A circadian biosignature in the Labeled Release data from Mars?

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ABSTRACT

Organisms on Earth commonly exhibit a circadian rhythm, which is synchronized to the 24-hour day-night (diurnal) cycle of the planet. However, if isolated from strong environmental time cues (e.g., light-dark, temperature, etc.), many organisms revert to a "free-running" rhythm that is close to, but significantly different from, the diurnal cycle. Such a free-running rhythm is a distinct biological feature, as it requires an endogenous pacemaker that is not just passively driven by rhythms in the environment. On Mars, a free-running rhythm (i.e., significantly different from the Martian diurnal cycle of 24.66 hours) would constitute independent proof of the presence of living organisms.

Evidence for such a circadian biosignature from Mars has been sought in the data sent by the 1976 Viking Labeled Release (LR) life detection experiment[1]. In the search for circadian rhythmicity, oscillatory fluctuations in the amount of radiolabeled gas in the headspace of the LR test cell of Viking Lander 2, test cycle 3, were studied. The cycle duration of the LR oscillations examined did not differ significantly from that of the daily cell temperature oscillations controlled ultimately by the Martian diurnal cycle. Thus, these specific LR oscillations produced no independent evidence for an endogenous biological origin. However, it was found that the amplitudes of the oscillations in the gas (presumably CO_2) were greater than could be accounted for by the most likely non-biological mechanism (i.e., temperature-induced changes in soil solubility of CO_2). The possibility thus remained that biological activity, synchronized to the Martian diurnal cycle, could be responsible for at least part of the oscillatory activity in the LR signals.

We now propose to consider all data from the nine active and control cycles of the Martian LR experiment. A comprehensive set of null and alternative hypotheses is proposed for statistical testing using the digitized data. Advanced, statistically rigorous methods of circadian rhythm analysis are laid out to determine whether an endogenous circadian rhythm was present. The data will be analyzed for any free-running rhythm deviating from the Martian diurnal cycle. The possibility that nutrient administration altered the phase (i.e., timing) of the LR oscillations (as has been observed in terrestrial microorganisms) will also be examined. Any indication that the signal may be of biological origin will be tested against the hypothesis that it was caused solely by temperature-induced changes (e.g., temperature-dependent changes in soil physical chemistry). The focus of this paper is to develop broadly accepted methodology to determine definitively whether the LR data exhibit circadian characteristics that imply the involvement of Martian biology.

Keywords: Life on Mars, Labeled Release Experiment, Circadian Rhythms, Harmonic Regression

1. THE LABELED RELEASE EXPERIMENT IN THE VIKING LANDERS ON MARS

In 1976, a variety of life detection experiments was performed on board the Viking Landers on Mars. One of these experiments, the Mars Labeled Release experiment[2], added 0.115 ml of a dilute aqueous solution of seven radioactive organic nutrients, uniformly labeled with C^{14}, to moisten a 0.5 cc sample of Martian soil placed in a test cell inside each of the Landers. The low nutrient concentrations, made possible by the sensitivity of the radioisotope method, were selected to preclude any toxic effects. No other ingredients were added in the presumption that any living organisms must already be obtaining such resources from their habitat. The nutrients were selected from among Miller-Urey compounds that were shown to be metabolized by the broadest possible array of microorganisms

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in tests of pure cultures, mixed cultures, and soils from normal and extreme environments; and by conducting *in situ* field tests in both types of environments. For chiral compounds, both isomers were included, in case the chiralty preference of Martian life differed from that of Earth life. It was proposed that, just as literature searches and direct experiments had shown for terrestrial microorganisms, any Martian microorganisms metabolizing one or more of the labeled nutrients would evolve radioactive gas.

Two solid state beta detectors, separated from the test cell by a 13 inch long, 1/8 inch diameter "swan neck" tube, were designed to detect any carbon-based gas evolved (CO_2, CO, CH_4, etc.), while the tube precluded carryover of radioactive aerosol or dust from the test cell. To determine whether a positive response was biological, a control was conducted in which another sample of the same soil was heated to a temperature designed to kill any microorganisms present without destroying chemicals thought capable of producing the result. After cooling, the soil received an identical dose of nutrient as had the test sample. If no response were obtained from this control, this confirmed that microorganisms had caused the initial response, and the criteria for life were deemed satisfied. Validated in laboratory and *in situ* field tests, the Labeled Release experiment was extremely rapid, sensitive, and accurate in detecting living terrestrial microorganisms. Laboratory and field tests also demonstrated that the control system was capable of culling out false positives.

On Mars, a total of nine experiments was conducted. In active tests, strong positive responses were found at both Viking landing sites. The heated control samples eliminated or significantly reduced the magnitude of the positive responses. All results, tests and controls, were consistent with biological activity. The results for two test experiments and a control experiment conducted at Viking Lander site 1 are shown in Figure 1. This figure shows rapid evolution of gas following the first nutrient injection (cycles 1 and 3). The control for this experiment (cycle 2) produced virtually nil response, thereby satisfying the pre-mission criteria for the detection of living microorganisms.

The rapid initial evolution of gas in the positive tests, beginning immediately upon moistening the soil with nutrient, appeared to slow after 3 to 4 Martian sols (i.e., Martian days). This is typical of responses seen in laboratory and field tests, as illustrated in Figure 2, and could represent near-stationary metabolism rather than growth[3]. The Labeled Release experiment did not explicitly depend on growth—which was one of its main attributes, considering that on Earth, less than 1% of soil microorganisms can be grown in culture[4].

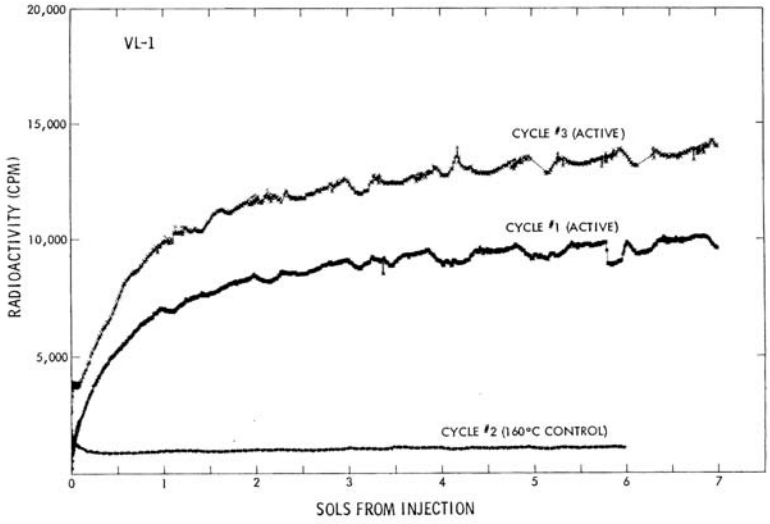

Figure 1: Comparison of radioactivity evolved as a function of time (in Martian days or "sols") after the first injection of radioactive nutrient to each of three analysis cycles of Viking Lander 1. A fresh surface sample of Martian soil was used for the active sequences of cycles 1 and 3. For cycle 2, a stored portion of the same sample used for cycle 1 was heated for 3 hours at 160 ºC before nutrient injection. Background radioactivity observed prior to nutrient injection was subtracted from all data. Figure taken from Levin and Straat (1977)[3].

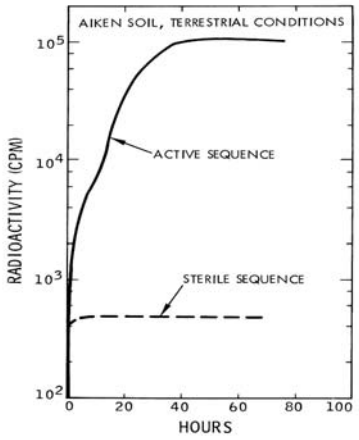

Figure 2: Radioactivity evolved from a terrestrial soil in the Test Standard Module (TSM, a flight-like Labeled Release instrument). Nutrient was added to a 0.5 cc sample of Aiken soil under terrestrial atmospheric conditions at room temperature according to a flight sequence. Radioactivity evolved after nutrient injection to either an active test sample (solid curve) or control sample (dashed curve) is shown as a function of time. The control soil was heated in the TSM test cell for 3 hours at 160 ºC before nutrient injection. Background radioactivity was subtracted from all data. Figure taken from Levin and Straat (1976)[5].

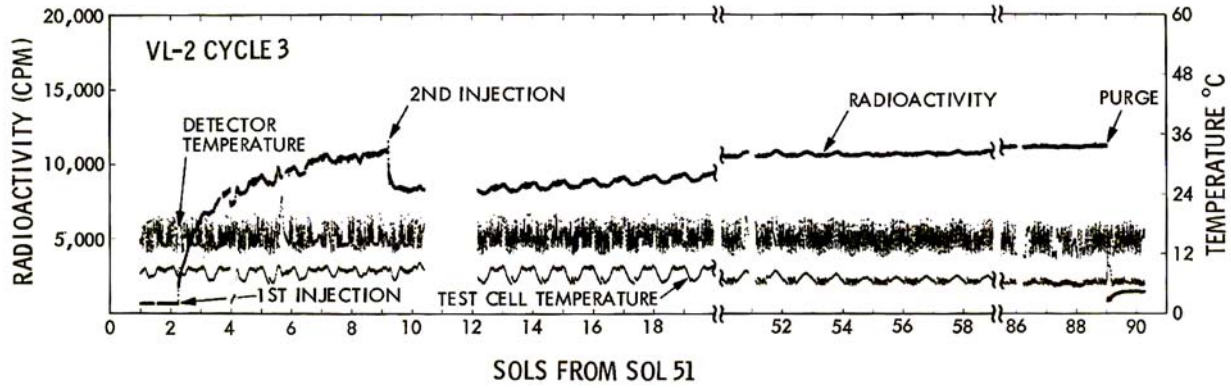

Figure 3: Radioactive gas evolved as a function of time (in Martian days or "sols") after nutrient injection to the third Martian soil sample (i.e., cycle 3) on Viking Lander 2. Test cell temperature and detector temperature are also shown. Radioactivity data include a background count of 659 cpm prior to the onset of the cycle. Note the small but consistent oscillations in radioactivity, starting about sol 12, which are similar to those seen in the test cell temperature and have a periodicity of approximately one Martian sol. Figure taken from Levin and Straat (1977)[3].

Figure 3 shows the Labeled Release results from Viking Lander 2, test cycle 3, and includes the head-end temperature in the test cell (HT) and the temperature in the detector (DT) in addition to the amount of gas evolved (LR). This active cycle was essentially the same as the active cycle seen in Figure 1 for Viking Lander 1, except that it was monitored for a longer period of time. Figure 3 also shows the LR results following a second injection of nutrient. The unexpected decrease in the amount of gas in the detector cell following second nutrient injection was attributed to re-absorption of the gas, thought to be primarily CO_2, by the soil when moistened by the second injection[6]. Less than one sol after the second injection, the gas began to evolve again, eventually reaching its original maximum, but arriving there at a much slower rate.

According to the pre-mission criteria for the detection of living organisms, the LR data provided convincing support for the presence of microbial life on Mars (Figure 1). However, while the differences between the active and control cycles were highly significant, the interpretation of the results has been controversial. Although Levin[7] and

subsequently Straat, concluded that the LR results demonstrated biology, others have suggested that a heat-sensitive non-biological oxidant could explain the Mars LR data[8]. Recent studies[9,10] cast doubt on the presence of a strong oxidant on the surface of Mars, though. Only recently has it been possible to produce a superoxide in the laboratory[11], and that superoxide was immediately destroyed in the presence of water. Yet, the LR evolution occurred for many Martian sols, in spite of the presence of the aqueous nutrient solution that had been added to the samples. Moreover, evidence is accumulating for the current presence of liquid water on Mars[12]. Other recent findings, such as the detection of considerable amounts of methane in the Martian atmosphere[13], also make it increasingly likely that life may exist on present-day Mars[14]. Even so, to achieve general acceptance of the life hypothesis by the scientific community, independent confirmation is still necessary.

Establishing evidence of endogenous circadian rhythmicity in the LR data would offer such confirmation, one likely to be accepted by all scientists familiar with this *sine qua non* biomarker. Therefore, the oscillations in the LR data (see Figures 1 and 3) are currently under investigation for evidence of endogenous circadian rhythmicity. Data segments selected for analysis are those where the evolution of gas seemed continuous. These segments range from the emergence of periodicity following the first injection to the point of the second injection; and from the re-emergence of periodicity after the diminution of gas following the second injection until the moment of purge.

2. CHARACTERISTICS OF CIRCADIAN RHYTHMS

Circadian rhythms are oscillations with a period of approximately one day (i.e., about 24 hours on Earth), which are found in virtually every physiologic and metabolic measure in living organisms[15]. Under normal circumstances, circadian rhythms are synchronized (entrained) to "zeitgebers" (i.e., environmental entraining stimuli) such as the light/dark cycle. However, circadian rhythms persist in the absence of zeitgebers, as they are driven by endogenous oscillatory mechanisms. When circadian rhythms are not entrained by zeitgebers, they typically show a free-running pattern with a period close to, but significantly different from, the diurnal cycle. Endogenous free-running circadian rhythms are a ubiquitous biosignature on our planet.

It is generally believed that the ability to detect and anticipate the light and dark portions of the diurnal cycle constitutes an evolutionary advantage[16]. For example, diurnal species may effectively forage during the day and avoid nocturnal predators by sleeping in a sheltered location at night. Endogenous circadian rhythmicity allows life on Earth to stay entrained to the cycles of the planet's orientation relative to the Sun, even in situations when environmental cues may be obscured (rainstorms or sandstorms, underground habitats, etc.). Thus, there may have been considerable selection pressure in evolutionary history to evolve an endogenous timekeeping mechanism (i.e., a circadian clock) as an alternative to direct sensing of light/dark alternation. Indeed, circadian clocks have been found consistently in organisms ranging from cyanobacteria to primates.

If life exists on other planets, it would be reasonable to expect similar evolutionary selection with regard to the rotational period of the planet in question. In the case of Mars, this period is similar to that of Earth; relative to the Sun, the rotational period of Mars is 24.66 hours. Any free-running circadian rhythms on Mars would be expected to have a period close to, but significantly different from, 24.66 hours. The detection of free-running rhythmicity on Mars would constitute evidence of the presence of endogenous circadian rhythmicity, and therefore life, on our closest planetary neighbor.

Ecologically meaningful stimuli exhibit the ability to phase-shift terrestrial circadian rhythms. While for most organisms light appears to be the strongest such stimulus, for non-homoeothermic organisms (ranging from cyanobacteria to reptiles) ambient temperature is a potent zeitgeber, too[17,18,19]. Other entraining stimuli, including food availability[20], have been documented to phase-shift circadian rhythms as well. It is not clear whether nutritive availability can be a zeitgeber in microbes, but pulses of nitrogen or nicotinamide adenine dinucleotide (NAD) have shown phase-shifting and period-altering effects[21], and in some circumstances the carbon source in the nutrient medium (acetate or maltose) has been found to determine whether circadian rhythmicity occurs[22]. Because the entrainment of circadian rhythms depends on the ability of zeitgebars to induce phase-shifting, a shift in circadian rhythmicity in response to a potential zeitgebar (such as a nutrient) would, just like a free-running circadian rhythm, constitute a biosignature for life on Mars.

3. RESEARCH HYPOTHESES FOR THE DETECTION OF LIFE ON MARS

Based on the design of the LR test cycles and the characteristics of endogenous circadian rhythmicity on Earth, we posit the following hypotheses concerning the detection of life on Mars:

3.1 Research hypothesis 1, "free-running circadian rhythmicity"

The presence of a circadian rhythm in the LR signal with a period deviating systematically from the circadian rhythm in the environment is evidence for life on Mars.

Thus, if at least one LR segment has a circadian period significantly different from the Martian sol (i.e., 24.66 hours), then this implies free-running circadian rhythmicity—which constitutes independent evidence for life on Mars.

Subsumed in this hypothesis is that circadian rhythmicity must be present in the LR signal (i.e., the circadian amplitude must be significantly greater than zero). Furthermore, the circadian period of the LR signal must be significantly different from the circadian period in the corresponding HT data in order for the LR rhythm to be considered truly free-running.

3.2 Research hypothesis 2, "stimulus-induced circadian phase shift"

The presence of an abrupt phase shift of the circadian rhythm in the LR signal, in response to external stimulation by nutrient administration, is evidence for life on Mars.

Thus, if at least one pair of consecutive LR segments, one recorded before the second nutrient injection and one after, exhibits a significant difference in circadian phase, then this demonstration of a phase shift induced by the nutrient administration stimulus would constitute independent evidence for life on Mars.

Subsumed in this hypothesis is that circadian rhythmicity must be present in the LR signal of both segments (i.e., the circadian amplitudes must be significantly greater than zero). Furthermore, the circadian period of the two segments must be the same, so that circadian phase is measured on the same scale. (Hypothesis 2 will effectively only be tested if hypothesis 1 failed to yield evidence for life on Mars.) Finally, the circadian phase shift in the LR data must not be caused by a coincidental phase shift in the environmental temperature cycle. Thus, there must also be a significant difference between the two consecutive segments in the circadian phase angle of the LR signal relative to the HT signal.

4. STATISTICAL HYPOTHESES FOR THE DETECTION OF LIFE ON MARS

The three research hypotheses posited above can be translated into a number of statistical hypotheses, to be tested with the LR and HT data at hand. These statistical (i.e., null and alternative) hypotheses can be formulated as follows:

4.1 Statistical hypothesis A

H_o: the amplitude of the circadian rhythm in a given LR segment does not differ from zero;
H_a: the amplitude of the circadian rhythm in the LR segment is greater than zero.

This hypothesis is an integral component of both research hypotheses posited above.

4.2 Statistical hypothesis B

H_o: the period of the circadian rhythm in a given LR segment is the same as the Martian sol (i.e., 24.66 hours);
H_a: the period of the circadian rhythm in the LR segment is not the same as the Martian sol.

This is a key statistical hypothesis underlying research hypothesis 1.

4.3 Statistical hypothesis C

H_o: the period of the circadian rhythm in a given LR segment is the same as the period of the circadian rhythm in the corresponding HT segment;

H_a: the period of the circadian rhythm in the LR segment is not the same as the period of the circadian rhythm in the corresponding HT segment.

This statistical hypothesis deals with a necessary check for research hypothesis 1.

4.4 Statistical hypothesis D

H_o: the phase of the circadian rhythm does not differ between two consecutive LR segments;

H_a: the phase of the circadian rhythm differs between the two LR segments;

This is a key statistical hypothesis underlying research hypothesis 2.

4.5 Statistical hypothesis E

H_o: the period of the circadian rhythm does not differ between two given LR segments;

H_a: the period of the circadian rhythm differs between the two LR segments;

This statistical hypothesis deals with a necessary check for research hypothesis 2.

4.6 Statistical hypothesis F

H_o: the phase difference between the circadian rhythm in a given LR segment and the circadian rhythm in the corresponding HT segment is the same as the phase difference between the circadian rhythm in another LR segment and the circadian rhythm in the corresponding HT segment;

H_a: the phase difference between the circadian rhythm in the LR segment and the circadian rhythm in the corresponding HT segment is not the same as the phase difference between the circadian rhythm in the other LR segment and the circadian rhythm in the corresponding HT segment.

This statistical hypothesis deals with another necessary check, concerning the circadian phase angle of the LR signal relative to the HT signal, for research hypothesis 2.

5. ANALYTIC PLAN FOR THE DETECTION OF LIFE ON MARS

The statistical hypotheses posited above can all be tested within the framework of harmonic regression analysis, using the digitized data of the Viking Lander test cycles. Figure 4 illustrates the calibrated LR data from active test cycle 1 of Viking Lander 2. Two selected LR segments, one before nutrient administration and one after, are marked by dashed brackets.

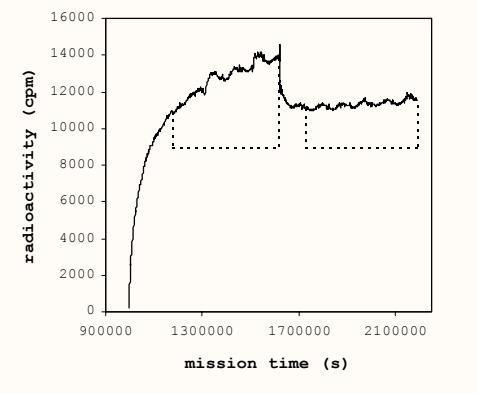

Figure 4: LR data from Viking Lander 2, test cycle 1, with two segments indicated by dashed brackets. The sharp transition in LR in the middle marks the second administration of radiolabeled nutrients.

Specific numerical analyses will be performed on the data in the LR segments and the corresponding HT segments to test each of the statistical hypotheses. First, the LR data of each segment separately will be subjected to harmonic regression analysis, as follows:

$$\mathrm{LR} = c + s\,(t-q)^{\theta} + a\cos\{2\pi\,(t - b - \delta\,t)\,/\,T\} + \varepsilon,$$

where t stands for time; T is the Martian sol of 24.66 hours; q is a constant; ε represents independent, identically distributed Gaussian noise with zero mean; and a, b, c, s, δ and θ are regression parameters. The parameter c is the intercept in the model. The term $s\,(t-q)^{\theta}$ models the overall trend (growth curve) in the LR data, using an approach published previously[23,24]. The parameters a and b represent the amplitude and phase, respectively, of the circadian rhythm superimposed on the growth curve. The parameter δ captures the deviation of the period of the circadian rhythm from the Martian sol, such that the period is $\tau = T\,/\,(1-\delta)$.

This analysis will allow the testing of statistical hypotheses A and B, by transcribing them as follows:

Statistical Hypothesis A:
H_o: $a = 0$;
H_a: $a > 0$.

Statistical Hypothesis B:
H_o: $\delta = 0$;
H_a: $\delta \neq 0$.

The testing of these two hypotheses will be performed with the likelihood ratio test for nested models, after repeating the regression analysis with the relevant parameter (a or δ, respectively) fixed at zero (reduced model). A χ^2 statistic is then computed as follows:

$$\chi^2[\mathrm{df}] = (-2\ln L_r) - (-2\ln L_f),$$

where L_f and L_r are the maximized likelihoods for the full (i.e., original) and reduced (i.e., nested) models, respectively, and df is the difference in the number of parameters between the full and reduced models (3 for testing a, and 1 for testing δ). If the magnitude of the χ^2 statistic indicates statistical significance at a type I error threshold of $\alpha = 0.05$, then the full model will be considered a significant improvement over the reduced model, and the null hypothesis will be rejected.

As appropriate with regard to the outcomes of the previous tests, the combined LR and HT data of each segment separately will be subjected to harmonic regression analysis, as follows:

$$y_k = c_k + k\,s\,(t-q)^{\theta} + a_k\cos\{2\pi\,(t - b_k - [\delta + k\,\Delta\delta]\,t)\,/\,T\} + \varepsilon_k,$$

where k equals 0 or 1, and y_k stands for the LR data if $k = 1$ and for the HT data if $k = 0$. Note that the overall trend (growth curve) should not be necessary to model the HT data, and thus cancels out for $k = 0$. The parameter c_k is the intercept for LR and HT depending on k. The parameters a_k and b_k represent the circadian amplitude and phase, respectively, for LR and HT depending on k. The parameter $\Delta\delta$ represents the difference between the LR and HT data in the deviation of the circadian period from the Martian sol. Furthermore, ε_k is independent, identically distributed Gaussian noise with zero mean and variance σ_k depending on k.

This analysis will allow the testing of statistical hypothesis C, by transcribing it as follows:

Statistical Hypothesis C:
H_o: $\Delta\delta = 0$;
H_a: $\Delta\delta \neq 0$.

The testing of this hypothesis will be performed with the likelihood ratio test for nested models, after repeating the regression analysis with $\Delta\delta$ fixed at zero (reduced model), equivalent to the procedure described above (1 degree of freedom).

As appropriate with regard to the outcomes of the previous tests, the combined LR data of two consecutive segments will be subjected to harmonic regression analysis, as follows:

$$\mathrm{LR}_j = c_j + s_j\,(t - q_j)^{\theta j} + [a + j\,\Delta a]\cos\{2\,\pi\,(t - [b + j\,\Delta b] - [\delta + j\,\Delta\delta]\,t)\,/\,T\} + \varepsilon_j,$$

where j equals 0 or 1, and LR_j stands for the LR data of the first segment if $j = 1$ and the second segment if $j = 0$. The parameters c_j, s_j and θ_j and the constant q_j are defined as above but specific to segment j. The parameters Δa, Δb and $\Delta\delta$ represents the difference in a, b and δ, respectively, between the two segments. Furthermore, ε_j is independent, identically distributed Gaussian noise with zero mean and variance σ_j depending on j.

This analysis will allow the testing of statistical hypotheses D and E, by transcribing them as follows:

Statistical Hypothesis D:
H_o: $\Delta b = 0$;
H_a: $\Delta b \neq 0$.

Statistical Hypothesis E:
H_o: $\Delta\delta = 0$;
H_a: $\Delta\delta \neq 0$.

The testing of hypotheses D and E will be performed with the likelihood ratio test for nested models, after repeating the regression analysis with the relevant parameter (Δb, $\Delta\delta$ or Δa, respectively) fixed at zero (1 degree of freedom for each test).

Finally, as appropriate with regard to the outcomes of the previous tests, the combined LR and HT data of two consecutive segments will be subjected to harmonic regression analysis, as follows:

$$y_{kj} = c_{kj} + k\,s_j\,(t - q_j)^{\theta j} + a_{kj}\cos\{2\,\pi\,(t - [b_j + k\,\{\Delta b + j\,\beta\}] - \delta_k\,t)\,/\,T\} + \varepsilon_{kj},$$

where k equals 0 or 1, and y_{kj} stands for the LR data if $k = 1$ and for the HT data if $k = 0$; and where j equals 0 or 1, and y_{kj} stands for the data of the first segment if $j = 1$ and the second segment if $j = 0$. Note again that the overall trend (growth curve) should not be necessary to model the HT data, and thus cancels out for $k = 0$. The parameters s_j, θ_j and b_j and the constant q_j are defined as above but specific to segment j. The parameter δ_k is defined as above for LR and HT depending on k. Note that there is no need here to allow δ_k to vary by segment j, as this particular regression analysis will not be performed if the circadian period for LR is not stable across the two consecutive segments. The parameters c_{kj} and a_{kj} are defined as above for LR and HT depending on k, specific to segment j. The parameter Δb represents the difference in circadian phase between the LR and HT data, which is referred to as the circadian phase angle. The parameter β, which is the one of primary interest here, represents the difference in the circadian phase angle between the two segments. Furthermore, ε_{kj} is independent, identically distributed Gaussian noise with zero mean and variance σ_{kj} depending on k and j.

This analysis will allow the testing of statistical hypothesis F, by transcribing it as follows:

Statistical Hypothesis F:
H_o: $\beta = 0$;
H_a: $\beta \neq 0$.

The testing of this hypothesis will be performed with the likelihood ratio test for nested models, after repeating the regression analysis with β fixed at zero (1 degree of freedom).

If any of the null hypotheses is rejected, an additional check will be necessary. The rhythmicity in the data may not be purely sinusoidal, and may involve higher harmonics. In addition, there may be serial correlation in the data, which is not accounted for in models assuming independent noise. These model misspecifications may lead to incorrect estimates of type I error. This will be checked using a procedure set forth in the circadian literature[25], involving the fitting of harmonic regression models with correlated noise implemented as an autoregressive moving average (ARMA) process, and estimating the number of harmonics d and the orders p and q of the ARMA process on the basis of Akaike's Information Criterion (AIC). We will apply this procedure to the reduced model corresponding to the rejected null hypothesis (with the parameters of any harmonics being restricted commensurately), verify goodness-of-fit graphically, and ascertain statistically that the residual noise is now sufficiently independent and Gaussian.

After estimating the variance of the residual noise, we will make use of the method of surrogate data[26] to create 1,000 new time series by means of Monte Carlo simulation based on the reduced model with any additional harmonics and the ARMA process. We will analyze these simulated time series using the original full model (and the corresponding reduced model), and assess the proportion of false rejections of the null hypothesis—which in this simulated case is known to be true. If the proportion of false rejections remains below 5%, the type I error threshold of $\alpha = 0.05$ used in the original evaluation of the full model was not inflated, and it will be concluded that the rejection of the null hypothesis was statistically justified.

All statistical analyses above assume that under the null hypothesis, there is weak stationarity (after controlling for the growth curve in LR). This assumption, which puts necessary constraints on the nature of the error variance, is challenged by the observation that the circadian rhythm in LR was not present from the outset, but gradually developed while overall LR levels were rising after the first nutrient administration (cf. Figures 1, 3 and 4). For this reason, LR segments selected for analysis capture only intervals with continuous profiles, as determined by visual inspection. These segments range from the emergence of periodicity following the first injection to the point of the second injection; and from the re-emergence of periodicity after the diminution of gas following the second injection to the moment of purge. The available data are free from artifacts, defined as data points exceeding the moving average over the previous and subsequent 10 data points by 4 standard deviations or more.

The numerical analyses will be performed in SAS (SAS Institute Inc.; Cary, NC) using the NLMIXED procedure (without using the random effects feature).

CONCLUSION

The 1976 Labeled Release life detection experiment on Mars provided support for the presence of microbial life on Mars. The interpretation of the results of the experiment has been controversial, but in the meantime other evidence has surfaced suggesting that life on present-day Mars would be possible. To achieve general acceptance of the life hypothesis by the scientific community, independent confirmation is now a research priority. Establishing evidence of endogenous circadian rhythmicity in the data from the LR experiment could offer such independent confirmation.

This paper explicated the methods we propose to determine whether an endogenous circadian rhythm was present in the LR data from the Viking Landers. A comprehensive set of null and alternative hypotheses for statistical testing was advanced. In addition, state-of-the-art statistical methods for circadian rhythm analysis were presented. We believe this methodology to be the most rigorous possible. If either of the stated hypotheses is supported by the proposed analyses, a biosignature will have been established confirming and independently proving the existence of present-day microbial life on Mars. Before engaging in the actual data analyses, we hereby solicit feedback regarding the methodology currently proposed.

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