Evidence for Compounds Hydrolyzable to Amino Acids in Aqueous Extracts of Apollo 11 and Apollo 12 Lunar Fines

Abstract. *Hydrolyzates of aqueous extracts of Apollo 11 fines, an Apollo 12 trench sample, and an Apollo 12 surface sample have been analyzed on an ultrasensitive amino acid analyzer. The total content of amino acids recovered ranged from 20 to 70 parts per billion of lunar soil. Amino acids are not recovered by the direct hydrolysis of lunar fines, presumably because of decomposition in the presence of the large excess of lunar mineral. As judged by retention time, glycine is the dominant amino acid found; alanine is secondarily present in each case in the profile. Only a few amino acids have been recorded in each analysis. The pattern is relatively consistent in the samples from the three locations; the pattern from either hydrolyzed or unhydrolyzed extracts differs markedly from that of hydrolyzed or unhydrolyzed handprints. The evidence is not consistent with contamination of the kind expected by many investigators.*

The lunar soil returned from the Apollo 11 and Apollo 12 missions provided the first opoprtunity to search potentially contamination-free extraterrestrial matter for evidence of prebiological molecular evolution (*1*). In a theory of macromolecular and cellular origins Fox and his collaborators have proposed that amino acids on Earth yielded protein-like polymers that were readily converted to microsystems having many of the properties of contemporary cells (*2*). Microparticles have indeed been found in the lunar dust, but these have been essentially siliceous glass in constitution (*3, 4*). In this report, the questions of the occurrence of amino acids and of precursors hydrolyzable to amino acids are examined. Amino acids are of unique interest in this context because of their special chemical nature as nitrogen-containing carbon compounds and because of their special significance with reference to an understanding of the origins of life.

The prospects for the existence of extraterrestrial amino acids have been enhanced by spectroscopic data indicating the presence of formaldehyde, ammonia, hydrogen cyanide, and other compounds in interstellar space (*5*). Some of these compounds have been converted in the laboratory to amino acids (*6, 7*). The conditions employed in the laboratory are relevant geologically even in the present era (*2*). Amino acids are of special diagnostic value in lunar organic chemistry because (i) they may be identified by sensitive methods of detection (*8*), (ii) they often appear in families of differing composition (*7*), thus permitting a high degree of discrimination, and (iii) they and their polymers have a relatively high degree of stability among organic compounds. The relative stability of amino acids is indicated by their high melting points and their solubility and is understood on the basis of their salt-like character (*9*).

Two free amino acids, glycine and alanine, have been identified in aqueous extracts from Apollo 11 fines by two investigative teams (*10, 11*). In addition, extracts of Apollo 11 material

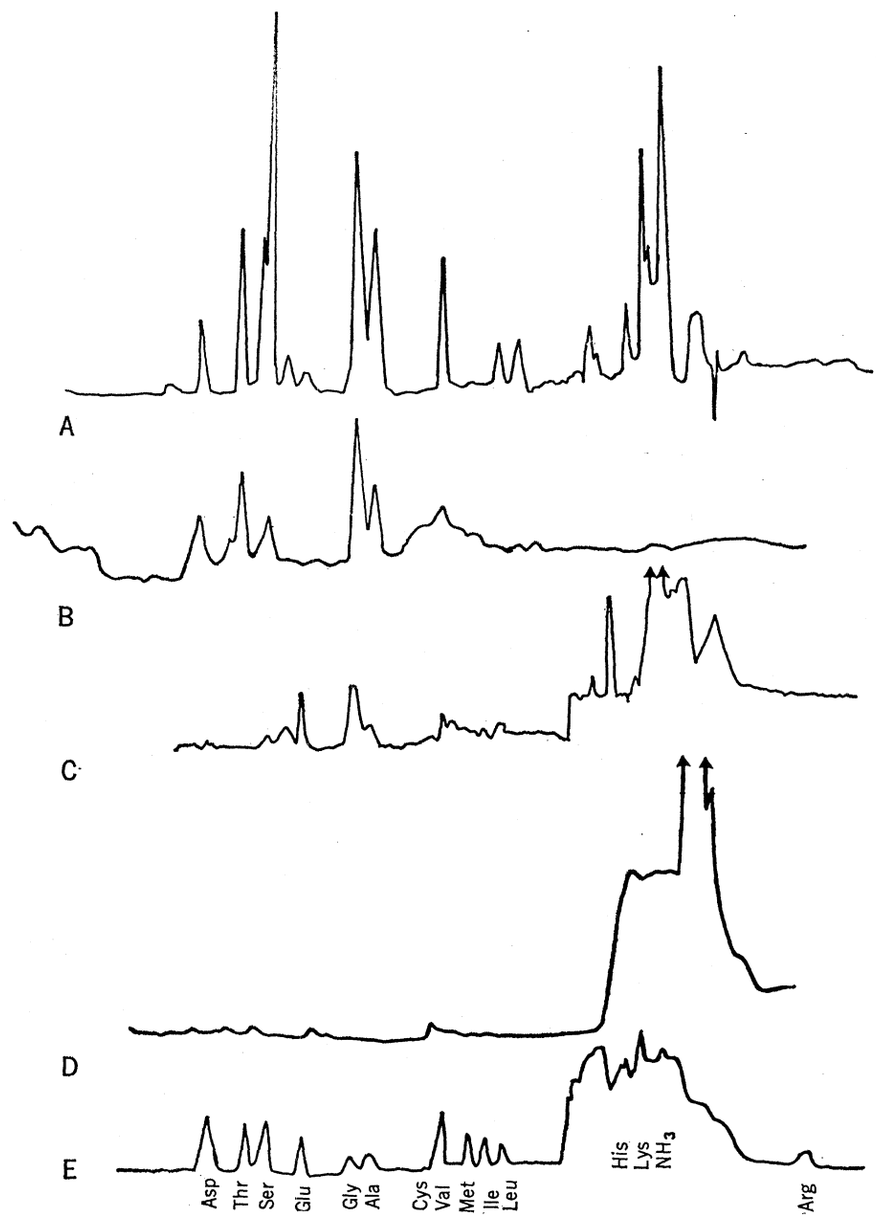

Fig. 1. (A) Amino acid profile of a human handprint; other human handprints and their hydrolyzates resemble this one. (B) Hydrolyzate of the aqueous extract of the Apollo 11 fines (sample 10086). In this analysis, the use of the column was terminated before the basic region. (C) Hydrolyzate of the aqueous extract of the Apollo 12 fines from below the surface (sample 12033). (D) Water blank carried through the entire analysis; it resembles other water blanks and the unhydrolyzed extract of sample 12033. (E) Standard amino acid profile; profiles (A), (B), and (C) were approximately lined up on the glycine and alanine peaks of this profile. The standard contained 0.10 nmole of each amino acid. Rates of elution varied between the analyses.

have been hydrolyzed and examined for precursors of amino acids in our study. Besides glycine and alanine, four other amino acids in lesser proportions were found in the hydrolyzed extracts. In all cases, the identity was established by means of the retention time (RT), in comparison with that of standard amino acids, in the usual fashion.

A specially collected subsurface Apollo 12 sample, sample 12033, was examined for free amino acids by Nagy *et al.* (*12*) and by us. Neither group found any amino acids present. The hydrolyzate of aqueous extracts of Apollo 12 fines prepared in the same way as the Apollo 11 samples (*4*), however, yielded in our laboratory principally glycine with also an alanine peak, as in the Apollo 11 samples (Fig. 1). Figure 1C shows much ammonia and a peak designated as BAA (basic amino acid range) in Table 1. The RT of the BAA peak does not resemble that of any basic proteinous amino acid. Although the absence of peaks for lysine, arginine, or histidine is in agreement with the inference of noncontamination (the presence of the strange peak also supports that inference), the BAA peak has not been identified. In all samples, the total amino acid content was three to ten times that of the water blanks carried through the entire procedure.

Investigators in other laboratories (*13, 14*) have failed to obtain for Apollo 11 fines the results we have reported (*4, 11*) for precursors hydrolyzable to amino acids. Of the three laboratory groups which sought amino acid precursors by hydrolysis, our team alone used extraction by hot water, followed by hydrolysis of the aqueous extract, followed, in turn, by estimation by ion-exchange chromatography with an especially sensitive amino acid analyzer (*8*). Our experiences with volcanic and other terrestrial samples led us to use hot aqueous extraction followed by hydrolysis of the aqueous extracts. This method was chosen to avoid decomposition in the presence of a large excess of mineral, and to minimize difficulties introduced by subsequent desalting. Such a need was accordingly anticipated for examination of the lunar samples as well. A controlled experiment (*11*) indicated that amino acids could be recovered from the hydrolyzates of aqueous extracts of fortified (*7*) water-extracted Apollo 11 fines, whereas these amino acid precursors were destroyed by direct hydrolysis in the presence of fines. Some of the workers who failed to report amino acids in lunar samples did record quantitative amounts of amino acids in hydrolyzates of extracts of the Murchison meteorite (*15*); in so doing they used the sequence of methods we employed on the Apollo 11 analyses (*4*), that is, extraction with hot water, hydrolysis of the aqueous extract, and estimation by ion-exchange chromatography. Any recorded failure (*13*) to obtain amino acids from Apollo 11 lunar fines (sample 10086) must, therefore, at the present time be ascribed to the use of a total method, including apparatus, that was inadequate for the finding of such compounds and their precursors at concentrations as low as 20 to 70 parts per billion.

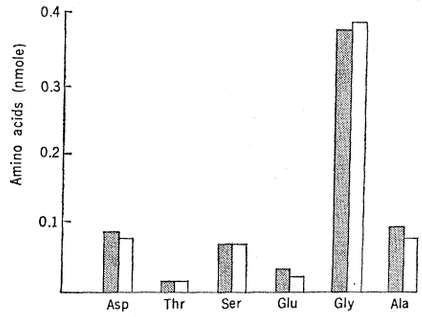

Fig. 2. Results of successive extraction by water of Apollo 11 fines. Open bars are for acid hydrolyzates of the first water extract minus the second water extract; solid bars are for acid hydrolyzates of the first water extract minus the third water extract.

The results from the analyses of Apollo 11 and Apollo 12 samples (Table 1) for precursors hydrolyzable to amino acids are in accord with those shown in Fig. 1. The possibility that the amino acids observed are due to oxidized rocket fuel has been largely ruled out on the basis of experiments with oxidized rocket fuel, from which no amino acid retention times or mass numbers have been observed (*4, 16*). The explanation that what is being examined is impurity introduced by handling at the Lunar Receiving Laboratory, or by the astronauts, appears unlikely in view of the fact that the amino acid profiles of Fig. 1 are not typical of terrestrial contamination. The profile from a rinse of human skin (*17*), for example, is shown in Fig. 1A. The original profile reveals virtually all of the amino acids typically present in terrestrial protein, and, in addition, citrulline and ornithine. This profile is for the free amino acids; the hydrolyzate of skin extract is very similar in appearance. Both the hydrolyzed and the unhydrolyzed extracts of the Apollo 12 trench samples thus show differences from this type of potential contamination (*11*). High proportions of glycine are, however, typical of the hydrolyzed products of synthesis (*7*).

The suggestion that some positive results and other negative results could appear from the same original sample might tentatively be explained as due to inhomogeneities in the sample. The accumulated results of Table 1 present values for two Apollo 11 samples and two Apollo 12 samples from two different collections (a total of three collections); this concordance from a variety of sources would appear to rule out an explanation of discrepancy due to inhomogeneity. Were inhomogeneity to be the explanation, the finding of dominance by glycine, with lesser amounts of alanine, could hardly have resulted from each of three independent samples examined. Also relevant to a judgment on homogeneity is the absence of free amino acids from the Apollo 12 trench samples as recorded by two

Table 1. Apparent amino acid contents of lunar samples in molar percent.*

Amino acids in hydrolyzate	Apollo 11		Apollo 12	
	Sample 1 (No. 10086)	Sample 2 (No. 10086)	Trench sample (No. 12033)	Surface sample (No. 12001)
Aspartic acid	5	5	<1	2
Threonine	2	3	<1	
Serine	9	10	<1	3
Glutamic acid	9	11	20	6
Glycine	50	52	37	70
Alanine	25	19	12	3
Valine		<1	<1	<1
Isoleucine		<1	<1	<1
Leucine		<1	3	<1
Tyrosine				2
Phenylalanine				2
BAA†			25	10
Total content of amino acids in hydrolyzate (parts per billion)				
	53	37	19	69

* Calculated without ammonia. Values for the water blank have been subtracted in each analysis.
† A dominant peak in the basic amino acid (BAA) region, which does not correspond to a proteinous amino acid, with an RT between those of phenylalanine and histidine.

investigative groups, and the fact that these same two groups both found glycine and alanine in the free state in hot water extracts of the Apollo 11 samples (*10, 11*).

A third sample of Apollo 11 fines was extracted three times with hot water, and the extracts were subjected to hydrolysis and analysis. The results are presented in Fig. 2. These results demonstrate that the first extraction was complete and that no contamination was introduced into the sample during processing in this laboratory. The ability to detect more amino acids, by types and amounts, might, however, result from further improvement in the methods. The small amounts of amino acids found and the fact that the proportions in the trench sample are the lowest examined suggest that the amino acid precursors may be products of the reaction of components of the solar wind (*18*), or from passage of the solar system through clouds of interstellar matter. Such an inference deserves to be tested by analysis of other subsurface samples.

The amino acids released by the method described are mainly similar to those reported for the Murchison meteorite (*15*). Although some reservations apply in each case and although the history of the meteorite poses especially difficult questions, the results from both sources may be viewed as supporting each other and providing evidence for the existence of extraterrestrial amino acids. The results are consistent with firmer data on the convertibility of components of interstellar matter to amino acids (*7*) and on laboratory models constructed under geologically relevant conditions (*2*).

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Photochemical Transformation of 5-Alkyluracils and Their Nucleosides

Abstract. *Irradiation at 254 nm of aqueous solutions of 5-ethyl-, 5-propyl-, and 5-isopropyluracils (or their nucleosides) leads to cleavage of the 5-alkyl substituents, via an intramolecular electrocyclic photoaddition intermediate, with formation of uracil (or its nucleoside). The photoaddition intermediates represent a new class of dihydropyrimidines, namely analogs of 5,6-dihydro-5,6-cyclobutanyluracil and its nucleosides; the biological significance is discussed.*

It has been demonstrated (*1, 2*) that 5-ethyldeoxyuridine (**1**, EtUdR), a nonmutagenic thymidine base analog with antiviral activity (*3*), undergoes two wavelength-dependent photochemical transformations in neutral aqueous medium. (i) At wavelengths longer than 265 nm, the principal reaction is a photodimerization, similar to that undergone by thymine or thymidine; (ii) at shorter wavelengths, 254 nm, irradiation leads to cleavage of the 5-ethyl side chain with formation of deoxyuridine (**3a**) in more than 80 percent yield (*4*). Photodimerization occurs also on irradiation at 254 nm; however, at this wavelength, the dimers exhibit absorption so that they are rapidly photodissociated and can be detected only in small quantity by paper chromatography during the initial stage of the reaction (*2*).

The behavior at 254 nm of compound **1** contrasts with that for dilute ($10^{-4}M$) aqueous solutions of **3a** and thymidine; compound **3a** undergoes mainly photohydration of the 5,6 bond; thymidine is relatively radiation resistant under these conditions (*5*). We have examined the behavior of two higher 5-alkyl analogs, 5-propyluridine (**4**) and 5-isopropyluridine, both of which were prepared on a small scale enzymatically from the corresponding bases with the aid of the uridine phosphorylase system (*6*).

Both the foregoing were only very slowly transformed to unidentified products on irradiation of 10^{-4} to $10^{-3}M$ neutral aqueous solutions at long wavelengths. But, at 254 nm, either in the presence or absence of oxygen, each readily underwent cleavage of the 5-alkyl substituent via an identifiable intermediate (**5**, see below) with formation of uridine (**3b**) in 80 to 90 percent yield. The quantum yields for these transformations were 5×10^{-3} for isopropyluridine and 9×10^{-3} for propyluridine, as compared to 21×10^{-3} for hydration of uridine. No photodimer formation was observed