## The Likelihood of Methane-producing Microbes on Mars

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The discovery of extraterrestrial life could impact biology in a manner not seen since the discovery of the double helix structure of DNA. And yet to date only one life detection mission has ever traveled to another planet, the Viking mission of 1976. One of the Viking experiments, the Labeled Release (LR) experiment, has been a source of controversy for over 30 years. In the LR experiments a small amount of a <sup>14</sup>C-labeled nutrient solution was added to a Martian soil sample and the evolution of <sup>14</sup>C-labeled gas was monitored via a beta detector. The rapid evolution of gas and the failure to see such evolution in sterilized soil was considered presumptive evidence for Martian microbiology,<sup>1</sup> although alternative explanations have dominated the interpretation of these data until recently. Our more recent analyses have suggested that the presence of circadian rhythmicity, a reliable biosignature, in the temporal structure of the gas release data further support the idea that the gas was of biological origin.<sup>2</sup>

The discovery of methane in the Martian atmosphere<sup>3</sup> and its steady replacement from equatorial sites that seem to overlap substantial sub-surface ice deposits<sup>4</sup> suggests a possible biological origin, methanogenic microbes. An apparent absence of Martian volcanic activity precludes one explanation for the methane. These findings suggest the possibility that methane, in addition to CO2, was evolved in the LR experiment (Levin and Straat).<sup>5</sup> Here we consider how much of the LR gas could have been methane and whether extrapolation of the amount released per gram of Mars soil could produce the measured levels of methane in the atmosphere. Finally, assuming terrestrial rates of methane production by methanogens we estimate the necessary size of the microbial population and the amount of water necessary to maintain that population in an aqueous state.

For a first approximation we will largely ignore temperature, pH and atmospheric pressure differences, and any correction for doubling rates of the microbes. Terrestrial rates of methane production by methanogens could easily account for observed methane in the Martian atmosphere. For instance, Takai et al.<sup>6</sup> have studied a hyperthermophilic methanogen at 85° C and 0.4 mMPa pressure (one ml of medium). First doubling occurred at 6 hr with 4 X 10exp6 cells and 37  $\mu$ mols/ml of methane released. Similarly, Kral has produced results in terrestrial experiments using a wetted Mars soil stimulant such that methane release was about 7  $\mu$ mols/ml with methane doubling times of 1-5 days. The estimates of methanogen density and methane production varied from 10exp4 to 4.3 X 10exp5 microbes/ml depending on the particular microbial species and the amount of water mixed with the Mars soil stimulant.<sup>7</sup> These estimates are similar to various reports of terrestrial methanogen soil densities of about 10exp5 microbes/gm.

In the Viking LR experiment (e.g. VL2C3, see Figure 1) evolved gas increased slowly over the first two weeks of the experiment resulting in about 30 nmols of a radiolabeled carbon-containing gas. However, about 1/3 of the gas was resorbed following second administration of an aqueous nutrient (.115 ml) to a 0.5 cc soil sample. Methane is not soluble at the temperatures and pressure of the LR experiment, but  $CO_2$  is. If the non-resorbed fraction were entirely methane, and in terms of methane/ml of added nutrient (for comparability to above), that would be about 174 nmols/ml. This is about 40 X lower than the maximum Kral result and thus might scale down to a population of 10exp4 microbes/ml, still well within the range of terrestrial methanogen soil densities.



Figure 1. First injection of aqueous nutrient causes a large elevation of radiolabeled carbon-containing gas (LR). Second injection results in mean absorption of about 1/3 of the total. Note oscillations persisting through 4000 intervals or about 40 sols.

The total amount of methane in the Martian atmosphere is 2.5 X 10exp8 kg. Without a renewal source that much methane would be broken down by UV photolysis in about 300 yrs. However, more recent estimates suggest that the actual methane lifetime is about 600 times shorter than that required by a photochemical process,<sup>8</sup> suggesting a global methane production rate of 10exp18 nmols CH4/2 wks. Extrapolating the Viking LR numbers suggests 5 X 10exp15 ml of a probably briny high osmolality medium would be needed to support an active global methanogen population of about 10exp20 microbes in 2.5 X 10exp15 gm of Martian soil. That much mass could be provided by a 10 meter deep shell around the planet. (Kral's data suggest water is rate-limiting for methane production which may have also been true of the Viking LR experiment. If so, there could be even larger numbers of inactive (sporiform?) methanogens in dry Martian soil).

The water content of Mars is now estimated at about 1/1000 of earth's or 4.4 X 10exp6 cubic km or 4.4 x 10exp21 mls. The aqueous requirement for a putative slow growing methanogen with the growth kinetics inferred from the Viking experiments would only require that about a millionth part of Mars water be in a liquid, presumably high osmolality, form. Liquid water can exist in microdomains in ice on earth and possibly on Mars. Alternatively, at a sufficient depth below the Mars ice layer temperatures may be high enough to allow water, particularly a brine, in liquid state. Prospects would be further improved if growth/cell division kinetics were assumed to follow those of Kral or Takai.

The remarkable result is that a population of methanogens existing at the surface to no more than 10 meters below the surface on average, and in a low concentration of Mars water similar to the Viking LR experiments, could easily replenish the methane content of the Martian atmosphere on the time scale required. These estimates are global averages by necessity-if methanogens exist they may be concentrated in geologically restricted patches or oases. In any event, the simulations and extrapolations discussed here will be improved by more realistic estimates of Mars soil pH, water content, temperature, atmospheric pressure and constituents. But considering the available data it is our belief that, after an interregnum of some 35 years, it is time for NASA to again fly an explicit life detection mission to Mars.

## References

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