Detecting Life on Mars: Reanalysis of the Viking Life Detection Experiments and the Role of H_2O_2 as a Possible Biological Agent.

J. M. Houtkooper[1] and D. Schulze-Makuch[2], [1]Center for Psychobiology and Behavioral Medicine, Justus-Liebig-University of Giessen, D-35394 Germany, joophoutkooper@gmail.com, [2]School of Earth and Environmental Sciences, Washington State University, Pullman, WA 99164, USA, dirksm@wsu.edu.

Introduction: The Viking Landers conducted the first ever life detection experiments on another planet. Although many hypotheses have been advanced, no particular hypothesis can explain all the observations from the conducted experiments. The main problem of a chemical explanation is that the putative strong oxidant has not been identified to date, while a biological explanation has the drawback that there are no Earth analog organisms which display the observed responses. Here, we examine a differing hypothesis based on the proposed existence of Martian organisms well adapted to current surface conditions, which use a H_2O_2-H_2O mixture as intracellular liquid [1,2]. This has critical advantages for these organisms such as a very low freezing point, a suitable metabolic pathway releasing oxygen, and the ability to assimilate water from the thin martian atmosphere due to hydrogen peroxide's hygroscopic properties. Contrary to common belief, H_2O_2 is used for a variety of purposes in terrestrial biochemistry, and would provide an intriguing and remarkably effective solution to the many remaining open questions about the Viking Lander biology experiments.

Hydrogen peroxide as a biological agent on Mars: Although H_2O_2 is well-known for its properties as a desinfectant, it has a variety of roles in biochemical processes in living organisms. These processes are for instance immune responses, metabolism (in *Acetobacter peroxidans* [3]), and quite spectacularly by the bombardier beetle, an insect which produces a 25% H_2O_2 solution to defend against predators [4]. The cell organelles involved in the H_2O_2-centered oxidative processes, the peroxisomes, are found in the great majority of eukaryotic cells [5].

If life would have gained a foothold on Mars during an early period with a more benign climate, it is not unlikely that it would have adapted to the present cold and dry conditions. The adaptation considered here is the replacement of the water in the intracellular fluid by a H_2O_2-H_2O mixture. The properties of this mixture are a low freezing point (-56.5^{o}C for the eutectic), a source of oxygen, which is a rare component of the atmosphere, and the mixture is hygroscopic, which means that it enables scavenging water vapor from the atmosphere in conditions significantly below saturation. Moreover, the H_2O_2-H_2O mixture will easily supercool at temperatures far below its freezing point [6]. Therefore, such organisms might well be able to survive under Martian conditions, including nighttime temperatures, and be able to thrive under conditions where liquid water would not occur. Therefore, the subpolar regions, with their relatively high concentration of water vapor in the atmosphere and relatively less harsh UV radiation, are a candidate for finding such organisms (see companion LPSC 2007 abstract by D. Schulze-Makuch and J.M. Houtkooper). The use of H_2O_2 in the intracellular fluid is not without some drawbacks: Although the reactivity of H_2O_2 is lower at lower temperatures, the organic components of the cell need to adapt to its presence, e.g. with an active stabilization mechanism. Another disadvantage of the H_2O_2-H_2O stratagem is the sensitivity for high concentrations of H_2O: these may lead to hyperhydrative stress and ultimately death. These factors are relevant for a new interpretation of the Viking experiments considering the "H_2O_2-H_2O hypothesis" [2].

The Viking Lander biology experiments: The responses of all three experiments, the Pyrolytic Release (PR), the Gas Exchange (GEx) and the Labeled Release (LR) experiments, were generally considered to be incompatible with biology, since 1) The Gas Chromatograph-Mass Spectrometer (GCMS) did not detect organic compounds in Martian soil samples through thermal volatilization; 2) The large production of O_2 after moisturizing a soil sample in the GEx experiment was unlike what is observed from any terrestrial soil or biota. The proposed explanation was the presence of an oxidant in the soil, such as a peroxide or superoxide.

However, the inorganic explanation was never completely satisfactory either, because: 1) The sensitivity of the GCMS has been drawn into doubt [7]; 2) The hypothetical Martian oxidant was never identified in simulation studies (being the methodological advantage over a biological explanation); 3) The "cold sterilization" control tests (at $<100^o$C) constricted the possibilities of an inorganic agent even further; 4) The long storage of samples at 10-20^{o}C led to diminished activity which is unlike what would be expected of an inorganic agent; and 5) inconsistencies remain between the inorganic explanations for the different experiments, such as between the hypothetical oxidant for the GEx and the synthesis of refractory organic matter in the PR.

Comprehensive Analysis of the Viking experiments: Here we focus on the relative magnitudes of the responses in the different tests. Therefore we normalized each of the three experiments relative to the maximum response achieved in any test in the two landers. The responses of the PR and LR experiments are the amount of radioactivity of the gas in the test cell; for the LR we took the maximum radioactivity before the second injection of nutrient; for the GEx experiment we took the maximum amount of evolved CO_2, since the O_2 response is less reliable as it reacts with the ascorbate in the nutrient solution. This normalization enabled us to do an overall analysis of the responses of all 23 tests (not including the wet tests after a moist test) in relation to experimental conditions such as the duration and level of preheating/storage and the addition of moisture. The response in "active" tests (i.e. with fresh soil) can be compared with different control conditions. The differences of the mean responses between categories of tests were very significant for the active tests (59%) versus the controls (20%), the hot (16%) and the cold (25%) controls, and the long-stored samples (15%). Surprisingly, the three PR-Utopia tests (8%) differed much from the 10 other active tests (74%), suggesting that this PR instrument failed before its fourth test [8]. Detailed results of our compararative analysis are given in [9].

Interpretation of the Viking experiments: The cold control tests and the long storage tests constrain the inorganic chemistry explanation of the Viking observations significantly. In fact, numerous hypotheses about the Martian oxidant have been put forward, but none of these has been shown to account for all the data. The two common arguments usually put forward against a biological explanation, the evolving of O_2 upon moisturizing the soil samples and the lack of detected organics by the GCMS can both be explained by the H_2O_2-H_2O hypothesis [2]. In fact, for the chemical viewpoint H_2O_2 has been put forward as an oxidant, purportedly created in the atmosphere from water vapor and precipitating onto the surface. However the stability of H_2O_2 in the ubiquitous UV flux makes this assertion untenable.

Life based on an H_2O_2-H_2O mixture as its intracellular solvent: As this is a biochemically tenable and evolutionary advantageous stratagem, it must be considered as an explanation of the Viking results. The GCMS result occurs because the samples were heated gradually. The organisms went through heat stress and metabolized their resources and then decomposed. The internal supply of oxidant could be more than sufficient to oxidize all organics to CO_2, N_2 and H_2O. The production of O_2 (and CO_2 and N_2) upon moisturizing the samples in the GEx is explained by the hygroscopicity of concentrated H_2O_2 solutions. In the Martian environment defense by native organisms against too much water is not to be expected. The experimental condition of 100% moisture at the relatively high temperature of 10°C is unmartian. Hyperhydration followed by death of the organisms resulted in O_2, CO_2 and N_2 as metabolites and/or decomposition products. This also solves the puzzle of CO_2 evolving upon moisturizing in the GEx, but being absorbed upon wetting in the LR upon second injection of nutrient at which time the organisms were no longer active. The other Viking findings are compatible with biological activity, although in a number of cases it has been shortlived. The PR experiment had the most natural conditions, but it involved a single detection of assimilated carbon, so no growth could be observed. The LR and GEx experiments involved moisturizing at relatively elevated temperatures, resulting in hyperhydration. No organisms would have survived for very long. However one puzzle remains: After hot sterilization there appears to have been some activity left in the PR and the GEx experiments. Some chemical activity in the samples might have been responsible, or the sterilization may have been less than perfect (at 145°C in the GEx and 175°C in the PR), or the putative active cell stabilization mechanism may have caused some of the organisms to be able to survive these temperatures for about 3 hours. Although the Viking results await further elucidation, the H_2O_2-H_2O hypothesis has to be reckoned with in future life detection experiments on Mars.

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