14 October 1960, Volume 132, Number 3433 SCIENCE

Sterilization of Interplanetary Vehicles

Earthly organisms can be kept from contaminating the moon and planets, but careful planning will be required.

Charles R. Phillips and Robert K. Hoffman

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

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

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

Why Contamination Must Be Avoided

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

By many, for example, the moon has been thought to have existed for several billion years as an airless, barren body with no biology of its own, but capable of sweeping up the debris of outer space and preserving it in its nooks and crannies, whence it can be recovered and examined to see if any of this interstellar material shows evidence of organic origin. Sagan and Firsoff, on the other hand, contend that simple organic compounds or even life may have arisen on the moon (*4, 6*). Whatever may be the true situation, if the moon's surface becomes contaminated with living microorganisms from earth, or even with considerable amounts of organic debris from earth, before the "moondust" (*2*)

The authors are staff members of the Physical Defense Division, U.S. Army Chemical Corps Biological Laboratories, Fort Detrick, Md. Dr. Phillips is chief of the Division.

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

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

Just over 100 years ago Darwin pointed out the constant struggle for the survival of the fittest which goes on between all life forms occupying the same environment. The perilous knife-edge balance maintained by such competing forms is never more evident than when a new form is suddenly introduced and a new balance must be attained. The results of the transfer of rabbits from Europe to Australia and of Japanese beetles from their homeland to the eastern seaboard of the United States are well-known examples. Another example is the unrelenting effort of crab grass to invade and overwhelm the bluegrass of American lawns. It has been stated (*3*) that, to prevent the possibility of any such biological accident, no probe that allows as much as a one-in-a-million chance of landing a viable organism on the planetary surfaces should be launched toward either Mars or Venus. It has also been pointed out (*5*) that if it is possible for earthly life to infect neighboring planets, the reverse is also true, and that here much more is at stake than the loss of an unparalleled opportunity for scientific investigation. In the not too distant future, interplanetary quarantine regulations may become even more necessary than present national and regional regulations.

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

Resistance of Life Forms in Space

If one grants that it is desirable to prevent contamination, the second question then needs to be answered. Won't the sterility required be automatically achieved by passage of the vehicle through interplanetary space? This is by no means certain. The conditions believed to exist in outer space were recently discussed by Newell (*8*). In the 1958 Leeuwenhoek Lecture before the Royal Society, Keilin (*9*) carefully reviewed the resistance of various life forms to harsh environmental conditions when in a state of suspended animation, or kryptobiosis, to use the term he coined. A comparison of the information in these two documents can lead only to the conclusion, which has been presented before in considerably more detail (*3*), that spores or other earth life forms could indeed survive such a journey. Briefly, the penetrating radiations of outer space are not of sufficient intensity to assure sterility. The ultraviolet radiation is intense enough, but so easily shielded that only organisms uncovered on the surface of the space vehicle would be exposed. Cold, even down to a slight fraction of a degree above absolute zero, has no lethal effect (*8, 10*). Heat is lethal, even if the resistance of organisms in an evacuated dehydrated state is greater than we have supposed (*11*), but the temperature within space vehicles is carefully controlled at more or less room temperature so that the instruments will perform satisfactorily. This temperature can be maintained with remarkable accuracy merely by having the surface of the vehicle contain a predetermined ratio of reflecting and absorbing areas.

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

Sterilization Techniques

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

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

Although ethylene oxide sterilization is a relatively new development, there have been an increasing number of new applications of this technique within the past decade. The method is slow, requiring up to six hours' exposure time, but it is effective with many types of materials and objects that would be hopelessly damaged by other methods of sterilization. The gas diffuses readily through many types of porous materials but cannot, of course, penetrate hermetically sealed areas. When mixed in proper proportions with fluorinated hydrocarbons, the product is nonflammable (*13*) and can be packaged in convenient light-weight metal cans. Although elaborate automatic ethylene oxide sterilizing equipment is now available commercially, particularly for hospital use, the method can be adapted for use in extremely simple exposure chambers; for example, a simple polyethylene bag tightly closed at the neck serves adequately as a device to contain the gas (*14*). Within such a plastic container, sterilization is achieved at ambient temperatures and relative humidities, and at essentially ambient pressures, since the bag can expand as the liquefied chemicals volatilize. The same technique can be used to sterilize objects of any size or shape by building about them a bag or tent of heat-sealed plastic sheeting and admitting the sterilizing mixture into this container. At concentrations of about 300 to 400 milligrams of ethylene oxide per liter of air and at room temperature, sterilization will occur in six hours or less (*14*).

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

A Hypothetical Case

Such a typical example might be a space vehicle atop a third-stage rocket, with both of them covered by a nose cone or fairing. The chief function of the fairing is to furnish environmental protection on the ground and, more particularly, during flight through the earth's lower atmosphere. The fairing in this assumed case would open and fall back to earth after the vehicle had reached a height (200,000 feet, for example) at which air pressure would be too low to damage the vehicle passing through it at high velocity. The third stage, we can assume, would separate from the space vehicle but would follow it throughout its flight and would be expected to impact any body upon which the vehicle itself might land. In such a hypothetical case, a sheet of plastic large enough to extend out beyond the fairing could be placed underneath the third-stage rocket during assembly. When the final check had been made on the assembly, a complete covering could be made by sealing other sheets of plastic to this base sheet, enveloping the third stage, vehicle, and fairing. Ethylene oxide gas would be admitted into this inclosure and, since the fairing would not present a hermetically sealed barrier, the gas would sterilize the space vehicle and the third stage as well as the fairing itself. After six hours' exposure, the plastic sheet could be cut away and removed, and the ethylene oxide gas would be dissipated in a matter of minutes. Before launching, the outside of the fairing would almost certainly become recontaminated, but it would act essentially as a petri dish cover does, preventing airborne organisms from entering underneath it and recontaminating the space vehicle or the third stage. To keep objects cool while the vehicle is still on the ground, conditioned air is sometimes blown under the fairing. This air can be kept sterile by the simple process of passing it through bacteria-tight filtering material, such as cotton, asbestos, or spun-glass fibers. Filters of the necessary efficiency are readily available. Upon launching, when the vehicle would be rising through the lower atmosphere, the fairing would protect the sensitive components of the payload against the heat and mechanical effects of atmospheric friction. With proper design, the fairing would continue to furnish biological protection in flight as well as on the ground, again acting like a petri dish cover. The sterile air under the fairing would diffuse outward as the atmosphere became less and less dense, and nonsterile outside air should not contact the probe. When the fairing was discharged, at the outer fringes of the atmosphere, the probe would be high enough in the air so that unshielded ultraviolet rays from the sun would prevent surface contamination from then on, if any microorganisms exist that high.

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

Once the decision has been made to perform the final sterilization with ethylene oxide at the last possible moment before launching the probe, one can proceed backward and design a probe suited to the ethylene oxide treatment, just as one designs the probe to withstand the forces of acceleration or vibration to which it will be exposed during launching.

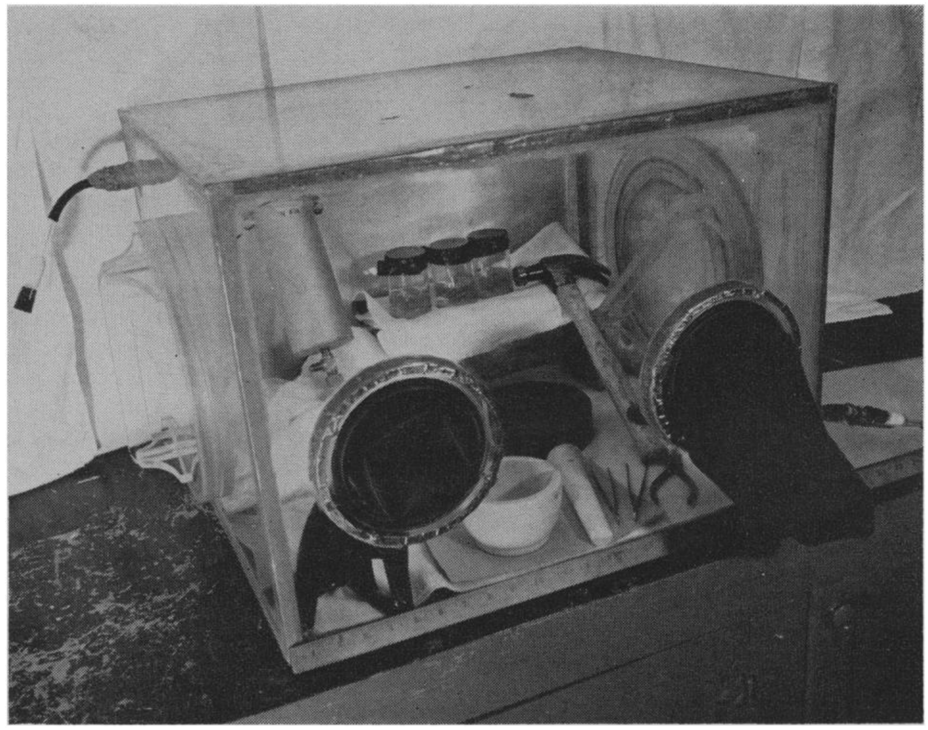

Fig. 1. Exposure chamber inside which electronic components are sterilized externally and then broken open so that they can be tested for internal bacterial contamination. A sterile hammer, anvil, pliers, and mortar and pestle may be seen, as well as the taped broth blanks into which the pieces from the components are placed. The empty ethylene oxide container is at the rear of the chamber, at left. The components to be tested are hidden behind the rubber gloves.

Design Considerations

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

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

This concern over possible contamination in hermetically sealed areas is based on the consideration that in a crash landing on a hard surface, the space vehicle might shatter completely. Any organisms that might be present in such inaccessible locations would be released. Bacteria might survive such a crash landing far better than metal or plastic objects. Thus, the requirement is not only that all accessible surfaces of the object should be sterile but that no viable organisms should be entrapped within the object which would be released if it were broken apart. Examples other than welded tubing of components in which organisms might be entrapped beyond the reach of a sterilizing gas are individual electronic components, assemblies of electronic components that have been encased or potted in plastic, tight metal-to-metal surfaces (particularly those held together with a sealing mastic), and aluminum or plastic honeycomb sheets.

Exploratory Experiments

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

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

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

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

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

Table 1. Electronic components with internal contamination.

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

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

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

Typical results obtained in these exploratory tests are given in Table 1. As is evident, transistors as a general class are more likely to be sterile internally than are capacitors, although at least one nonsterile example was found in each class. Considerably more investigations of this type will be necessary before definitive answers can be given concerning the biological status of all types of electronic components. Such data are now being routinely collected. Also, the ability of these components to perform satisfactorily after they have received various types of sterilization treatment is under investigation.

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

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