterilization at the launch site appears to be preferable to sterilization at the assembly site (ref. 1). This requires that the canister serve as the shipping container for the lander, that a separate shipping container be manufactured which will maintain the lander in a bioclean condition from the manufacturing site to the launch site, or that the lander canister assembly be encased in a special shipping container. The usual flow plan at the launch site must be altered to accommodate the sterilization requirement; a study of this is in progress but will not be reported in this paper.

Pilot Assembly Operation

A spacecraft pilot assembly operation will be performed inside a bioclean room in order to evaluate the problems caused by the

requirements for biocleanliness of planetary landers. Two operations subsidiary to the complete pilot assembly have been performed. One consisted of elementary electrical assembly operations both at a standard workbench and inside a glove box, with a comparison of the times required. The other consisted of assembling an electrical module under a variety of bioclean conditions and comparing the levels of biological contamination.

Glove-Box Operation

The following five operations were performed in a glove box on the commercial 19-pin, crimp-type, electrical connector shown in figure 1: (1) wires cut to length; (2) insulation stripped;

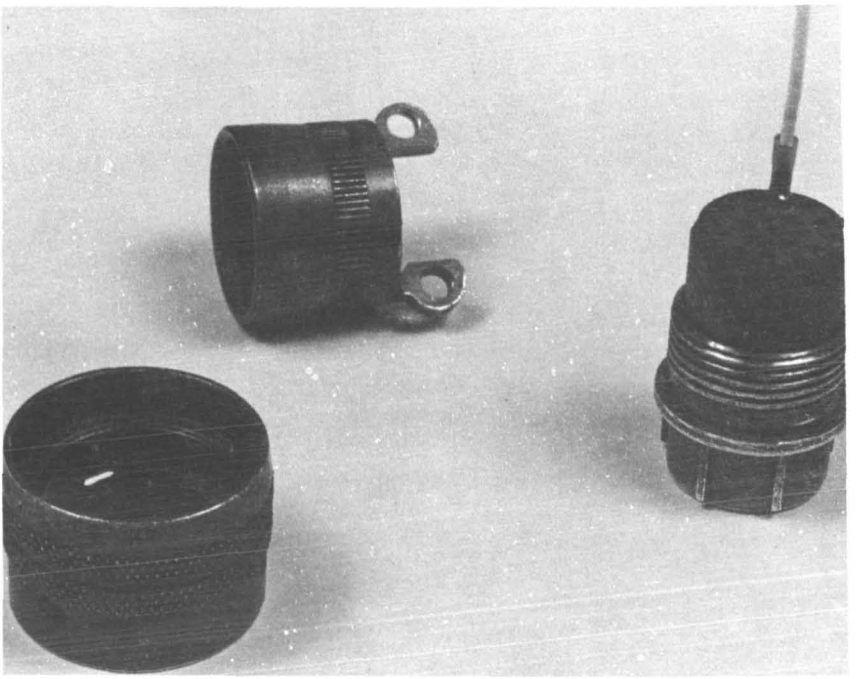

FIGURE 1.—*Crimp-type connector body.*

(3) pins installed and crimped; (4) pins inserted in connector; and (5) pins removed from connector.

The connector consisted of four major pieces: a two-part outer shell, a combination metal-plastic pin receptacle, and a snap ring. The shell and ring were simple in shape and had readily accessible surfaces, but the pin receptacle was complex and contained numerous hidden pieces. The metal-plastic structure of the connec-

tor has buried in it duplicate pieces of brass shaped so that they provide solder cups on one end of the connector and flexible receptacles at the other end into which crimped pins can be snapped; the number of these brass pieces depends upon the number of wires to be attached to the connector. The assembly operations required the insertion of parts, wires, and tools into all of these interstices, so there were many opportunities for depositing contamination in inaccessible crevices. This connector was therefore selected as representative of the types of hardware which might benefit from glove-box assembly.

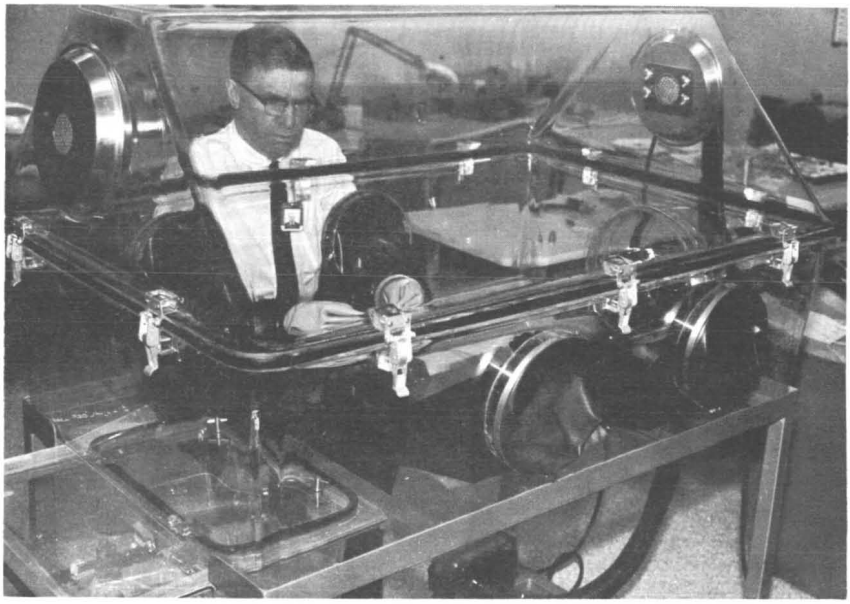

FIGURE 2.—Overall view of glove box.

The glove box used (see fig. 2) had an enclosure made of heavy plastic molded in two parts. The lower part provided a 48- by 30-inch working surface and four access ports. The upper part acted as a cover and was held in place by 10 toggle clamps which compressed a soft rubber gasket. A small blower produced a slight vacuum which assisted in the insertion of hands and arms into the gloves. Not all parts of the work area were within arm's length for the average operator; thus, in the absence of holding fixtures or restraints of some kind, parts could roll or slide on the smooth surface to a point where they were beyond reach. A more serious drawback in using this glove box was the distortion introduced by the thick, optically poor, molded plastic. This handicap became par-

ticularly noticeable during the use of the pin removal tool. From certain angles of viewing, the instrument appeared to be bent just as a pole immersed in water seems to bend below the surface. These drawbacks are cited to indicate that some of the retardation of motion was the result of using a glove box which had not been designed for this purpose (but for germ-free animal research) rather than the result of a barrier as such.

The major objectives in the glove-box operation were to (1) establish the additional time required to perform specific operations in the glove box; (2) ascertain in what manner and to what degree typical "transport" and "get" values employed in time and motion studies would have to modified; (3) determine the effect of the glove box on the execution of certain manipulations; and (4) observe and record the responses of the participants in the study of the glove-box situation.

The usual tools employed in the assembly of a crimped pin connector were used (fig. 3). They included side cutters, a

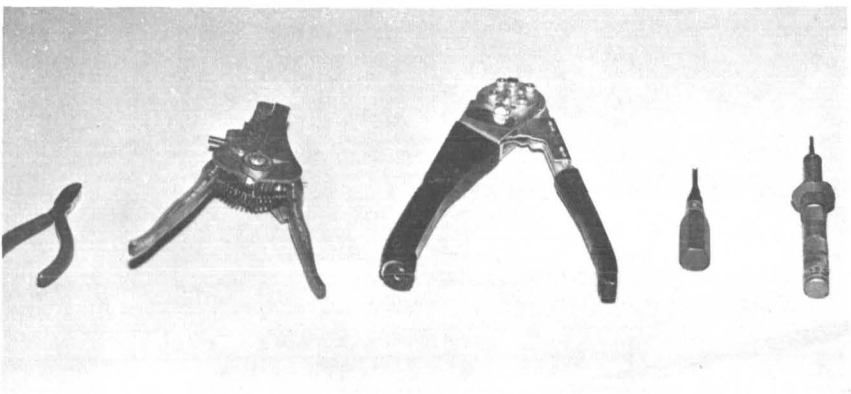

FIGURE 3.—Tools used in glove box operations.

mechanical wire stripper, a tool for the removal of the crimped pins from the connector, and tools for the insertion of the crimped pins into the connector. (Details of some of the operations are shown in figs. 4 to 7.) The same operator performed each operation six times on a standard open bench, and then six times inside the glove box. No account was taken of the time employed in setting up the work stations. This required little time on the open bench; the glove box, on the other hand, introduced additional manuevers such as stopping of the blower, unclamping, lifting, and displacement of the lid, return of the lid, reclamping, and restoration of the vacuum. The time needed to accomplish these steps as

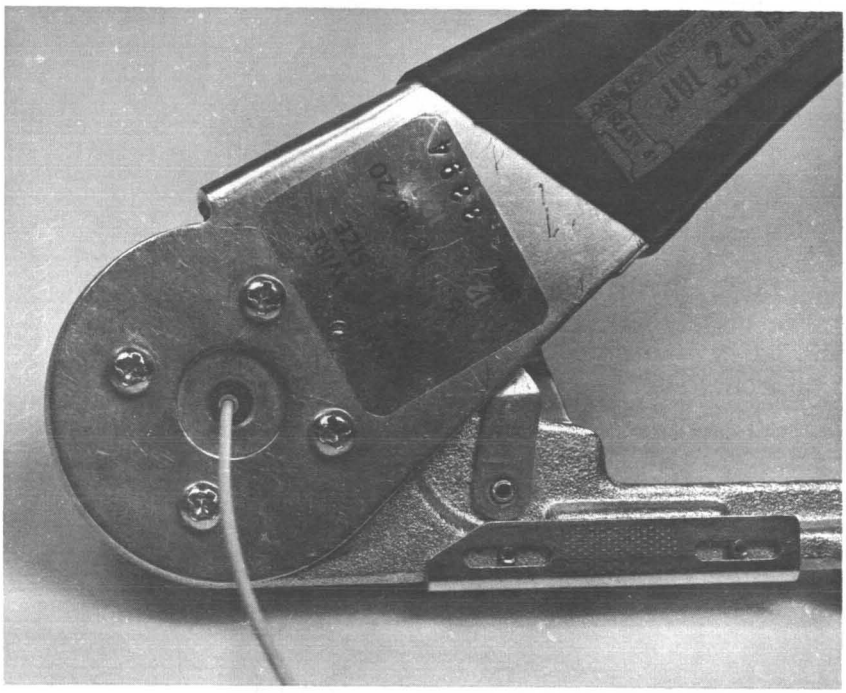

FIGURE 4.—Crimping tool with wire inserted.

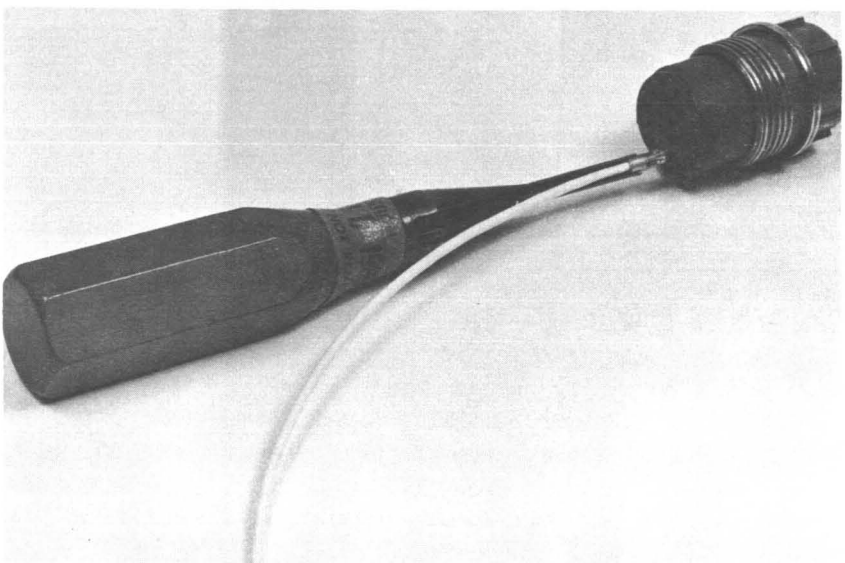

FIGURE 5.—Insertion of crimped pin into connector.

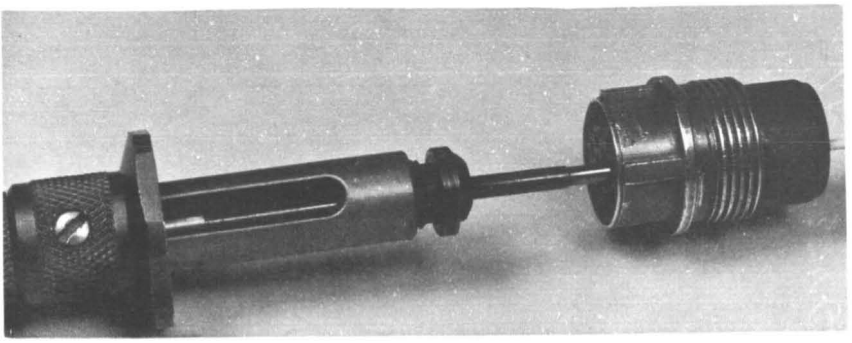

FIGURE 6.—Removal tool inserted in connector.

FIGURE 7.—Removal of pin from connector.

well as that to prepare the parts and tools so that they would not contaminate the glove box are not included in the following data.

Results of four operations performed on an open bench and in the glove box are plotted in figures 8 to 11. The figures show that a learning process occurred both on the open bench and inside the glove box, though the learning curve is very flat for the pin removal operation. Table I summarizes the results of all the curves. It shows that each operation took more time to perform in the

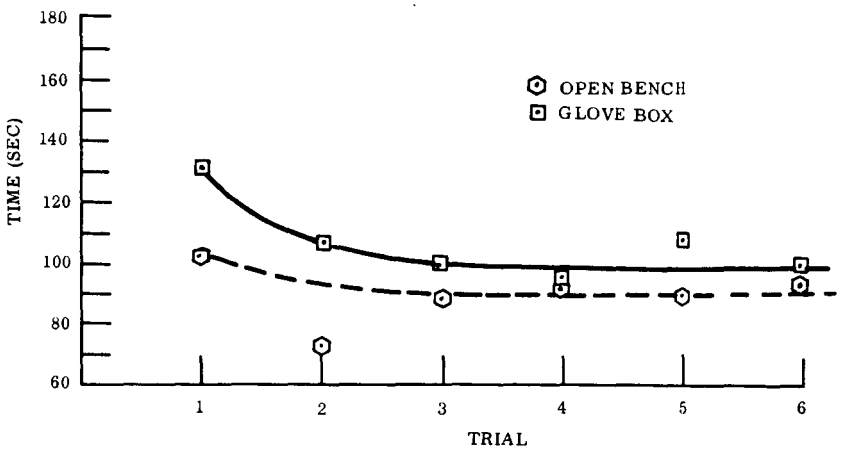

FIGURE 8.—Times for insulation stripping on open bench and in glove box.

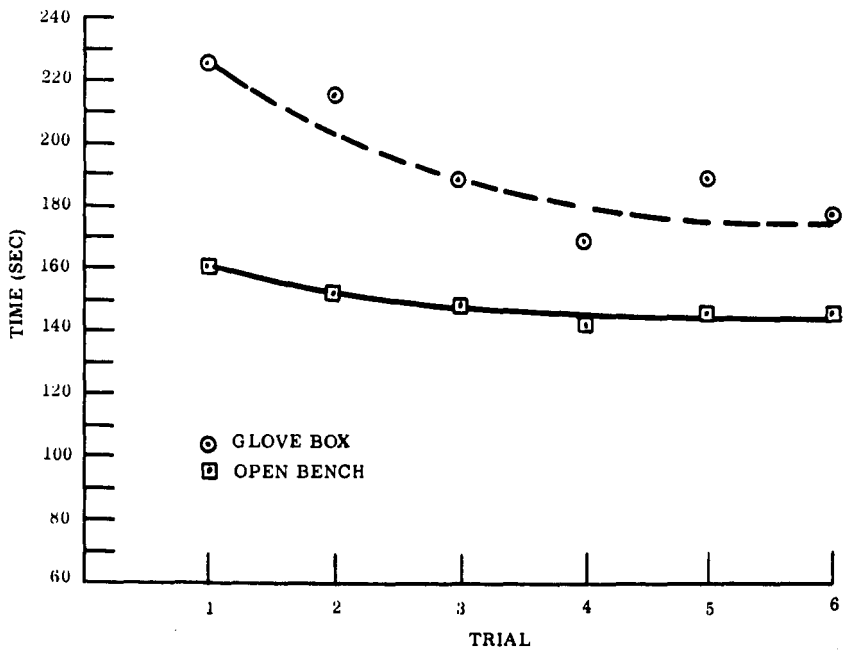

FIGURE 9.—Times for pin crimping on open bench and in glove box.

glove box than on the open bench. The increase ranged from 7½ to 58 percent if all six trials for each operation are averaged, and from 3 to 21 percent if the learning curve is extrapolated. The latter values are probably more significant. If the times required for the four operations are added (which may be representative of a

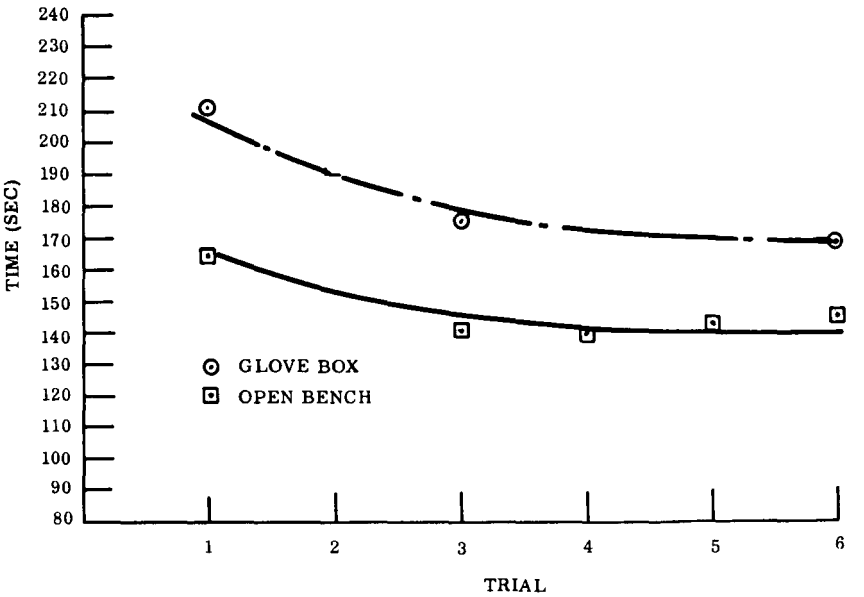

FIGURE 10.—Times for pin insertion on open bench and in glove box.

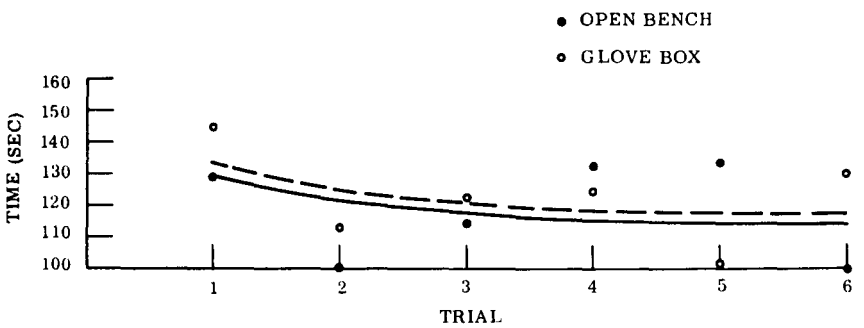

FIGURE 11.—Times for pin removal on open bench and in glove box.

TABLE I.—*Increase in Time Required To Perform Assembly Operations Resulting From Use of Glove Box*

Operation	Increase in required time when glove box is used, percent	
	Average of 6 trials	After learning
Strip insulation	58	10
Crimp pins	30	21
Insert pins	29	19
Remove pins	7.5	3
Total for 4 operations	29	14

connector assembly), the increase amounts to 14 percent after learning has taken place. This may be a typical figure to be used for elementary operations of this type. As mentioned above, it does not include the additional setup and cleaning time required for glove box operation, nor does it reflect increased operator discomfort and fatigue under glove box conditions. The total operating time for this experiment was too small to include those effects.

Operational time for the assembly of a crimp-type connector was secured by an entirely different approach. Nine separate individuals performed identical tests on an open bench and inside the glove box. Out of this group, two were experienced operators; the other seven were unfamiliar with the tools and procedures. Each in turn performed the 5 operations previously defined for a specified number of times which varied from 10 to 19. No provision was made for learning. The operator had no opportunity to practice to develop skill but simply familiarized himself with the various manipulations involved, and immediately undertook the performance of the prescribed tasks on the open bench. This approach is representative of actual conditions where an operator is asked to perform a task in a glove box which he previously performed on an open bench. The sequence chosen tends to decrease the time increments for glove box operation.

The results of this phase of the experiment are summarized in table II. The two experienced operators (Nos. 1 and 2) showed the largest percent increase in operational time because they performed the open-bench operations fastest. However, neither of the skilled operators was fastest for the glove box operations. The additional times required to perform operations inside the glove box are fairly consistent, with only a few exceptions. The average

TABLE II.—*Increase in Time Required To Perform Elementary Operations Resulting From Use of Glove Box*

Operator	1	2	3	4	5	6	7	8	9	Average of all trials	After learning
Increase in time required when glove box is used, percent:											
Cut wires	82	17	43	15	59	59	25	14	9	38	
Strip insulation	127	49	16	8	21	32	13	48	48	40	10
Install and crimp pins	179	76	88	18	56	51	24	13	21	64	21
Insert pins	56	41	9	25	24	21	50	9	−27	26	21
Total for 4 operations	115	57	37	18	39	38	29	17	8	43	

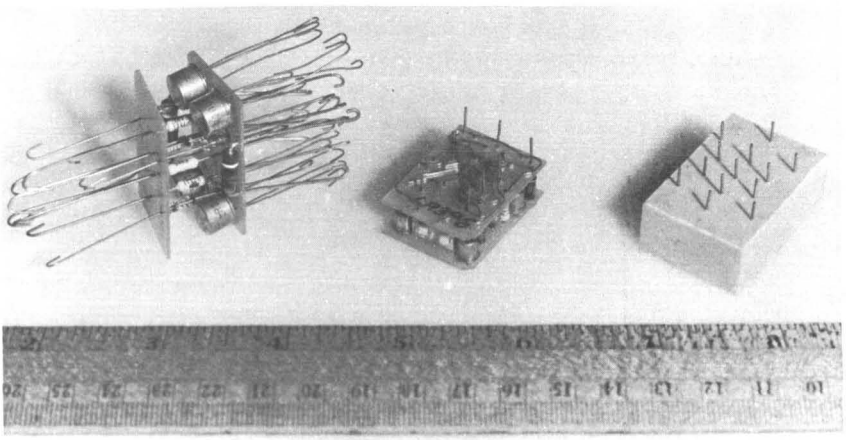

FIGURE 12.—Assembly of electronic module BX.

increase in required time is 43 percent, but this number decreases to between 10 and 21 percent after learning. These results bear out those previously presented.

Electronic Module Assembly

Five identical electronic cordwood modules BX (fig. 12) were assembled in five different ways, and their biological contamination was measured in order to determine the effect of different assembly methods on biological cleanliness. Each module consisted of six resistors, three diodes, three capacitors, two transistors, and six terminals. The components and terminals were mounted between two 1$\frac{3}{16}$-inch-thick glass epoxy boards spaced $\frac{7}{16}$ inch apart and were interconnected with 0.010- by 0.022-inch nickel ribbon by resistance welding. The tools required to perform the assembly are shown in figure 13.

The following procedures were ordered:

Module BX–1: Sterilize all parts. Recontaminate parts to contain approximately 10^6 spores of *Bacillus subtilis* var. *niger*. Assemble on open bench by standard process (fig. 14). Clean with fluorocarbon solution.

Module BX–2: Sterilize all parts. Recontaminate parts to contain approximately 10^6 spores of *B. subtilis* var. *niger*. Assemble on open bench by standard process. Clean with solution containing 5 grams of crystal phenol in 100 milliliters of fluorocarbon.

Module BX–3: Sterilize all parts. Assemble on open bench by standard process. Clean with fluorocarbon solution.

Module BX–4: Sterilize all parts. Assemble on open bench. Have operator wear finger cots.

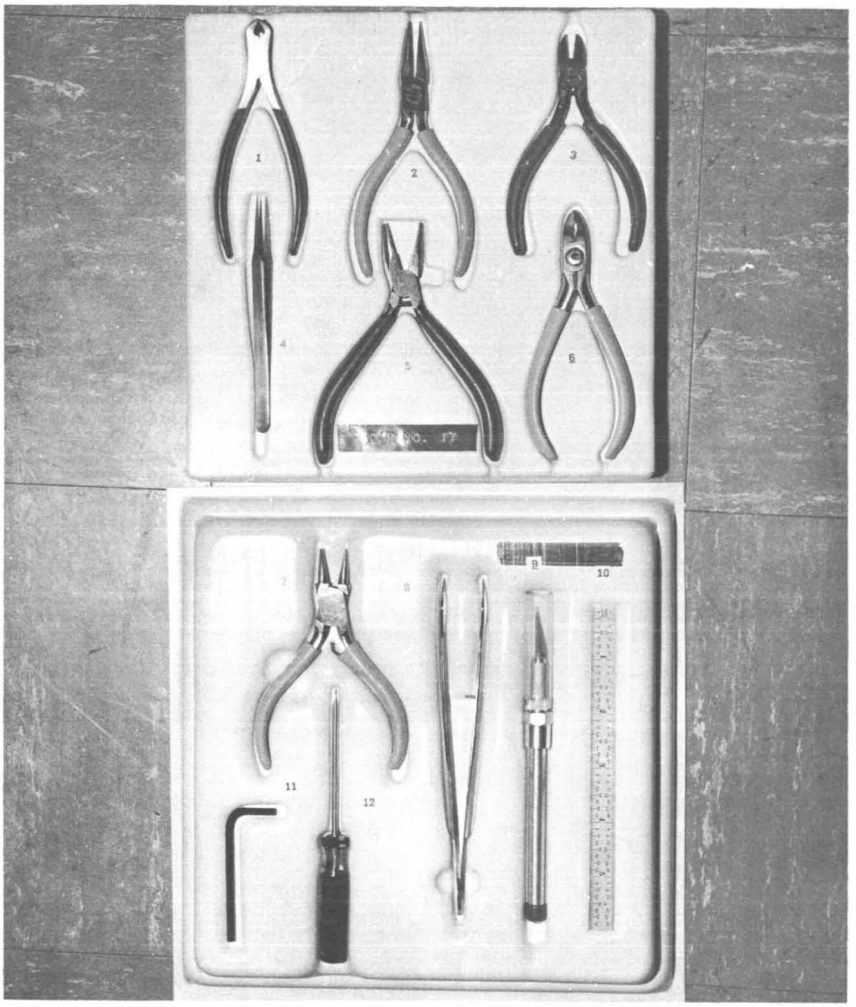

FIGURE 13.—Tools used to manufacture BX modules.

Module BX–6: Sterilize all parts. Assemble on laminar flow bench using sterilized tools and fixtures (fig. 15). Principal operator has an assistant who opens and closes all sealed containers (fig. 16). Principal operator wears Dacron smock over street clothes. Both operators wear face masks during actual assembly.

All parts of all modules were packaged in sterile Pyrex or glass tubes or jars prior to assembly (see fig. 14). The completed modules were again put in the jars and taken to the biolaboratory for assay. All modules were microbiologically assayed using stand-

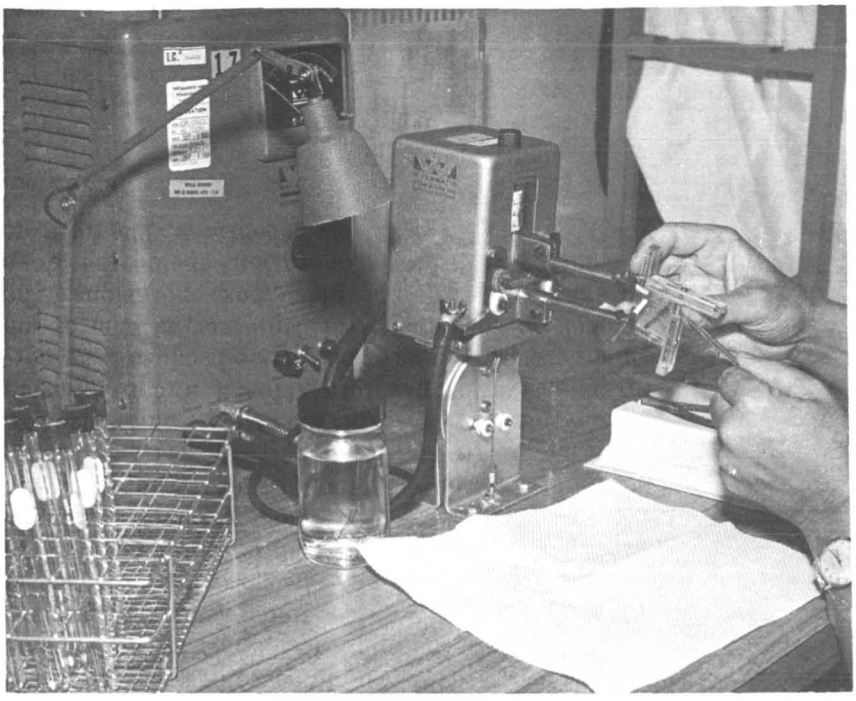

FIGURE 14.—Welding in open-bench operation.

ard procedures. The diluent was sterilized water; the nutrient was trypticase soy agar. The sterilized tools and welding fixture used in the fabrication of module BX–6 were assayed for sterility after completion of the module by rinsing the cutting or working edges directly in the sterile culture broth. No contamination was found on any of them.

The times required for the various assembly steps of the five modules are itemized in table III. The results of the bioassays are given in table IV. The following conclusions can be drawn from these data:

(1) The addition of a bactericidal rinse to the standard manufacturing process decreases the bioload 1 logarithm at an additional manufacturing cost of 18 percent.

(2) Presterilization of all parts and subsequent standard assembly decrease the bioload by 2 logarithms at an additional manufacturing cost of 60 percent.

(3) The use of finger cots with presterilized parts reduces the bioload by 1 additional logarithm; the manufacturing incremental cost increases from 60 to 70 percent. It appears that the use of finger cots is highly efficient; they

FIGURE 15.—BX-6 manufacture on laminar flow bench.

reduce the bioload by 1 logarithm at a very small increase in cost.

(4) The assembly of presterilized parts on a laminar flow bench reduces the bioload to a barely measurable level. The manufacturing cost is 2.5 times that of standard assembly.

Flexible-Wall Clean Enclosures

The proposed stipulation that all planetary spacecraft which are to be sterilized by heating must be assembled in Class 100 laminar downflow clean rooms necessitates extensive reconstruction of

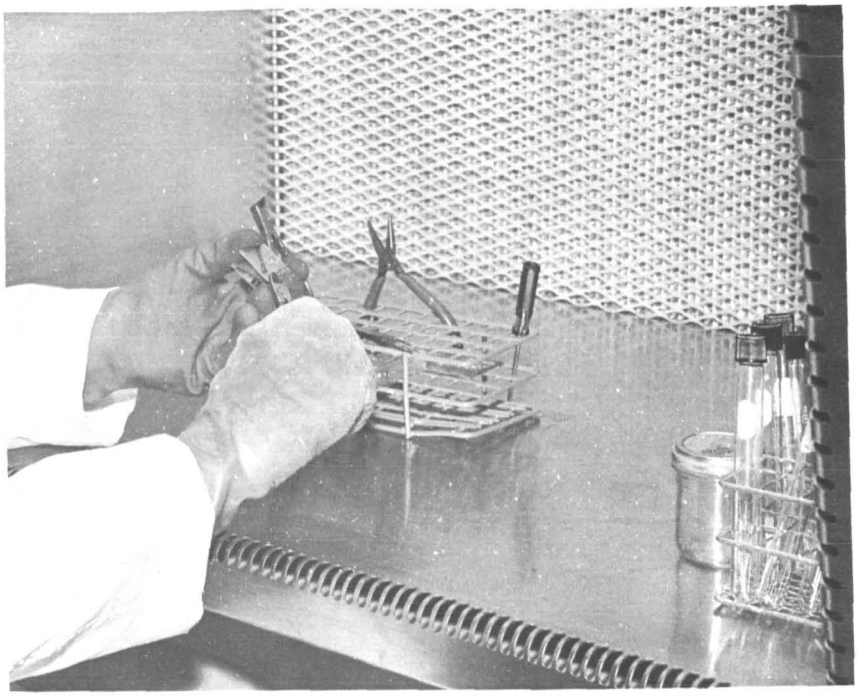

FIGURE 16.—BX-6 manufacture by two-man team.

TABLE III.—*Times and Costs Required for Assembly of Modules*

Module	BX–1	BX–2[a]	BX–3	BX–4[a]	BX–6[a]
Time required, min:					
Sterilization	0	0	30	30	45
Assembly	7	7	7	10	[b]30
Weld	35	32	32	34	36
Clean	1	11	0	0	0
Total	43	50	69	74	111
Cost based on $3/hr labor	$2.15	$2.53	$3.45	$3.70	$5.50

[a] Additional costs not considered: BX–2, cost of bactericide; BX–4, cost of finger cots; BX–6, cost of laminar flow bench, smock, surgical gloves, and sterilization of tools.

[b] 2-man team, 15 minutes each.

existing conventional clean rooms by the aerospace industry. Replacing these facilities by fixed, laminar flow rooms would require large expenditures of funds. An alternate method of providing downflow clean rooms appears to be the use of flexible-wall

TABLE IV.—*Number of Colonies After 72-Hour Incubation at 32° C*

Dilution	BX–1	BX–2	BX–3	BX-4	BX–6
¼	TNTC[a]	TNTC[a]	TNTC[a]	81	5
¼	TNTC[a]	TNTC[a]	TNTC[a]	78	2
1/20	TNTC[a]	TNTC[a]	133	17	0
1/20	TNTC[a]	TNTC[a]	156	8	0
1/200	1200	128	15	2	.....
1/200	1200	128	16	3	.....
None (estimated)	2.4×10^5	2.7×10^4	3.7×10^3	3.3×10^2	0.14×10^1

[a] TNTC: Too numerous to count.

enclosures. The General Electric Co. has had favorable experience with such enclosures over the past few years. Moreover, the cost of installing these enclosures is only a small part of the cost of rigid-wall laminar flow rooms. A considerable cost saving would therefore occur if these flexible-wall enclosures were to satisfy the planned NASA requirements for bioclean facilities.

The particulate and biological contamination of two such enclosures was determined and compared with the latest available NASA interim requirements. Pilot spacecraft assembly operations will be performed in the future in these enclosures and in a conventional clean room to evaluate any differences in the contamination levels of the hardware.

Description of Enclosures

Both of the enclosures investigated are of similar construction and use nylon-reinforced plastic film for the walls and the air-duct manifolds. The supporting structure is steel coated with an epoxy paint. Each enclosure is equipped with a remote air-handling system capable of delivering air through high-efficiency particulate air filters. The enclosures have a downward flow of air, although the flow is not laminar. Air is taken in through prefilters, compressed, passed through HEPA filters, and introduced into a plenum chamber above the enclosure proper (fig. 17). The many circular holes in the ceiling of the enclosure allow the air to enter from the plenum; it then flows in a generally downward direction and escapes through a peripheral 3-inch gap between the floor and the bottom of the enclosure walls.

Enclosure A measures 45 by 20 by 15 feet high; enclosure B is 12 by 4 by 8 feet high. The air velocities in the facilities average 10 to 15 and 50 ft/min, respectively. During the evaluation, both flexible-wall enclosures were located in a medium-high manufacturing area which is temperature and humidity controlled.

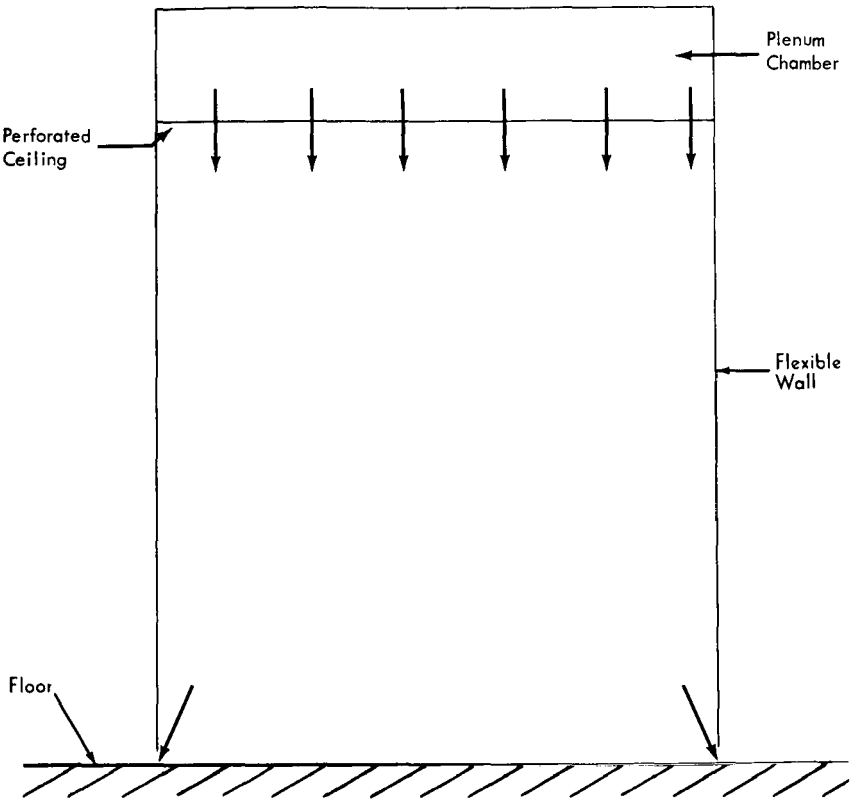

FIGURE 17.—Cross section of flexible-wall enclosure.

Particulate Contamination

Airborne contamination was monitored with a particle counter to determine the total number of particles with diameters greater than 0.5 micron. Sampling was performed under varied conditions over a period of 4 hours to determine (1) cleandown time, (2) quiescent level of operation, and (3) operational level under adverse conditions.

In order to establish cleandown time, the air-handling system of enclosure A was shut down. A maximum level of 3.5×10^6 particles/cu ft was measured for approximately 15 minutes, indicating equilibrium, before the air-handling system was turned on. After 30 minutes the level had receded to 1000 particles/cu ft and after 1 hour, to 100 particles/cu ft. For the next 2 hours the level oscillated between these two values. Cigarette smoke was then introduced into the enclosure, and the total count soared to 3.5×10^6 particles/cu ft.

Cleandown time was thus measured to be one-half hour. The contamination at the quiescent level was established to be between 100 and 1000 particles/cu ft as evidenced by the 2-hour period of relative stability within the facility. The high peak caused by the introduction of smoke presented another opportunity to measure cleandown time. As in the first case, it measured approximately 30 minutes.

Contamination under operating conditions was established by deliberately contaminating the facility with a small quantity of dirt (claylike) and then performing vigorous calisthenics in order to pulverize the dirt and stir it up. The time period for this exercise lasted 30 minutes, while the particle concentration varied from 1000 to 10 000 particles/cu ft.

The airborne particulate contamination of enclosure B averaged 50 particles/cu ft in the quiescent condition as measured with the same counter as that used for enclosure A. No readings were taken under other conditions.

Microbial Contamination

The sampling techniques employed to assess the level of airborne microbial contamination present in the enclosures were the Reynier slit sampler and the Andersen sieve sampler. In addition to these samplers, settling plates containing solidified agar were exposed as a supplementary monitoring technique. The Andersen sieve sampler was not employed in a standard manner. It was operated with one set of plates for times longer than normally recommended, namely, 1 and 3 hours. It was thought that two types of information might be obtained which would be pertinent to the overall problem: (1) a better estimate of the level of viable microbial spores would be obtained (assuming that most of the vegetative cells would be sacrificed due to the longer sampling time), and (2) some estimates of the frequency of the airborne particles and their sizes would be obtained to supplement the other monitoring techniques. The assumptions made in selecting the Andersen sampler for these reasons remain to be corroborated.

The enclosures were unoccupied except for the two persons operating the sampling equipment. The ventilation system was always in operation except in the case of enclosure B, in which sampling was performed prior to turning the air system on in an attempt to obtain a baseline of the level of the natural contamination present to which the operating level might be compared. Enclosure A was assayed on 2 successive days with a total of 38 separate samples, not including control samples. A similar number of samples was taken in enclosure B on a single day.

Table V shows the averages for the three sampling methods employed for the two enclosures. The results employing the Andersen sampler indicate that, even though the number recovered is low, the only viable particles were greater than 2 microns in size, since organisms were recovered only on stages with holes larger than that size. As long as the air-handling unit is operating, both enclosures have microbial contamination levels of less than 0.1 particle/cu ft of air. Turning the air off in enclosure B multiplies the microbial count by a factor of 5 for the Reynier slit sampler and by a factor of 4 for the settling plates.

TABLE V.—*Microbial Contamination at Tabletop Level of Flexible-Wall Enclosures*

Condition of filtered air	Type of sampling	Recovery culture medium	Average number of colonies counted	
			Enclosure A	Enclosure B
Air on	Reynier slit	Trypticase soy agar	0.11/cu ft air	0.07/cu ft air
		Sabouraud's agar	0.07/cu ft air	
	Andersen sieve	Trypticase soy agar	0.03/cu ft air	0.02/cu ft air
	Settling plates	Trypticase soy agar	0.9/plate/hr	0.6/plate/hr
		Sabouraud's agar	1.0/plate/hr	
Air off	Reynier slit	Trypticase soy agar		0.35/cu ft air
	Settling plates	Trypticase soy agar		2.4/plate/hr

In addition to the results presented in table V, which were taken at tabletop level, samples were taken from settling plates placed on the floor of enclosure A. These plates showed approximately twice the microbial count shown by the plates placed on the table. A partial explanation for the higher level on the floor is that the filtered air is essentially clean, but that the organisms fall out and remain on or near the floor and are not "washed" from the enclosure. Any activity in the area would, of course, stir up such concentrations with a resultant further increase in contamination at the floor level.

Discussion

Enclosure A may be classified as a Class 5 000 to 10 000 clean room and enclosure B as a Class 100 room. Both of them, however, appear to satisfy the microbial cleanliness specifications of the NASA interim requirements for bioclean facilities which specify a level of no more than 0.1 viable organism per cubic foot of air. The above tests, however, are not sufficient by themselves to prove

TABLE VI.—*Clean-Room Cost Comparison*

[Navy data from study performed by Y. Nakazawa for Bureau of Ships, Department of the Navy, under contract NBy-55641 and Mooney data from study performed by J. J. Mooney Associates at request of General Electric Co.]

Design	(1) Conventional	(2) Conventional	(3) Vertical laminar flow	(4) Vertical laminar flow	(5) Vertical laminar flow	(6) Vertical laminar flow	(7) Vertical laminar flow	(8) Vertical laminar flow	(9) Flexible-wall enclosure A	(10) Flexible-wall enclosure B
Source of data	Navy	Mooney	Navy	Navy	Navy	Navy	Mooney	Mooney	Mooney	Mooney
Area, sq ft	3000	512	3000	3000	3000	3000	512	1024	900	48
Filter location	Air handling unit	Air handling unit	Ceiling	Air handling unit	Ceiling	Air handling unit	Ceiling	Ceiling	Air handling unit	Air handling unit
Total air volume, cu/ft min	15 000	2250	150 000	150 000	75 000	75 000	25 600	51 200	12 000	2250
Air velocity, ft/min	500–800		50 av	50 av	25	25	50	50	10–15	50 av
Air changes per hr	30	34	300	300	150	150	384	384	25	240
Cost, dollars/sq ft	$42.43	$30	$102	$98.50	$85.15	$76.60	$65	$55	$13	$40
Ratio of cost of design to cost of conventional design:										
Determined by Navy	1.00		2.38	2.29	1.98	1.78				
Determined by Mooney		1.00					2.17	1.83	0.43	1.33

whether flexible-wall enclosures in general are sufficient to satisfy the requirements. They merely point out that there may be facilities other than laminar downflow rooms which could be suitable for the performance of certain bioclean manufacturing operations. It is planned to perform similar tests in a laminar downflow room in the future.

Cost Comparison

Table VI presents data from two independent studies, one performed by Yosh Nakazawa for the Bureau of Ships of the Department of the Navy under contract NBy–55641, and the other performed by J. J. Mooney Associates in response to a request from the General Electric Co. The data clearly show the considerable increase in facility construction cost of fixed-wall, laminar-flow clean rooms over that of conventional clean rooms. In the light of these results, the flexible-wall enclosures are of considerable interest.

Columns 1 and 2 of table VI show data for the conventional, rigid-wall clean rooms which are used as baselines. In columns 3 and 4, two types of laminar downflow rooms are compared; in one of them the filters are located in the air-handling system, and in the other they are in the ceiling. Both of these rooms are comparable in size to the conventional clean room of column 1. The table shows that comparable laminar flow facilities may cost up to 2.4 times as much as conventional clean rooms. The facilities listed in columns 5 and 6 are the same as those shown in columns 3 and 4, except that the velocity of the airflow has been reduced from 50 ft/min to 25 ft/min. The 50 percent reduction in airflow reduces the cost 20 percent only.

Columns 7 and 8 are shown to illustrate that, for small rooms, doubling the size of the room does not double the total cost because the cost per unit area decreases for the larger room. In the example given, this decrease is approximately 15 percent. The reduction in unit cost is due to the fact that many items (for example, engineering) are constant or vary only slightly with the size of the room.

Columns 9 and 10 show the data for the flexible-wall enclosures A and B, respectively. They show that enclosure B is too small for optimum construction because its unit cost is three times that of enclosure A and even exceeds the unit cost of a conventional 512 square-foot clean room (col. 2). Enclosure A, however, has by far the lowest unit cost of all the clean rooms under comparison, a cost less than one-quarter of the unit cost of a similar-sized, fixed-wall laminar downflow clean room.

The following conclusions can be drawn from table VI:

(1) Laminar flow, low-headroom, clean rooms cost approximately $100 per square foot.
(2) The ratio of initial construction costs for laminar flow to those for conventional clean rooms is in excess of 2.
(3) Placing the air filters with the air-handling unit instead of in the ceiling of the laminar-flow room reduces the cost between 3 and 10 percent.
(4) Reducing the laminar airflow velocity by 50 percent reduces the unit cost by only 20 percent.
(5) Larger rooms have a somewhat lower unit cost than smaller rooms.
(6) The cost of some flexible-wall downflow enclosures is not more than one-quarter the cost of fixed-wall laminar-flow clean rooms.

Vendor Requirements

The importance of vendors in any aerospace program is readily apparent. Most aerospace prime contractors are system, assembly, and test organizations, and they purchase the bulk of their hardware. On a recently completed $27 million program, General Electric Co. purchased items in excess of $10 million (just under 40 percent) from 10 major suppliers. Most of the remaining 60 percent went for software, and only a small part for inhouse manufactured items. On another current program, the bought hardware accounted for over 60 percent of the total hardware cost and for 35 percent of the hardware items. This indicates that the larger items are bought and the smaller items (harnesses, brackets, etc.) are made inhouse. This situation is fairly typical in the aerospace industry.

The problem of how to translate cleanliness and sterilization requirements into vendor procedures thus appear to be of major importance. It may be considerably simpler to educate a dozen or so prime contractors on planetary lander programs than to educate hundreds or thousands of second- and third-tier suppliers of these prime contractors.

For reliability reasons, the need for clean rooms and for meticulous control of particulate contamination is fairly well established among vendors in the pneumatics and fluids fields. However, these requirements are not well understood by some of the many vendors of electronic equipment, which comprises the majority of purchased spacecraft parts. A large segment of the electronic parts industry has yet to accept and act on the need for any but the most rudimentary forms of particulate contamination

control. If the requirement for biological cleanliness is added to that for particulate cleanliness, some vendors may become discouraged and may refuse to bid on purchase requests, or they may add an inordinately large amount to their quote price to cover themselves against this "unknown" specification. Either of these actions would result in increased costs for planetary spacecraft to be sterilized by heating.

There remains a large task of education to be performed on those suppliers unfamiliar with the techniques for contamination control. In many cases these vendors could profit from the know-how of that segment of the industry which has had experience with strict cleanliness requirements, either by adopting its procedures or by using those vendors as "cleaning subcontractors" to decontaminate purchased items.

Prime Contractor Responsbilities

In setting forth requirements to be imposed on his suppliers, a prime contractor's first thought is to satisfy the obligation to his customer by purchasing commodities which fully assure the quality of the end product. Secondly, he has a responsibility to his subcontractors to establish meaningful criteria by which the vendors' products can be measured to determine the degree of compliance. In order to facilitate the selection of the necessary controls on materials and processes, two basic questions must be satisfied: (1) is there a measurable phenomenon involved? and (2) can this measurement be validated to assure compliance with the imposed requirements? Unless the answer to both of these questions is affirmative, no meaningful specification to control this particular phenomenon can be written and enforced. Without clearly defined and rigidly enforceable and enforced specifications, no bioclean hardware program can succeed.

Poor definitions and work statements complicate vendor surveillance. Some of the major reasons for the inadequacy of many current cleanliness specifications are: (1) the absence of well-established relationships between cleanliness specifications and needs; (2) insufficient experience in the control of contamination; and (3) inadequately developed techniques for monitoring.

The first of these reasons causes engineers to resort to unrealistic cleanliness requirements, so that they will be "safe" through the imposition of excessively stringent specifications. The second leads to purchaser-supplier misunderstandings and disagreements. In establishing requirements for the manufacture of sterilizable spacecraft, every attempt must be made to avoid these pitfalls. The postulation of excessively high cleanliness requirements would

seriously jeopardize the successful manufacture of a planetary lander.

The necessity of manufacturing a bioclean product is a major new requirement for most vendors. The achievement of biocleanliness now becomes a major operation. Should responsibility for this remain with the supplier, or should cleaning operations be performed by the purchaser? There is probably no single general answer. The decision on when, how, and where to clean depends on the hardware, its design, the materials of construction, the handling required, the assembly procedures involved, the manner of its installation in the vehicle, and the expected reliability. From the prime contractor's point of view, preference should be given to centralizing all cleaning operations in his plant. In this way he can best assure compliance of all his vendors' products with the bioclean requirement. On the other hand, this presupposes that the prime contractor is well equipped to perform cleaning operations thoroughly and without compromising the functional reliability of the cleaned items.

Assurance of Vendor Quality and Biocleanliness

There are two ways of assuring and monitoring the satisfaction of bioclean or sterility requirements on the part of vendors: (1) use and expand the existing quality control (QC) organization to include biocleanliness control; or (2) set up a new sterility control organization which is to monitor bioclean or sterile quality of all vendor-procured parts and have QC monitor all other aspects of these parts.

At the vendor level, it seems preferable to use the first method for the following reasons:

(1) QC organizations are in existence, and thus there is no need to establish a new organization.
(2) Biocleanliness is just one of the many requirements which a part has to meet. It is the present task of QC to assure that all vendor parts meet specifications. The assurance that biocleanliness specifications are met is therefore a natural QC task.
(3) In cases where levels of particulate cleanliness are specified on present contracts, QC already has the task of assuring compliance.

It is, therefore, recommended that existing prime contractor QC organizations be given the task of assuring compliance with bioclean or sterility requirements on the part of vendors. This recommendation is limited to vendors. No recommendations are

made at the present time that QC alone be responsible for the sterility of the completed lander.

QC personnel must be educated and trained in monitoring biocleanliness, since this task is far more difficult than checking a blueprint dimension with a scale on a piece of hardware. Presumably microbiological technicians may be required within QC for certain monitoring functions. It is, however, not conceivable to have all biocleanliness checks performed by microbiologists only, and ordinary QC workers and technicians must be trained in the science of measuring biological contamination.

By its very nature, biological contamination control must take place during manufacture. It cannot be accomplished by merely measuring the contamination level of the finished product. In-process control and inspection are therefore vital parts in the chain required to assure satisfaction of the biocleanliness specification; they are more vital here than for the assurance of most other specifications. This makes it necessary for the quality assurance personnel to be present in the vendor's shop during most operations and to bioassay at critical stages of subassembly. On October 6, 1965, the NASA Office of Planetary Quarantine, in a letter to the Director of Lunar and Planetary Programs, stated that no item of hardware more complex than a piece part can be reliably bioassayed. It is expected that this limitation will eventually be overcome, and that spacecraft components and subassemblies can then be assayed with confidence.

Vendor Problems

The purchase of hardware necessitates vendor surveillance. One method for minimizing the difficulties of monitoring vendor operations is for the prime contractor to purchase nonbioclean vendor parts and to clean and assemble as many of them as possible within his own facilities. Implementation of this approach would entail drastic changes in the management and subcontracting arrangements of a number of important programs. For example, in the General Electric Co.'s current plans for a major planetary spacecraft program, approximately 70 percent of the pieces of hardware are to be fabricated by vendors. This figure is fairly typical for the industry.

Generally speaking, it is more efficient and reliable for the supplier to employ his own techniques and controls than to have the purchaser attempt to impose his methods and assurance procedures. However, the burden of proof to establish that a given method does perform the job satisfactorily falls upon the supplier. On the other hand, some vendors prefer to work toward specific

requirements imposed on them by the purchaser, but insist that the burden of proof that noncompliance exists fall on the purchaser. Needless to say, such an approach cannot be tolerated in a program involving sterilizable, bioclean hardware.

The "walkthrough" is undoubtedly the most efficient method of discovering possible leaks in a fabricating procedure, especially for novel requirements such as biocleanliness and sterilization for which no storehouse of experience exists. It should certainly be used at the initiation of any program for the manufacture of a sterilizable vehicle. The pilot manufacturing and assembly operation discussed elsewhere in this paper was undertaken with this in mind.

The problems of assuring biocleanliness are not unlike those encountered in attempts to achieve reliability. A review of the techniques employed in the enforcement of quality provisions on vendors may, therefore, provide ways and means applicable to a program aimed at biological cleanliness.

Reference

1. General Electric Co.: Design Criteria for Typical Planetary Spacecraft To Be Sterilized by Heating. Final Report, Contract NAS 8–11372 Phase I, GE Document 65SD4384, Dec. 15, 1965, pp. 6–36.

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Sterilization Facilities

A small experimental assembly and sterilization laboratory (EASL) was recently designed and constructed at the Jet Propulsion Laboratory (JPL). This EASL provides a facility wherein the effects of rigorous constraints on personnel and material during the fabrication of small modules and subassemblies could be evaluated.

The final design of a sterilization and assembly development laboratory (SADL) was just completed, with construction scheduled to start in January 1966, and completion scheduled for mid-August 1966.

The SADL facility features a 35-foot-high bay area of 1200 square feet which utilizes laminar downflow to obtain a Class 100 clean room in accordance with Federal Standard No. 209. Supporting features of SADL include a personnel-processing area containing a Class 100 laminar crossflow dressing room; a complete microbiological assay laboratory; receiving, cleaning, and decontamination spaces; and an OSE area. SADL will also contain two significant mechanical features: (1) a large sterilization oven which will be heated by gaseous nitrogen, and (2) a combination ethylene oxide decontamination chamber and airlock. Both of these units will be used in developmental experiments conducted with a full-scale model of the Voyager capsule. The SADL facility will permit approximately 2 years of developmental work on sterilization and hardware problems prior to fabrication of a proof test model for the 1971 mission in the ultimate assembly facility.

It should be noted that the EASL facility is involved with experiments at the component and module level. On the other hand, SADL is designed to handle a full-scale model of the capsule. In addition to resolving certain scaling problems in extrapolating from EASL results, SADL is expected to assist in the development of criteria for such items as handling gear, hoists, fixtures, jigs, and similar devices.

Both EASL and SADL employ laminar downflow to obtain a Class 100 clean-room environment in the working area. However, it cannot be emphasized too strongly at this point that the specific facility environment for the assembly of actual flight hardware is still subject to further definition based on results of project studies which are presently in progress. For example, one rigorous approach to a controlled facility environment is based upon the sterile concept which has been successfully employed by the gnotobiologists in the raising of germ-free animals. At the other end of the facility scale, recent project investigations indicate it may be possible to capitalize on the surface decontamination and dry-heat sterilization cycles to which all flight hardware will be subjected as part of required flight approval testing. In this concept, hardware assembly operations would proceed from subsystem fabrication through system assembly and test followed by disassembly and microscopic inspection, all in a "controlled clean environment," which has been tentatively identified as similar to that used for the Ranger and Mariner hardware. After the foregoing inspection, all hardware could be delivered to an ultra-clean facility (such as that provided by a laminar downflow design) at the launch site where it could be decontaminated, reassembled, tested, and terminally heat sterilized.

Essentially then, there are three basic approaches to the facility design under consideration; namely:

(1) Severe environmental and personnel controls (sterile facility concept)
(2) Intermediate environmental controls (laminar downflow clean room)
(3) Controlled clean environment (similar to Ranger and Mariner facility)

Irrespective of requirements for initial assembly and test operations on the capsule, adequate facilities appear to be required at the launch site for such operations as propellant loading, retro motor and parachute mortar insertion, special pyrotechnic loading, spin and balance tests, and similar terminal operations which should be accomplished in an explosive safe area.

In the case of the early Voyager capsules, which may be assembled at JPL, the capsule size is estimated at about 12 feet in diameter and perhaps about 3000 pounds in weight. A capsule assembly and sterilization addition (CASA) to the existing spacecraft assembly building (No. 179) is under consideration (fig. 1). The CASA design is presently based upon a laminar downflow design; however, the requirements for this addition are being reexamined at this time.

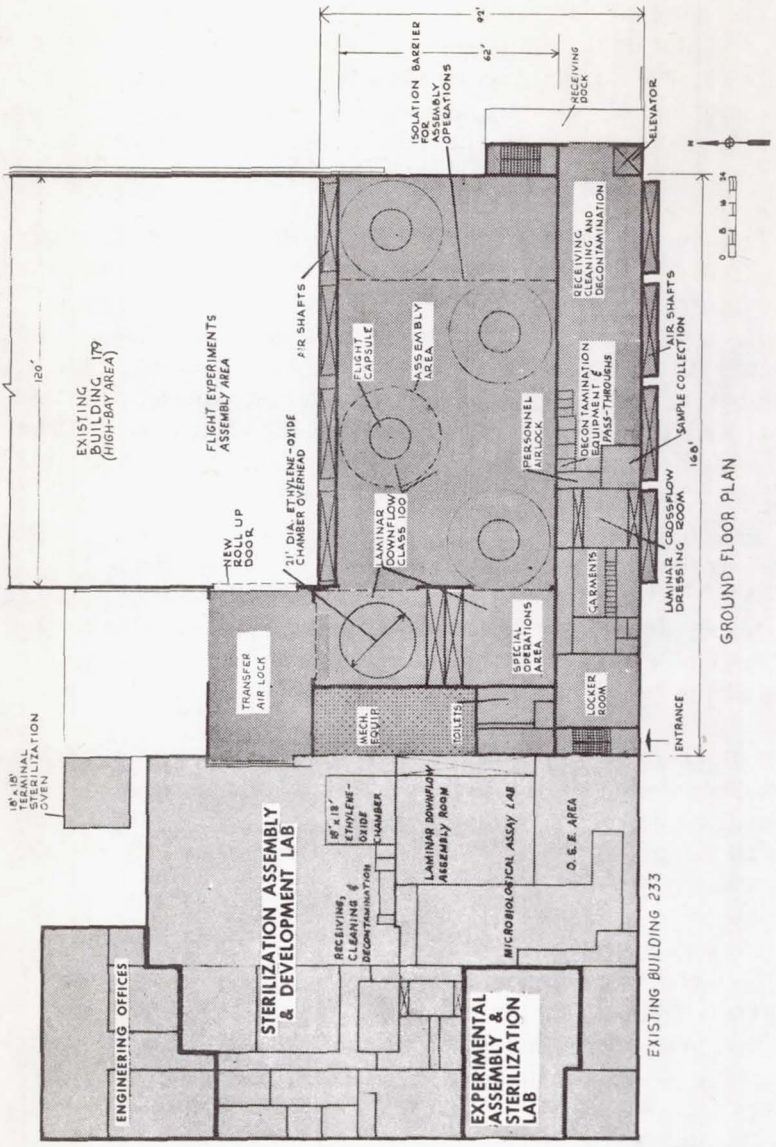

FIGURE 1.—Proposed capsule assembly and sterilization addition to building No. 179.

Under the direction of JPL, an architectural engineering firm has made preliminary engineering studies of the laminar flow and sterile concepts for the ultimate Voyager capsule configuration. Initial data from these studies, based on common design conditions, indicate the following:

(1) The sterile facility is less costly to build; it is about 20 percent cheaper.

(2) The laminar-flow concept requires less costly technical collateral; it requires about 10 percent less.
(3) The sterile facility may be expected to require a longer and more costly cleanup and certification period.
(4) The maintenance of the laminar-flow facility can be a serious consideration because of the extensive amount of mechanical equipment.
(5) The human factors in the sterile concept may seriously reduce personnel efficiency and markedly increase the project assembly and test costs.

Facility acquisition schedules are primarily a function of the anticipated tenor of capsule operations for each mission; that is, the number of flight capsules, the number of spares, the test schedule, and the assembly and test time for each capsule. The facilities themselves may be expected to conform to the following time allowances:

Final design	6 to 9 months
Construction	14 months
Activation	3 to 6 months

In general, the facility for assembly should be available to the project about 30 to 36 months prior to the mission. The facility complex at the launch site would normally follow by approximately 1 year.

While a final decision has not been made as to the specific facility design concept for the ultimate Voyager assembly and test operation, it should be noted that certain elements are common to all designs; only the degree or method of control of these elements vary. Among these are:

(1) Environmental controls of assembly area
(2) Personnel processing controls
(3) Material flow controls
(4) Tools and test equipment controls

The keyword in the foregoing is *control.* In addition to adequate provisions and spaces for these controls, the facility should provide:

(1) Cleaning and decontamination equipment
(2) Sterilization equipment
(3) OSE areas
(4) Microbiological assay capability
(5) Instrumentation and controls for proper monitoring and surveillance of all operations so that acceptable certification will ultimately be provided

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Special Problems in Spacecraft Sterilization

The present paper discusses special problem areas which require additional effort as an aid in the development of a broadly based sterilization technology. Consideration is given to the conflicting requirements of sterility assurance and equipment reliability and to the development of a rationale for achieving a practical balance between the two. It is shown that the present requirements for heat decontamination of nonassayable subsystems are too conservative and probably would produce needless degradation of reliability. It is pointed out that the decision not to sterilize Mars flyby vehicles is not consistent with the sterility assurance required for landers.

The role of thermal effects and compatibility testing is discussed in terms of the information which is needed to support the development of sterilizable hardware and the specification of appropriate sterilization procedures for Mars landing missions. Emphasis is placed on the need for more research on the physics of thermally induced failures and the correlation of component performance parameters and failure rates with the time-temperature cycle. The development and use of accelerated test methods are considered and encouraged as an integral part of the sterilization technology program.

A preliminary discussion of radiation as an alternate for heat sterilization is presented, including a discussion of the factors which enter into determining the feasibility and practicability of radiation sterilization by the use of gamma rays or X-rays. Comments are made on the need for improved communication and dissemination of information in the field of spacecraft sterilization.

The material presented herein is based primarily on the results of a study contract entitled "Definition of Requirements for Advanced Heat-Sterilizable Components for Planetary Spacecraft" which was recently completed for the Lunar and Planetary Programs Office of the National Aeronautics and Space Administration (NASA). Comments are also included on a study of the feasibility of radiation sterilization which is now being conducted for NASA.

It is by now clear that a variety of different procedures for manufacture, assembly, test, and terminal sterilization can be considered, all of which will meet the sterility assurance requirements specified. It is therefore important to analyze and evaluate these procedures in order to select the most practical and economi-

cal way to achieve the desired sterility assurance within the permissible time schedule and with an acceptably small reduction of the probability of attaining the scientific mission objectives.

Development of Rationale for Sterilization Program

As is well known, the terminal sterilization cycle is based on dry-heat destruction of spores in garden soil (ref. 1), and it is assumed that 12 decades of spore reduction are produced by the specified temperature cycle (e.g., 135° C for 22 hours). Since there are approximately 2 million spores per gram of garden soil, the specified terminal cycle is apparently adequate to sterilize a capsule containing 10^8 micro-organisms which are contained in 50 grams, or 0.1 pound, of soil.

If we assume that all spacecraft subsystems are to be subjected to a flight acceptance test which includes a heat cycle of 10 D-values as specified for decontamination of unassayable subsystems, then within the limits of perfectly sterile assembly, the total spore population in all the subsystems prior to the flight acceptance test could theoretically be 10^{10} times larger than 10^8, namely, 10^{18} spores, and we could still achieve a 10^{-4} probability of survival of one spore. This corresponds to a calculated mass of 10^9, or 1 billion pounds of garden soil. It should be possible to agree on some practical guidelines to set an upper limit to the contamination of unassayed subsystems. For example, if a subsystem is not assayable, perhaps it could be accepted that the total biological contamination will not exceed the equivalent of a percentage (say 10 percent) of the subsystem weight in the form of garden soil containing 2×10^6 organisms per gram. Such an assumption would permit a more realistic basis for specifying heat decontamination requirements. If a flight acceptance test on subsystems is to be required in addition to terminal sterilization, one can see that bioclean assembly of a capsule would be superfluous because the overkill capability provided by 2 heat cycles would total 22 decades of destruction.

Although it seems clear that the present decontamination requirement (10 D-values) is overly conservative, it is not clear that the terminal sterilization requirement of 12 D-values is conservative if one uses the quoted D-values for garden soil (e.g., 1.8 hours at 135° C). The reason is that mixed spore populations exhibit a "tail" on the logarithmic plot of survivors versus time which corresponds to survival of the most heat-resistant strain, which may have a D-value much greater than the average for a mixed population. Therefore it is important to identify the popu-

lation and D-value for the most heat-resistant spores which may be present in a capsule.

It is apparent that more analysis is needed to place the decontamination and sterilization requirements on a more firm, rational foundation. In order to select an optimum set of procedures, more attention should be paid to analyzing the possible tradeoffs between mission success probability and sterility assurance because they are interdependent. More test data are needed on the failure rate of components following heat cycling.

A related subject is the question of what impact probability limit should be set for an unsterilized flyby vehicle. It seems evident that accidental impact on Mars of a flyby vehicle containing a large number of heterogeneous organisms (say 10^{10}) would be much more likely to produce a growth and contamination of the planet than the presence of one heat-resistant spore inside a landing capsule on the surface. Therefore it seems inconsistent to specify 10^{-4} probability of one spore in a capsule while permitting 3×10^{-5} probability of a crash by an unsterilized flyby vehicle containing a wide variety of micro-organisms.

Qualification Tests and Design Margins

Until recently, most heat-sterilization compatibility testing has been conducted using three qualification cycles of 145° C for 36 hours. This qualification test was chosen by the hardware people somewhat arbitrarily to provide a "safety factor" in excess of the 135° C and 24-hour terminal sterilization cycle which had been specified by the biologists. Some of the questions which need answers are: (1) What is the magnitude of this safety factor for the various components and what units should be used to define it? (2) Is the safety factor an adequate and reasonable value or does it impose unnecessary hardships and costs on the developers of hardware and instruments? (3) If it is unreasonable, what is a more appropriate temperature and time cycle for the qualification test?

Even though the answers to these questions are not yet clear, the problem has recently been given an interesting additional degree of freedom by the specification of 12 alternate terminal sterilization cycles varying from 3 hours at 160° C to 336 hours at 105° C. The specification was made in a sterilization-cycle memorandum to the Director of Lunar and Planetary Programs for NASA on July 21, 1965. In principle, this additional flexibility should provide more latitude to the engineer and instrument developer to qualify his hardware. However, we must determine whether the capsule as a whole would be more reliable with a higher or lower temperature

cycle. Also we are confronted with the question, "What is an appropriate qualification cycle for use with the optimum terminal sterilization cycle?" In order to approach such questions, it is clear that it is desirable to have a great deal of experimental data covering the effect of various temperature-time cycles on the potential failure of all critical space capsule parts, components, and instruments.

A consequence of the 12 alternate heat cycles is the implication that credit for spore killing may be allowed for the heatup and cooldown portions of the terminal sterilization cycle at temperatures in excess of 105° C. Bruch (ref. 2 and a paper in this conference entitled "Dry-Heat Sterilization for Planetary-Impacting Spacecraft") has shown how the time-integral effect of temperature on spore survival can be correlated by an Arrhenius rate relationship. We are now attempting under a contract with the Office of Biosciences, NASA, to refine this analysis and to extend it to correlate component degradation by thermally induced failure mechanisms with the temperature-time cycle. An objective is to identify an optimum sterilization cycle for various critical components. We believe that much more effort is needed to understand the physics of thermally induced failures and to develop rational methods of engineering analysis which will assure safety margins which are adequate but not so excessive that they scuttle or delay

TABLE I.—*Outline of Primary Thermally Induced Failure Mechanisms and Modes*

Failure mechanisms	Failure modes
Thermal stress	Fracture Distortion (yielding or creep)
Outgassing (evaporation and sublimation)	Corona discharge Change in mechanical or electrical properties
Ethylene oxide absorption	Corona discharge Change in mechanical or electrical properties
Condensation of outgassed vapor	Changes in optical properties of surfaces
Chemical reactions (e.g., corrosion, depolymerization, crosslinking)	Changes in mechanical or electrical properties (e.g., batteries)
Physical and metallurgical reactions (diffusion)	Changes in mechanical or electrical properties (e.g., solder joints and capacitor dielectric)

the entire planetary exploration program. Table I lists some of the failure mechanisms and modes which need more investigation.

The data to be acquired by research on the physics of failure will aid the design engineer in material selection and component design and will provide a rational basis for specifying appropriate thermal acceptance (or screening) tests, appropriate heat sterilizability qualification tests, and an optimum terminal sterilization cycle. The research will also validate the extent to which accelerated testing techniques might be used to predict component life.

Reliability

In the past, life testing of components has often been conducted for periods much in excess of the expected mission operating time. However, for long-duration space missions, the operating life may be of the order of 1 year. For a large program such as the Voyager program, the number of spacecraft components is so large, the mission duration so long, and the system reliability required so high that individual parts must have a failure rate of the order of 0.001 percent per 1000 hours.

The 10 000-hour life tests which are now being conducted by the Jet Propulsion Laboratory on electronic parts following heat sterilization were designed to demonstrate a failure rate of less than 1 percent per 1000 hours with a lower confidence limit of 90 percent. It is recognized that there is approximately a three-order-of-magnitude difference between the desired part reliability and what can practically and economically be demonstrated. It is our opinion that the sensitivity of such life tests is probably not sufficient to permit quantitative determination of the effect of the heat sterilization cycle on part reliability.

Since a statistical demonstration of the desired reliability level does not appear to be feasible within the time and budget constraints of the Voyager program, it will be necessary, as in the Apollo program, to rely on techniques other than the usual life tests to assure reliability. Success will require adequate safety factors and excellent quality control. The subject of quality control will not be discussed, although, of course, it is a crucial element in reliability and entails material control, process control, inspection, and screening procedures.

In mechanical design, the safety factor is the ratio between an allowable stress and the working stress. Not all spacecraft failures are mechanical, although at least half of them probably are. In principle, mechanical failures by yielding, creep, fatigue, or fracture under applied or thermal stresses can be avoided by good mechanical design, selection of materials, and quality control.

However, there is another category of failure mechanisms which leads to failure modes other than mechanical stress failure. This category includes a variety of physical and chemical phenomena which occur at elevated temperatures, such as outgassing, evaporation or sublimation of materials, chemical or metallurgical reactions at interfaces, and diffusion of materials which may change electrical properties until performance parameters are outside tolerance. These thermally induced failure mechanisms are being investigated in varying degrees, but in many cases there are inadequate data to assure that parts have been designed with adequate safety factors against these types of degradation. Indeed, the quantitative definition and measurement of safety factors are rather nebulous until sufficient research and testing has been performed on specific devices to characterize the thermal degradation phenomena quantitatively with respect to the particular device in question. A good example is the tape recorder, where it was found that outgassing from one component caused failure of another. Therefore research on physics of failure is a prerequisite to the definition of safety factors.

Until such safety factors can be defined, measured, and predicted, the field of heat sterilization technology will remain an art rather than a science. In order to improve the reliability of heat-sterilized spacecraft and to permit valid prediction of reliability, more research is needed on the failure modes which are induced by exposure of parts to elevated temperatures.

Potentialities of Accelerated Test Techniques

One of the most promising techniques for correlating the magnitude of failure phenomena with the time and temperature of thermal treatment is the accelerated test technique (refs. 3 and 4). There is now substantial evidence that accelerated test techniques can be useful in reducing the test time needed to qualify parts or components and to predict their life under operating environments.

The basic requirements which are necessary to validate accelerated testing are:

(1) The failure modes induced by the accelerated test environment must be the same as those which will predominate in the operating environment.

(2) The failure rate must be correlated with the magnitude of the environmental stress parameters which induce failure (such as temperature and operating voltage) so that accelerated test data on failure rate may be extrapolated to predict failure rates at the lower environmental stress values of operation.

In order that accelerated tests be valid, it is necessary that the

quality control, inspection, and screening procedures be adequate to remove from the part population most of those parts which would otherwise fail from random causes. This, of course, is necessary in any case in order to achieve the reliability goals for long-life space missions. The primary purpose of accelerated tests is to validate a prediction of the wearout life of parts in order to assure the desired mission reliability. As examples of accelerated test methods, one can consider the work performed in correlating failure rate of electronic parts with the operating temperature by using either the Arrhenius rate relationship or the Eyring model. Thomas and Gorton of the Battelle Memorial Institute have shown (ref. 5) that the decrease in resistance of carbon composition resistors can be correlated with temperature in the range from 70° to 125° C by an Arrhenius rate equation with an activation temperature constant of 6500° K. (The activation temperature constant is equal to the activation energy in calories per gram-mole divided by the gas constant 1.98.) Their data validate the use of accelerated tests at high temperature to predict degradation of electrical resistance at lower operating temperature. Thomas and Gorton also were able to correlate the degradation of transistor parameters by an Arrhenius equation with an activation temperature constant at 1096° K. They also correlated the degradation of silicon-controlled rectifiers under combined temperature and voltage stresses by using the Eyring model. They found that three different values of activation energy were required to correlate degradation by three different mechanisms over the temperature range from 100° to 235° C.

Sikora and Miller (ref. 6) correlated the median life of silicon mesa transistors against junction temperature by means of an Arrhenius equation with an activation temperature constant of approximately 13 000° K (corresponding to an activation energy of 1.24 eV). They showed that failure was determined by the transistor junction temperature and that short-time accelerated testing was valid for predicting median life values in excess of 10 000 hours.

Walsh and others of the General Electric Co. (GE) (refs. 3 and 4) have conducted extensive work for the U.S. Air Force on accelerated testing and have recently obtained data which show the extent of applicability of the "step-stress" method for accelerated testing of resistors, capacitors, transistors, and diodes. The step-stress method utilizes sequential stressing of parts at successive steps of greater environmental stress (e.g., higher temperature) until failure or measurable degradation has occurred. The GE data (ref. 4) show that the decrease in resistance of metal film resistors

in the temperature range up to 250° C can be correlated by the Arrhenius equation and that the use of two steps of fairly short duration (12 and 160 hours) in a step-stress test can give a quick evaluation of the part sensitivity to temperature. The GE group has recommended that step-stress testing be used to determine the maximum temperature at which constant temperature accelerated tests can be performed without changing the predominant failure mode. The use of constant stress accelerated tests is then recommended to obtain data which will permit prediction of life under lower operating temperatures.

It appears that further evaluation of the applicability of accelerated testing to heat sterilization technology is needed, since it offers the potential of predicting thermal degradation during long-time operation from short-time tests. Also, a knowledge of thermal failure mechanisms occurring in the temperature range from 105° to 160° C will aid in selecting an optimum heat sterilization cycle.

Feasibility of Radiation Sterilization

An analytic study was recently initiated by Exotech, Inc., to examine the feasibility of using ionizing radiation for future spacecraft sterilization and to compare the relative merits of radiation and heat, with consideration being given to their comparative effects on system reliability. The starting point was the assumption suggested by Bruch in a personal communication, March 16, 1965, that a radiation dose of 2.5 to 5 megarads will provide the required sterility assurance. This dose is to be provided by electrons or photons (X-rays or gamma rays) to avoid nuclear reactions in the spacecraft. Nuclear reactor radiation is tentatively being eliminated from consideration, since it contains neutrons that would make the spacecraft radioactive and interfere with scientific experiments.

Both electrons and photons are presently in use for sterilization of pharmaceuticals and surgical sutures, for radiation processing of plastics and chemicals, and for sterilization and pasteurization of foods. As a result, the technology of their use is becoming well established and presents no major problems.

Sterilization by electrons is practical only with thin, flat packages because electrons lack the power to penetrate, and therefore the use of electrons alone is not being considered seriously for spacecraft sterilization. Of the photon sources, gamma-emitting radioisotopes and electron accelerator X-ray machines are appropriate. Cobalt 60, because of its availability, long life, and energetic photons, is the most popular radioisotope used for sterilization by gamma rays. Electron accelerators are used to produce bremsstrah-

lung photons by electron interaction with a target material. Accelerator machines include electrostatic (Van de Graaf) generators, resonant transformers, betatrons, and linear accelerators.

Van de Graaf generators and resonant transformers produce photons in the 0.5-MeV range but become unwieldy above about 3 MeV. Betatrons and linear accelerators are more practical for radiation from 3 MeV to 30 MeV (ref. 7). The size of the capsule to be irradiated might require development of a machine with a capacity larger than that of those presently available. A severe problem in radiation sterilization is lack of uniformity in the dose deposition. In order for all parts of the capsule to receive the required minimum dose of 2.5 to 5 megarads, some parts, such as the skin and outer components, will receive higher doses because of the attenuation of the radiation as it penetrates. Using radioisotopes, one can investigate such possibilities as temporary insertion of cobalt 60 rods in thimbles through the capsule to sterilize it. This would provide more uniform radiation but would require design modification and probably complicate the canister separation problem.

The attenuation of radiation can be minimized, and the uniformity of dose enhanced, by a proper selection of photon energy. Around 25 MeV, there is a minimum in the attenuation by aluminum. Such high photon energies are not available with radioisotopes, and an accelerator must be employed. In such a case, the capsule would be rotated to irradiate it from all directions.

With X-ray energies above about 20 MeV, photoneutrons are produced in light elements such as aluminum in sufficient quantity to produce radioactivity which would later disturb counting experiments and might provide a handling hazard after the irradiation.

We also are studying the problems of radiation damage to components, particularly semiconductor devices, polymers, and optical materials. Much of this information is available from the Radiation Effects Information Center at Battelle Memorial Institute in Columbus, Ohio. Much progress has recently been made in developing components and circuits which are more radiation resistant. We plan to compare the practicability and economy of radiation sterilization with that of heat sterilization in order to recommend whether further effort on radiation sterilization is warranted for potential application to future planetary landers.

Information Dissemination

In any new area of technology, particularly in one which is as interdisciplinary as spacecraft sterilization, there is a great need for effective communications. This conference fills a great need in

this regard. However, the problem of information dissemination is a continuing one which is best fulfilled by the timely publication and distribution of technical papers, reports, and documents which are available for review and criticism by all interested parties. It is our opinion that there is a need for more complete and timely documentation of much of the work now underway on spacecraft sterilization. In the past, the lack of adequate documentation has made it very difficult for aerospace contractors and university research personnel to acquire the information needed for effective participation in the planetary exploration program. The absence of a specialized documentation and information center presently detracts from the rate of progress and education in this new field. It is recommended that a sterilization technology information center be established to provide effective abstracting, review, evaluation, and dissemination of pertinent microbiological and engineering information which is being developed under this program.

Conclusions

In conclusion, we believe that a balanced evaluation of both the biological and engineering aspects of this problem of heat sterilization is desirable in order to evolve a rationale which can be understood and supported by both biologists and engineers.

To optimize the selection of time-temperature cycles for decontamination, both in qualification and terminal sterilization, more research is needed on the physics of thermally induced failures of critical components. Accelerated test techniques should be developed where applicable to permit prediction of the effect of heat sterilization on part failure rates.

It appears at this early stage of our study that the use of high-energy X-rays ($\sim$ 10 MeV) may be a feasible means for sterilizing large spacecraft by radiation. However, more effort is needed to assess the critical problems and to compare the merits of radiation and heat with respect to practicality and economy.

We believe that the sterilization technology effort would be more effective if there were more rapid and complete documentation and dissemination of research and development results. An effective information center would be the preferable means of handling this problem.

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Plan for Sterilization of Voyager Capsule

At the outset, let us describe the bounds of the subject that concerns us in this paper. "Planetary quarantine" deals with the overall problem of preventing contamination of the planets with Earth life forms until such a time that satisfying biological investigation of the planets can be conducted. It is therefore concerned not only with the sterility of items intended to enter the atmosphere or land on a planet but also with maintaining a sufficiently low probability that unsterile objects not directed at the planet will accidentally impact it. Such considerations as spacecraft guidance errors, orbital decay, and booster trajectory must be taken into account.

We are concerned herein only with the sterility of capsules or probes intended to land on or enter the atmosphere of Mars. Furthermore, we are not considering the design and probability aspects of recontaminating the capsule after launch. Presented is a plan for the sterilization of the Voyager capsule, which has as its objective the launching of a capsule of demonstrated reliability and certified sterility.

We have set out for ourselves the following ground rules:

(1) The overriding constraint is for the capsule to meet its sterility requirements.
(2) Within this constraint, we must develop equipment that will operate reliably.
(3) Sterilization of the assembled capsule will be by means of dry heat.
(4) Ethylene oxide may be used as a surface decontaminant to reduce microbial population.

In many instances, we are working at cross-purposes in trying to conform to the first two ground rules. Reliability and sterility are the inseparable parameters of a two-sided coin. Heating of equipment to produce sterility has an inherent degrading effect on reliability. Although we must develop equipment that will survive heat sterilization and operate reliably over its required lifetime, we

should be sure that excessive conservatism in sterilization requirements is not making the achievement of reliability unnecessarily difficult and costly. Many readers of this paper will be specifically concerned with hardware reliability; we hope the paper will convey to you that we are acutely aware of the sterilization-reliability interaction.

Background

Digressing into past history, we have some object lessons on the detrimental effects of heat sterilization. The failures of Rangers III, IV, and V may well be attributable to equipment degraded by sterilization temperatures. This experience pointed out the need for a long-range program specifically designed to develop sterilizable hardware.

Emphasis up to this time has been on developing the technology of basic types of capsule hardware capable of withstanding the rigors of sterilization. We are now at that point in the Voyager project where these technologies must soon be channeled toward the design and production of specific items of capsule equipment. A technology cutoff date of January 1, 1967, has been set as a milestone in the schedule. This gives approximately one more year to fill the gaps in our technology for a 1971 capsule.

An operational plan is now required for the manufacture and testing of Voyager capsules that will result in flight capsules that can be certified to meet sterility requirements and that have demonstrated reliability. This plan must take into account the realities of the equipment, procedures, and controls that are needed to produce sterile capsules. Such items as monitoring and control of subassembly vendors, ability to assess the contamination occurring during assembly and testing, capability and control of sterilization ovens, and thermal lag characteristics of the assembled capsule must be considered. These must in turn be fitted into the framework of schedule and available funds. Obviously there are many trade-offs to go through in developing such a plan.

The first item that demanded attention was a decision on a time-temperature cycle for sterilization toward which future effort could be focused. In the early stages of the sterilization program it was necessary to fix on a sterilization cycle for use in developing sterilizable hardware technology. Since adequate information was not available at that time on acceptable ranges of time-temperature combinations, a single cycle of known efficacy was chosen; this cycle was 135° C for 24 hours with a qualification temperature of 145° C and was predicated on a low organism count prior to heating. Fortunately, compatibility with ethylene oxide was also made a requirement.

Subsequent effort by our biological coworkers has presented us with the range of time-temperature combinations from which to choose a sterilization cycle shown in table I. On the basis of the

TABLE I.—*Terminal Sterilization Cycles*

Temperature, °C	*D*-value, hr	Sterilization time, hr
155	0.31	4
145	.73	9
135	1.8	22
125	4.4	53
115	11.0	132
105	28.0	336

work done thus far in the development of sterilizable hardware, in the best judgment of those who have performed this work the cycle of 125° C for 53 hours was selected. Consistent with this will be a qualification temperature of 135° C, with appropriate times depending on the level of assembly being considered. Work in the development capsule hardware that may be used by Voyager should now be directed toward including these levels.

Flight Acceptance

In keeping with the test philosophy that the Jet Propulsion Laboratory (JPL) has found to be associated with successful flight missions, the selected sterilization cycle has been specified as a series of type-approval and flight-acceptance tests. Type-approval tests are intended for verification of design integrity and are conducted at environmental levels exceeding those used for flight equipment; they are conducted at progressively more severe levels as we go back to lower stages of assembly. Flight-acceptance tests are intended to demonstrate readiness for flight and are conducted at the same environmental levels that the equipment is expected to experience. In planning these tests, terminal sterilization has been considered essentially as another element of the launch environment analogous to launch vibration, and the test program has been structured accordingly.

Figure 1 shows the series of type-approval and flight-acceptance heat cycles that are planned. On the left is the terminal sterilization cycle of 125° C for 53 hours. This cycle will be used for flight-acceptance testing of subsystems prior to capsule assembly. The next block indicates the type-approval tests that will be performed at the system level with the completely assembled capsule—three

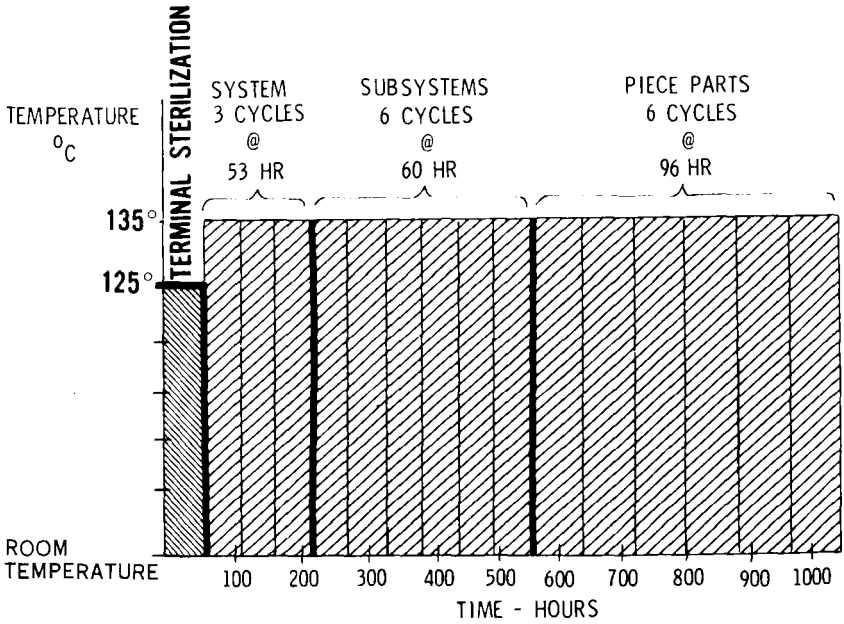

FIGURE 1.—Planned type-approval and flight-acceptance heat cycles for hardware qualification. System, subsystem, and piece part tests will not use same hardware.

cycles at 135° C. These will be longer than 3×53 hours, as we shall see. Stepping back further in assembly, subsystems will be type-approval tested at 135° C for six 60- to 70-hour cycles; the increased time per cycle allows for the additional time consumed by a subsystem in reaching a total capsule stabilized temperature. On the right of the chart, piece parts will be subjected to 135° C for 96 hours per cycle for 6 cycles. A reason similar to that given for the subsystems accounts for the longer piece part times. Where practical, we are attempting to specify times in multiples of 24 hours to aid testing.

In addition to the heat cycles described, it is planned to include exposure to ethylene oxide (ETO) as a part of the type-approval and flight-acceptance tests, since it is a vital part of the overall sterilization plan. Flight-acceptance testing will consist of one 24-hour cycle of ETO at 40° C, the same conditions that will be used on the flight equipment later. Type-approval testing at the system level will be three 24-hour cycles at 50° C and, at the subsystem level, six 24-hour cycles at 50° C. Piece parts will be qualified by similar testing.

Verifying Operations

Type-approval testing at each level obviously must include

verification of satisfactory operation after heat exposure. These tests must be performed for a long-enough time with respect to expected operational lifetime and with sufficient quantities of hardware at each level to demonstrate acceptable reliability. We are now going through the trade-offs of developing a plan that will balance mission risk against the astronomical costs of rigorous statistical demonstration of reliability. It will be keyed to the approach shown here of more rigorous testing at the lower levels of assembly where better statistical demonstration of operational lifetime can be achieved.

In our previous reference to a selected terminal cycle of 53 hours at 125° C, the time specified is elapsed time after the entire capsule has reached, within tolerances, thermal equilibrium. Since the capsule design may be such as to produce a large thermal lag, and one that may be difficult to verify analytically at all of the subsystem locations, the first type-approval heat cycle at the system level has been planned to provide additional information for use in flight-acceptance testing. As you can see from figure 2, we will first heat

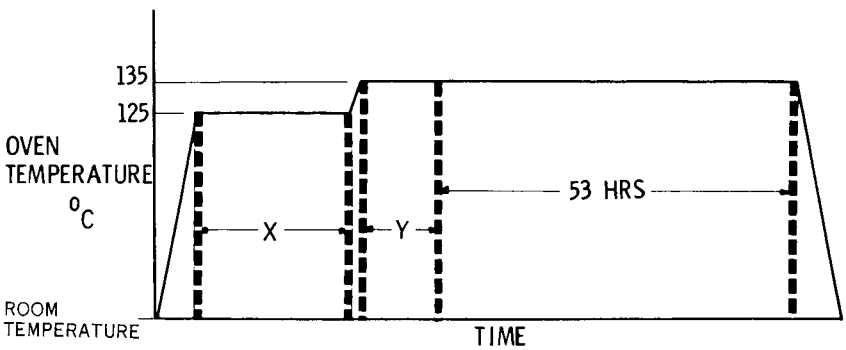

FIGURE 2.—First system type-approval cycle. Times X and Y are dependent on thermal characteristics of capsule.

the capsule to 125° C and measure the temperatures at various subsystem locations before finally raising the temperature to 135° C, where we will again measure the thermal inertia time. This will then give us actual heat exposure times that these subsystems will experience during terminal sterilization, and we can conduct the flight-acceptance tests on this basis. Indeed, we plan to certify flight hardware sterilization by inference. As an example, in a solid propulsion system we would employ thermocouples in the propellant grain of units identical to but certainly not in the flight units. The second system type-approval test will consist of a 53-hour stabilized 135° C temperature; the third test will be for 53 hours,

plus a 26-percent factor to assure a satisfactory margin for the system configuration.

Figure 3 shows the overall concept that we plan to use for sterilization of Voyager flight capsules. Subsystem manufacture will be conducted in a normal working environment having cleanliness requirements consistent with the production of reliable space hardware. However, in addition to the usual quality controls (QC), signoffs, facility surveys, etc., a sterility analysis will be conducted to give us as much information as possible on subsystem contamination levels as a means of locating and correcting sources of excessive contamination. Detachable coupons for microbiologi-

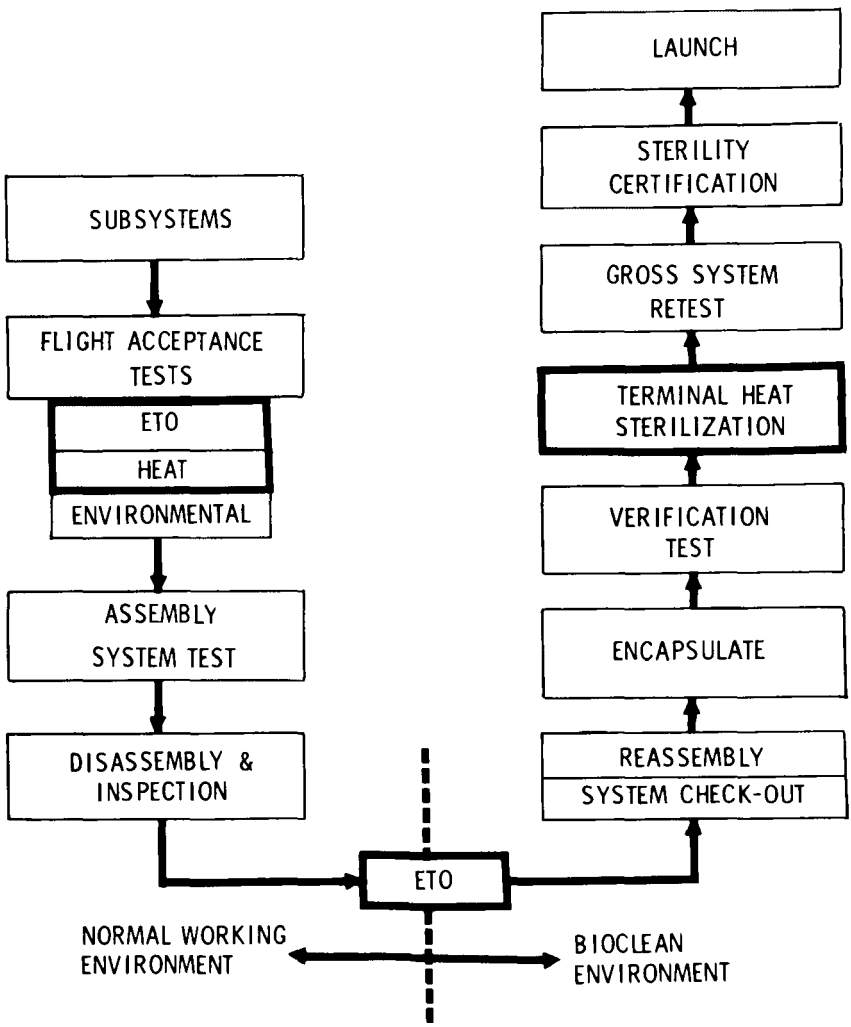

FIGURE 3.—Sterilization concept.

cal assay will be incorporated in the design of the subsystems to facilitate this task; these coupons will be carried with the equipment up to final assembly as a means of accumulating contamination data.

Initial capsule assembly and system testing will also be conducted in a normal working environment with cleanliness and traffic requirements consistent with the assembly of high reliability equipment. However, additional emphasis will be placed on the control of personnel practices to eliminate sources of excessive contamination, on controlled handling, and on monitoring procedures to provide data for later assessment of contamination level.

Flight Acceptance Testing

The first step after receipt of the subsystems is to subject them to flight-acceptance tests and complete functional checkouts. In addition to the usual environmental tests, these will include a dry-heat cycle equal to the terminal sterilization cycle and an ETO cycle duplicating the ETO cycle to be used later. The flight-acceptance tests will thus serve two purposes. First, they will demonstrate that the flight hardware is ready for assembly into the capsule system. Second, because sufficient surveillance will have been exercised over subsystem manufacture to keep the contamination within acceptable levels, the tests will render the equipment internally sterile.

At this point we introduce additional controls to minimize contamination and to provide a reliable detailed history of each hardware item. Containers will be used for all equipment handling prior to assembly into the capsule. Visual monitoring of all operations will be instituted and a careful plan of microbial sampling of the environment will be carried out. Information from these procedures will be accumulated in a data bank and used to maintain a continuous analytical assessment of contamination level.

After flight-acceptance testing and subsystem buy-off, the subsystems will be assembled into a complete flight capsule and environmental and functional tests will be conducted at the system level. Conventional environmental facilities such as vacuum chambers and shakers will be used, but with careful environmental sampling and special attention to conditions that might produce excessive contamination.

ETO Decontamination

Following system testing, the capsule will be disassembled to the level of assembly at which the equipment was subjected to the

flight-acceptance sterilization cycle. By the use of suitable design precautions, the hardware at this assembly level will have remained internally sterile; that is, sterile in all internal areas that cannot be reached by ethylene oxide. Now, if we decontaminate all exposed surfaces with ethylene oxide and conduct all subsequent handling and assembly operations in an ultraclean environment, we should be able to reach terminal sterilization with an acceptably low micro-organism count.

ETO decontamination will be performed on entry into the final assembly facility, and, as indicated on the diagram, marks the transition from what we have called normal working conditions to a controlled bioclean environment. This final assembly facility is envisioned as a laminar downflow facility with provision for thorough microbiological environmental control, decontamination of working tools and equipment, and appropriate personnel practices. All remaining operations prior to terminal sterilization will be performed in this environment; however, this will involve a relatively short period of time. Environmental sampling and control procedures similar to those described earlier will be continued, but at an enhanced level of detail. The capsule will be reassembled and system checkouts performed. It will be sealed in the sterilization canister and given another functional verification test. It is then ready for the final steps of terminal heat sterilization, a gross system test, and conditional certification of sterility for launch.

This plan accomplishes the objective of enabling us to perform the more difficult phases of assembly and all the environmental tests under reasonably normal working conditions. If environmental testing had to be performed under the bioclean conditions of final assembly, a manifold increase in time and cost would be incurred. By incorporating ethylene oxide capability into the final assembly area, we not only complete the job of decontaminating the already internally sterile subsystems but we have a working tool at our disposal for coping with accidental occurrences of contamination. For this reason, we feel the requirement that all capsule hardware be compatible with ETO and its inclusion as part of type-approval and flight-acceptance tests to be well worth the effort.

Concluding Remarks

A few final words on Voyager's continuing needs and schedule are in order. Our current planning calls for launching two planetary vehicles per opportunity on a single Saturn V booster, beginning in 1971. The first capsule science mission is scheduled

for the 1973 opportunity and, if feasible, two test capsules will be launched in 1971. As I mentioned earlier, we have set a technology cutoff date of January 1, 1967, as the latest time compatible with launching the 1971 capsules. Whether we develop a survivable lander for 1971 or something less ambitious, such as an atmospheric probe, we will need to have most of the same sterilization technologies available by the cutoff date.

We will mention briefly a few of the more critical areas requiring additional effort. Obviously we must have an adequate selection list of qualified sterilizable piece parts. Further development is needed on an analytical sterility model for maintaining control of capsule contamination. We urgently need more work on the efficiency of ethylene oxide and on development of better and more reliable collection and assay techniques. We need to work out techniques for sterile insertion as a backup to permit more flexibility in repair at the launch site. Much more work is needed on the operational problems involved in clean-room assembly of large capsules. Add to these the many areas of sterilizable equipment technology that will be needed by the technology cutoff date, and you can appreciate the size of our task for the coming year.

Session VI—Discussion

Burnett, *Electro-Optical Systems*: Dr. Cooley, Electro-Optical Systems has recently finished a job for the Air Force in a radiation-tolerant ion propulsion system. In this program we did quite a bit of gamma testing at a level of 100 megarads and encountered little difficulty with the electronic components; the same was true with electromechanical systems and batteries. I wonder if you could tell the amount of gamma radiation dose depression obtained between the edge and center of the spacecraft and if you had considered a 4-pi solid angle source and the dose factor buildup in this?

Cooley: I am interested in the data on the 100-megarad circuit. We are now in the process of calculating dose depression rates through simple geometry. We are considering 4-pi isotropic sources of isotopes such as cobalt 60. I don't have any numbers which I could report now. It is a real problem to get less than a 10^1 dose variation. One may have to accept numbers bigger than that. It means that certain components may have to withstand at least 50 megarads.

Varga, *Hughes Aircraft Corp.*: Mr. Kautz, I did not fully understand the type-approval concept as to how one bears out the adequacy of the total sterilization plan. I noticed that in your last figure you had the flight-acceptance test, but I didn't see the type-approval test. What plans do you have for type approval of the sterilization requirements?

Kautz: I indicated that we will have the severe environment at the piece parts level for 96 hours. For example, this will be six cycles of heat and six cycles of ETO at 135° C. This gives a 10° cushion over the flight-acceptance and terminal sterilization levels. On the subsystem level we will require six cycles again at the elevated temperature for the qualifying hardware. At 135° each cycle will be between 60 and 70 hours, depending upon the timelag for various configurations of the capsule. In the system configuration we will have the three full qualification levels which I indicated.

Varga: In the three full systems tests will you have actual micro-biological samples that you will monitor through the cycles to see that these sterilization processes are adequate?

Kautz: Absolutely. We will include those capsules that Mr. Irons discussed. This work will be done on a pilot basis by running a PTM which will be designated for sterilization use throughout the full sequence.

Varga: In the piece parts and subsystems cycles, you show 53 hours at 135° C. I was wondering how you arrived at the 53 when your first official chart showed that the 135° C sterilization cycle would only require 22 hours of actual exposure.

Kautz: The chart of the selected time temperature curves was provided by NASA. The spectrum ranges from 105° for 336 hours to 160° for 3 hours. As a hardware supplier, we have a choice of this spectrum, Our choice is 125° for 53 hours. If you plot the hours of exposure at varying temperatures against your confidence in mission performance, you find the highest confidence level at 125°. Now, as you go over 125° the confidence level falls off very rapidly; below 125°, it falls off more slowly.

Varga: I thought from what you had on the chart that you could go through a sterilization cycle in 22 hours at 135°.

Kautz: We are qualifying by adding a margin to the 125° which is what the flight equipment will use in terminal sterilization, and we expose for 53 hours because we need that as a cushion. The choices that you have are equivalent from the biological standpoint, not from the reliability standpoint of the hardware.

Schalkowsky: I gather that the basis for the flight-acceptance test is the notion that the terminal sterilization should be considered as part of the launch environment. Now this is a choice but, physically, it certainly is not part of the launch environment. There are tests that you specify following terminal sterilization so that you really do not have to consider it as part of the launch environment. It may not be the most desirable choice. You must have a rationale which I would appreciate hearing.

Kautz: We do, indeed, consider this terminal sterilization as a part of the launch environment. We certainly would not want to launch hardware that had not been tested for the booster environment.

Connelly, *Decontamination, Inc.*: Mr. Lorsch mentioned the use of portable or plastic clean rooms, using a plenum in order to get something like vertical laminar flow conditions. Generally speaking, particularly in contamination control, we have avoided the use of plenums because we can have spontaneous generation of this kind of contamination. I had not given them any thought in the biologically clean rooms. What is NASA policy regarding this concept?

Hall: This is a problem that has plagued engineers working in the hospital field for some time: whether there is actual generation of contamination within the plenums. As far as the literature goes, there are several reports of this occurring. In the experience that I have had in 10 years' association with this work, our staff never

detected a situation in which contamination could be confirmed. We are not concerned with it, although we would prefer to be aware of it. In the biological field, this would be a problem only if there were a high level of humidity in the plenum. These are the only conditions under which it has been reported. Where steam or other nebulized water was introduced into the plenum for humidity control, that might provide enough water for growth of microbial life.

Lipson, *General Electric Co.*: Mr. Kautz, concerning the chart showing the flow of activities from subsystem to launch (the point of ETO application which divided normal working environment from bioclean environment), was that supposed to indicate shipment to the Cape?

Kautz: No; it was not.

Lipson: Does the contractor at his home facility require bioclean environment under your present subassembly and assembly procedures?

Kautz: If you mean does a prime contractor on the capsule need bioclean assembly facilities, this answer has not yet been determined. We are continuing evaluations and discussing trade-offs associated with the problem and we expect to have an answer soon.

Hedén, *Karolinska Institute (Stockholm)*: Dr. Bruch, I was interested to note that you had problems in sterilization of media. I noticed also that this problem was not solved, actually, by your sterilization of dried powders. Were the dried powders in the form of a mixture of all the media components or were the different chemical components separated by powder coating or similar techniques which could make a difference? Also, I noticed your difficulties with sugar; do you mean monosaccharides? What about subjecting polysaccharides to hydrolysis after sterilization? Have you considered that aspect?

Bruch: I understood that the media were sterilized as a complete media; in other words, all the reagents were mixed together into the total powder.

Opfell, *Aeronutronic Div.*: They were done two ways, with and without water in the standard medium. We had the complete medium with water and the complete medium without water. As for the polysaccharides, the purpose of this experiment was to identify critical sterilization problems. We made no attempt to resolve those problems. We simply confirmed what was expected: that the complex media cannot survive 135° C for 24 hours and perform in the manner in which they are conventionally used.

Riggs, *Abbott Laboratories*: I have a suggestion related to terminology. I notice that we talk about certifying sterility; I wonder if

it might be made comparable to quality. Substitute sterility assurance for quality assurance and then certify that the sterility assurance requirements have been met.

Trischler, *Narmco*: The requirements stretch from minute parts up through the finished capsule with safety margins of twofold and threefold. One bar graph illustrates a piece part receiving 135° C for 1000 hours. I do not recall any piece part being tested for such a long time.

Kautz: You must understand that the qualified piece parts are not the ones that go into the qualified subassembly which goes into the qualified capsule. These are not necessarily cumulative. As a matter of fact, except for demanding screening requirements for piece parts which ultimately go into flight, there is none of this accumulation in the total. I might clarify this one thing; if you look at a configuration of the capsule, somewhere down in the center is the worst point (the most remote point), that has to receive 125° C for 53 hours. At the present time we do not know the configuration of the subsystems. In starting out with piece parts we have to consider that the most remote one might well have the temperature applied for 53 hours. By the same token, that is why we have picked 60 to 70 hours for a subsystem. A subsystem may be very close to the canister and take this temperature for the increased length of time.

Miles: Would it be fair to say that the total cumulative time of heat treatment would be the FA cycle plus the final terminal sterilization cycle of these parts?

Kautz: On piece parts the qualifying time will be six times the 96 hours. On the qualification of subsystems, it will be six cycles at 60 to 70 hours; roughly, 360 to 400 hours.

Miles: How many hours of heat do flight items get?

Kautz: On the flight items, about 2 times 53 hours.

Dixon, *TRW*: Mr. Spencer noted that one desirable mode of separation had the possible consequence that the capsule was exposed to cross-contamination and that it might be good if you could rotate it and get it exposed to ultraviolet from the Sun. Mr. Crawford noted that different surface finishes on stainless steel made no difference in the ability to sterilize. I wonder if we could get together and propose a smoother outside vitreous surface to facilitate ultraviolet sterilization after separation from the spacecraft.

Miles: I would remind you that the surface about which we are speaking is the capsule heat-shield surface. Therefore, you may not have the flexibility of accepting any surface coating that you want.

It must be compatible with the heat-shield material which would be utilized for protection during the Mars entry.

Shull, *General Electric* Co.: Mr. Spencer, you quoted some information concerning the killing action of ultraviolet light. You mentioned that this had been computed to be in the neighborhood of a few minutes in the area of Mars. Can you give me the source of the ultraviolet flux that was used in the calculation of this information?

Spencer: I cannot give you the source offhand. The calculation by Dr. Horowitz in a forthcoming article in *Science* indicates a maximum time of 10 minutes' exposure at a mean Mars distance.

Comment: Dr. Silverman said that a spore could hide under a little colloidal particle and not get exposed to ultraviolet. One could assume they might survive.

Session VII

General Summary and Panel Discussion

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General Summary and Panel Discussion

SUMMARY

Hall: The first Session may be summarized briefly as follows Dr. Atwood took a fresh new look at the possibility of contaminating Mars. In his evaluation, the probability of terrestrial life infecting Mars is very low, but the penalty for doing so is very high. The penalty is too great a risk and must be avoided by doing the best possible job of planetary quarantine. Dr. Newell reviewed the NASA organization and emphasized that NASA management is determined not to infect Mars with life. Dr. Reynolds pointed out the relative tasks of the Bioscience Programs and of the Lunar and Planetary Programs Offices. I would like to reemphasize his point that the Lunar and Planetary Programs Office proposes measures and the Bioscience Programs Office approves those measures. In my paper, I stated that the planetary quarantine requirements had been developed as broad guidelines within which Lunar and Planetary Programs can develop these proposals. I emphasize again that these requirements are under continuous review, and will be relaxed as knowledge permits, but they will *not* yield to pressures of convenience or expediency. In Session II, it was brought out that the problems of planetary quarantine are more than just those of biological sterilization. The probability of impact of the bus and other hardware exists, and must be controlled within the constraints specified by NASA. Dr. Craven clearly defined a number of these possibilities and recognized that numbers cannot be assigned to many of these factors. The technology of control of microbial burden has developed markedly in the past year, to the extent that it exceeds requirements of current surgical practice. The justification for these practices rests not only in the control of numbers of organisms, but also on species selection and on the prevention of biological accidents. It was recognized by two authors that man is the source of most contaminants in controlled environments. However, it was also pointed out that man *can* be isolated from the environment by topological techniques. Also, in

Session II, it was pointed out that visual monitoring is less expensive than bioassay. Microbial recovery techniques, as far as surfaces are concerned, are accurate within the order of magnitude required. The real bottleneck in evaluation of bioburden is our inability to quantitate adequately the internal contamination. Research is being supported in this area. The value of sterility indicators has been questioned, but will be resolved as the matter is given further study. In Session III, the authors stated that nonthermal methods of sterilization are possible, and much is known about their capabilities. However, each method has its disadvantages and for the present, heat sterilization is preferable. Dry-heat sterilization has been studied, and sufficient knowledge is available to justify our standards at the present time. Value judgments have been necessary; however, refinement of results will permit more accurate judgments. Gaseous and liquid sterilants are available. They can be used for decontamination, but need more thorough study before they can be depended upon to produce the same reliability as heat. Filters are available for the processing of air and liquids. Reliabilities range from zero to complete removal of all particulates. They may be usable for sterilization of liquids for spacecraft work, but must be evaluated in each particular case. We acknowledge that viable contamination deposited by human contact is a problem, but it can be prevented, and when it does occur it is capable of quantitation. Very serious engineering problems are amenable to solution. We believe that the engineering profession can solve these problems. In Section IV it was pointed out that mathematical models can be developed that will be of great aid in estimating the biological burden, probably within the limits of confidence that will be required. In general, a commentary on the session so far has been that there is a tendency for the engineers to insist on a systems approach to biology. Biologists, on the other hand, do not understand or appreciate the value of a systems approach. Such an approach has much to offer, but it has limitations in its application to biology. The meeting has resulted, however, in an exchange of information and viewpoints between biologists and engineers, although they are still far from understanding the capabilities of each other. I plead for understanding, a full realization that biologists and engineers have been devoting their lives to their respective specialties. It would be presumptuous for anyone to assume that a brief exposure to a given technology makes him as capable as one who has made it a career. Wisdom is not housed exclusively in any one group or profession. Let us use the best that is available, wherever it is found.

Goddard: The following principal points were made in Session V. The discussion by Mr. Lockyear concerned the all-important and ubiquitous problem of electronic piece parts that thread their way through the spacecraft. Briefly, he pointed out that a substantial continuous program had been undertaken for the systematic testing of all possible categories of electronics piece parts most likely to be used in the Voyager lander device. In general, it has been found that most electronic parts will survive the sterilization treatment. A bible, so to speak, of approved electronic piece parts is in process of compilation and will be available just as soon as results from the long life reliability studies following heat sterilization are completed. The piece parts list will be worked into the basic specifications and requirements of the Voyager project. He stressed the great importance of quality control and process knowledge in the fabrication of piece parts. I should make clear that the program's discussion was based on a particular heat cycle used for the past 3 years for the SR&T Program: namely, type approval of three cycles at 145° C for 36 hours and flight approval of one cycle at 135° C for 24 hours. As the Voyager program becomes better defined, the specifications of the piece parts program will benefit accordingly.

The next area discussed was that of the polymer class of materials. It was stated that about three-quarters of the polymer materials studied thus far would survive the sterilization treatment, while the remaining one-quarter were marginal or still under consideration. It was stated that no insurmountable problem would be encountered in finding a successful material in this category for a given spacecraft need. It was also stated that plastics, generally, were not a problem, but that some polymer films were damaged by ethylene oxide. Mention was made of a master list of polymeric materials, that is, those surviving the sterilization environment, which would be out soon. Mr. Holtz, speaking of other materials and processes involved in spacecraft, stated that the situation was generally satisfactory with regard to finding materials capable of meeting the sterilization environment. Mr. Lutwack, in regard to batteries on board spacecraft, described very well the considerable problems that exist in this particular area. Important problems under consideration include separators, the case, and seals. The battery problem is of paramount importance and needs considerable attention from all parties concerned. Mr. Soltis described the silver-zinc cell, and other backups or alternates to the regular battery.

In the area of guidance and control, Mr. Nicklas mentioned four problem areas under study: (1) actuators, (2) optical sensor

devices, (3) gas-bearing gyrosystems, (4) electrostatically suspended gyrosystems. It was felt that sufficient progress had been made in guidance and control engineering to meet the Voyager requirements. Mr. Arens of JPL Telecommunications talked about equivalent suspect items in the communications and data acquisition area, with emphasis on pressure transducers and tape recorders. He noted that the belts and magnetic heads of the tape recorders were the most significant problems. It is felt that the problems are not insurmountable, and we believe that they can be solved with continued effort. Mr. Gin of the JPL Propulsion Division stated that the solid propellant and rocket motor could be satisfactorily sterilized. However, liquid propellants present problems of a more difficult nature, but continuing work should result in a solution acceptable to the Voyager requirements.

Miles: In Session VI, Mr. Spencer dealt with the problem of separation of the lander without recontamination of sterilized hardware. Work in progress covers heat shields, impact limiters, and parachutes. He mentioned particularly that balsa wood, a most notable candidate for its physical properties, is presenting some problems with regard to its sterilizability. In the case of parachutes he mentioned two materials that seem to be satisfactory: Nomex and Dacron. Nomex is preferred to Dacron. Mr. Crawford dealt with design problems. The preliminary problems concerning spacecraft designers were those of joint materials, thermal stresses, and heat exchange. Significant was the fact that a system under vacuum, as illustrated in the figure he showed, requires approximately 8 hours for the innermost component to come to temperature. He also mentioned that the organization of the spacecraft is extremely important, since the outside parts are going to get hot before the inside ones. Therefore, the most resistant parts should be externally located. He indicated that they had determined the thermal characteristics of a typical model and that thermocouples would probably be required in the finished product. The end result would be design manuals organized for the use of those concerned.

Dr. Bruch described some of the activities that are coming to light in his work with ABL. Our work in systems, he said, deserves a great deal more emphasis than we are giving it, because parts do not necessarily represent the whole when it comes to heat performance.

Dr. Lorsch estimated an increase in cost of approximately 70 percent in changing from aerospace clean to biologically clean assembly. He produced some figures with relationship to bioassays which had been performed and left doubt as to whether the need for this higher quality clean room can be sustained.

Mr. Cohen described the status of the JPL efforts to develop a pilot sterilization and assembly facility. The first one will be called the experimental EASL; the second one, SADL, will be more of a prototype. He noted that it would be approximately 36 months from go-ahead to construction of the final facility.

Dr. Cooley discussed several unrelated problems and described some of the deficiencies in our program. The problems he described were centered around the conflicting requirements of sterility assurance and equipment reliability. He questioned the applicability of the soil curves to the decontamination cycle and to the terminal sterilization cycle. He pointed out a notable deficiency in the method of long life testing of piece parts: namely, the time and expense involved to determine reliability of parts following the heat cycle. Rather than subject parts to 10 000 hours of reliability testing, he suggested stressing, accelerated aging, and failure analysis. He stated that we are reopening the question of radiation as an alternate to heat as a means of sterilization. He made the point that we need to develop an expanded system for distribution of information about the sterilization program.

Mr. Kautz discussed a plan for his sterilization activities for the 1971 mission. Discussion centered about the type-approval tests (TA) and the flight-acceptance tests (FA) being applied in addition to the terminal cycle. The conclusion was that an FA cycle would be added to the terminal sterilization cycle. The FA cycle would be equal in time and temperature to the terminal cycle. He implied that if the requirements can be reduced to those which are needed, then both cycles could be lowered appropriately.

QUESTION PERIOD

Question: Will NASA consider the use of sterile bioclean portable barriers to conduct black-box replacement in order to avoid reheating the flight capsule?

Hall: Definitely. We are considering this possibility. We have not conducted or funded much research in this area, but we have recognized a need for it. The techniques that can be used for this must have reliability equal to that of heat sterilization and, at present, the entire matter hinges on this one point. If they can be shown to have this reliability, then they will be acceptable; if they do not have this reliability, then they must be improved or cannot be used.

Question: The present criterion for planet biological contamination is 1 in 10 000 per launch. I do not see how this extrapolates to a 1-in-10 000 chance of a living organism being onboard a lander. An organism located internal to a part must be liberated. What is

the chance of this occurring? It must not be killed during liberation. What is the possibility that this occurs? Many organisms could be aboard and landed, provided the probability that they are liberated alive is 1 in 10 000. Should we not consider this probability and thus ease the component qualification tests?

Bruch: These are good questions, and my answer will not be complete. The chance of an organism being liberated, I agree, is quite low, based upon some preliminary information that we have. When the Bioscience Subcommittee of the Space Science Steering Committee of NASA considered this problem, they felt that the scientific risk was of sufficient priority that we should state the probability in terms of not landing an organism. The lack of information on the probability of release from solids did not allow the committee any other approach. A total analysis of the probability of contamination of Mars by unmanned landers should include the probability of release of any residual contaminants from a heat-sterilized lander. A decision on this aspect must be based on the advice of international and national scientific groups concerned with planetary exploration.

Question: Assuming the Voyager will incorporate heat-sensitive components, will the development of an alternate method of sterilization be funded by NASA or be the responsibility of the contractor?

Miles: First, there is work underway now in regard to sterile insertion. Second, work that we are carrying on at the Langley Research Center is looking into the feasibility of going through a tube into a suit from outside of a chamber and making repairs or assembling the spacecraft inside the chamber—a human glove box. The SR&T people of both Bioscience Programs and Lunar and Planetary Programs are working on it, and both recognize the responsibility. Mr. Hall has said that it will have to be proven that this is of equal reliability to the heat cycle that he has prescribed and, indeed, this is no less than we have expected. All parties concerned will be working on this. I do not think that the Voyager contractor will have sole responsibility, but that is a matter that I believe the Voyager people will have to face when they get there.

Question: What criteria were used in making the decision to dismiss the role of infectious nucleic acid carried on the spacecraft in altering the genetic material or metabolic processes in extraterrestrial organisms?

Bruch: Well, I think this goes back to the same question that Dr. Héden has asked about the possibility of metazoan viruses reproducing in *Bacillus subtilis*. I agree that this possibility can occur if it is assumed that the biochemical pathways in the

extraterrestrial organisms are the same as those that exist in the terrestrial system. From the practical viewpoint, a spacecraft free of nucleic acid is not within the capability of our technology. Our assay system cannot tell with much precision the number of viable organisms inside a solid. I know of no system that will tell the total amount of nucleic acid in a solid.

Question: Until now, it has been impossible to get JPL material on approved parts which are compatible with heat and ETO. Is this information now going to be released?

Goddard: There are two documents. One is entitled *The Preferred Parts List of Reliable Electronic Components.* The other is called *Electronic Parts Sterilization Candidates for Spacecraft Application,* which is the beginning document of the sterilization parts list. Both of these documents are available through the JPL Technical Document Control Center. Cite in the request a contract you have that indicates a need to know, and you will receive copies of these documents. The program has been underway for about 3 years; 10 000 hours is about 1.2 years. The piece parts have been bought in large batches and have been put through the processes as rapidly as possible. This has been done piecemeal as funds have become available, so that testing of some parts has just begun. Some are a quarter of the way through; some are finished; but most are not. In other words, it is clearly an accumulating list which, during the coming year, will certainly produce many results which will be analyzed and then added to the final list. The JPL preferred parts list has the following identification: JPL–Spec zpp–2061–ppl–g. The second one, *Electronic Parts Sterilization Candidates for Spacecraft Application,* has this number: JPL–Spec zpp–2010–spl–a.

Question: I am uncertain whether suppliers of electronic components for the capsule (tape recorders, power amplifiers, etc.) will be required to fabricate and test this equipment in a class 100 clean room equipped with biological assay facilities. Can you specifically state that these suppliers will be required to have these facilities?

Tarver: A class 100 clean room would not be a requirement. We cannot, however, second-guess each and every piece of hardware; we may find that there are some specific pieces of hardware which we would want to assemble under cleaner conditions. Nominally, however, parts would be put together under conditions of cleanliness that are commensurate with reliable assembly of high-quality equipment.

Question: What are the contemplated or existing requirements for manned spacecraft sterilization?

Hall: This is too far away. At the present time, we do not really

consider the problem because it is quite possible that when man lands on the planet, we will have to accept contamination. Certainly I am not qualified by either knowledge or authority to speak on this subject, nor is anyone else.

Question: Because of the limitations of the microbiological assay in confirming the success of the sterilization process, has consideration been given to selection of other biological tests: for example, integrity of nucleic enzymatic activity, which could be employed to establish the state of the micro-organisms after sterilization?

Bruch: I feel that this is one approach in answering the previous question about the concern for infectious nucleic acid. This could be a way to attack the problem not only of infectious nucleic acid on a sterilized spacecraft but also to establish the upper limits of contamination inside various piece parts and solids before sterilization. We can employ the tools of the molecular biologist by assaying the nucleic acid from both the living and dead organisms and thus establish an upper limit for initial microbial contamination. This concept is very good and we should give more consideration to it.

Question: I have noticed that considerable reliance has been placed on heat and gas sterilization but practically none on X-ray techniques. Why?

Miles: The answer is that we are not going to depend on it until we know we have a specific need for it. We are pursuing it, however, and we will carry this as a backup effort in SR&T until we can prove that it is both economically and technically feasible.

Question: What measures were taken with regard to Mariner III and IV spacecraft to reduce the possibility of biological contamination of Mars?

Craven: The aim point was biased to insure that the spacecraft would miss the planet. In fact, some people were quite concerned that the aim point was biased so far away that the altitude was higher than the TV cameraman wanted. In addition, the attitude control gases were filtered to assure removal of contaminants that might have reached the planet by going into an intercept trajectory during the control maneuvers.

Question: When using such numbers as 10^8 and a probability of 10^{-4}, are you speaking of the discrete living organism or discrete colonies of organisms? If the latter, what is the significance of 10^8 colonies of many discrete organisms as a biological load?

Bruch: We are measuring a combination of discrete organisms and discrete colonies; but, more correctly, it is the colonies that appear on the plate. If one is trying to kill clumps of organisms, the kinetics of the death-rate curve will not be first order. One

has initially a lower estimate of total organisms when clumps are present. I do not know how many people have noticed this point, but the heat cycles are specified for spores, while the biological loading limit is set for organisms. The thinking right now is to state that 10^8 organisms is the limit rather than the number of spores because of this clumping phenomenon and uncertainties in assays for spores. Even if one had clumps of vegetative cells, these clumps would not have the resistance of one bacterial spore. It would take quite a clump of vegetative cells to equal the heat resistance of one spore.

Question: Has the aerospace microbiological state of the art reached a definite stage that would allow the development of methods and techniques of microbial survey that are sufficiently standardized to allow an industrywide exchange and comparison of data with a minimum of confusion?

McDade: Mr. Hall has a task force working now on a set of procedures for the microbiological examination of spacecraft. We hope that this preliminary method will be circulated and evaluated under a number of situations in an attempt to standardize. So, the answer is that proposed methods have been prepared and will be distributed shortly.

Question: Will the planetary quarantine program include consideration of chemical contamination of sampling sites or experimental equipment?

Hall: The Planetary Quarantine Office will definitely include the consideration of chemical contamination of the planet, but it is not being given tremendous importance now because chemicals in general do not have the property of reproduction. This is the hazard of the biological contaminant: not that a single organism or five million organisms are deposited in one spot, but the fact that given time and the right conditions, they will multiply until they cover the entire surface of the planet. The National Academy of Sciences, in its early deliberations, recommended that an inventory be made of all organic materials on board each spacecraft in order that those materials found on the planet later could be identified as having come from the spacecraft. As I recall, this was considered to be a monumental job; however, the Planetary Quarantine Advisory Group is looking into this in the near future, but it is not being given a high priority.

Question: What is the significance of the plateau that appears on your graphs when sterilizing with dry heat at 135° C for 24 hours?

Bruch: I think that this refers to the figure on the spore indicator tablets which illustrated a constant ratio of tablets with survivors during 10 to 18 hours of dry heat exposure. A rapid decrease in the

number of survivors occurs during the period of 2 hours to 10 hours' exposure, a plateau of 25 percent of the tablets with survivors until 18 hours, and then sterilization of all tablets after 23 hours.

Question: What is the kill lag or plateau?

Bruch: Each of the spore tablets has between 10^9 and 10^{10} spores. The data show that quite a few of these spore tablets have no survivors after short exposures at 135° C; however, some of the tablets show survivors over a long period of time. Our problem is to determine what we are measuring with the spore tablets. I have a suspicion that maintenance of a critical moisture level inside the spore tablet enables some of the organisms to withstand the sterilization temperature. The theory as I understand it from Dr. Pflug's forthcoming review paper, is that a certain moisture level (activity of water of 0.2) enables organisms to show maximum resistance to dry heat. This situation can exist in some of those spore tablets. Dr. Pflug used some of our data on spore resistance in solids to suggest this relationship, and that is why I want to extrapolate them further to find what is occurring in the spore tablets. The second point that has been raised is the possibility that the spores are going into dormancy during this exposure to dry heat. The spore tablets showing no growth would contain viable spores, but the spores are not able to grow out. This seems a little difficult to accept because, as Dr. Stumbo mentioned, you usually use moist heat to get heat activation of the spores. Whether dry heat has the same effect, I do not know. It is a little difficult to expect that after you have exposed a spore to dry heat, you would have to shock it with moist heat to bring it out of dormancy. The exposure of the spore to two lethal agents should be more damaging than exposure to a single agent. I am very skeptical about the dormancy argument.

Question: In data of the plateau phenomenon showing fallout of micro-organisms on stainless steel strips, are you talking about total number of organisms or are you considering only spore formers?

McDade: The answer is total aerobic mesophiles. We include both spore forming and asporogenous organisms, having determined first the spore forming ratio of vegetative cells on stainless steel strips over an extended period; and there is no shift unless you put strips exposed to the environment in the chambers and do not allow any more material to accumulate. If you expose these surfaces to the environment, then you will have a shift in the ratio of the spore population to vegetative cells.

Question: Nowhere in the sterilization specifications does it

mention pressure other than atmospheric. Does pressure increase the activity of ethylene oxide? Will pressure eventually be specified and what work has been done on this topic?

Phillips: There are several ways of measuring concentration of gases. Pressure is one; milligrams per liter is another. As far as the gas is concerned, pressure is merely an indication of its concentration. The reason we are not talking about pressure, but rather about milligrams per liter of ethylene oxide, is that given a mixture of gases, all are contributing to the pressure, but only the ethylene oxide is contributing to the sterilization; therefore, it is meaningless to talk about 7 pounds per square inch on the gage of a hospital-type ethylene oxide autoclave unless you say how much is subtracted for oxygen, nitrogen, carbon dioxide, and Freon. On the other hand, you can express ethylene oxide concentration as so many pounds per square inch, millimeters of mercury, or any other way you want, but only if you are expressing its chemical concentration in terms of pressure; however, ethylene oxide is not used alone very much, and as the pressures are cumulative, you only want to know how much of it is ethylene oxide.

Question: We have postulated the prelaunch terminal sterilization for the capsule and its barrier that might produce mechanical stress which may cause failures during launch, acceleration, and landing impact. Would it not be wise to include, after terminal sterilization, a flight environment vibration test requirement, particularly since the integrity of the sterilization barrier itself may be in doubt?

Tarver: There are several aspects to this. Mr. Kautz's flow chart showed, after terminal sterilization, a gross system retest after the capsule is buttoned up in its biological barrier, the amount of functional checking performed will be very sketchy. It will be, in fact, a gross system retest. If we were going to conduct a vibration test, we would want to be able to do a much better systems test than this to be sure that we had not caused any malfunction. There is another aspect: namely, after we have sealed the biological barrier and subjected the whole capsule to heat sterilization, would we want to subject the sealed capsule to vibration tests with no very good way of ascertaining that we had not breached the biological barrier? The answer is "No."

Question: Present tests for living organisms are based on potency or ability of the organisms to divide and reproduce. Is it possible that the organisms are not dead but merely dormant?

Bruch: Dormancy has always been a problem with the bacterial spores. One is never certain in conducting spore viability tests, after sterilization, that destruction has been achieved. According

to C. F. Schmitt, canned foods have been held for as long as a year to bring out some thermophilic anaerobes. There is the possibility that when we say that the spores are dead, the sterility tests are unable to stimulate spore germination. The average sterility test is incubated for 3 weeks; if we wanted to incubate longer, we would have to hire more people and purchase more equipment and more facilities to make these assays.

Question: Have sterilized items been put away for a year in space environment and tested for sterilization at that time?

Bruch: As far as I know, no.

Phillips: The question of whether nucleic acid is alive or dead has come up several times, and I would like to add a few words. People used to argue about whether viruses were chemical or living organisms because you could crystallize them. Stanley got a Nobel Prize in the twenties for crystallizing the tobacco mosaic virus. We know a lot about the actual structure of nucleic acids. We know that the smallest things that contain them and replicate are still called viruses. We know that infective nucleic acid, be it phage or animal virus or plant virus, has little more to it than nucleic acid. We also know that chemical and physical procedures will kill bacterial spores. As far as I know, none of the viruses remain infective, and I am talking about viruses, not nucleic acid. So, we use procedures that kill bacterial spores and the more resistant viruses. The only sterilizing treatment wherein viruses are more resistant in certain cases than bacterial spores is ionizing radiation. The virus, being smaller, is less apt to be hit. We have only a few cases where viruses are more resistant than bacterial spores; however, some of the viruses are fantastically fragile. Few are quite resistant. Infectious nucleic acid is a virus, but I do not think we will have any live viruses if we sterilized to that extent. We do not know what life is, and none of the bacterial spores exhibit what we think life to be.

Question: Biologists are concerned with sterilization of interplanetary landers in order to preserve our once-in-history chance to investigate possible life evolution on other planets. Assuming a low probability of encountering and bringing to Earth anything dangerous to terrestrial life, is not postlunar return quarantine mandatory?

Hall: My answer is that a year and a half ago the National Academy of Sciences assembled a select committee that considered this question. They prepared and transmitted to NASA a report with recommendations. That report is in NASA's hands and is under study. When we know the answers, we will inform all interested persons.

Question: How can Mars be kept free of terrestrial micro-organisms for 50 years when manned landings are planned during this period?

Hall: The Bioscience Subcommittee of the Space Science Steering Committee took the position that 50 years was a reasonable, if pessimistic, period to be expected before man could land and contaminate the planet. Meanwhile, we do not want to take a chance that failure to land and explore the planet in less than 50 years would prevent fruitful examination of Mars because of prior contamination; therefore, 2021 is only an estimated figure that can be revised when we have further knowledge of manned Mars landings.

Question: I noticed a serious omission with respect to considerations of the possibility of accumulating large numbers of viable particles on electrically charged surfaces in view of the fact that such surfaces can accumulate high charges by reason of the nature of the clean room constraints. Please explain.

McDade: There are many excellent studies on the physics of such systems. There seems to be a definite paucity of data of the effect of surface charges on the loading and distribution of viable particles in spacecraft assembly and clean room environments. In the studies that were conducted by our group, as well as studies conducted by Douglas, Phoenix, and Minnesota, no differences were noted in recovery from the stainless steel surfaces, glass surfaces, or Lucite surfaces exposed to the environment. We realize other surfaces may be involved, and I believe Mr. Vesley mentioned that an evaluation was being made of some seven or eight different surface types. As the results become available, we may have some idea of the importance of different surfaces. Right now, the only thing I can say in general is that we did not notice a difference in Lucite, glass, or stainless steel in our work.

Miles: A microbe lying on the surface of the spacecraft may be released from the spacecraft and attracted to the sterile capsule if there are sufficient unbalanced electrostatic charges on either the spacecraft or the lander. There may be considerable static charge on organic matter, particularly on some of the plastics. This seems to me an important area of investigation that we must study very soon.

Question: Have investigations been made to heat-cycle the organisms being studied—that is, bring the organisms to 135° C for a few hours, then cool, then flush with high velocity inert sterile gas to stir up the surface contamination, and then heat-cycle again?

Bruch: I can introduce here the concept of additive sterilities. If a few *D*-values of dry heat are given, the object then cooled, and a few more *D*-values of dry heat given, the *D*-values are cumulative. The results are similar to the case when all the *D*-values of heat are given in one cycle. I cannot see what we would gain in terms of dry heat by stirring up the surface, because heat penetration of surface contamination is not a problem. This approach might be applicable when we try to sterilize a surface with ethylene oxide. Also, we have been looking at the possibility of increasing the activity of the ethylene oxide molecules by combining them with ultrasonic energy in the gaseous phase. Preliminary data obtained at St. John's University show increased kill rates of bacterial spores on surfaces when ultrasonic energy is used with ethylene oxide in the gaseous phase. Insofar as dry heat is concerned, this approach has little merit.

Phillips: Ultrasonics is one method of destroying micro-organisms. When two different lethal factors are applied, sometimes cumulative effects are obtained, sometimes synergistic effects. Unfortunately, one of the main things they are interested in is radiation combined with chemical sterilization. Sometimes measurable effects of 5 to 10 percent, say, are obtained. I do not imagine the effect is very pronounced, is it?

Bruch: The response from the combined agents in the St. John's test is significant. I cannot give from memory a percentage value for this synergism.

Question: There were very few remarks about cleaning of inspectors, stamps and the unnecessary markings on parts. Could this area be commented on?

Crawford: We have not completely forgotten this problem. The presentation on this subject was a brief summary of the paper itself; and the paper is a brief summary of what has been done. There will be some recommendations on this.

Question: So far, has proof or even good reason been given to keep the biological contamination of a spacecraft below 10^8 at terminal sterilization?

Bruch: This goal is a value judgment relative to establishment of policy. We can apply the additional *D*-values if the microbial load is higher than 10^8. Whether the equipment will be able to take additional heat stress, I do not know. Preliminary shots from aerospace assembly environments show that the predicted accumulations from limited assays will be less than 10^8 organisms. Only 1 to 10 percent of these are spores.

Question: A real analysis of trade-off and reliability versus terminal sterilization testing appears likely to show that the added

time to remove an additional factor of 10^6 and 10^{10} organisms would not reduce the reliability adversely. Please comment.

Bruch: I can't answer. For each log of contamination above 10^8, we would add another *D*-value. I have often been asked, "If we made the spacecraft under the worst possible conditions, how many *D*-values would be required for sterilization?" If a gram of bacterial spores is approximately 5×10^{11} spores, I cannot envision more than a hundred grams of pure dry spores on a 10 000-pound lander. It seems to me that you would need a bacterial fermentor and a spray layer next to the spacecraft to achieve the population of dry spores. If you take the argument that Dr. Cooley developed, these organisms in a soil menstruum would require 50 000 pounds of soil. We need to reexamine our approaches and the expected levels of contamination in spacecraft during assembly. I have to admit that.

Question: The two stage heat cycle seems to have a potential danger—the first heat cycle will kill the weak bugs and cause the survivors to build immunity to further heat and thus increase the probability of surviving terminal sterilization. A pro-and-con discussion is requested.

Bruch: As we apply heat, we do have a selection process. In a mixed population, the more resistant fraction will be selected out; but that resistant fraction is being exposed to the degrading effects of dry heat. The resistant fraction has a thermal decay rate. Our sterilization cycle should be based on the heat kinetics of these resistant fractions. I have never seen in heat sterilization studies any evidence that the last organism to survive is inherently different from those who died earlier in the process. In other words, I have never seen data for a genetic relationship that demonstrates that the last survivor is hereditarily heat resistant.

Question: The applicability of measuring the viable cells carried on the surface of the detachable coupon is dependent upon the assumption that 100 percent of the viable cells do indeed survive the washing, shaking, transfer, plating, and incubation procedures. Is this a valid assumption? If not, then what is the predicted loss of micro-organisms prior to actual identification of the colonies?

McDade: No, it is not a valid assumption. I don't know what the predicted loss is.

Question: Has anyone considered vital staining as a technique for identification of living cells?

McDade: Yes, but there is reason to believe it's not applicable. When you have an environmentally stressed micro-organism, you may or may not be able to recover it. It depends upon the medium

on which you plate the organism and on the procedures that you use. We have put the organisms that we want to recover from the strips into the most suitable medium, and we hope to recover many of the sublethally damaged organisms as well as those that are viable. The 100 percent figure is not based on a 100 percent recovery. When you expose these organisms to different conditions, you can recover up to 90 to 95 percent of the organisms in a given washing of the strip with peptone water. The use of vital staining is fine. Tetrazolium chloride uptake or fluorescent antibody tests are fine if you have large numbers of isolated organisms. One has to see the organism before one can see this staining. So, with large populations it's all right, but when the number of organisms is reduced, then you have a problem finding the organism before determining whether it is viable; therefore, we think culturing is one of the best ways.

Question: Cost reduction in vertical laminar flow rooms could be effected by use of the plenum approach. They are not ordinarily satisfactory for particulate contamination control use due to weathering of surfaces and allied problems. Let us assume that such a system has its blowers, chillers, reheat coils, humidifiers, and other accessories upstream at its HEPA filter. Could the HEPA filter, ducts, and plenum be ETO sterilized when the room is placed in service to provide the required assurance of a sterile air supply?

Hall: It is conceivable that a plenum, filters, and associated equipment could be sterilized by ethylene oxide; but it is not practical due to the ability of the gas to escape from all but the most tightly sealed containers. Though decontamination of equipment upstream from HEPA filters is theoretically desirable in practice the filter will remove all but a trace of upstream contamination. Therefore, emphasis should be placed on the efficiency of the HEPA filters and on the integrity of their seals.

Question: Regarding the problem of outgassing, oxidation, and other deleterious effects on materials, these might be alleviated in certain circumstances. For instance, the tape recorder we were shown evidently carried its own atmosphere. Perhaps oxidation sensitive films could be used if a reducing atmosphere (such as gaseous hydrogen) were provided. Such an environment should also minimize the observed degradation of lubricants, rusting, and other observed changes. Perhaps other subsystems could similarly be protected. Is there any reason that they would not be expected to function?

Miles: This presents an interesting idea and should be examined. A question that comes to mind is related to safety problems. Also, the outgassing that caused the problem in the recorder was

hydrochloric acid, so a hydrogen environment would not have solved that problem; nor would it have solved the corrosion problem where water serves as the electrolyte for galvanic action. However, the idea may have merit.

GENERAL DISCUSSION

Stumbo: There seems to have been a bit of confusion concerning the mathematics involved in obtaining the survivor curve, so to speak, as to what should be considered the initial number in the equation for calculating the sterilization time. I believe the confusion started when the spore tablets were introduced, and it was stated that any number of tablets could be added at the same temperature and all the organisms in the tablets destroyed. I take objection to that and I think I can demonstrate why; the death of bacteria, that is, the reduction in number by heating, is an exponential function of time. What we call the survivor curve has a definite equation, an absolute equation which can be put to it.

The general equation is

$$t = D\ (\log a - \log b)$$

in which t is time at a constant temperature; D is the time required at that temperature to destroy 90 percent of the cell population; a is the initial number of cells present in the total population; b is the number of cells remaining in the population after heating time. In any given set of conditions, such as we are talking about here, t is constant and D is constant. If you increase a, and t and D are constant, something has to increase or decrease correspondingly. If you can increase a, it is obvious that b has to increase. And if you add more bacteria to a population, you are going to increase a, the initial number. With the same given time and the same given rate of destruction described by D, you are going to have greater survival. It's a mathematical fact. If you pick out individuals, some say that you get different results. You don't. It's the entire population that's important. That's the a value in the equation.

A few questions have come to my mind. Reference has been made to a decontamination period equivalent to 10 D-values, then a terminal sterilization, or disinfection period—a 10 D period followed by a 12 D terminal sterilization period. That makes a 22 D total. Now this combined heat process, if it is carried out as proposed, would be adequate to reduce 10^{18} organisms down to 10^{-4}. I know that a load of 10^{18} is a little hard to imagine. I made a few quick calculations on the basis of weight. Now, unfortunately, we are measuring total numbers of bacteria, counting all types that will grow in the limited conditions provided: yeasts, small vegeta-

tive bacteria, non-sporeformers and spores alike. I have reduced this to an average value. If you consider all these organisms as spheres, which they are not, you have an approximate mean diameter of about 2 microns. The cubic volume ($4/3\ \pi\ r^3$) of one of these organisms would be $4/3\ \pi$ times 1 cubed. There are 10^{12} cubic microns in the cubic centimeter. If we take $4/3\ \pi$ times 10^{18} divided by 10^{12}, you get 4.2 times 10^6. As specific gravity is 1, that would be 4.2×10^6 grams. Divide this by 454 grams per pound. You come out with the figure that has been specified for the combined heat cycle. I understand that the spacecraft may weigh about 2000 pounds, but the combined heat process would reduce the round figure of 9300 pounds of spores to 10^{-4}. Now if the purpose of that decontamination treatment is material testing rather than spore reduction, well and good; but if it is not, don't call it decontamination. If you call it decontamination, you are combining the two to reduce the spore load, and you have a process which will reduce 9300 pounds of spores down to 10^{-4} spores. I think that someone should give this a bit more consideration.

One of the other things that bothered me was that reference was made to a time and a temperature cycle: 135° C for a period of 22 hours. I haven't heard any consideration given to the lethality of the temperatures existing when coming up to the desired temperature or the temperature that exists during cooling. I would suspect that the lethality of the temperature during ascension and descension from 135° C is greater than has been specified. It seems to me unscientific not to consider the lethality of temperatures existent during heating and cooling phases of the heat cycle. That is standard practice in sterilization procedures, and it can be very significant. In fact, in food sterilization you never reach the temperature that you are specifying. You get sterilization before reaching that temperature. If you have the temperature profile, it is a simple job to integrate the lethality of all the temperatures existent in the heating cycle and in the cooling cycle. Why it has not been done here, I don't know. I would suspect that if you want to ease the damage to the spacecraft by reducing the severity of heating you could evaluate the process and materially reduce the proposed amount of heat and still get the sterility required.

One other point I'm bothered about concerns bacteriological methods and techniques. I am amazed that, from the standpoint of sterilization of a spacecraft, only the total count is being considered. Yeast cells, vegetative cells, and viruses have virtually no resistance compared to that of most spores. If you are going to arrive at an a value in the equation, it should be in terms of numbers of the most resistant spores of the total count. The total

count is meaningless in this respect from the standpoint of sterilization.

Also, I am amazed at the choice of medium that has been selected as a standard. I don't think it is a good medium for anaerobic spores. But I was even more surprised at the incubation period of 72 hours. There are many spores, both aerobic and anaerobic, but more particularly anaerobic, that won't even appear in 72 hours. There will be no count. You will have to incubate them 1, 2, or 3 weeks in order to get the spores developed into colonies. With the technique which has been described here, it is my feeling that you are missing most of the spores that might be present.

The uniformity of heating throughout a spacecraft is also of concern. The point was made that one should take the slowest heating point. This is incorrect. Scientifically, one should get the temperature profile for the different locations in the spacecraft, integrate the lethality of the heat received by those, and use that integrated value to determine the probability of survival in each spot. Then the total probability of survival will be the summation of the probability in all spots.

Hall: I believe that these points could stimulate much discussion. Let me offer some points by way of explanation. I quite agree with you that 22 D values are far more than necessary. The reason that this appears to be the requirement is the fact that we are in a transition period. It is only a short time since we seriously considered that we would have more than one heat cycle. So, with the decision or proposal of more than one heat cycle we have not automatically nor quickly changed all our other concepts. I anticipate that there will be a revision downward of the heat cycles in view of this double exposure. However, we are not jumping at decisions without thorough study nor making any changes until we are sure of the desired direction. I assure Dr. Stumbo that his words will be very thoroughly weighed and discussed, and that undoubtedly they will be translated into action.

Iandolo, *JPL*: We have acted under the constraints of the present D values of 1.8 hours at 135° C. We found that come-up times at the slowest point for any particular object have to be somewhat less than 5° per hour. The engineers say that this is too slow. So the decreasing time that you actually buy by using this analysis is not significant over the entire curve.

Schalkowsky, *Exotech, Inc.*: I made some calculations on the heating and cooling time using some of the GE data. The heating rates of the components are roughly equivalent to about 30 to 40 percent of the sterilization at the specified constant temperatures.

Koesterer: I can corroborate that with a couple of numbers to give a feel for the times. In this case the overall come-up time was something like 50 to 60 hours. This was experimental and did not follow the prescribed temperature, 50 to 60 hours of which were at 135° C or higher, so that some 6/14ths of a cycle is warm-up and cool-down. I did not use these long times. The times that I used for the drafts of theoretical curves were more realistic come-up times in the order of maybe 4 to 6 hours, and even those are quite effective in reducing total time at 135° C.

Kautz: We have analyzed this problem to see what we could gain from an integration. The difficulty is that we don't know at this time what the come-up thermal times are. We have obtained from NASA permission to use this approach and hope we get benefit from it.

Kretz, *Wilmot Castle*: I think in integrating the total time some specification concerning a lethal baseline temperature level must be given as a guide factor, since in any integration with biological systems, you must specify a lethal temperature above which you may begin your integration. I would also suggest in the specifications being prepared, that we specify rate of temperature rise, a delta T for piece parts, subsystems, and proof test models.

Stumbo: The integration methods available take into account all lethal temperatures. You don't have to specify any particular temperature. You construct a lethal rate paper, and you go from nonlethal temperatures up through the lethal range. All you have to do is plot the temperatures on the paper, the area underneath the graph, and equilibrate that in terms of the equivalent in minutes at any one temperature. At 135° C or 130° C it makes no difference. It can be equilibrated to anything. But the whole method automatically takes care of the entire lethal range.

Neumann, *Aeronutronic Div.*: I would like to comment in view of this attempt to utilize the buildup and tailoff temperatures in heating the capsule that they are not as important as thought earlier. I did not see any indication in the Marshall or GE data relative to internal heating of the capsule. But we took a look at this, with regard to the Automated Biological Laboratory (ABL) study, and a complex payload is going to have to have a temperature conditioning system. The nature of the heat transfer problem in a landed payload is one of heat production; so you will have to have some heat producing source on board. In our case, we were using the waste heat from the Radioisotope Thermal Generator (RTG) supply for this. This can automatically form a convenient heat source to increase the internal temperature of the caspule during sterilization time. Even though you may not want to do this from

the standpoint of the kill mechanism, it might be desirable from the standpoint of reducing thermal gradients.

Miles: Concerning this Marshall work, practically all possible means of bringing up the temperature were investigated in the typical capsule available. Rather than try to summarize this, let us just say that your observations are entirely correct. I believe it was found that internal gas with a blower combined with internal heaters gave us the minimum time, and that the distribution of heat by internal heaters and blowers was of sufficient advantage to components for their additional weight. The results of this are pretty conclusive for the particular vehicle which we had in the program. A second item that I would suggest is the fact that we make many parts before they ever reach the room where the final assembly and terminal sterilization will occur. I mention transistors as an example. These parts undergo a lot of heat in the normal course of their manufacture. Presumably an assay might show that the total number has been reduced to a point that would cause a change in the whole philosophy. The proposals that have been made, concerning which heat cycle would be used and how it would be interwoven into our program, is a process that undergoes continuing review. Sufficient questions have been raised to cause us to review the matter.

McDade: Dr. Stumbo, are you specifically referring to anaerobes that have been exposed to thermal stress or are you concerned with environmental isolates?

Stumbo: I was concerned with the method used to establish this value of 10^8 that was being used to establish the process. If you want to go into the recovery of the thermally damaged organism, you must have a more fastidious medium than used before. Organisms severely damaged by heat, although not dead, require a more complete medium and considerably longer incubation period of growth. I don't think you can specify one medium and one incubation period to cover this whole thing.

McDade: Well, there are problems that we didn't mention in the presentation of our assay technique. For example, when making cultures of heat-shocked organisms, one selectively tries to recover sporeforming organisms. Since a spore plate usually has surface moisture within 36 hours, a single organism is often overgrown by a spreader. So, we traded off the incubation time after we had done some preliminary experiments; e.g., incubation periods of 7 days, 10 days, and so on. We have evaluated other media and have developed a working system that we want to evaluate under all types of situations. It is not intended to be the standard procedure. It is subject to upgrading as soon as we get any information that

merits incorporation into this system. What is the medium that you would consider for anaerobes?

Stumbo: I think the choice of spore plates is not right. You can't get much more than a 72-hour incubation period on spore plates without dehydration or overcrowding. Why don't you use tubes? Then you can have a long incubation period. If you want the protracted incubation period for obligate anaerobes and facultative anaerobes, seal the tubes and incubate them for 6 months. Now, as to the medium for anaerobes, we found the best medium thus far developed for the cultivation of anaerobes in general is pork-pea infusion agar. It's in the literature, and there are others that are almost as good, but certainly the one that you proposed is not a good medium for most anaerobes though it's good for facultative anaerobes.

McDade: We have incubators that are humidity controlled so we do not have the problem of dehydration as far as the aerobic plates are concerned. At least our plates do not dry out. The other thing you touched on was the anaerobic genus *Clostridium.* You say pork-pea infusion is a good medium for the genus but not for the specific member species. Perhaps you can come up with one or two media that are better for one or two members of the other species. If you continue in this manner, then you might end up by using 26 or 27 kinds of media looking for any number of organisms, and that becomes a problem. To reduce the problem to manageable proportions, Trypticase Soy Agar has been selected, but we may use other media to supplement it. This again, I wish to emphasize, is just a try at a preliminary working system. These procedures will probably be circulated and criticisms, both constructive and destructive, solicited.

Burnett, *Electro-Optical*: The fact was mentioned that there isn't a great deal of concern over chemical contamination. Could a comment be made on radioisotope contaminations, such as plutonium 238, or oxide, or carbide?

Miles: I am informed that in the selection of the experiments, those which have nuclear or any radioactive sources will be seriously considered from the contamination point of view. This is being considered, for example, in making our plans of whether to use batteries or RTG units for landers.

Riely, *Fairchild-Hiller Corp.*: In the method shown for recovery of bacteria by the shaking of the stainless steel strips for 5 minutes in peptone water, did the choice of this method include precoating the strips with nonsporulating anaerobes of fecal origin and PPLO to determine their recovery rate?

Favero, *Communicable Disease Center:* Let me answer it in two ways. We have tested the recovery of organisms from stainless steel strips by shaking and find that we get 95 percent removal. We have not coated with PPLO organisms. I would find it very difficult to assay this reliably. We have not put on any of the nonsporeforming anaerobes such as *Bacteroides*. We have put on sporeforming anaerobes such as *Clostridia*, and we find about 90 to 95 percent recovery. We also find that if the surface is very rough, as with frosted glass, shaking in a fluid menstruum only removes 40 to 50 percent of the organisms. A method such as ultrasonic vibration removes many more organisms. Also, if you pretreat the surface with heat, which tends to hold the contaminants on the surface, you will have much more difficulty in removing the contaminants. In the experimental stage, however, ultrasonic vibration seemed to be the superior method.

Riely: In environmental sampling, the choice of a single medium would seem to reduce greatly the possibility of total recovery of those organisms shed by man. We have found that nonsporulating anaerobes, PPLO, and a number of the actinomycetes are present in relatively large numbers and that certain of the diphtheroid-like bacteria have exacting nutritional requirements. Since resting forms are formed by some of these bacteria, their detection seems important. Have quantitative studies of organisms recovered in microbiotank experiments been made?

Riemensnider, *Communicable Disease Center:* No, we have not. The main purpose of the biotank when first built was to recover certain organisms of humans, mainly the stephylococcus. That is the reason we have not used any other media to recover other organisms of humans, mainly the staphylococcus. I think from some 18 to 20 experiments we have recovered less than 1 percent of any given type of anaerobe. Diphtheroids present no problem. We get so many that we cannot identify all of them. At this time, media for staphylococcus isolation is what we are primarily using.

Neumann: Most of the philosophy that has been discussed is in regard to sterilization as related to the whole planetary problem, but most of the specifics that we have been talking about should be related to Voyager and Mars at the present time. If our present knowledge of Venus holds up, do you see anything in its environment that would change in any essential way our work in regard to sterilization of payloads for that planet?

Hall: I am sure that before we seriously plan a mission to Venus this matter will receive very serious consideration by international and national scientific bodies and that a set of requirements will

be established as they have been for Mars. It is true that we have one reading indicating a temperature of around 800° F; it may or may not be in error. If it is correct, then somewhere between outer space and the planet at 800° F, there is a temperature in the atmosphere that is somewhat suitable for terrestrial life. It is conceivable that terrestrial forms of life might live at some level in the atmosphere of Venus; but to the best of my knowledge, this problem is not under serious consideration at the present time.

Neumann: In the Automated Biological Laboratory work, we came across a significant number of materials principally related to wet chemical processing that appeared to be possible sterilization problems. Rather than eliminating these possibilities immediately, we looked at techniques for thermally isolating these in a completed payload, assuming that they would be sterilized in some alternative procedure during manufacture. In one of our models an isolated chemical antral storage system was incorporated. This does appear to be a significant mechanization problem. It requires some engineering solutions. Could the work that is going on in the area of thermal isolation or sterile insertion be summarized briefly?

Miles: For several reasons we have gotten the notion that we will isolate within a hot body some item that will be heat labile. Evidently this originates because some component of the Mars lander might fall in this category. Once a Venus mission is agreed to it might be possible, through some heat exchange principle, to keep scientific instruments functional for a while. In 1962 we started the development of a thermoelectric cooler at Marshall for this purpose (Venus landers). There may be other ways to keep heat labile parts cool while the spacecraft is being subjected to terminal sterilization. Bottled gases might be used. Through umbilicals, one could bring power into the spacecraft to operate coolers and blowers. A current investigation is being carried on at Marshall to determine what system would be optimum and what developments would be required to produce the hardware involved. While sterile insertion is a much-needed technology, it has not been proven to have the same biological reliability as heat. Research is now underway to determine if this can be done with the required degree of reliability. It was extremely hard to devise an experiment that would prove this.

Riggs, *Abbott Laboratories*: In relation to the proposed NASA standard procedure for microbiological examination methods, I wonder what collaboration exists or is proposed with such compendia as the National Formulary or the United States Pharmacopeia which at the present time describes official test methods.

Hall: We have no relationship to those organizations at the present time but the American Society for Testing Materials is interested in coordinating with our work. Would you recommend that we coordinate with these other bodies?

Riggs: I recommend that you consult with them simply because those two compendia exist under Federal statute. The Food and Drug Administration, for example, specifies an enforceable procedure of methods for microbiological examination for sterility. I think that the degree of collaboration would be of value both ways. Also, another question, what are the source availability and composition of the spore tablet?

Irons, *JPL*: At this time, we have a source but we don't have a supply. I think we are missing sight of one thing. And perhaps Dr. Stumbo can clarify this. We speak of population, and we don't seem to realize that each tablet is a population and an entity in itself and is not influenced by the other spore tablets around it. As an example, if this were true, we could never have replicate testing. If we put a rack of 10 tubes in a sterilizer, in the case of the values of which we speak, it would automatically increase the time 1.8 hours at 135° C. We don't find this to be the case. Now, if these were combined in one population, then they would be reflected in his equation. My point is that, instead of one large population, we have 10 smaller ones. Therefore, the equation for the one-tenth or the one-tenth smaller population would apply.

Stumbo: No, this reasoning is wrong as I think a study of the problem will show.

Brauninger, *McDonnell Aircraft Corp.*: Under what conditions of change and process would you consider foregoing your flight acceptance cycle?

Kautz: At this point, we hadn't considered making exceptions to any subsystems. We fully appreciate that the scheme we are presently contemplating is a nominal arrangement. I feel, based on our past experience with hardware, that it is inevitable that we are going to have exceptions. I want to emphasize that the word "waiver" is not in the JPL vocabulary, although I am sure that we are going to have to make compromises on some of these subsystems.

Brauninger: I really was referring less to waivers for specific subsystems than I was to the fact that the process which you described was a process. I assume that you are still thinking of various other conditions that might prevail here, and I thought that possibly you might have some comments on those changes in the process, which you would accompany with relaxation of this flight acceptance heat cycle.

Kautz: We do have some problems and we think we have some solutions. I might cite two problem areas to which we anticipate giving special scrutiny. One of these is the battery; I am confident that we are going to come up with a satisfactory sterilizable battery. However, if you look at the operational cycle involved in getting one of these spacecraft ready to fly, it goes without saying that one runs all the tests with a battery which does not fly, since you need a fresh power supply on launch. Now, if this battery undergoes only one heat cycle, there is no particular reason to qualify a battery for six cycles or three times the normal expectancy of the heat cycle that you'll need for flight gear. We are not considering this approach, but we've got to keep it in the back of our minds. Another problem area is that of solid propulsion if, indeed, we go to solid propulsion. A large retromotor weighing about 600 pounds has a larger thermal delay time. If, at the subsystem level, we heat at 125° C for 53 hours at its innermost point, there will be a time lag of maybe 200 hours. This solid propellant engine is never going to get the final heat sterilization in its innermost portions. For these reasons we've got to treat this problem differently.

Martin, *JPL*: It seems that the fundamental tenet that the total planetary quarantine effort is based upon is the probability of landing a viable organism is less than 10^{-4}. Who is presently charged with completing this probabilistic specification by adding the confidence interval about this probabilistic number?

Hall: Research sponsored by NASA is being conducted in the area of confidence limits at this time by the Illinois Institute of Technology Research Institute. We also expect to have other people considering it.

Martin: Is it possible that by landing a capsule that has a great many dead organisms on it, we are providing the nucleic acid which is the missing link in a Martian life cycle, and we are perhaps defeating our purpose this way?

Bruch: It's a definite possibility, but I don't know how probable. If we chase nucleic acids, it calls for a major reanalysis of the problem, and it might wipe out the flight mission altogether.

Schull, *General Electric Company*: In the calculation of the heat sterilization cycle, the number has been given as 10^8 micro-organisms on the spacecraft at the time, the limit, and the calculations have been made to reduce this population by 10^{12} so the probability of 10^{-4} remains. Now, on the other hand, the constraints on the total mission are stated as having a probability of 1×10^{-4} of contamination of the planet. These two seem to be incompatible. Are they?

Craven: The sterilization cycle is calculated to reduce the probability of surviving micro-organisms to 10^{-4}; yet this is the total mission constraint for the lander to the surface of Mars. Does this modify our sterilization cycle? We still have not had the official numerical constraint put on the program, to this date. However, Dr. Newell has said that items concerned with the overall capsule would be limited to this constraint of 1×10^{-4}. All other things, such as the launch vehicle, would have a numerical constraint of something like 3×10^{-5}. This is not a significant change, since you still have, for each planetary vehicle, a constraint of 1.3×10^{-4}, on the same order of 1×10^{-4}.

Cooley, *Exotech, Inc.*: Are there any technical data on specific components of the capsule which will withstand the 125° cycle better than the 135° C and might therefore justify a change?

Kautz: In response to that previous question, our whole scheme, I might point out, is predicated on 2×10^{-5}. We have not based the scheme on the 10^{-4}, so we have already bettered the mathematicians on this. As far as a specific response is concerned, I can't give one in quantitative terms. However, in the area of the battery, for example, the older technologies would seem to indicate that we would have a much better chance of meeting the lower temperature than the higher.

Miles: A more specific answer might be given to the question that was asked about the probabilities of 10^{-4} being considered as one of the ground rules. On the first Voyager there will be a vehicle from which some things will fly off and also gases that will be given off. On top of this will rest a bus. Next will rest two capsules. Each of the capsules will have 2×10^{-4} probability. All the rest of these items have 3×10^{-5}. As to the reply that was made about the batteries, I have followed the battery for quite a long time, and I am strictly unfamiliar with any data stating that 125° C for 53 hours is better or worse than 135° C for 24 hours. I believe the decision to go to 125° C for 53 hours was strictly intuitive, and we are hoping that we can put together a program that will determine the best cycle; but at this moment it must be recognized by what it actually is—a judgment.

Craven: A group of things that do stand the temperature better at 125° C are the nylons that might be used in parachutes and some of the polymers. There is an area that is really of great concern to the entry of the Voyager lander.

Dixon, *TRW Systems*: What planetary quarantine policies are being followed by the U.S.S.R. in its planetary exploration program? To what extent are the Russian and American policies cooperative or coordinated in this area? Are we prepared to revise

our policies in the event of an intentional or unintentional violation of Mars or Venus by either the United States or Russia?

Hall: The answer to this is that there is a degree of cooperation in this area between the two countries, as well as in international bodies such as COSPAR. You are probably aware that direct exchange of official information is not possible at the present time. We have the open literature and contacts in the international organizations that give us general objectives of the other launching countries and approximately how they are going about it, but we do not have the details. As for the contamination of Mars by another country, I can only give you the answer that was arrived at in the Bioscience Subcommittee. The recommendation was that such an accident should not influence the program of this nation at the present time. The reasoning was that even if an impact took place, it might not contaminate the planet; or if it did contaminate the planet, the result might not be so serious that we could not achieve useful results by continuing our program.

Clarke, *General Electric Co.*: Is there any limitation on dry heat sterilization concerning the humidity that might be permissible? What is dry?

Pflug, *Michigan State University*: To go back a little on sterilization, we all know what wet heat is. That's basically saturated steam, or we have equilibrium between water and water vapor. But dry heat is a great many things. It can be superheated steam, air, or nitrogen, and each of these conditions will have its own water activity. It's the general belief that spores in equilibrium with a water activity of around 0.20 show the maximum resistance, while with extremely dry spores, you find a considerably decreased resistance. Of course, if the water activity increases to a point of about 0.60 to 0.65, then we have wet heat conditions even though we are below this equilibrium point of water and vapor.

Scheir, *Douglas Aircraft*: Dr. Bruch, you mentioned the St. John's report using sonics to enhance the ethylene oxide sterilization cycle. Can you tell us what frequency range is effective and is this report available?

Bruch: The report is available. I think the frequency range was about 10 to 30 kilocycles per second.

Cooley: The point was made that parachute material or Nomex is better off at 125° for 53 hours than at 135° C for 24 hours. Is that correct?

Craven: Yes.

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