BIOMARKERS IN ALH84001 ??? Allan H. Treiman, Lunar and Planetary Institute, 3600 Bay Area Boulevard, Houston TX 77058-1113 USA. <treiman@lpi.jsc.nasa.gov>

Summary: D. McKay and colleagues suggested that four sets of features in ALH84001 were biomarkers, signs of an ancient martian biota that once inhabited the meteorite. Subsequent work has not validated their hypothesis; each suggested biomarker has been found to be ambiguous or immaterial. Nor has their hypothesis been disproved. Rather, it is now one of many hypotheses about the alteration of ALH84001.

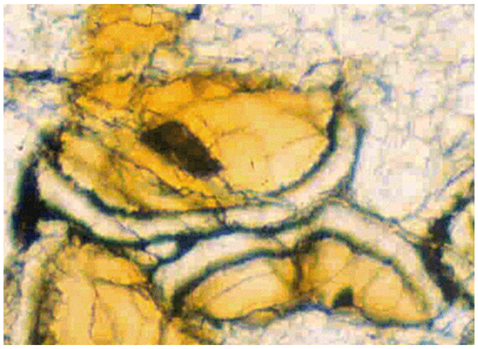

Figure 1. Carbonate globules in ALH84001, thin section view, plane polarized light, 0.2 mm across. Globule cores, brown Fe-rich carbonate, are surrounded by black layers (rich in magnetite and pyrrhotite) sandwiching Mg-rich carbonate.

Background: McKay et al. [1] reported four possible markers of ancient martian life in meteorite ALH84001, all related to chemical alteration of this ancient igneous rock. These biomarkers, all associated with carbonate mineral discs in fractures, are: organic compounds, a disequilibrium mineral assemblage, nanophase magnetite crystals resembling products of terrestrial bacteria, and bacteria-shaped objects. No single marker was considered definitive, but the combination of all four was held as clear evidence of ancient martian life in the meteorite.

Here, I will evaluate these four putative biomarkers and their combination in light of subsequent studies. I will assume that the carbonate minerals and associated materials formed at temperatures conducive to life.

I. Organic Compounds: McKay's group found that the carbonate globules contained polycyclic aromatic compounds, PAHs [1]. These PAHs were claimed to be like those formed during thermal decomposition of bacterial matter, and so were claimed to be biomarkers.

The PAHs Are There, but Where? ALH84001 does contain organic carbon, in the sense of carbon-carbon bonding [1-5]. McKay's group used a very sensitive technique that detects only PAHs [1], and found that the PAHs were abundant only on fracture surfaces that contained carbonate discs. The discs contain organic carbon [2,3], although some contain very little [4]. PAHs may be distributed widely in ALH84001, and are reported to be scarce in carbonate globules <u>outside of fractures</u> [5]. This difference between carbonates in and outside fractures is puzzling, as the carbonates are otherwise apparently identical.

Are the PAHs Martian? McKay and co-workers showed that PAHs were less abundant near the fusion crust of ALH84001, suggesting that the PAHs are indigenous to the meteorite and thus martian [1,2]. Contamination by terrestrial carbon is a critical concern, and was the subject of several experimental studies [2,6]. Most or all of the organic carbon in ALH84001 is terrestrial, as it has live ^{14}C [7] which must have entered the meteorite during its ~13,000 years in Antarctica. A small portion of the carbon without live ^{14}C could be from Martian organics, but could also have come from occluded carbonate grains. Amino acids in ALH84001 are clearly terrestrial [8].

Could PAHs be Biogenic? The claim that the PAHs are similar to biogenic products has not been fully documented; other origins are possible. The PAHs are similar to those in CM chondrites and micrometeorites [9], which presumably formed by inorganic mechanisms (e.g., [10,11]). However, terrestrial weathering of all PAHs removes their characteristic side chains and heterocycles, leaving generic PAH core molecules that could have formed by many processes [12].

Conclusion. It is not proven that the PAHs in ALH84001 are relics of a martian microbiota. The vast bulk (perhaps all) of the organic matter in ALH84001 is terrestrial, but the PAHs could still be Martian. The spatial distribution of PAHs appears inconsistent with terrestrial contamination (at least by bulk diffusion). In their molecular simplicity, the PAHs in ALH84001 do not require either a biotic or abiotic origin. The PAHs are comparable to those in CM meteorites (inorganic), but could also have formed by other inorganic mechanisms, or by weathering of biogenic PAHs (terrestrial or martian).

II. Disequilibrium Minerals: McKay et al. [1] cited the chemical zoning of the carbonate discs and the discs mineral assemblage siderite–magnetite–pyrrhotite (with evidence of carbonate dissolution) as representing likely products of biological chemistry. However, the chemical zoning pattern and the mineral assemblage are common and expected in inorganic systems, and so are not strong evidence for biology

[10,13]. The sulfur isotope composition of the pyrrhotite is not consistent with common bacterial metabolisms [14]. Greigite (Fe_3S_4), a common bacterial product, has been reported in ALH84001 [1]. However, the report has not been confirmed [15] and greigite itself cannot be considered a biomarker [16].

Conclusion. The chemical zoning and mineral assemblage of the carbonate discs could be consistent with martian life. However, they arise abiotically too, and can hardly be used as biomarkers.

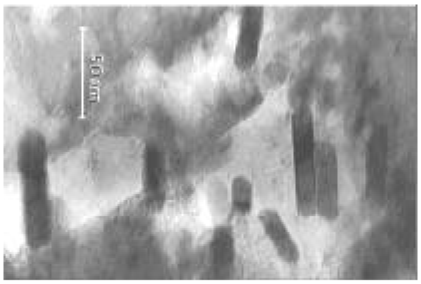

Figure 2. Whisker-shaped magnetite grains from ALH84001, epitaxially aligned on carbonate [17]. Scale bar is 50 nm.

III. Nanophase Magnetite Grains: Sub-micron magnetite grains are concentrated in two sharply-bounded layers near peripheries of carbonate discs and globules in ALH84001 (Fig. 1). Because some of these grains are similar in size, shape, structural perfection, and composition to magnetites produced by some magnetotactic Earth bacteria, McKay and Thomas-Keprta suggested that these martian magnetites were formed by a martian magnetotactic microbiota [1].

Multiplicity of Magnetites. Sub-micron magnetites from the carbonate discs occur in many shapes: whisker, ribbon, cuboidal, irregular, and parallelepiped [1,17-20]. Ribbon and whisker magnetites (Fig. 2; some with screw dislocations) may have formed at high temperature [17]. Irregular magnetites have aspect ratios near 2:1, do not show euhedral crystal forms, and are reported to contain detectable chemical impurities. Inclusion magnetites [21] may belong here, although [20] appear to attribute the irregular magnetites to oxidation of pyrite. Parallelepiped magnetites have aspect ratios near 2:1, show fully euhedral crystal forms, and are reported to contain nearly negligible chemical impurities. This latter group, ~25% of the nanophase magnetite grains, are indistinguishable from some magnetites produced intracellularly by some magnetotactic bacteria [18-20, 22]. Most of the magnetites, of all classes, have lengths and widths consistent with being magnetic single domain (SD) particles, a requirement for efficient magnetosome magnetites.

Magnetite Chains: "Virtually all magnetotactic organisms arrange their magnetite crystals into linear chains…." [22]; chains of magnetite grains have been recognized in ALH84001 and inferred to be biogenic [18,23]. Some of these chains may be chance associations of magnetite grains distributed at random. Others may be artifacts of sample preparation. Evidence for magnetite chains is not fully developed, especially considering that a chain of oriented magnetite grains "… is dynamically unstable…" [22].

Problems: Magnetotaxy seems of limited value to bacteria living in rock, and McKay's group has hypothesized that the irregular and parallelepiped magnetites washed into ALH84001 with the water that deposited the carbonate discs and globules [24]. But the sharp boundaries of the magnetite-rich layers seems inconsistent with the vagaries of fluid flow through a fractured rock.

The sizes of the ALH84001 magnetites are a problem. A significant proportion (up to 46%) of the parallelepiped magnetites in ALH84001 are smaller than single magnetic domain size ([15,18,19], presentation of [20]), and so are unlikely as bacterial magnetosomes. It is also puzzling that the parallelepiped (biotic?) and irregular (abiotic?) magnetite populations in ALH84001 appear to have identical size ranges (presentation of [20]).

Conclusion. It seems reasonable that the parallelepiped magnetites in the ALH84001 carbonates could be products of a martian magnetotactic microbiota; the irregular magnetites, abundances of sub-SD magnetites, and the sharpness of the magnetite layers would still need explanations. On the other hand, an abiotic (perhaps high-temperature) origin for all the magnetites has not been disproved [15,17,21]. Biogenic magnetites have been studied extensively, but there has been less effort devoted to abiotic growth of nanophase magnetites. Much work remains on ALH84001 (e.g., cataloging shapes and compositions of many submicron magnetites, verifying presence of chains) and on terrestrial analogs (e.g., cataloging submicron magnetites from abiotic settings).

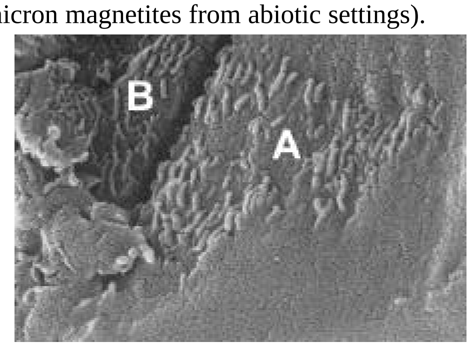

Figure 3. Bacteria-shaped objects (BSO) in ALH84001. SEM image from McKay et al. (NASA S96-12299). Image is ~2.5 microns across. Aligned BSOs at A are no longer believed to be bacterial. Branching and curving BSO at B.

IV. Shapes Resembling Bacteria: McKay's group found small (20 – 200 nm long) bacteria-shaped objects (BSOs) on fracture surfaces in ALH84001, and suggested that they were mineralized remains of bacteria [1]. Additional images of these objects were released to the press (Fig. 3). The BSOs have been compared to biogenic forms in Earth basalts [25].

Some are Inorganic? Many of the BSOs have sizes and shapes similar to those of whisker magnetite grains, and may be these magnetites [17,21]. In particular, the aligned BSOs of Fig. 3 may be whisker magnetites that grew epitaxially onto a carbonate grain (Fig. 1, [17]), or might be lamellar protrusions from the carbonate mineral surface [26,24]. McKay's group has disavowed aligned BSOs as biogenic [27], emphasizing larger objects with "intersecting alignments" and "significant curvature."

Some are Terrestrial? The BSOs are in or on martian materials, but might be terrestrial artifacts or contamination. Sample preparation for electron microcopy can produce BSOs or can modify inorganic surface features to resemble BSOs [26]. Earth bacteria have grown in ALH84001 [28], and look like some BSO (Figs. 3, 4). BSOs like those in ALH84001 are also present in some non-martian meteorites from Antarctica [29], suggesting that both acquired BSOs on Earth.

Some are Too Small? The BSO's originally figured were 30-50 nm in diameter and up to 100 nm long [1]; these objects are almost certainly too small to contain the requisite molecules for (Earth-like) bacterial metabolism [30,24], but might be bacterial appendages [25]. BSOs in other images ranged to 750 nm long and 100 nm wide [25,27], and are within the size range of terrestrial bacteria.

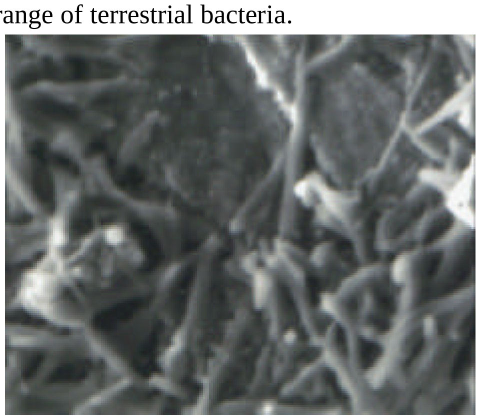

Figure 4. Terrestrial fungus in ALH84001. SEM image from [28]. Image ~ 2.5 microns across.

Conclusion: Are Any Just Right? BSOs are the most photogenic and intuitively appealing line of evidence for ancient martian life in ALH84001, but external shape is the only available evidence for a biogenic origin. BSOs can be produced by known abiotic processes [17], known biotic processes [28], and unknown terrestrial processes [29]. In my opinion, much work would need to be done before BSOs could be accepted as markers of a martian biota.

V. Additional Arguments: Other interesting issues have arisen, but have not been critical in validating or refuting McKay's group's hypothesis [1].

Carbon Isotopes. It was claimed that some organic matter in the ALH84001 carbonates had $\delta^{13}C \sim -60‰$, enormously depleted compared to carbon in the carbonates discs ($\delta^{13}C \sim +40‰$), and suggestive of extreme isotope fractionations produced by some terrestrial organisms [31]. However, this preliminary result was not reproduced, and may have represented a laboratory contaminant [32].

Biofilm. McKay's group described some unusual textures on fracture surfaces in ALH84001, and attributed these morphologies to biofilm, politely called extracellular polysaccharide slime [33]. This preliminary result has not been pursued, and there is now evidence that terrestrial bacteria and fungi can inhabit meteorites rapidly and secrete their own slime [28,34].

Conclusions: *Observations Individually…*

I. There is no proof that the PAHs are biogenic or necessarily associated with carbonate discs. ALH84001 is heavily contaminated with other types of organic matter [7,8], and one might reasonably infer that the PAHs were also terrestrial.

II. There is no evidence that the disequilibrium mineral assemblage is a marker for biology. Although those minerals could form together through bacterial metabolism, they could also form abiotically.

III. Some submicron magnetite grains in the carbonate discs are quite similar to magnetosome magnetites. Yet a significant proportion of these grains are too small to be magnetic single domains, and thus may not be biogenic. Nor is it clear how magnetosome magnetites could come to form such sharply bounded layers in the carbonate discs and globules.

IV. Bacteria-shaped objects can form by many terrestrial and abiotic processes, not only by mineralization of martian biota.

Thus, no individual line of observation supporting ancient martian life in ALH84001 has been fully validated; if anything, arguments based on I, II, and IV appear weaker now than they did in 1996. Study of the submicron magnetites (observation III) has continued, observations have been refined, and some ALH84001 magnetites are clearly similar to magnetosome magnetites. Serious issues (noted above) have inhibited widespread acceptance of the parallelepiped magnetites as magnetosomes magnetites.

… *And as a Whole.* Recognizing the potential ambiguity in a single biomarker, McKay's group suggested that the combination of their observations presented a strong case for relics of ancient martian biota in ALH84001 [1]. They suggested that the single hypothesis of martian biological activity was simpler and more credible than a separate hypothesis for each observation. Can scientific arguments be treated like sticks – four weak sticks tied together equal one strong stick? The main issue seems to be the plausibility and self-consistency of alternate hypotheses to explain the data. If the best non-biological explanation for feature A is ludicrous, then a biological origin for all features seems more plausible. What if features B and C were known to be contemporaneous, but non-biological explanations of B required silicate magma, while non-biological explanations of C required dry ice? Again, the biological hypothesis would become attractive.

For ALH84001, it is not clear that ludicrous or contradictory processes are needed to explain the four possible biomarkers. For instance, one need invoke only two processes to explain the "martian biomarkers." On Earth, microbial infestation along fractures could produce BSOs (whole cells and appendages) and PAHs as their decomposition products. On Mars, the carbonate-magnetite-pyrrhotite assemblage, with submicron parallelepiped magnetites, could form by rapid precipitation from aqueous solution. Perhaps the rock was suffused by waters of different compositions, forcing the rapid deposition (in elongate crystal shapes) of minerals at chemical disequilibrium, and forcing the oxygen isotopic variations in the carbonate [35]. Are these two processes less likely than martian biota?

Lack of Proof is not Disproof. The hypothesis that ALH84001 contains traces of ancient martian life [1] has not been proved [36]. Neither has the hypothesis or any part of it been disproved, at least in my opinion and that of its authors [36]. The PAHs in ALH84001 could be martian and biogenic (until C and H isotope measurements show otherwise). The parallelepiped magnetites could be from magnetosomes (until a conclusive test for abiogenic magnetite is found). Etc.

A Plurality of Hypotheses: On its publication in 1966, McKay's group's paper [1] was both the sole source of exciting new data on ALH84001 and the sole published explanation of the data. Since then, most new data have not seemed consistent with McKay's hypothesis, and have inspired explanations ranging from high-temperature shock [15] to cold, abiotic groundwater [37]. McKay's groups' hypothesis has (in effect) been demoted to being one hypothesis among many, all vying to explain this wonderful meteorite and its enigmatic clues to Mars' distant past.

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