Reprinted from *BioScience*, Vol. 19, No. 8, 1969, pp. 712-715

Physical, Chemical, and Biological Activities at the Lunar Receiving Laboratory

W. W. Kemmerer, Jr., J. A. Mason, and B. C. Wooley

The possibility of life on the moon has intrigued man since the earliest recordings of history. However, with today's scientific findings of wide temperature variations, radioactivity, meteoric bombardment, lack of atmosphere, and apparent lack of water, this possibility seems extremely remote. Nevertheless, rudimentary life-forms could exist, and until proof to the contrary is established, they must be anticipated in planning lunar investigative activities.

On 20 July 1969, Apollo 11 landed two men on the moon, obtained lunar samples, and returned both men and samples safely to earth for scientific observation.

A prime concern of this mission was the prevention of introducing any contaminants into the earth's biosphere until scientific personnel could ascertain that they posed no threat to terrestrial life.

The authors are affiliated with the Lunar Receiving Laboratory, Manned Spacecraft Center, Houston, Texas

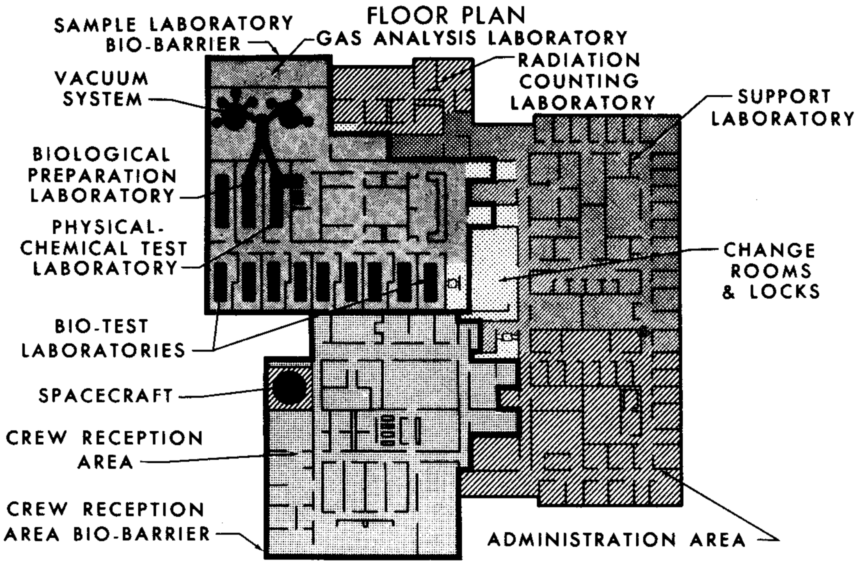

Fig. 2. Lunar Receiving Laboratory.

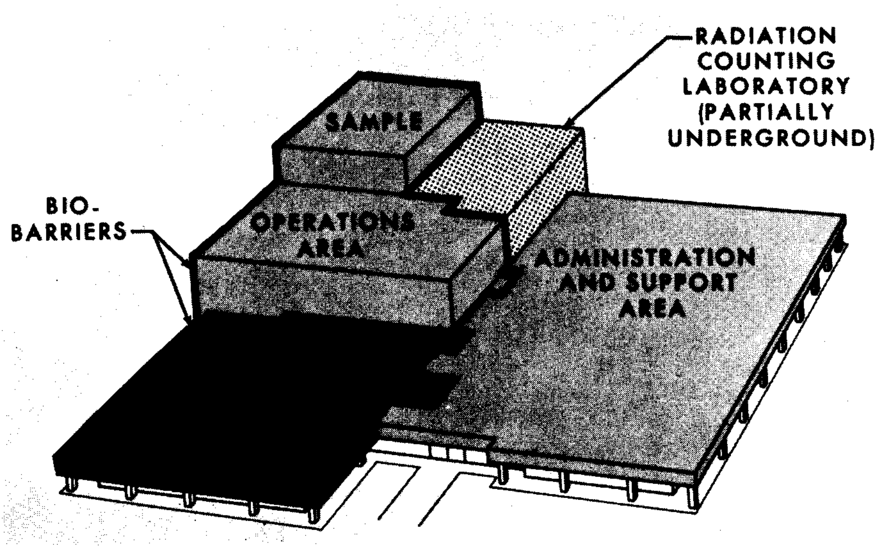

Fig. 1. LRL Functional Areas.

Early in this decade, the Space Science Board of the NRC-NAS issued a report recommending measures to protect the earth's biosphere from material returned from the moon. In response to these recommendations, the NASA Deputy Administrator announced a policy to comply. Subsequently, in mutual agreement, the Secretaries of Agriculture; Health, Education and Welfare; Interior; and the President, National Academy of Sciences advised NASA that all technically feasible steps must be taken to minimize or eliminate the possibility of introducing extraterrestrial (alien) life forms into the earth's biosphere; this process is known as back contamination. An Interagency Committee on Back Contamination (ICBC), with membership drawn from the agencies listed above, was formed to provide policy guidance for quarantine

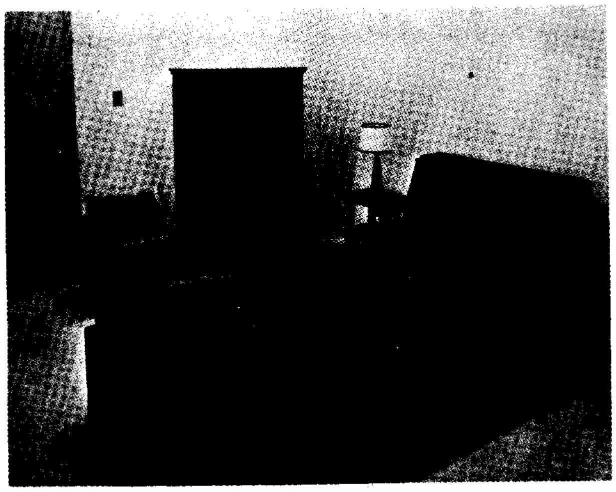

Fig. 3. Astronaut bedroom in Crew Reception Area.

and release plans for crew and lunar samples.

In compliance with this advice, NASA personnel constructed, equipped, and staffed[1] the Lunar Receiving Laboratory (LRL), which is located at NASA Manned Spacecraft Center (MSC) in Houston, Texas. This multidisciplinary facility is divided into three functional areas (Figs. 1 and 2). The three areas are the Sample Operations Area, the Crew Reception Area (CRA), and the Administration and Support Area.

The Sample Operations Area and the CRA, both confined within the unit-biological-barrier system, assume six major tasks: (1) physical-chemical testing of lunar samples; (2) time-critical and scientific investigations that must be completed within the proposed quarantine examination of lunar samples; (4) biomedical examination of the astronauts; (5) permanent storage under vacuum of a portion of the samples; and (6) sample distribution to the scientific community.

Physical-Chemical Testing

Analyses of residual effluent gases in the returned lunar sample containers and sample bags are conducted in the gas analysis laboratory. Such analyses are extremely important in determining the atmospheric conditions of the moon, the geochemical history, the sample volatility, and the amount of earth contamination, if any, of each sample. These analyses also include the search for volatile organic molecules and evidence of lunar life and lunar life-related compounds.

The physical-chemical test laboratory area (area B on Fig. 4) of the Sample Operations Area consists of gas-tight, negatively-pressurized, dry-nitrogen atmosphere, Class III biological cabinetry which is connected to the vacuum laboratory as shown between areas A and B on Figure 4.

The preliminary examinations performed in this area provide detailed microscopic, limited petrographic, and compositional data on selected lunar samples. Then these data are referenced to the geological data obtained by the astronauts during their short lunar exploration.

Biological Testing

To satisfy the requirements of the Federal Regulatory Agencies, portions of the lunar samples are distributed to personnel in the biological test laboratories (Fig. 2). The personnel in these laboratories use extensive quarantine test procedures to determine if the returned samples contain detrimental, replicating alien life forms. All biological quarantine analyses are performed within the Class III biological cabinetry in accordance with prescribed quarantine requirements. Here, many test systems are used. They can be divided into three categories: microbiological, zoological, and botanical.

Microbiology

Microbiological testing (for viruses, bacteria, and other agents) are performed on specimens from the crew, on lunar samples, and upon test species exposed to lunar material.

To produce baseline data for comparative studies, a preflight microbial profile was developed for each astronaut in the crew. Sampling intervals were 30 days, 14 days, and 0 days before the flight. Postflight examinations began soon after the astronauts entered the Mobile Quarantinetine Facility (MQF) aboard the aircraft carrier. Samples for microbiological analyses were shipped immediately in special containers to the LRL.

After the departure from the lunar surface, illnesses occurring in the astronauts must be presumed to be of lunar origin until proven otherwise. Therefore, much emphasis is placed on ascribing specific etiologies to illness events in the shortest possible time. The host systems (tissue cultures, organ cultures, and in vivo host's) used for the isolation of viruses from crew members and contacts within the CRA provide the capability to isolate and identify member viruses representing essentially all known groups capable

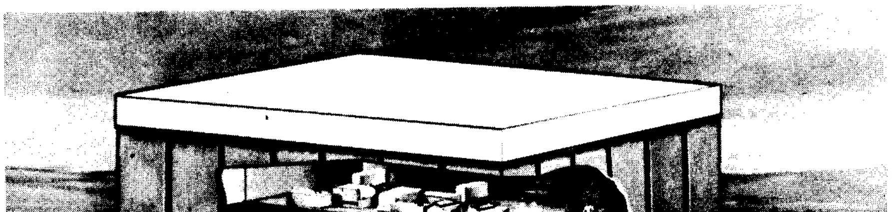

Fig. 4. LRL Sample Operations Area.

[1]Staffing is mainly NASA civil service. However, the technical staff is comprised of personnel from the Departments of Air Force; Agriculture; Interior; Health, Education, and Welfare; as well as support contractors.

of producing acute illnesses in human populations. In that any replicating "agent" must be presumed to be of lunar origin until proven differently, great care has been taken to obtain the host systems free of latent or indigenous viruses.

The host systems (in vivo and in vitro) used by LRL microbiologists for detection of alien life forms in lunar soil sample do not differ significantly from thos in current use. In addition to those host systems used for crew virology, supernatant fluids from lunar soil suspensions are being tested in tissue cultures of mammalian, avian, and poikilothermic species.

Because of the necessity for quickly characterizing any agent grown from the lunar soil or from the astronauts as terrestrial or lunar in origin, the preflight microbial data on each astronaut are of prime importance. Bacteria emanating from the astronauts during lunar surface operations are a probable source of contamination of the lunar samples with terrestrial microorganisms. Samples are streaked on several slant and growth media and incubated under a variety of temperatures and oxygen tensions. Resulting isolants undergo biochemical analyses for identification of genus and species. The resulting morphological and biochemical characteristics are then compared with those obtained for lunar isolants as an attempt to establish the carrier source. The computerized data system developed within the LRL provides for quick and detailed comparisons of agents isolated from the lunar soil with the preflight microbiological profile of each

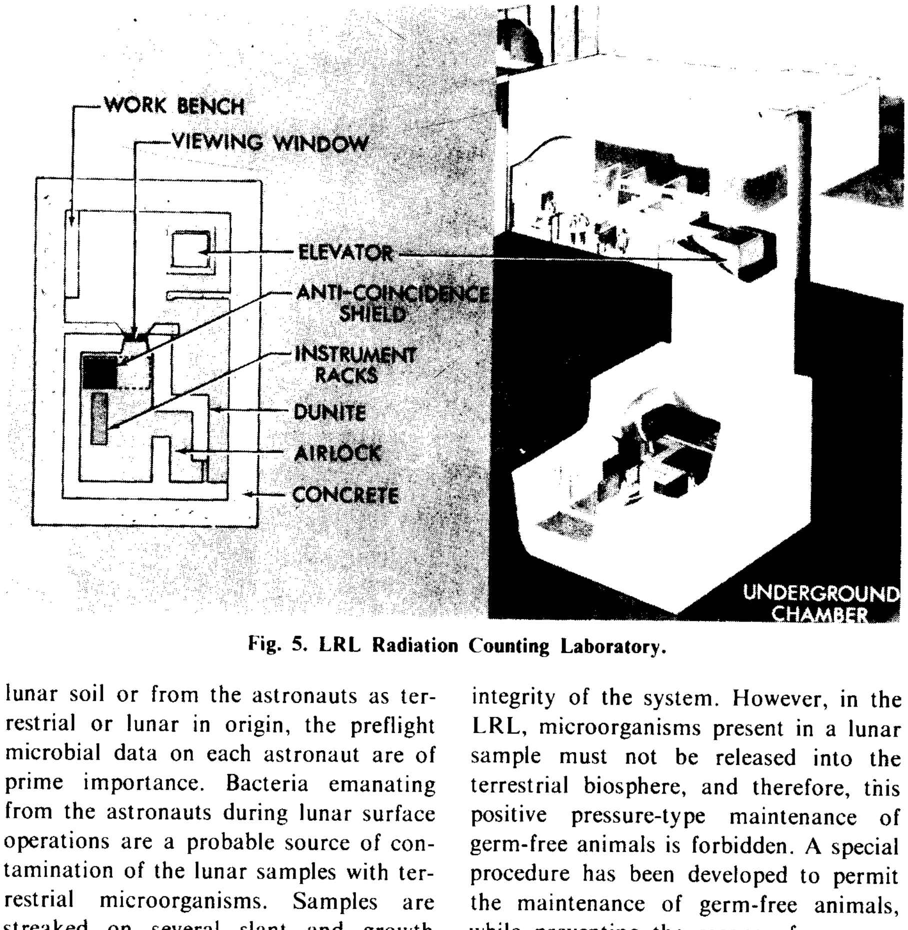

Fig. 5. LRL Radiation Counting Laboratory.

astronaut. Thus, rapid judgments may be made on whether an agent is of probable terrestrial or lunar origin.

Zoology

To represent the mammalian portion of the zoological program, the germ-free mouse was chosen as the prime test system. Normal procedures (i.e., those used in the scientific community) for maintaining a germ-free environment support the germ-free mouse within a positively-pressurized, germ-free isolator. The positive pressure in the isolators prevents contamination inside by allowing an outward flow of air if small holes or tears break the integrity of the system. However, in the LRL, microorganisms present in a lunar sample must not be released into the terrestrial biosphere, and therefore, this positive pressure-type maintenance of germ-free animals is forbidden. A special procedure has been developed to permit the maintenance of germ-free animals, while preventing the escape of any possible hazardous organisms in the lunar samples.

Basically, the procedure consists of a double biological barrier. The mice are maintained within a plastic isolator which, in turn, is housed within stainless-steel-glass Class III cabinetry. The cabinet is maintained at a negative pressure relative to room pressure. The plastic isolator is also maintained at negative pressure with respect to the room, but is maintained at a positive pressure relative to the Class III cabinetry. This double cabinet system has necessitated the development of unique transfer procedures for the introduction of food, water, and other supplies required for animal support. The use of these procedures has broad applications; germ-free animals can now be used for testing hazardous terrestrial microorganisms without danger of investigator exposure.

Another test system used in the LRL zoological program is the Japanese quail, whose small size and prolific nature lends itself ideally to laboratory test conditions.

Veterinary pathologic techniques have been modified to work in Class III quarantine cabinets in order to detect changes in the test systems which will indicate an altered host reaction caused by disease.

A second category of the zoological program is the exposure of various aquatic species to the lunar material. These test systems include both marine and freshwater species and range from protozoa to the oyster, shrimp, and various types of fish. Actually, the unique aspect of this program is that most species have not been domesticated. Attempts have not been made previously to adapt these organisms to controlled laboratory conditions. Indeed, the source of many of the species is limited to their natural environment, the ocean or freshwater streams and lakes.

An accelerated program has been conducted to develop procedures for the movement of aquatic test species from their natural environment into the laboratory, maintaining them under laboratory conditions and actually developing procedures for their propagation under laboratory conditions. After domestication is successful, techniques and procedures might be developed to detect altered host reactions that may be an indication of disease resulting from exposure of aquatic test systems to the lunar sample.

A third category of the Zoological Program is the terrestrial invertebrate which is represented by the insects. It is impossible to cover all of the taxa in this enormous group, but three major orders have been represented by the German cockroach, housefly, and greater wax moth.

Botany

The botanical section was developed according to recommendations from the U.S. Department of Agriculture emphasizing exposure of 33 plant species to lunar samples for detecting possible plant pathogens. Sterile cabinets are maintained to grow pure cultures of algae and diatoms, germinate surface sterilized seeds, grow pathogen-free seedlings, and develop tissue cultures. The development of equipment, instruments and techniques to grow large numbers of plants allow botantists to eliminate as many as possible of the uncontrolled variables, while maintaining pathogen-free plants. The potentiality of lunar pathogens requires that all materials be sterilized on exiting these cabinets. This could impose an additional problem in collecting data of living materials.

Freshwater and seawater algae and diatoms are challenged to determine effects on reproduction and maturation. The four species include blue-green, green, and red algae and a diatom. Eight types of seed are exposed to the lunar sample (including fern spores) to observe germination rates and development while in direct contact with the inoculum. The development of 13 seedling species will be examined after direct foliar application of the lunar material followed by a water slurry on the roots. Dry lunar material is sprinkled onto the surface of eight species grown in tissue culture to note effects on division and differentiation of callus.

Fig. 6. Transplanted seedlings in BR-8 growth support medium (Plant Sample Laboratory).

All systems are maintained under aseptic conditions incorporating controlled light, temperature, carbon dioxide and nutrition. The test design includes: (1) challenged toxicity control with dry-heat-sterilized lunar material; (2) challenged control consisting of dry-heat-sterilized earth material; (3) challenge with lunar sample; and (4) an unchallenged group. Plant life is being examined with both light and electron microscopy to collect anatomical data regarding the four treatments (Fig. 6).

Plant-holding colonies are being used to maintain a reserve of pathogen-free plants to serve as back-up material.

Crew Reception Area Quarantine

The Federal Regulatory Agencies issued quarantine requirements directly applicable to the CRA (Fig. 3). The crew, medical, and support personnel quarantine requirements can be summarized as follows:

1) Release from the CRA will occur 21 days after ascent from the lunar surface if no alterations in general health are observed and in the absence of an infectious disease attributable to lunar exposure.

2) If significant alterations in general health do occur, release from the CRA will still be indicated if alterations are diagnosed to be of terrestrial origin or as noncommunicable.

3) If alterations are apparent and are not diagnosed, some delay in release from the CRA would be indicated with the final action to be recommended by the NASA medical team.

To anticipate every eventuality in any set of quarantine requirements is impossible; however, provisions are made so that the Quarantine Control Officer can extend the original quarantine to meet various contingencies. Also, provision is made for quarantine of personnel from the sample laboratory should a break occur in the primary biological barrier.

Vacuum Laboratory

The initial processing of the returned lunar samples is performed in the vacuum laboratory of the Sample Operations Area (area A on Fig. 4). The vacuum laboratory contains an ultraclean vacuum system that is designed to preserve each lunar sample in a pristine or near-pristine state. The system is also designed to prevent *back contamination*. Samples were prepared in vacuum conditions according to plan and sent to the various laboratories within the building. After completion of the quarantine, other samples will be placed in containers under vacuum and sent to 125 lunar sample principal investigators for exhaustive scientific analyses. The LRL will be a permanent repository for a portion of the collected samples. It will safeguard the collection and insure sample integrity.

Another important function of the vacuum laboratory is to decontaminate sample equipment prior to the lunar missions (that is, prepare sterile tools and containers to be taken onto the lunar surface) in order to minimize the amount of contamination to the lunar surface by various earth microbes.

Radiation Counting Laboratory

The levels of radioactivity in a given lunar sample may be comparable to the low levels of radioactivity that occur in meteorites. Because of short half-life, a capability for rapidly measuring low-level activities is integral to the LRL. This capability is afforded by the Radiation Counting Laboratory. This underground section (Fig. 5) of the LRL is unique and should provide many research opportunities for visiting scientists during short-term and long-term studies.

Summary

The validity and reproducibility of the data gained in the physical, chemical, and bioscience facilities of the LRL is directly proportional to (1) the competence of the scientific staff working under containment conditions, and (2) the ability of the facility to sustain two-way protection; that is, product protection from earth-borne contamination and personnel protection from lunar material. Thus, it is necessary that personnel in the LRL understand the importance of the problems inherent to a quarantine facility, as well as the necessity operational techniques required to maintain quarantine.

The successful conduct of the biological containment program can be realized only when each procedure, technician, or piece of equipment works precisely. One "weak link" will compromise a part or all of the lunar sample quarantine requirements. Moreover, it is incumbent upon all who participate to evaluate and re-evaluate their efforts in light of new "break throughs," "spin-offs," and imaginative analysis in order to improve or update techniques and procedures for future space flights. Knowledge gained, if not applied, has little impact and denies bold new steps in extraterrestrial exploration—be it lunar or planetary.

In carrying out the immediate studies and continuing efforts of the laboratory, NASA will rely on interagency support and the support of visiting scientists to supplement a small resident staff.