CHAPTER 2
QUARANTINE TESTING AND BIOCHARACTERIZATION OF LUNAR MATERIALS

by

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Introduction

The objective of the quarantine testing and biocharacterization portion of the Apollo medical program was to test appropriate representative lunar samples for the possible presence of agents that might be infectious or toxic for plants, man, and other animals. The goal of the laboratory was to provide safety clearance for lunar samples within a period of approximately 30 days. Lunar materials were analyzed in an isolated environment. These analyses were performed immediately after the lunar samples were unpacked in the Lunar Receiving Laboratory (LRL) at the Johnson Space Center. Small but representative samples of lunar material were used to assess whether they contained microorganisms, and to ensure that the lunar materials were nonhazardous to the selected test species.

The quarantine testing included a wide variety of biological species. Approximately 500 gm of lunar material were required for each investigation. Analyses of data from the Apollo 11, 12, and 14 missions indicated that no microbial life forms had been recovered from the lunar material. For subsequent missions, the containment aspects of the postflight quarantine were omitted and the biocharacterization or preliminary biomedical evaluation of lunar materials was initiated. The aims were to characterize the lunar material with respect to its ability to stimulate biological activity, and to measure possible microbial contamination of lunar samples. For the Apollo 15 mission, the number of biological tests was reduced to one-third of those performed on previous missions. Further reduction in the scope of the program occurred after the Apollo 15 mission.

Except for the exposure of animal tissue culture cells to suspensions of lunar material, animal test systems were omitted from the Apollo 16 and 17 protocols.

All test protocols were extensively reviewed by the scientific community, the American Institute of Biological Sciences, and the Interagency Committee on Back-Contamination. The Interagency Committee was formed of representatives of various agencies of the Federal Government for the purpose of reviewing protocols to assure that the biosphere would not be contaminated with organisms from the moon. The aim was to use as many different kinds of organisms as possible. The organisms chosen were well known research tools, including mice, oysters, paramecia, and fishes.

The results of the first experiments in several completely new fields, namely lunar agriculture, lunar soil microbiology, and ecology of lunar soil on contact with terrestrial organisms, are presented in this chapter. It should be stated at the outset that the implication of the findings reported here are largely speculative because of limited experimentation. However, findings are consistent with the generally accepted hypothesis that the lunar surface is now, and has always been, sterile.

Botanical Investigations

The botanical quarantine studies at the Lunar Receiving Laboratory were designed to determine whether lunar material contained any agent capable of generating an epidemic disease in representative species of the plant kingdom. These tests were conducted under conditions which would ensure confinement of any infectious agents that might be found in the lunar materials or generated in the lunar-exposed plants (Walkinshaw et al., 1970). Class III biological glove boxes were used to achieve the required protective containment (Kemmerer et al., 1969).

A total of 35 plant species were exposed to lunar material returned during the Apollo 11 and 12 missions (table 1). Four test systems were employed. These included liquid or solid cultures of algal cells, germinating spores and seeds, actively growing seedlings, and tissue cultures on solid media.

Lunar samples used in Apollo 11, 12, and 14 studies were composites of representative rock fragments and surface fines; samples used in Apollo 15, 16, and 17 postflight studies were composites of surface fines. The samples were handled and analyzed as described by Johnson and co-workers (1972). Descriptions of the terrestrial controls may also be found in the work by Johnson and his associates.

Treatment of algal cultures with lunar material inhibited growth in dense cellular suspensions and stimulated growth in cultures grown on semisolid mineral media. Growth promotion was evident by marked increase in cell density in areas adjacent to lunar particles. Treatment of algal cells by exposure to lunar material suspended via gentle agitation resulted in cultures having higher respiration rates than untreated controls. Microscopic examination of treated cultures revealed no significant differences between lunar- and terrestrial-treated cells.

The fern, *Onoclea sensibililis L.*, which was tested with each composite sample, appeared to be the most sensitive plant for demonstrating that lunar material can act as a source of nutrients for plants. Clumps of spores germinating on lunar material placed within a well cut into mineral agar showed a severalfold increase in mass. The resulting

Table 1
Plant Species Challenged With Lunar Materials
in Apollo 11 or 12 Quarantine Studies

Species	Common Name	Challenge System*
Allium cepa L.	Onion	SG
Anacystis nidulans (Richt) Drouet	Blue-green alga	A
Brassica oleracea L.	Cabbage	SG, S
Capsicum frutescens L.	Pepper	SG, S
Chenopodium amaranticolor Coste and Reyn.	Weed	S
Chlorella pyrenoidosa Chick	Green alga	A
Citrullus vulgaris Schrad.	Watermelon	S
Citrus limonia L.	Lime	S
Cucumis melo L.	Cantaloupe	S
Cucumis sativus L.	Cucumber	S
Glycine soja (L.) Sieb and Zucc.	Soybean	TC
Haplopappus gracilis (Nutt.) Gray	Weed	TC
Helianthus annuus L.	Sunflower	TC
Lactuca sativa L.	Lettuce	SG
Lycopersicum esculentum Mill.	Tomato	S
Lycopodium cernuum L.	Clubmoss	G
Marchantia polymorpha L.	Liverwort	G
Nicotiana tabacum L. (albino)	Tobacco	TC
Nicotiana tabacum L. (habituated)	Tobacco	TC
Nicotiana tabacum L. var. Samson	Tobacco	SG
Nicotiana tabacum L. var. Xanthi NC	Tobacco	S
Onoclea sensibilis L.	Sensitive fern	SPG
Oryza sativa L.	Rice	TC
Phaeodactylum tricornutum Bohlin	Diatom	A
Phaseolus aureus L.	Mung bean	SG
Phaseolus vulgaris L.	Common bean	S
Pinus elliottii Engelm.	Slash pine	S
Pinus lambertiana Dougl.	Sugar pine	TC
Pinus palustris Mill.	Longleaf pine	TC
Prophyridium cruentum (Ag.) Naeg.	Red alga	A
Raphanus sativus L.	Radish	SG, S
Saccharum officinarum L.	Sugarcane	S
Solanum tuberosum L.	Potato	S
Sorghum vulgare Pers.	Sorghum	S
Spinacia oleracea L.	Spinach	SG
Todea barbara (L.) Moore	Fern	G
Triticum vulgare Vill.	Wheat	S
Zea mays L.	Corn	TC
Zea mays L. var. *everta*	Popcorn	S

*A = algal culture, G = gametophyte culture, S = seedling, SG = seed germination unit, SPG = spore germination unit, TC = tissue culture.

(Walkinshaw et al., 1970).

gametophytes were also greener than those treated with terrestrial basalts. Other lower plants, such as *Lycopodium cernuum L.* and *Marchantia polymorpha L.* (liverwort), exhibited similar stimulation. Measurements of chlorophyll *a* in the treated plants showed significantly higher concentrations of that pigment than of chlorophyll *b* or carotenoids.

Seeds germinated in the presence of lunar materials grew vigorously and absorbed significant quantities of aluminum, chromium, iron, titanium (Walkinshaw & Johnson, 1971), and a variety of elements including rare-earth elements. In addition, cabbage and brussels sprouts absorbed large amounts of manganese. Lettuce seedlings generally thrived in the presence of lunar material. Germ-free bean, citrus, corn, sorghum, soybean, tobacco, and tomato plants showed no deleterious effects when their leaves or roots were treated with 0.2 gm/specimen of lunar material (figure 1). Citrus, corn, and soybean plants appeared to grow consistently better if treated in the sand-water culture system originally described by Walkinshaw and co-workers (1970). Histological specimens taken from lunar-treated plants revealed no deleterious effects.

Figure 1. Corn treated with lunar material from the Apollo 17 mission.

The twelve plant tissue culture systems used in the biocharacterization program appeared to be the most useful for studying cell/lunar particle interactions (Walkinshaw et al., 1973). Lunar-treated tobacco cells accumulated approximately 30 percent more total chlorophyll *a* than did untreated ones (Weete & Walkinshaw, 1972). Relative and absolute concentrations of fatty acids and sterols were changed by lunar treatment

(Weete, Walkinshaw & Laseter, 1972). Pine cells, on the other hand, exhibited a remarkable increase in accumulation of tannin but not of fatty acids or sterols. Both stationary and suspension cultures of tobacco tissue cultures treated with lunar material exhibited an increased maturation of chloroplasts and apparent secretory activity (Baur et al., 1973).

In summary, a number of beneficial effects were observed to be associated with the use of lunar soil cultivation, and none of these effects was found to be associated with an infectious process. The absence of microorganisms or any harmful substance suggests that lunar material could be used as a support medium for the growth of many plants. The tests conducted at the Johnson Space Center indicate that ferns, liverworts, and tobacco cultures utilize lunar material as a source of nutrients (Walkinshaw et al., 1970).

Virological Investigations

Virological studies of the lunar material obtained during the Apollo missions consisted primarily of analyses for replicating agents, principally those able to reproduce. The materials tested and the systems challenged are presented in table 2. The fluid obtained from centrifuging 50 percent weight per volume (W/V) suspensions of lunar material in sterile media was used to inoculate the test systems. Mammalian and avian cultures were re-inoculated ten and twenty days later. Fish cell cultures were re-inoculated in 15 days. Cell cultures in the final passage were tested for infection. All systems were tested to make sure they would react with known viruses. African green monkey kidney (GMK) cultures were challenged with enteric cytopathogenic human orphan virus type 11; mammalian and avian cultures were challenged with pancreatic necrosis virus. Embryonated eggs were inoculated by way of the yolk sac, the chorioallantoic membrane, and the amniotic and allantoic sacs. Extracts of lunar material were inoculated into the brain and the body cavity of mice (figure 2). Materials from tissue cultures, embryonated eggs, and suckling mice were tested for hemaglutinins using chicken, guinea pig, and human type O red blood cells. Viral passage materials were processed for light- and electron-microscope examinations. Standard mycoplasma isolation procedures were used. No evidence of replicating agents was found in any of the systems used.

Additional studies were performed on the Apollo 15 lunar material to measure changes in the ability to infect the host cells. The green monkey kidney cell cultures were exposed to extracts (20 percent W/V) of lunar material and were challenged with parainfluenza and rubella viruses. The ability of the cell cultures to support virus replication was not affected. To determine the effect on growth, metabolism, and colony morphology of *Mycoplasma pneumoniae*, the organism was grown in suspensions of lunar material (ten percent W/V), in mycoplasma broth medium, and in agar containing 0.75 percent lunar material. No significant differences were observed between terrestrial basalt used to simulate lunar material and lunar material suspensions. Colonies grown on agar containing lunar material were similar to those grown on agar medium alone or on agar containing simulated lunar material.

Table 2

Systems Challenged in the Virological Analyses of Lunar Material Obtained During the Apollo Missions

Apollo Mission	Number of Samples Tested	Tissue Cultures	Systems Challenged		
			Embryonated Eggs	Suckling Mice	Mycoplasma Media
11	3	GMK, HEK, WI-38, BEK, PEK, DEF, RTG-2, FHM, GF	X	–	X
12	2	GMK, HEK, WI-38, MDBK, PK_{15}, DEF, RTG-2, FHM, GF	X	–	X
14	6	GMK, HEK, WI-38, MDBK, PK_{15}, DEF, RTG-2, FHM, GF	X	X	X
15	1	GMK, HEK, WI-38	X	X	X
16	1	GMK, HEK, WI-38	X	X	X
17	1	GMK, HEK, WI-38	X	X	X

GMK = African green monkey kidney
HEK = Primary human embryonic kidney
WI-38 = Diploid human embryonic lung
BEK = Primary bovine embryonic kidney
PEK = Primary porcine embryonic kidney
DEF = Primary duck embryonic fibroblast
RTG-2 = Rainbow trout gonadal tissue, *Salmo gairdneri*
FHM = Fathead minnow, *Pimephales promelas*
GF = Grunt fin, *Haemulon sciuras*
MDBK = Heteroploid bovine kidney
PK_{15} = Heteroploid porcine kidney

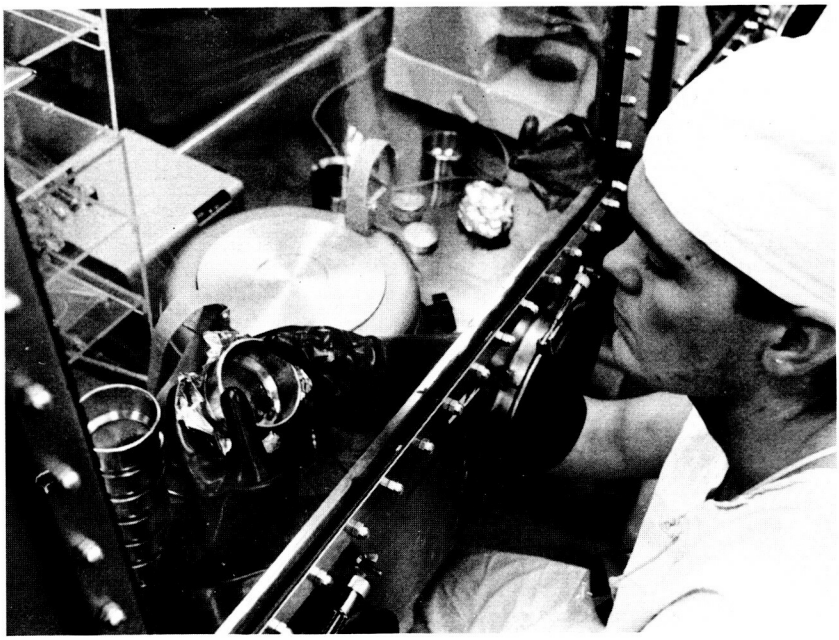

Figure 2. Mice, inoculated with lunar sample material, are examined by NASA technician.

Another study was performed to determine the effect of lunar materials on the stability of poliomyelitis virus. Fifty-percent suspensions of lunar material from the Apollo 11, 12, 14, and 15 missions were inoculated with poliomyelitis virus and incubated at 277°K (≈4°C). Virus-inoculated balanced salt solution and suspensions of simulated lunar material served as controls. Aliquots were removed for viral assay periodically. The number of virus particles in the suspensions of the lunar material was significantly lower than the number in the balanced salt solution. However, no significant differences were detected between simulated and lunar material suspensions.

Zoological Investigations

Following the Apollo 11, 12, 14, and 15 missions, 15 species of animals representing five phyla were exposed to untreated lunar material (table 3). These tests were complementary to the other protocols and were designed to detect any viable or replicating agents capable of infecting and multiplying in animals. The lunar material used for these tests came from the pooled biosamples (Long et al., 1972).

Because of the differences in maintenance techniques for the aquatic and terrestrial species, the methods of providing exposure to the lunar samples differed. The aquatic and protozoan species were exposed by adding lunar material to the medium in which the animals were living. For the Apollo 14 tests, oysters were exposed by introduction of lunar material into the shell cavity through a 0.32 cm (1/8 in.) hole drilled in the shell. Exposure of the insect species was accomplished by mixing the lunar samples with their food. The mice were exposed by inoculation into the body cavity (intraperitoneally) or the skin (subcutaneously). The guinea pigs used for evaluating pulmonary response to lunar material were exposed by inoculating this suspension into the respiratory tract (trachea). The quail were exposed by intraperitoneal inoculation.

Results of exposure of the various animal species were uniformly negative (Simmonds et al., 1972; and Benschoter et al., 1970). No viable or replicating agents, other than identifiable terrestrial microorganisms, were ever recovered or observed in the test animals. Exposure of the animals to the lunar material resulted in some minor and temporary inhibition or toxicity.

Following relaxation of the quarantine requirements after the Apollo 14 mission, lifespan studies were initiated with germ-free mice inoculated with lunar material. The response of these mice to both intraperitoneal and subcutaneous injections of aqueous suspensions of lunar material was evaluated on a long-term basis (Holland & Simmonds, 1973). Classical inflammatory reactions were noted in both intraperitoneal and subcutaneous inoculations, and the lunar material was observed to persist for the life of the animal (20 months). A low-grade inflammatory reaction and the absence of significant fibroplasia (fibrous tissue development) characterized the lesion. These observations suggest that the lunar material was relatively insoluble in tissue and that, although acting as a low-grade irritant, it has little tendency to evoke reactive fibrosis. The significance of such a chronic low-level stimulus and the various factors governing the retention, the elimination, and the turnover of lunar material in mammalian tissue have yet to be determined.

Table 3
Summary of Species Conditions and Procedures Used in Quarantine Testing and Biocharacterization of Lunar Materials

Genus and Species (Common Name)	Lunar Material from Apollo Mission	Results
Euglena gracilis (euglena)	11	Slight reduction in locomotive ability after exposure and a return to normal activity by the fourth day. All groups had normal morphologic features.
Paramecium aurelia (paramecium)	11, 12, 14	Initial reduction in fission rates after exposure, rapidly increasing to normal after 4 to 5 days. All groups had normal morphologic features.
Dugesia dorotocephala (planaria)	11, 12, 14	No significant gross or histopathologic changes.
Crassostrea virginica (commercial oyster)	11, 12, 14	During Apollo 11 and 14 missions, large numbers of deaths were encountered in all groups but correlation could not be shown between the deaths and exposure to lunar material. During Apollo 12 mission, all oysters remained in excellent health.
Penaeus aztecus (brown shrimp)	11, 14	No abnormal behavior or significant gross or histopathologic changes.
Penaeus duorarum (pink shrimp)	12	Considerable fighting in all groups early in test. No significant gross or histopathologic changes.
Blattella germanica [German cockroach (gnotobiotic)]	11, 12, 14	No unaccountable gross or histopathologic changes.
Musca domestica (house fly)	11, 12, 14	No unaccountable gross or histopathologic changes.
Galleria mellonella (greater wax moth)	11, 12, 14	No unaccountable gross or histopathologic changes.
Lebistes reticulatus (guppy)	12, 14	No unaccountable gross or histopathologic changes.
Pimephales promelas (fathead minnow)	11	Sporadic deaths in all groups because of sodium hypochlorite spill. No unaccountable gross or histopathologic changes.
Fundulus heteroclitus (mummichog minnow)	11, 12, 14	With the exception of a few fish in each group during Apollo 12 mission (lost because of gill congestion from exposure to sodium hypochlorite), all mummichogs remained in excellent health, and no unaccountable gross or histopathologic changes were found.
Coturnix coturnix (Japanese quail)	11, 12	No unaccountable gross or histopathologic changes. Several deaths attributed to inoculation, laceration of internal organs or self-inflicted trauma.
Mus musculus [motobiotic CD-I mouse (Charles River)]	11, 12, 14, 15	No indication of any infectious-disease-producing agent or acute toxic component in lunar material. Some evidence of long-term irritative effect; however, resolution of this point must await complete analysis of data obtained from long-term test groups.
Cavia porcellus (guinea pig)	14	No unaccountable gross or histopathologic changes.

Bacteriological and Mycological Investigations

A variety of samples from all six lunar exploration missions was examined for the presence of biological forms or viable organisms (Taylor & Wooley, 1973). To evaluate lunar material for the presence of viable organisms, aliquots of each sample were inoculated into an array of culture media and incubated at several temperatures [277°, 297°, 308°, and 328°K (≈4°, 24° 35° and 55°C)] in three gaseous environments (sterile nitrogen, 10 percent carbon dioxide in air, and air) (Taylor & Ferguson, 1970). No evidence of viable organisms was obtained from any of the analyses.

Following incubation of the lunar material in the culture media complexes, microbial growth dynamics studies were performed with known test species to evaluate the possible presence of toxic factors. Only extracts of culture media that had been in contact with a mixture of lunar material from both Apollo 11 core tubes proved to be toxic to all species tested (Taylor et al., 1971; and Taylor, Ellis et al., 1970). Attempts to reproduce this toxic effect with individual Apollo 11 core samples obtained at other parts of the core tube and analyzed under somewhat different conditions were unsuccessful. The mechanism causing this microbial death has not been determined. In all, 48 different lunar samples, collected to a depth of 297 cm (117 in.) from six different landing sites, were examined.

Summary

The likelihood that life existed on the moon was considered quite remote by most members of the scientific community and by NASA officials, but the extensive testing described above was conducted to ensure the safety of all life on Earth. The plants and animals which were exposed to lunar material were carefully observed for prolonged periods to determine if any mutation or changes in growing characteristics and behavior occurred. The quarantine testing was terminated after the Apollo 14 flight when it became apparent that previously returned lunar material contained no potentially harmful agents. Further biological experimentation with the lunar material was conducted to determine its chemical, physical, and nutritional qualities.

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