References and Notes

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Analysis of Lunar Material for Organic Compounds

Abstract. *A sample of lunar material from Apollo 11 was subjected to analysis by several techniques, which included mass spectrometry, gas chromatography, liquid chromatography, and nuclear magnetic resonance and their variations, in an effort to detect the presence of organic compounds. None were found. On the basis of the sensitivity ascribed to certain of the methods employed, it is assumed that if organic matter were present it would exist in concentrations less than 1 part per million.*

Three sample-handling procedures were utilized during efforts to determine the existence of organic compounds in the Apollo 11 lunar sample 10086.5 (class D). First, an aliquot of untreated sample was directly analyzed by gas chromatography and mass spectrometry. Second, the sample was subjected to extraction by certain organic solvents (*1*), the solvents were removed, and portions of the residue, if any, were analyzed by gas chromatography, mass spectrometry, and nuclear magnetic resonance. Third, an aliquot of the sample was acid-hydrolyzed, and the hydrolyzate was extracted and subjected to analysis by high-performance liquid chromatography on pellicular resins, by gas chromatography, and by mass spectrometry.

All solvents were of spectroscopic grade and were redistilled when necessary. A series of blanks was run prior to assay of the material in all instances.

For the nuclear-magnetic-resonance analysis a 23-g sample of lunar material was placed in an extraction thimble and extracted with a solution containing 80 ml of a 4:1 benzene-methanol (spectrograde, redistilled solvents) mixture in a Soxhlet extraction apparatus for 24 hours and then was stirred as a slurry for 24 hours. The contents were decanted into four test tubes and centrifuged for 2 hours. The supernatants were combined, and an aliquot was evaporated to dryness in a stream of pure nitrogen. The contents were triturated 18 hours with 0.8 ml of spectrograde carbon tetrachloride, and the carbon tetrachloride solution was added to a Wilmad Imperial 507PP sample tube (5 mm). A small amount of Matheson tetramethylsilane was added as a reference signal for the field-frequency lock. A blank was determined with identical materials and apparatus.

The nuclear-magnetic-resonance study was conducted with a Bruker HFX-3 spectrometer operating at 90 Mhz (H^1). The sensitivity was increased by use of a 4096 channel computer of average transients (CAT). The spectrum was determined over a sweep range from 30 to 1166 hz downfield with tetramethylsilane. The sweep speed, determined by the CAT, was 80 msec per channel, and the signal was filtered with an input integrator (time constant = 80 msec per channel).

After 27 hours, the spectrum showed peaks at 133 hz and a multiplet at 305 hz. The multiplet was also present in the blank and is a solvent impurity. No other peaks were present. The peak at 133 hz was probably due to the intense tetramethylsilane peak due to repeated scans by the signal-averaging device as evidenced by the spectrum of the blank.

The sensitivity of the Bruker HFX-3 spectrometer for a 1-percent (by volume) ethylbenzene solution, using a 5-mm sample tube, is given as a 50-to-1 signal-to-noise ratio. The CAT further increases the signal-to-noise ratio by a factor of 40. Therefore the sensitivity of a sample with the same molecular weight and number of hydrogen atoms as ethylbenzene will be approximately 5×10^{-4}. Any material present in a concentration of less than 1×10^{-4} will be difficult to detect.

An analysis of the sample extract was made with a high-performance liquid chromatography system (*2*) equipped with an ultraviolet detector. With this technique, minute quantities of substances, such as nucleic acid constituents, which have absorbtivity at 250 nm, can be separated and determined.

Ten grams of the finely ground lunar sample were digested with 50 ml of 2.5*N* hydrochloric acid at 50°C for 2 days. After centrifugation the extract was lyophilized. Then 1 ml of distilled water was added to the vessel in order to prepare the sample solution for the chromatographic investigation. Another portion of this sample solution was then processed for analysis by mass spectrometry and gas chromatography.

The LCS 1000 liquid chromatograph (Varian Aerograph) with 1-mm inside-diameter columns packed with pellicular cation exchange or anion exchange resins (particle diameter 50 μm) was used in a fashion described earlier (*2*). The length of both columns was 150 cm. Dilute potassium hydrogen phosphate solutions having different *p*H values were used as eluents. In some experiments gradient elution (concentrated KH_2PO_4 served as strong eluent) was employed. The flow rate was 10 ml/hour, and the column temperature was 60°C in all experiments. A 10- or 20-μl sample solution was injected onto the chromatographic columns with a Hamilton No. 701 microsyringe.

Chromatographic runs were made on both cation exchanger and anion exchanger columns with eluents of various *p*H values in the range 2 to 7. No peaks attributable to retarded solutes have been found. In gradient elution with both columns small peaks appeared regularly at a certain point of the chromatogram, but these peaks were also obtained when a solvent blank was analyzed. Thus it is concluded that under the experimental conditions described no nucleic acid constituents or ultraviolet-absorbing materials of similar chromatographic behavior have been extracted from the lunar sample in an appreciable quantity. Considering the sensitivity of the technique, therefore, the amount of individual nucleic acid constituents, if such were present, must be less than 10^{-10} mole per gram of sample.

For the gas-chromatography analysis a 25-mg sample of the lunar material without prior treatment was placed in a small quartz tube containing quartz wool and attached to a supported coated open tubular (SCOT) column, 15.1 m long with a 0.058-cm inside diameter, containing silicone fluid (SF96, 1000 cs) and Igepal CO-880 (nonylphenoxy-polyethyleneoxy-ethanol). The column was connected to a flame ionization detector (sensitivity 10^{-12} g/sec), and the entire system was

flushed with carrier gas (nitrogen) for 30 minutes. The sample was heated to 150°C, then to 250°C, and finally to 500°C in the flash heater zone of the gas chromatograph. The temperature of the sample tubes was maintained at each temperature level while the column was temperature-programmed from 50° to 180°C at the rate of 3°C per minute. A control containing solvent-washed glass beads was substituted for the lunar sample and analyzed in a similar fashion. There was no evidence of organic compounds' being eluted from the sample. Similar results were obtained when these analyses were repeated on a 1.5-m by 0.058-cm SCOT column coated with Apiezon L and programmed to 210°C.

Aliquots from both the acid hydrolyzate and the organic solvent extracts of the sample and reagent blanks were concentrated and then subjected to the gas chromatographic procedures described above. Again, the findings were negative.

In all mass-spectrometry experiments, the spectrometer (M-S 9, Associated Electronics Industries, Manchester, England) was operated at 70 ev and at 100 or 300 μa trap current. The resolution was approximately 1:1000.

All samples, extracts, and reagent blanks were introduced via the direct insertion probe. Prior to sample introduction, the operating parameters of the instrument were optimized with perfluorotributylamine as a reference compound. After this material was pumped off, the ion source was baked at 250°C for 4 days.

A 10-mg portion of the lunar sample was loaded into a special quartz capillary tube attached to the direct-insertion probe. The probe was inserted into the mass spectrometer and spectra were recorded at various temperatures as the temperature of the ion source was increased from 50° to 250°C. The various spectra were dominated by peaks due to H_2O, N_2, O_2, and CO_2. The mass spectral patterns of the remaining peaks were indicative of very small quantities of low-molecular-weight hydrocarbons consistent with that noted as background. A repeat analysis gave similar results.

Studies of the extracts and reagent blanks by mass spectrometry did not reveal the presence of any components which could be considered to be indicative of endogenous material derived from the lunar sample.

In conclusion it is assumed that the principal components of the various spectra were contaminant gases and traces of low-molecular-weight hydrocarbons which had adsorbed onto the surface of the lunar sample during handling and exposure to the atmosphere.

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Micropaleontological Studies of Lunar Samples

Abstract. *Optical and electron microscopic studies of rock chips and dust from the bulk sample box returned by Apollo 11 and of petrographic thin sections and acid-resistant residues of lunar material have yielded no evidence of indigenous biological activity.*

Although the present lunar environment is inimical to known biological systems, more favorable conditions may have existed in the geologic past. Urey (*1*) has suggested that the moon may have become "contaminated" with terrestrial organic matter early in the evolution of the earth-moon system. If this suggestion is correct, and if life became established, evidence of fossil organisms might be detectable in lunar rocks. It is even conceivable that such organisms might have been the progenitors of an extant biota, adapted to the harsh conditions of the lunar surface; such organisms probably could not survive in the terrestrial environment and therefore would not be recognized in studies designed to detect vital processes (such as metabolism, growth, and pathogenicity). The approach and techniques successfully used in Precambrian paleobiology (*2*), and the criteria developed to establish the indigenous and biogenic nature of Precambrian microfossils, seem well suited for the detection, characterization, and interpretation of any fossil or recently dead microorganisms that might occur in lunar materials (*3*).

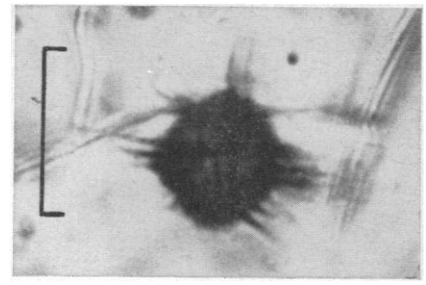

Fig. 1. Optical photomicrograph showing actinomorphic pseudofossil, apparently produced by partial devitrification of the surrounding glassy matrix, in a petrographic thin section of a microbreccia (sample 10046,56); the scale represents 10 μm.

In an effort to detect evidence of lunar organisms in the Apollo 11 samples, studies were made with a light microscope (L) at magnifications ranging from 4 to 1500, and, after the specimens had been coated with a thin gold-palladium film, with a scanning electron microscope (SEM) at magnifications ranging from 30 to 30,000. I examined samples as follows: (i) lunar dust (sample 10086,18 from the bulk sample box), divided into four size-fractions by sieving (>246 μm, 246 to 124 μm, 124 to 74 μm, and < 74 μm)—L and SEM; (ii) residue resulting from dissolution of lunar dust in hydrofluoric and hydrochloric acids—L; (iii) surfaces of rock chips from the exterior and interior of a microbreccia (sample 10002,54 from the bulk sample box), and fragments of these chips—L and SEM; (iv) petrographic thin sections of microbreccias (samples 10019,15, 10046,56, 10059,32, 10059,37, 10061, 27, 10061,28, and 10065,25)—L; (v) as a member of the Ames Lunar Sample Consortium, I studied (L) samples being investigated by Ponnamperuma *et al.* (*4*) (sample 10086, bulk A fines); (vi) as a member of the Lunar Sample Preliminary Examination Team for the Apollo 11 mission, I studied (L) rocks, chips, dust, and bioquarantine samples (including portions of both cores) (*5*).

Several thin sections (among them 10046,56, 10059,32, and 10061,27) contain elongate, spheroidal, spinose, or actinomorphic structures (Fig. 1) that superficially resemble terrestrial microfossils; many of these mineralogic