istry. Each of these two is necessary for life, but neither alone is sufficient.

Our results may be relevant to the formation of judgments of morphological entities from data which are to be telemetered to the earth from the Viking missions in the absence of returned samples.

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Organic Compounds in Lunar Samples: Pyrolysis Products, Hydrocarbons, Amino Acids

Abstract. *Lunar fines and a chip from inside a rock pyrolyzed in helium at 700°C gave methane, other gases, and aromatic hydrocarbons. Benzene/methanol extracts of fines yielded traces of high molecular weight alkanes and sulfur. Traces of glycine, alanine, ethanolamine, and urea were found in aqueous extracts. Biological controls and a terrestrial rock, dunite, subjected to exhaust from the lunar module descent engine showed a different amino acid distribution. Interpretation of the origin of the carbon compounds requires extreme care, because of possible contamination acquired during initial sample processing.*

The amount of organic matter in lunar samples returned during the Apollo 11 mission is very small. Various sources of possible contamination (*1*) make a significant analysis difficult. Pyrolysis, solvent extraction, and microscopy were applied to lunar fines (10086) and chips (inside and outside) from a lunar breccia (10002,54) (*2*).

Lunar fines (148.6 mg, untreated) were pyrolyzed for 5 minutes at 510°C. Trace quantities of methane, CO_2, ethylene, propylene, benzene, and toluene were detected. Pyrolysis at 700°C, for 7½ minutes of (i) untreated lunar fines (623.4 mg), (ii) solvent-extracted fines (717.2 mg), from which remaining traces of solvent were removed by heating for 6 hours at 134°C and pressure of 35 mm-Hg), and (iii) a powdered rock chip (675.8 mg), from inside a rock, yielded these same compounds in higher concentrations, and additional products. The product distributions from these three pyrolyses at 700°C were almost identical and amounted to less than 100 parts per million. Again methane was the main component, followed by benzene, toluene, CO_2, and smaller amounts of saturated hydrocarbons of low molecular weight, alkyl benzenes, thiophene and methyl thiophenes, indene and methyl indene, naphthalene and methyl naphthalenes, diphenyl, styrene and methyl styrene (Fig. 1).

In these experiments a Hamilton pyrolysis unit consisting of a quartz tube (43 cm by 4 mm internal diameter), heated externally by an electric furnace and utilizing a helium flow of 3 to 5 ml/min, was connected by a heated transfer line to a Perkin-Elmer model 226 gas chromatograph coupled (*3*) with a Watson-Biemann molecular separator to a Hitachi RMU-6E single-focusing mass spectrometer. During pyrolysis, products were collected in a liquid N_2 trap, which was then heated rapidly to 250° to 300°C with an adjustable oven for instantaneous introduction of the trapped products onto the gas chromatographic column [50-foot by 0.02-inch internal diameter, capillary; polyphenyl ether). Individual components were identified by mass spectrometry.

The pyrolysis products may result from several sources, including degraded polymeric substances, the synthesis from low-molecular-weight compounds, or release by heat treatment from mineral enclosures. Scanning electron and light microscopy suggested possible sources of the pyrolysis products. Two 0.5 mg quantities of untreated lunar fines, and one HF- and HCl-treated sample were distributed on the surface of polished aluminum pedestals with a drop of absolute ethanol, which dispersed the samples and facilitated adhesion to the metal surface. An outside chip of lunar rock was fractured into two pieces, one of which was mounted on the sample pedestal by means of double adhesive tape. Sample mounting for scanning electron microscopy was performed in the clean room facilities of the Naval Weapons Center, China Lake, California. After mounting, the specimens were coated with gold-palladium alloy approximately 500Å thick, in vacuum. Coating artifacts, such as granular surfaces, were absent on the lunar samples. After coating, the samples were examined with a Stereoscan Mark II (Cambridge Instruments Company, Ltd.) electron microscope. Light microscopic studies were performed with a Leitz Ortholux microscope; untreated fines as well as samples pyrolyzed at 700°C were examined in Permount preparations under transmitted light.

The light and the scanning electron micrographs of Fig. 2 show enclosures in glass beads (Fig. 2B) and broken beads (Fig. 2, A and C). Electron micrographs of the interior of a small crater, taken at different magnifications (Fig. 2, D, E, and F), show hollow, bubble-like structures, which suggest that perhaps a positive vapor pressure was behind the once molten surface. Figure 2G shows a particle of similar character. The insoluble residue from lunar fines treated with hot 48 percent HF and hot 6*N* HCl showed a folded, sheet-like object, (Fig. 2H). This has been best seen on examination of stereopairs of photographs.

For the analysis of soluble organic substances, 22.81-, 22.61-, and 16.44-g samples of lunar fines were removed from the top, middle, and bottom of the sample container. It appeared that coarser-grained material, including a small rock, was distributed nearer the bottom of the container. The fines were

extracted with benzene and methanol (4:1, vol/vol) for 1 hour with 28-kilocycle ultrasonic energy yielding 1.7, 0.3, and 0.2 mg of residue, respectively. The yellow extracts were chromatographed on prewashed thin-layer plates coated with Silica Gel G, with two solvent systems: (i) *n*-hexane and (ii) *n*-hexane, diethyl ether, acetic acid (95:5:2, vol/vol/vol). The plates, visualized with aqueous Rhodamine 6G (0.0005 percent) under ultraviolet light (*4*), revealed traces of saturated hydrocarbons (2.0, 0.5, and 0.3 parts per million, respectively), elemental sulfur, and a yellow fluorescent substance which remained at the origin even in the more polar solvent system. Procedural controls showed less than 0.05 parts of hydrocarbons per million.

Approximately half of the total extract described above was inserted into the heated, direct-inlet system of the mass spectrometer. At a probe temperature of 100°C it was possible to identify sulfur and hydrocarbons in all three extracts. As the temperature was raised to 110° to 115°C, the series of alkyl ions, C_nH_{2n+1}, appeared in the extract of the 22.81-g sample; at this and increasingly higher temperatures, C_{25} to C_{40} ions were noted. Prominent odd-mass ions suggesting branching of carbon skeletons were absent. The mass spectra of the second and third extracts showed substantially lower quantities of alkyl ions. A second analysis of each extract gave duplicate results, while sulfur and alkyl ions were absent in procedure controls. Optical rotation measurements made on the second extract showed no optical activity above the limits of detection of the polarimeter (0.3×10^{-3} degree of rotation).

Standard ion exchange chromatographic procedures of high sensitivity

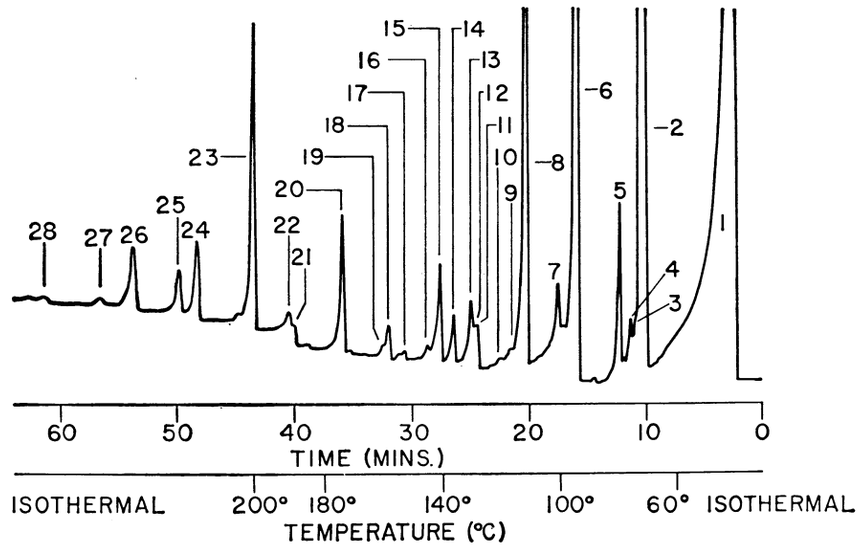

Fig. 1. Gas chromatogram of the pyrolysis products of a 675.8 mg powdered chip from the inside of a lunar breccia (No. 10002, 54), heated to 700°C in He. Peak 1, CH_4; 2, CO_2, C_2H_4, and C_2H_6; 3, 4, and 5, C_5H_6, C_5H_8, and C_5H_{10}; 6, benzene; 7, thiophene; 8, toluene; 9 and 10, methyl thiophenes; 11, ethyl benzene; 12, 13 and 14. *p*-, *m*-, and *o*-xylenes; 15 and 18, styrene and methyl styrene; 16, 17, and 19, C_3 alkyl benzenes; 20, indene; 21 and 22, methyl indenes; 23, naphthalene; 24 and 25, methyl naphthalenes; 26, diphenyl; 27 and 28, C_2 alkyl naphthalenes. Identifications made with a Hitachi RMU-6E mass spectrometer connected to a Perkin-Elmer 226 gas chromatograph, (polyphenyl ether capillary column, 50-foot by 0.02 inch, internal diameter).

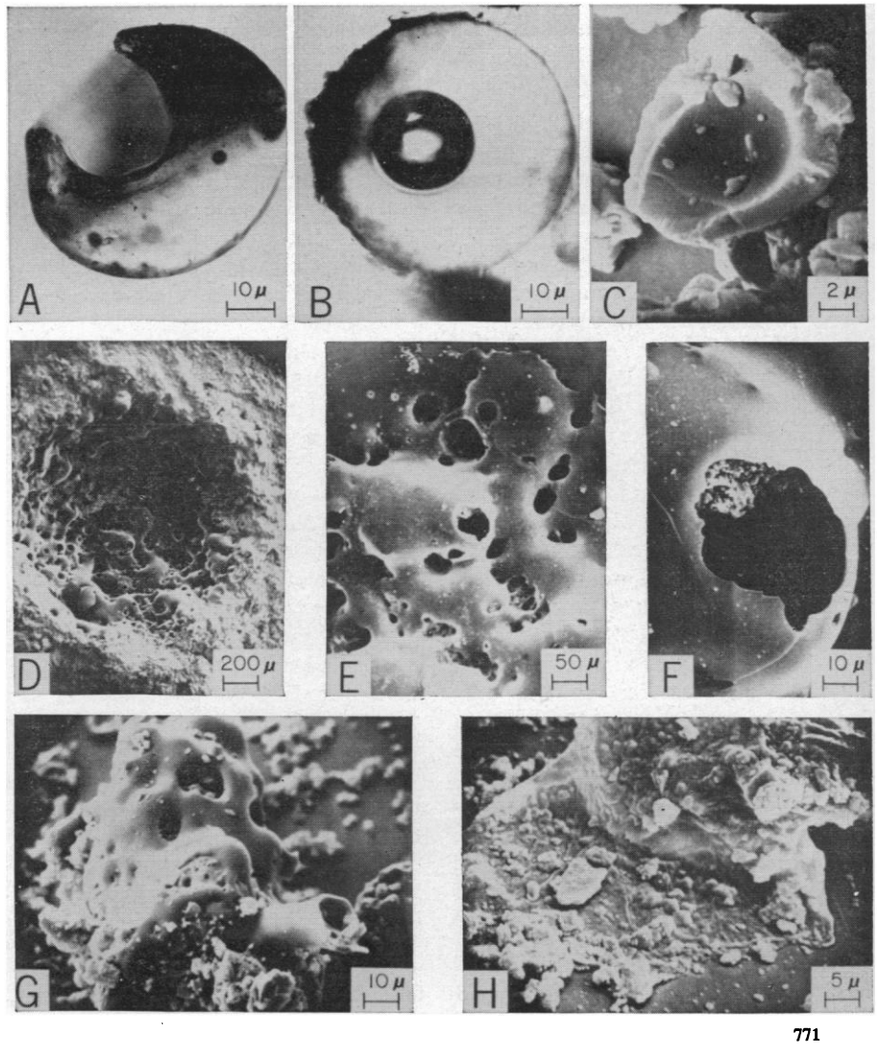

Fig. 2. Photomicrographs (A and B) and scanning electron micrographs (C–H) of lunar fines (10086) and the outer surface of a lunar breccia (10002, 54). (A and B) Particles that had been heated to 700°C for 7½ minutes; (A) broken hollow glass bead with the cavity exposed, (B) hollow glass bead with the interior intact. (C) Scanning electron micrograph of broken, hollow glass bead. (D, E, and F) Small crater on surface of lunar breccia showing hollow, thin-walled bubbles indicating an initial, positive vapor pressure behind the once molten surface. (G) Glass bead showing "bubble-like" structure. (H) Sheet-like folded material, with mineral enclosures, resembling possible polymeric substances, in the residue of fines treated with 48 percent hot HF and hot 6*N* HCl.

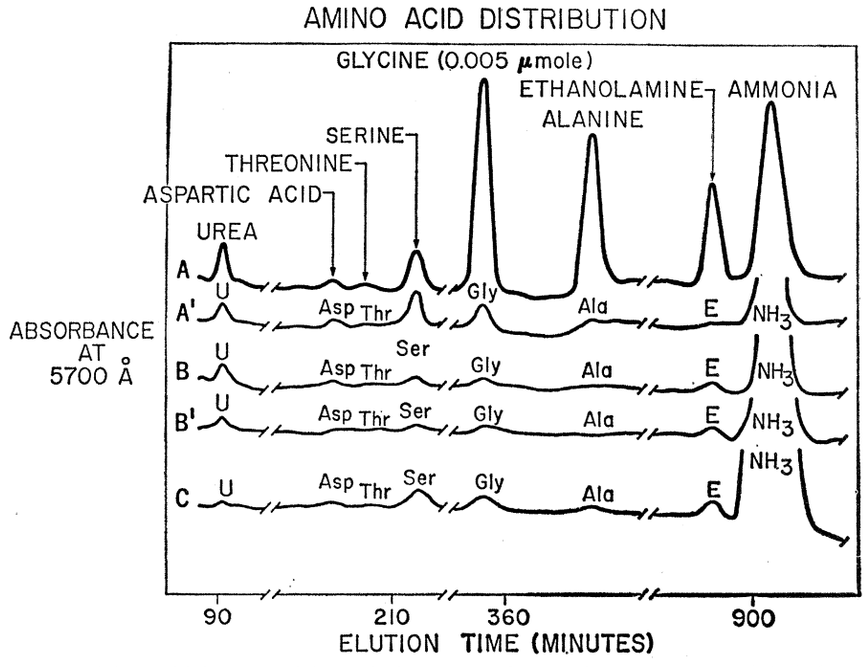

Fig. 3. High-sensitivity and high-resolution ion exchange chromatograms of (*A*) lunar fines (10086) extract; (*A'*) corresponding procedure blank; (*B*) extract of terrestrial dunite rock subjected to the exhaust from the lunar module descent engine in an initial vacuum; (*B'*) corresponding procedure blank; (*C*) hand contamination at 10^{-10} mole level.

and high resolution (*5*) were used for the analysis of free amino acids from 32.66 g of lunar fines extracted in a Soxhlet with 150 ml of freshly triple-distilled water. Precautions were taken to prepare and keep water and glassware free from hand and microbial contamination. After extraction, the contents of the flask were dried on a rotary evaporator. The supernatant in the thimble was transferred to a separate flask and evaporated similarly. In a procedure blank, the thimble contents were added to the extraction flask. The same lunar material was extracted a second time, and the corresponding procedure blank was obtained.

Immediately prior to chromatographic analysis, 2.0 ml of deionized water passed through a 0.22-μm pore filter was added to the flask, and 0.1 ml was removed for bacterial colony count by plating; all transfers of fluid were carried out aseptically. Three flasks showed no bacterial growth, and three flasks had very low colony counts. The source of this contamination was proven to be the added water. The remaining 1.9 ml of water was then evaporated. Subsequently 1.0 ml of 0.1*N* hydrochloric acid was added to the flasks to dissolve soluble residues, and 0.33 ml of the acid solution was placed on the ion exchange column. Prior to and between analyses of the lunar material, the analyzer was tested to show that amino acids were not originating from within the column, that recoveries of known amino acids were satisfactory, and that total operational contamination could be consistently kept at approximately 10^{-10} mole of each amino acid. Cysteic acid, taurine, aspartic acid, threonine, serine, glycine, alanine, and ornithine were readily detected at the 10^{-10} mole level, whereas other common amino acids were barely discernible or were not detected at all.

The only findings of interest appeared in the analysis of the first lunar Soxhlet extract. The following compounds (corrected for blank values) were detected: urea, 65 μg per kilogram of lunar material; glycine, 32 μg/kg; alanine, 36 μg/kg; ethanolamine, 22 μg/kg (Fig. 3). Apparently the first Soxhlet extraction of the lunar sample was essentially quantitative.

Although the position of a peak on the chromatogram is of great qualitative value, it is not an absolute criterion of identity. Neither is symmetrical overlap by a known compound co-chromatographed with a peak of interest an absolute criterion of identity, but it does provide confirmatory evidence. Accordingly, 0.065 ml, or one-fifth of the first extract, was co-chromatographed with a known amino acid mixture at the 10^{-9} mole level. Symmetrical peaks were obtained, and glycine, alanine, and ethanolamine completely overlapped the corresponding peaks in the lunar sample. Moreover, the net positive differences between the lunar sample plus test mixture and the test mixture were approximately one-fifth those determined in the original run. The above identifications are therefore reasonable. Identity must still remain tentative pending confirmation from other laboratories by different techniques.

The presence of these four substances (if the above identifications are correct) is inconsistent with either hand (*6*) or microbial contamination. Furthermore, it does not seem likely that they have arisen from presently existing materials of biological origin. In abiotic syntheses, simulating primitive earth conditions in the laboratory, glycine and alanine have usually been formed in greater abundance than other amino acids (*7*). One is led to consider abiotic synthesis of trace amounts of these substances from the exhaust from the lunar module descent engine, or abiotic synthesis on the lunar surface from indigenous organic materials under the influence of ultraviolet or ionizing radiation. Neither the hot aqueous extract of a 27.3-g sample of the terrestrial rock dunite, subjected by NASA (*8*) to exhaust from the lunar module descent engine in an initial vacuum of 10^{-4} torr, nor a portion (one-third) of the 150-ml extract, analyzed by the identical procedure, showed the same amino acid distribution as the lunar sample (Fig. 3). Furthermore, no exhaust gases from the lunar module descent engine were found in the lunar samples analyzed during the investigation at the Lunar Receiving Laboratory (*1*).

During the extraction of the lunar fines with hot water a strong odor, resembling H_2S, was noticed, and a positive $AgNO_3$ test was obtained. In one experiment, a spot approximating the position of sulfur, or of certain sulfur compounds, was observed on a thin-layer chromatogram when the plate was placed under the effluent gas.

Interpretation of the origin of the organic compounds in the lunar samples requires extreme caution because of the possibility that contamination occurred during acquisition and initial processing (*1*). It has been shown that the

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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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

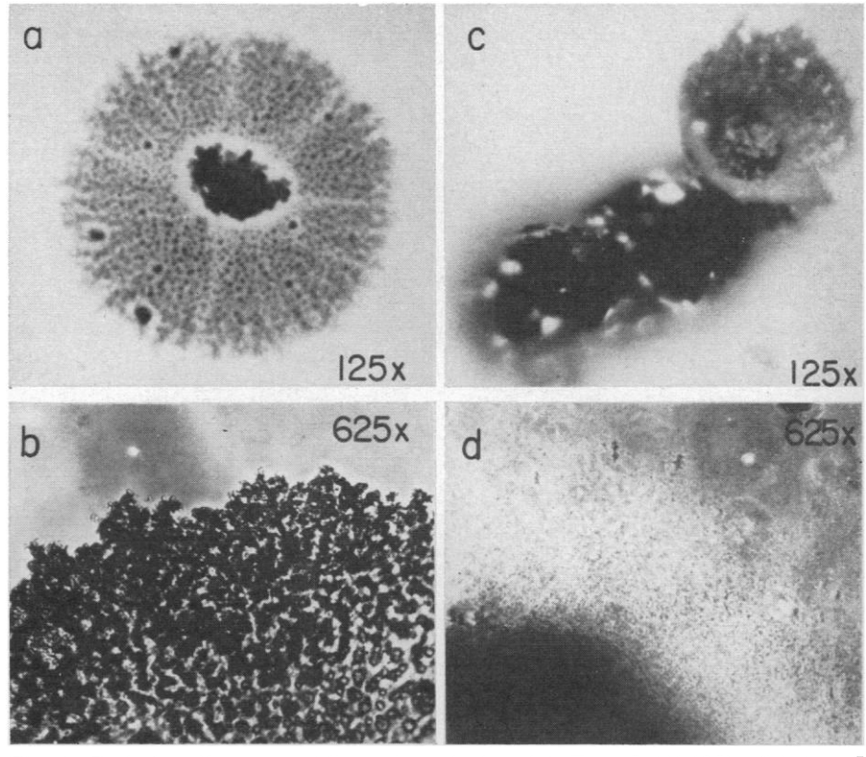

Fig. 1. Typical artifacts resembling microcolonies. (a) Type I (× 125); (b) type I (× 625); (c) type II (× 125); (d) type II (× 625).