pyrolysis products found in lunar fines can be synthesized by the pyrolysis of methane in the laboratory (*9*). It is also possible that these compounds were synthesized from methane on the primitive moon. A definite conclusion is not warranted until the results are confirmed by subsequent analyses.

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4 January 1970

A Search for Viable Organisms in a Lunar Sample

Abstract. *The hypothesis that the moon could harbor viable life forms was not verified on analysis of the first samples from the Apollo 11 mission. Biological examination of 50 grams of the bulk fines confirm the negative results obtained by the Manned Spacecraft Center quarantine team. No viable life forms, including terrestrial contaminants, were found when the sample was tested in 300 separate environments. Only colored inorganic artifacts, resembling microbial clones, appeared around some particles.*

This study to elicit the growth of organisms from lunar matter is the first in a series to determine the existence of extraterrestrial life. The discovery of extraterrestrial life and its characteristics will be important in constructing theories regarding the origin, evolution, distribution, and frequency of life in the universe, when these findings are related to cosmology and the physical and chemical nature of the particular body from which the samples are derived.

The moon is a testing ground for the concept of panspermia—that is, the theory that life can be disseminated through space without the intervention of man (*1*). The moon is hardly capable of the kind of chemical evolution, proposed for the earth, that might result in the synthesis of organic matter sufficient in amount and residence time to allow life to evolve; because of its mass, the moon cannot hold an atmosphere for long (*2*). At the time of its formation the moon must have been a sterile body, and it must subsequently have served as a repository—it appears from its impact history—for the collection of debris from space.

At the NASA Ames Research Center, we designed and supervised the construction of a biological barrier system to perform microbiological experiments on extraterrestrial samples. The system is maintained at a positive pressure (0.2 inch of water) to protect the sample from terrestrial contamination and is housed in a class-100 room [particle (> 0.5 μm) count less than 100 per cubic foot of air] to protect personnel from exposure to potential hazards of the sample.

The biological barrier system contains a preparation area and an incubation area. Twelve adjoining glove boxes serve as the preparation area. In this

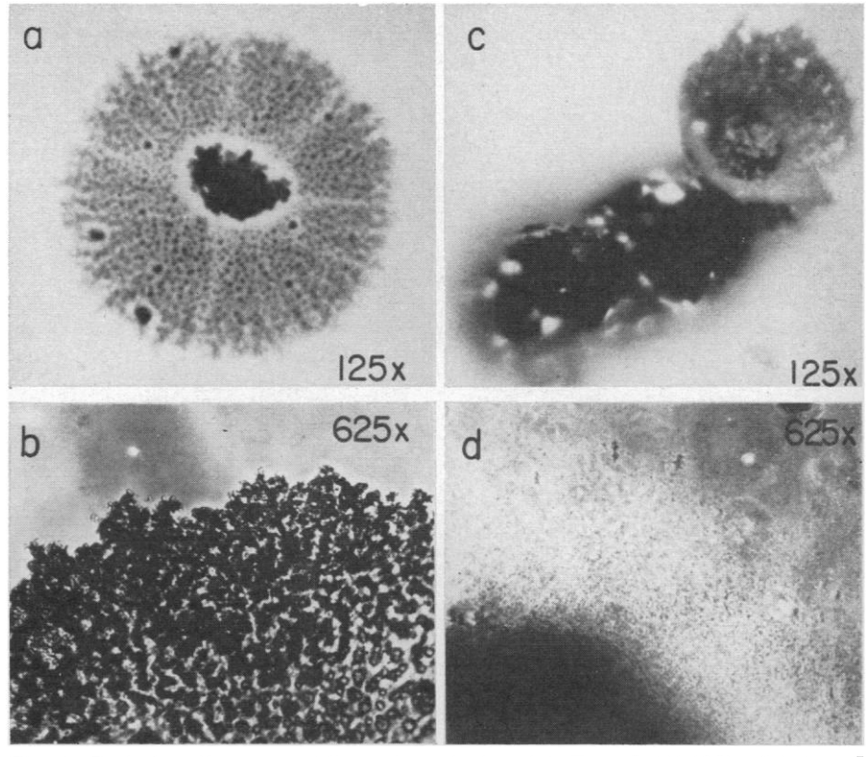

Fig. 1. Typical artifacts resembling microcolonies. (a) Type I (× 125); (b) type I (× 625); (c) type II (× 125); (d) type II (× 625).

Table 1. Atomic absorption analysis of extracts of lunar sample.

Extract*	Total in extract (mg/g of sample)						
	Li	Na	K	Fe	Mn	Ti	Cr
Water	<0.0001	1.76	0.062	<0.001	<0.0006	<0.002	<0.0003
1*N* HCl				8.75	.112	1.50	.256
6*N* HCl				8.00	.106	1.99	.120
1*M* sodium citrate				2.07	.0115	0.077	.0115

* Extractions of a 1-g portion of sample were made with 10 ml of water followed by 1*N* HCl, then by 6*N* HCl. A fresh sample was used with 1*M* sodium citrate. Extractions were performed with shaking for 30 minutes, at 80°C for water and sodium citrate, at room temperature for the other extracts. The citrate and 6*N* HCl extracts were yellow; the others were colorless.

area molten agar media are dispensed and hardened; the sample is weighed, sieved, and distributed onto the surface of the agar media. To this preparation area, aseptic means of removing material from, or introducing it to, the preparation area are provided by a pass-through sterilizer, equipped for steam or gaseous sterilization; a transfer box, equipped for gaseous or liquid sterilization; a dunk tank containing a solution of sterilant; and a sterile filter assembly, for passing thermolabile solutions into the barrier system. The preparation area is equipped with gas lines for introducing ethylene oxide for sterilizing, and dry-filtered nitrogen for purging and maintaining an oxygen-free working environment.

The incubation area consists of three wings of incubator glove boxes arranged in banks of four each. Each wing has an incubator set at 10°, 20°, 35°, and 55°C. Every incubator is divided vertically into three compartments for maintaining humidity. Each compartment has nine removable trays, each of which contains ten petri dishes, and a Teflon-lined bottom tray with a wick assembly for humidity control by means of water or saturated brine solutions. Fluorescent lamps, mounted behind each incubator, illuminate the petri dishes. The compartments are moved by an elevator; each tray can be removed or inserted through an opening into a glove-box working area. This working area has built-in objective lenses, to which a stereoscope body can be mounted. Magnifications up to × 32 are obtainable, and photographs can be taken. Each incubator has valved gas lines for the introduction of ethylene oxide, nitrogen, or mixed gases.

The gases provided pass through a pair of biological filters and through sterile tubing before entering the biological barrier. All gases leaving the barrier system, with the exception of ethylene oxide, are exhausted to the atmosphere through a furnace.

Prior to receipt of the sample, the barrier system was treated for 24 hours with a mixture of ethylene oxide and Freon, and purged with dry sterile nitrogen. These gases were monitored with a gas chromatograph during the purging cycles. The barrier system was then tested for sterility. All interior surfaces were swabbed, and the swabs were incubated in trypticase soy broth and fluid thioglycollate broth for 7 days. No viable organisms were found.

Nine media containing 2 percent agar were sterilized in the connecting autoclave, and 30 ml was dispensed into sterile glass petri dishes. The media selected were a synthetic complex medium with a low *p*H (*3*), a neutral synthetic complex medium (*4*), a synthetic complex medium with a high *p*H (*5*), a dilute synthetic complex medium (*6*), a basal medium containing only sea salts (*7*), a medium containing only inorganic forms of nitrogen, phosphorus, and sulfur (*8*), a medium containing elemental sulfur (*9*), a medium containing formose sugars (*10*), and a medium containing spark-discharge products (*11*). Three different concentrations of a synthetic sea salt formulation (*7*) were used in the preparation of all media; the concentrations selected were 0.1, 3.0, and 20.0 percent.

The 3000 petri dishes containing media were placed on trays and incubated at 10°, 20°, 35°, and 55°C under one of three different gas mixtures: 78 percent nitrogen, 20 percent oxygen, 2 percent carbon dioxide; 97 percent nitrogen, 1 percent oxygen, 2 percent carbon dioxide; and 98 percent nitrogen, 2 percent carbon dioxide. After a minimum 72-hour incubation, all the petri dishes were examined and found to be sterile. The barrier system was then purged with dry sterile nitrogen.

Upon delivery to Ames Research Center, the sample container was wiped with 5 percent peracetic acid solution and treated with ultraviolet light before insertion into the barrier system. The sample container was opened and the sample (10089,1) was weighed and sieved, and the bulk density was determined (*12*). During the week of 6 October 1969, 12 g of the sample were distributed uniformly on the 3000 petri dishes, by means of a small settling tower within the preparation area. The inoculated petri dishes were returned to the incubators, which were again flushed with one of the three gas mixtures. These gas mixtures were maintained for the duration of the incubation period. The plates were observed for evidence of colony formation until the week of 8 December 1969.

By the week of 1 December 1969 no microorganisms had been detected in the 12 g of lunar sample. An enrichment procedure was next used on the remainder of the sample. A disk of sterile filter paper was placed upon the surface of agar media [the media of (*4*), (*8*), (*10*), and (*11*), combined], and a 1-g sample was placed on the center of the filter paper. These plates were incubated at 20°, 35°, and 55°C. After 10 days the sample and filter paper were moved to a plate containing fresh medium. No microorganisms were detected, but incubation is continuing.

Although no microorganisms were detected in the 12 g of sample distributed on 3000 petri dishes, numerous artifacts resembling microcolonies were seen after 4 days of incubation. Two types were distinguished. Type I was circular, flat, granular, and yellow-to-brown, surrounding a central lunar particle (Fig. 1a). Type II was irregularly lobed, raised, moist, and orange-to-dark-brown, and often completely covered a lunar particle (Fig. 1c). Observations at higher magnifications revealed the type I artifacts to be composed of a more coarsely crystalline material than the type II artifacts (Fig. 1, b and d). After 6 weeks of incubation, transplants were made to identical media and environmental conditions, but they did not give rise to similar artifacts.

A series of spot tests (*13*) was performed on both types of artifacts for those elements present in the lunar material (*14*) which might give rise to colored inorganic precipitates. Type I artifacts gave negative results when tested for Ti, Mn, and Cr, but equivocal results for Fe. The artifacts were insoluble in 3*N* HCl, 6*N* HCl, 6*N* H_2SO_4, 0.05*N* NaOH, and concentrated NH_4OH. They dissolved partially in NaOBr, but complete dissolution was achieved when NaOBr treatment was followed by treatment with 6*N* H_2SO_4. Type II artifacts gave a negative result when tested for Ti but gave strong positive reactions for Fe when tested with

$K_4Fe(CN)_6$ or α,α'-dipyridyl in thioglycollate.

Extracts of the lunar sample were prepared and analyzed by atomic absorption spectrophotometry. The results (Table 1) showed that minerals containing Fe—the most abundant of the ions analyzed—were not solubilized by water alone, but required H^+ or Na^+. The results are consistent with cation exchange phenomena. The spot-test results obtained on the Type II artifacts support the cation exchange results. The reason for the equivocal response by the type I artifacts to the iron reagents is not known, but it may be that the brown color arising around the particle is an iron mineral at concentrations barely detectable by the two reagents. We believe that the higher concentrations of Fe in some lunar particles may well produce the lobular structures of the type II artifacts.

We conclude for this sample of the moon that there was no viable life present. Our conclusion agrees with that of the biological quarantine team at the Manned Spacecraft Center, Houston.

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3. The medium contained 1.0 g/liter of glycerol, sodium salts of lactate, pyruvate, and acetate, methanol, ethanol, K_2HPO_4, KNO_3, and $(NH_4)_2SO_4$; 0.2 g/liter of 18 DL-amino acids, glycine, 4-hydroxy-L-proline, β-alanine; 0.01 g/liter of seven purines and pyrimidines; and trace quantities of 16 vitamins; *p*H adjusted to 3.0 with HCl.
4. Same as described in (*3*), but with *p*H 7.0.
5. Same as described in (*3*), but with *p*H adjusted to 10–11 with NaOH.
6. Ingredients same as (*4*), but at 1/10 concentration.
7. Synthetic sea salt mixture (Aquarium Systems, Inc., Wickliffe, Ohio).
8. Same as (*7*), supplemented with 1.0 g/liter each of K_2HPO_4, KNO_3, and $(NH_4)_2SO_4$.
9. Same as (*8*), but with *p*H adjusted to 3.0 with H_2SO_4. After the agar solidified, 0.5 ml of a solution containing elemental sulfur (100 g) dissolved in Na_2S solution (180 g of $Na_2S \cdot 9H_2O$ per 330 ml of H_2O) was spread on the surface; elemental sulfur then precipitated.
10. Same as (*8*), supplemented with 10 g of formose sugars per liter. (Prepared by Dr. J. Shapira, NASA SP-134, 1967).
11. Contained 500 ml/liter of a solution prepared by the reaction of CH_4, NH_3, and water vapor in a spark discharge apparatus [J. Rabinowitz, F. Woeller, J. Flores, R. Krebsbach, *Nature* 224, 796 (1969)] consuming 90 watt-hours. The solution contained (in grams per liter) organic carbon, 2.3; cyanide carbon, 1.3; NH_3–N, 7.3; other N, 1.9.
12. The sieve distributions of the 50-g sample (10089,1) were as follows: 16/35, 6.7%; 35/60, 6.3%; 60/115, 13.1%; 115/250, 15.8%; 250/325, 12.4%; and <325, 45.7%. The sample color closely matched Munsell color index 5YR.
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5 January 1970

Micropaleontological Study of Lunar Material

Abstract. *Samples of the lunar dust, rock chips, and thin sections of rocks from Tranquillity Base have been examined by use of white light and electron optics. In transmitted and in dark- and bright-field incident light and in the scanning electron beam the material reveals no indication of biological morphology. It is inferred that the lunar regolith has always been devoid of life.*

The hope of finding evidence of a preexistent biosphere, or even of stages in chemical evolution of organic systems, on the moon began to fade even as Astronauts Armstrong and Aldrin's early observations began to return to earth from Tranquillity Base. The assumption that no such evidence would be found has been fully confirmed, at least with respect to microorganic structures that might represent extinct or extant microorganisms on the lunar surface. The conclusion bears the limitation that the lunar maria, of which the Sea of Tranquillity is regarded as typical, are representative of the moon as a whole during its entire history. It can be inferred that the lunar regolith has never possessed life and is inimical to life.

The samples investigated in this study consisted of the following: (i) lunar fines (10086,8) from the bulk sample container, sieved to various size fractions; (ii) rock chips of microbrecciated structure, from an outside chip (10091,6) and an inside chip (10091,7), respectively; and (iii) thin sections of microbreccia (10059,32; 10065,25; 10046,56; 10021,29).

The lunar dust was examined as free powder, on the untreated surfaces of glass slides, by normal and polarized, transmitted, and indirectly reflected light (dark-field illumination), and by transmitted light on whole mounts employing various mounting media, such as microscope immersion oil, gum damar, and diaphane.

Rock chips were examined by scanning electron microscopy after deposition of a 300- to 500-Å layer of gold.

Thin sections were examined by polarized and nonpolarized transmitted light, by polarized and nonpolarized direct incident light, and by polarized indirect reflected light (dark-field illumination). (Thin sections bearing cover glasses can be examined by transmitted light only.)

The morphology and optical properties of discrete objects in the lunar fines and in the thin sections of the rocks, at all magnifications accessible to white-light microscopy, indicate total absence of structure that can be interpreted as biological in origin.

Studies of the rock chips, both of outside and inside surfaces, with the scanning electron microscope likewise demonstrate complete absence of biological morphology.

It is appropriate to note that certain of the ubiquitous glass beads in the Apollo 11 dust, especially those of <10-μm size range and of spheroidal and ellipsoidal shape, curiously resemble microorganisms. These "pseudofossils" occasionally appear to possess double "walls," owing to spherical refraction of light around their surfaces and false "organelles" resulting from minute inclusions within the glass and to minute particles of adhering dust. Their mineral origin can readily be determined by optical properties in different combinations of light response in the white-light microscope.

All samples examined in this study were virtually devoid of terrestrial contaminants, with the exception of occassional birefringent fibers occurring in the bulk dust sample and occasional chemically induced artifacts within the epoxy mounting medium of the thin section preparations.

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References and Notes

1. We express our appreciation to Umesh Banerjee, Harvard University, for assistance in this investigation, and to Sheldon Moll, Advanced Metals Research, Burlington, Massachusetts, for assistance in the scanning electron microscopy studies. Supported by NASA contract NAS 9-8060.

4 January 1970