Solving the Problems with Chirality as a BioMarker for Alien Life

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ABSTRACT

The basis for chiral biomarkers that have been increasingly proposed to obtain evidence for life is reviewed. Specific problems in accepting them and other biomarkers as proof of life are cited. A new chiral method is offered to overcome these difficulties, a method that can make an unambiguous determination of extant microbial life.

Key Words: astrobiology, extraterrestrial life, chiral biomarkers, chirality and life detection, TWEEL, TWEEI 2

1. BACKGROUND

One of the strange and unexplained mysteries of life is that all forms examined to date express a strong proclivity for only one of the two enantiomers of stereoisomeric organic compounds[1]. Figure 1 illustrates the stereoisomeric forms, or "mirror images," that occur for the essential amino acid, alanine, and the carbohydrate, glucose.

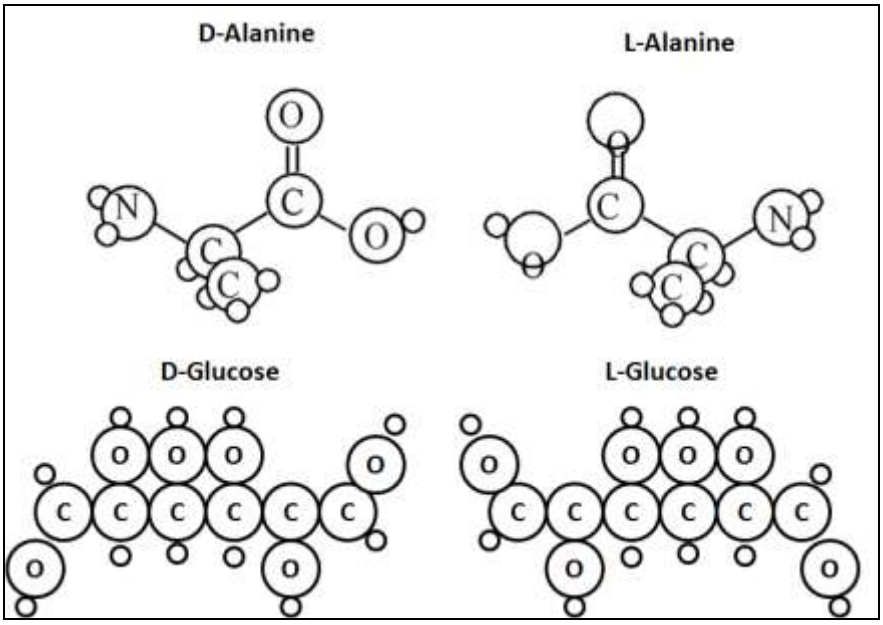

Fig. 1. L- and D-Forms of Amino Acid Alanine and Carbohydrate Glucose

Terrestrial life shows a complete or strong preference for the L-isomer in the anabolism and catabolism of sterioisomeric amino acids. The opposite selection (for the D-isomer) is true in the metabolism of sterioisomeric carbohydrates. In the pre-biotic evolution of life, once a selection of an isomeric form had been made, it became a fundamental property of life. The opposite stereoisomer would no longer fit the topology required for the enzyme-substrate pairing so selected (whether selected by chance or by some still unknown law of nature). Since the 1700s, it has become increasingly clear that living systems control virtually all of their activities through enzymes. While recent research[2] has identified microorganisms that can catabolize D-amino acids, this is accomplished by a racemase that first converts the D- to the L-form, which is then utilized in the conventional manner. The direct use by microorganisms of a D-amino acid or an L-carbohydrate is being sought[3] as evidence of a "shadow life," the product of a separate genesis from ours, be it found on Earth or elsewhere.

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2. CHIRALITY AS A BIOMARKER, AND ITS PROBLEMS

The mere finding of an excess of one chiral isomer over its mirror-image sister isomer of an organic compound has been advanced, e.g.[4], as evidence for life. While broadly endorsed, however, this "biomarker" approach faces a number of problems: 1. rapidly-improving methods are demonstrating abiotic synthesis[5] of chiral amino acids with strong excesses of one isomer; 2. racemization of ancient samples over time may elude the method; 3. the chiral test may produce a false negative should the alien life not be based on a preferential chiral biochemistry; 4. the method provides only a "snapshot" of the chirality found, and cannot determine whether the putative organism in which excessive chirality is detected is alive or a fossil; 5. like all biomarkers, the finding of chiral excess in a target suffers a fundamental hazard: chiral preference, and all other biomarkers, will likely fail the test of Occam's Razor. The case of the Viking Mission's "Labeled Release" (LR) Experiment, discussed below, strongly illustrates this point. Any and all biomarker evidence will probably be deemed as more likely to have occurred through abiotic happenstance rather than having required the much more unlikely and difficult development of a living entity to produce it. Thus, in view of these realizations, the mere detection of chiral asymmetry in an organic compound within a putative life form has become less convincing as unambiguous evidence for life. The purpose of this paper is to illuminate and circumvent these problems. A method utilizing chiral preference is proposed for the detection of extant microorganisms, which method convincingly distinguishes the activity of any living microorganisms found in a soil sample from any abiotic source of that signal.

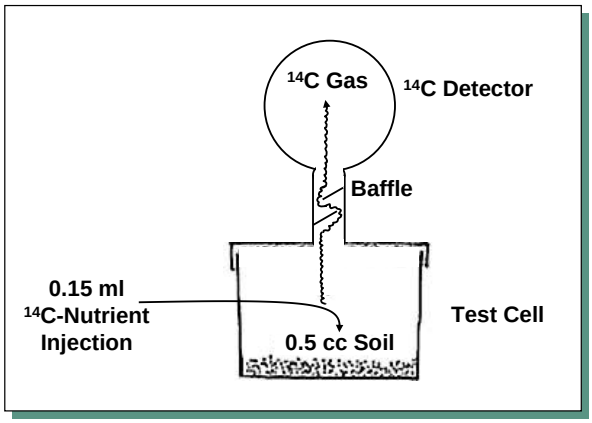
3. THE VIKING LR EXPERIMENT ON MARS

The first use of the chiral properties of organic compounds in connection with life detection was proposed[6] in 1962 in the course of the development of "Gulliver," subsequently renamed the LR experiment[7] when instrumented for flight on the 1976 Viking Mission to Mars. This experiment injected soil samples with a droplet of the simple, dilute aqueous solution of ^{14}C-labeled Miller-Urey products as shown in Table 1. As seen in Figure 2, the headspace above the soil was then monitored for the appearance and accumulation of any ^{14}C-labeled gas as evidence for on-going metabolism of one or more of the Miller-Urey compounds by living microorganisms in the soil.

Table 1. Labeled Release Substrates

Labeled substrate	Formula	Concentration ($\times 10^{-4}$M)	μCi mL^{-1}*	Specific Activity (Ci/Mole)
^{14}C-glycine	NH_2CH_2COOH	2.5	4	16
^{14}C-DL-alanine	$CH_3CH(NH_2)COOH$	5.0	12	48
^{14}C-sodium formate	HCOONa	2.5	2	8
^{14}C-DL-sodium lactate	$CH_3CHOHCOONa$	5.0	12	48
^{14}C-calcium glycolate	$(CH_2OHCOO)_2Ca$	2.5	4	16

All carbons uniformly labeled, *Total = 34 μCi, which yields 6.8×10^7 dpm ml^{-1}



Mars Test Conditions:

- **Martian atmosphere, pressurized with He to 85 mb**
- **Central injection to provide wet to moist soil**
- **7°C-10°C**
- **Dark**

Fig. 2. Schematic of LR Instrument

This initial search for life was based on the only sample of life available, terrestrial, with the assumptions that Martian life would likely also be carbon-based, aqueous, and would function with a biochemistry similar to ours. Miller-Urey compounds were chosen because it was widely accepted that they had formed from the primitive Earth's atmosphere and under conditions believed similar to those on early Mars. It was presumed that, as had happened in the formation of life inhabiting Earth, the Miller-Urey compounds on Mars would have participated in the development and evolution of life, making them good candidate nutrients for Martian life detection. This theoretical aspect of selection was rigorously confirmed empirically for each selected substrate by massive field and laboratory testing with a wide variety of terrestrial microbial species and viable soils, including *in situ* field tests performed at extremophile locations. Although this rationale for nutrient substrate selection seemed sound, a doubt quickly arose concerning those selected nutrient compounds that occurred as stereoisomers. Up to this point, only the terrestrial life chiralities had been included in the nutrient solution. It was now realized, however, that, while alien life forms were likely to exhibit chiral preferences, there is no reason to presume these preferences will be the same as ours. Enzyme-substrate systems might have developed about isomer preferences different from ours. It was thus proposed that the experiment be modified to apply each isomer to its respective soil sample to detect which isomers might be preferred. Unfortunately, the complex changes required in the instrumentation exceeded its budget and weight constraints. Therefore, in order to preclude missing life of a non-terrestrial chirality, both optical isomers of the isomeric amino acid and the carbohydrate selected were included among the nutrient substrates to be injected onto the samples of Martian soil. This chiral approach differs markedly from the more recent chiral biomarker methods proposed. The Viking LR looked for on-going, long-term (7 sols) metabolic-type reactions, as opposed to the recent "snapshot" methods which seek only a strong predominance of one isomer of a chiral compound over its enantiomer in a targeted sample.

Positive LR results were obtained at both Viking landing sites. They were confirmed by the pre-mission control that tested a duplicate sample after it had been heated to "sterilization" temperature (160^{o} C for three hr), a negative response confirming that the first response was biological. Several *ad hoc* controls were also run. Table 2 presents the thermal profile for the active agent detected. No abiotic experiment has duplicated these results, nor has any abiotic theory proposed proved tenable, but the scientific community generally attributed the positive responses to a chemical reaction, not life. Had the original intent of including chiral discrimination in the LR been carried out, it is highly probable the life issue would have been immediately resolved, rather than lingering on for more than a third of a century, as it has. However, with new data from Mars, and the discovery of extremophiles on Earth living under conditions vying those on Mars, renewed interest has been developing in the LR.

Table 2. Thermal Profile of Active Agent in Martian Soil: LR Data

- Soil in LR 2-5 Sols: results similar to terrestrial soils
- On 2nd second nutrient injection, ~ 20% of gas already evolved left chamber (probably absorbed by wetted soil); gradually re-evolved over two months
- Soil* heated to 160° C for 3 hr produced nil result
- Soil** heated to 51° C for 3 hr produced several small sporadic peaks (~ 5%-10% of positive)
- Soil** heated to 46° C for 3 hr produced kinetics of positive, but ~ 70% reduced in amplitude
- Soils maintained 3 or 5 months in soil distribution box in dark at ~ 7-10° C under ambient Mars atmosphere, pressure and humidity, produced nil responses after 1st or 2nd injection
- Soil** protected from UV by overlying rock produced typical active response

THIS ENTIRE PROFILE WAS NEVER MATCHED ABIOTICALLY

*Run at VL1 site only **Run at VL2 site only

4. FURTHER DEVELOPMENT OF CHIRALITY LIFE DETECTION

The first effort[8] to include chirality in a life detection experiment, as described above, was primarily defensive in that it sought to preclude missing a life form with a chirality different from ours, such preference absent in strictly chemical or other abiotic reactions. However, in 1987, a distinct method specifically designed to detect extant life based on chiral preference was proposed[9]. Since then, the concept and its instrumentation have gone through a number of iterations. The most prominent of these, the "Twin Wireless Experiment for Extraterrestrial Life" (TWEEL)[10], was based on the legacy of the 1976 Viking LR experiment. Named after the mythical Martian bird[11] that landed beak first, the TWEEL was to be launched from the landed spacecraft, and falling to the Martian surface nose-first, would scoop up soil in twin sample chambers through the impact of landing. As seen in Figure 4, TWEEL ampoules containing single isomers of a

test substrate were smashed by the incoming soil, and the substrate and soil mixed thereby to initiate the test. Any gas evolved rose and entered the counting chamber after passing through a gas-permeable barrier, which kept out any radioactive dust and aerosol. The amount of gas evolving was monitored continuously and cumulatively by a beta detector. The TWEEL included a light source and distribution pipe to permit the monitoring of possible photosynthetic organisms. Turning the light on and off for intervals would produce a corresponding response in gas production and absorption were phototrophs present.

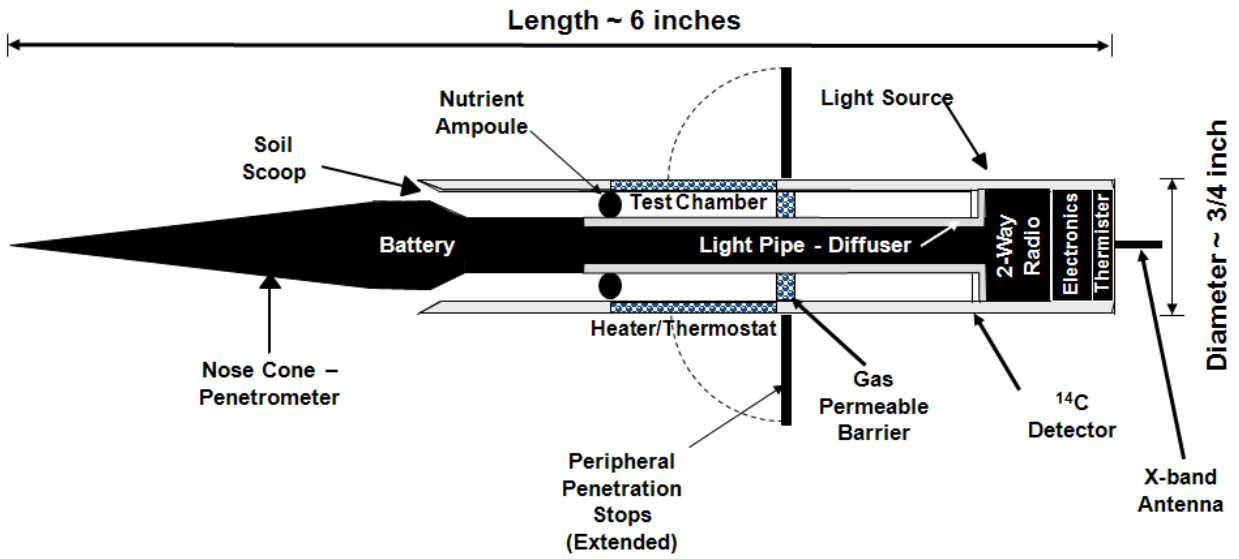


Fig. 4. Concept for the Original TWEEL

Data were to be collected over a period of days, as had been done with the Viking LR. Should something in the soil show a strong preference for one isomer over its enantiomer in the other test chamber, this was to be considered strong evidence for life. Multiple TWEELs were to be deployed by ejection through the lid of a canister of TWEELs that had been heat-sterilized and attached to the lander prior to the mission. A variety of isomeric substrates would be deployed in this manner, with the TWEEL trajectories being into the wind to avoid possible terrestrial microbial contamination from the spacecraft. Each TWEEL was independently powered and operated, with its data radioed back to the lander for relay to Earth. The chiral LR concept has been tested[12] on terrestrial soils using non-radioactive chiral substrates. Differential chiral preference was measured by substrate uptake from the dosed sample. The tests validated the concept. However, it is now realized that this probe, like biomarker probes mentioned above, could report a false negative. In view of our lack of knowledge about the origin of homochirality in life, it seems possible that an alien life might have evolved with an achiral biochemistry. A new approach is now given to overcome this difficulty.

5. THE NEW CONCEPT, "TWEEL 2"

The major nuance of this iteration of the TWEEL design, as seen in Figure 5, lies in the concept of a more rigorous control system.

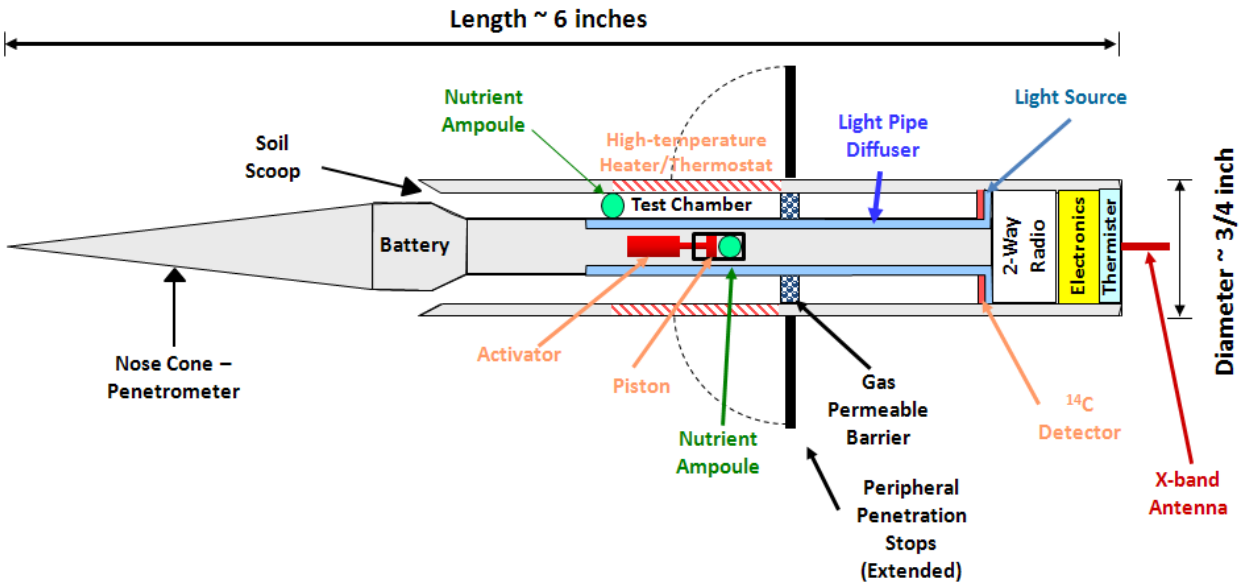


Fig. 5. Currently Proposed TWEEL 2

Multiple controls are proposed to augment the determination of chiral preference. As already discussed, each of the chiral substrates contains its own control in that extant biology would be strongly indicated by a preferential, continuing, metabolic-type response to only one of the mirror-image molecules. To overcome the possibility of being deceived by a non-chiral life form, a series of controls has been added by placing a third ampoule in the TWEEL 2. That ampoule is not broken by the incoming soil, but can be remotely broken at any time during the test run. A piston can then be activated to force the content of the ampoule into a test chamber, and the effect on gas evolution monitored. As seen in Figure 5, only one test chamber contains a ampoule that would be broken by the inrushing soil. The second chamber permits entry of the soil without treatment. That soil can then be heated to provide a Viking-type "sterilized" control. After the heat treatment, the sample is allowed to cool to the LR test temperature, and the remotely controlled ampoule system is activated to inject a desired substrate into the heat-treated soil for monitoring.

This design permits imposing other substances or environmental conditions as important controls for TWEEL 2. These could include imposing moisture, humidity, atmospheric composition and the like on the sample through appropriate contents or environmental control administered by the remotely operated system. Additional controls could consist of adding anti-metabolites to the samples at the initiation of the LR test or at any desired point during it. While these are more Earth-centric, a variety of them could offer the evidence sought. A reaction to only one of them would supply strong additional evidence for life. Toxic metals, cyanide, antibiotics and enzyme inhibitors are candidates, as is the de-coupling agent 2,4-dinitrophenol. A thus-supported chiral preference could indicate whether the life detected were likely related to terrestrial life, or of independent origin. Results from the various tests and controls could begin a study of comparative biology with terrestrial forms.

The deployment of multiple units provides two types of redundancy. Should one unit fail, a duplicate would be available. Alternatively, another of the units with different substrates could obtain the definitive result sought. The units are thus independent, mutually supportive and functionally redundant. Additionally, it is possible to imagine a rover mission deploying TWEEL 2s at multiple locations. Pre-mission studies would establish priorities for the test and control TWEEL 2s. Availability of instrument weight would determine the final package.

TWEEL 2, thus, inverts the normal concept of an experiment. Multiple controls are applied to each test. A positive result combined with more than one nil control would be sufficiently difficult to explain away abiotically so that the results might survive Occam's Razor.

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