

NOTE

Iron(VI) Seems an Unlikely Explanation for Viking Labeled Release Results

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Tsapin *et al.* (2000, *Icarus* 147, 68-78) propose the strong oxidant ferrate(VI) to explain the Viking Labeled Release Mars life detection results. However, their data do not support that theory. Further, sensitive IR searches for oxidants on Mars found none, and Viking produced physical evidence against an oxidizing surface. Finally, Tsapin *et al.* (2000, *Icarus* 147, 68-78) report no precautions to prevent microbial contamination from confounding their results.
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Tsapin *et al.* (2000) say that ferrate(VI) reacts with water in a manner "qualitatively similar to the Viking Gas Exchange Experiment (GEx) results," and produces a reaction "in line with the results of the Viking Labeled Release (LR) Experiment."

The difficulty of creating ferrate(VI) is acknowledged by the authors: "via wet oxidation of Fe(III) with hypochlorite," or "elevated temperature reactions ... with some peroxides." The authors choose the latter method, citing four papers in "the strong belief" that "...peroxides and other active oxygen species are formed under UV radiation in the Martian atmosphere." No mention is made of where the elevated temperatures to replenish the highly labile ferrate(VI) come from on the surface of present-day Mars. And, no reference is made to the two exquisitely sensitive, direct observations that found no peroxide on Mars (Hanel *et al.* 1970, Krasnopolsky *et al.* 1997).

Tsapin *et al.* (2000) need "a highly alkaline environment for Ferrate(VI) to be stored" (emphasis added). The references they cite indicate only the likelihood of mildly alkaline conditions on Mars. Thus, the references do not support the authors' statement: "Overall, the formation of ferrate(VI) and its preservation in soil are consistent with present knowledge of the chemical composition and environmental conditions at the Mars surface."

Claiming simulation of the GEx data also, Tsapin *et al.* (2000) show oxygen arising from ferrate(VI) exposed to water vapor (their Fig. 8a.). However, in GEX, 72% of the oxygen released evolved as a spike within 2.78 hours of exposing the soil to humidity (Klein *et al.* 1976). Tsapin *et al.* (2000), describing their results, state "The reaction develops slowly and steadily ..." Yet, they state, "the observations were qualitatively similar to the Viking results."

Turning to the LR, they cite Levin and Straat (1981) experiments on UV-irradiated martian analog soil (based on Viking analysis) as "evidence" for their theory. They neglect to report that the Levin and Straat (1981) article showed that UV irradiation could not explain the Mars LR results. Also, they incorrectly attribute formation of oxygen to the LR experiment. The LR instrument had no way of measuring oxygen.

The "chemical" experiments conducted by Tsapin *et al.* (2000) investigated the reactions of ferrate(VI), first with formic acid alone, and then with all the LR nutrients, including formate. Their first experiment cannot be compared to the Mars LR data because the LR did not apply formate alone to the soil on Mars. Even so, one wonders at Tsapin *et al.*'s (2000) choice of dousing their sample with 0.2 ml 2.4-M formic acid (see legend, their Fig. 9), 25,000 times stronger than the formate in the LR nutrient solution! Their evolved CO₂ peaked at 2 h and plateaued at 7 hr, very unlike that in the LR Mars results, even if these results were caused by formate alone, as some have inferred. When the complete LR nutrients were used by Tsapin *et al.* (2000) (probably omitting the optical isomers of two of LR's seven nutrients since the two are not mentioned), the dose was 2 ml of the solution containing each constituent at 0.24 M. This is more than 10,000 times the amount of nutrient applied in the Mars LR experiment, again making any comparison difficult.

Tsapin *et al.* (2000) pre-heated Fe(VI) before testing, in an attempt to simulate the Viking LR controls. Graphs 4-7 in their Fig. 9 give the results. Each test absorbed headspace CO₂ in amounts equal to 50% of the maximum CO₂ released in the positive tests. It is not explained how more CO₂ was absorbed from the headspace than was present in the atmosphere that originally filled it (presuming the unstated headspace volume was less than 1 liter = 300 μL CO₂). The LR pre-heated soil samples never showed absorption of CO₂ originally in the headspace. Nonetheless, Tsapin *et al.* (2000) state that "its (K₂FeO₄) relative lack of sensitivity (emphasis added) to pre-heating, as well as carbon dioxide release and absorption, together with pre-heating effects, are all in line with the observations made by the Viking landers." This internally contradictory statement in no way agrees with the graphic data they presented. The "in line" results include complete inhibition of the ferrate(VI) after heating to 145 or 170°C (although earlier in the article, they state the ferrate(VI) begins "decomposing starting at ca. 200°C," and elsewhere in their article, "This decomposition proceeds slowly, starting about 50°C and becoming complete by 300°C").

However, their efforts to explain the "biology" experiments by "chemistry" may have been ill starred from the outset. Their "chemistry" may well include biology. No mention of sterile materials, equipment, and procedures is made. Perhaps they were unstated, but without such precautions, which are key to these experiments, ubiquitous microbial contamination would have contributed to the evolution of CO₂ from the rich nutrients. In fact, their data could be explained by LR experiments conducted with terrestrial soils that have yielded over 300,000 cpm (Levin and Straat 1976) over essentially the same time span as that of the ferrate(VI) experiments. Each 15,000 cpm released equates to 29 nM CO₂. Thus, 300,000 x 29 = 580 nM CO₂ were released. The ferrate(VI) experiment with the full LR nutrient yielded approximately 1000 mL CO₂ (Tsapin *et al.* 2000, Fig. 9). This equates to 1000 x 10³, 22.4 = 4.46 x 10⁴ nM CO₂, some 77 times as much CO₂ as the LR produced. However, normalizing the ferrate(VI) result for the amount of nutrient applied yields 4.46 x 10⁴, 10⁴ = 4.46 nM CO₂, only 4.46 / 580 = 0.77% as much as the LR product. Normalization for sample size should also be applied. The LR sample was 0.5 cc. Assuming the generally accepted density of 1.5, this provides a sample of 750 mg. Viking soil analysis showed the total iron content to be about 10%. If all the iron were Fe(VI), this would permit the LR sample to contain 750 x .10 x 198 = 56 = 265 mg Fe(VI). Allowing for all of the LR samples to have been contacted by nutrient (highly unlikely), normalizing adjusts the Fe(VI) yield to a maximum of 0.77 x 265 = 50 = 4.08% of the LR yield. It can be seen that even minor microbial contamination could have produced all or part of the Fe(VI) results.

Putting the above demonstration of the inapplicability of the Fe(VI) data to the LR results aside, the assumption that all of the iron on Mars is Fe(VI) seems rash. Tsapin *et al.* (2000) acknowledge this by stating: "According to Viking results, the content of oxidant (which would include ferrate(VI)) should be rather low, most likely in the ppm range." Factoring in a lesser ferrate(VI) concentration in the Mars soil would proportionately further weaken the already weak signal strength of their experiment. Finally, the high percentage of their proposed ferrate, K₂FeO₄, would likely have revealed its dark purple/black color in the images of the Viking Lander sites. No such evidence appeared.

After 25 years, the Mars LR data still excite attempts at a chemical explanation, three within the last year. This indicates that none of the 30 non-biological explanations offered to date has been completely convincing. New findings concerning the existence of liquid water on the surface of Mars, and extremophile microorganisms on Earth, are consistent with my conclusion that the LR detected living microorganisms in the soil of Mars (Levin 1997), which may explain the difficulties with the non-biological theories.

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NOTE

Viking's Experiments and Hypothesis that Fe(VI) Is a Possible Candidate as a Martian Oxidant

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The note submitted by Dr. Gil Levin was to be expected⁴one always wants a forum for arguing for one's ideas. In suggesting that Fe(VI) in some form might be part of the explanation for the Viking Labeled Release (LR) experiment, we hoped to open a dialogue with those who seek to understand the past and plan for the future.

Dr. Levin's interpretation of our report is that we want to explain all of the Viking results via Fe(VI) oxidative chemistry. While this might be an outcome (we personally believe it is unlikely to be the sole explanation), nothing of the sort was or is on our minds. We sought to report the oxidative properties of a few Fe(VI) adducts, to show that qualitatively they mimicked some of the Viking results, and to suggest that if they are on the surface of Mars, they will be easy to identify by any of several different techniques, specifically by the Moessbauer spectrometer to be carried by the '03 MER rovers.

Our paper does not deal with the question of whether there is (or was) life on Mars. Our paper was written simply to point out that there are potential oxidants that may not have been previously considered and that these can be easily measured *in situ*. Why not do it? As we pointed out in our paper, what we really need is more data, and some experiments with controls. These will be forthcoming, and if real evidence to support the presence of life, oxidants, or both comes forward, we will welcome it.

Belief in the oxidizing nature of martian soil, and the contribution of active oxygen forms such as peroxides and superoxides to these oxidizing properties, is widespread. In a recent publication (A.S. Yen *et al.* 2000; see also rebuttal by Levin 2001), some additional chemical simulations and spectral data have been presented in favor of the formation of superoxide radical ions in martian soil at UV irradiation. Thus, both superoxide and iron(III) may be there. Lack of IR evidence for the presence of hydrogen peroxide in the atmosphere of Mars (Krasnopolsky *et al.* 1997) has little to do with the composition of martian soil. A further question is what is the fate of this active oxygen. It may stay as, say, potassium superoxide, or another metal superoxide, or transform into other chemical forms. We explored one such possibility; formation of higher oxidation state iron, ferrate(VI).

There is an important point to be understood here, one that perhaps deserves some discussion. The Viking experiments were remarkable achievements of technology, especially for their time. The fact that they remain to be explained some 25 years later is testament to the complexity of the situation. If life had been abundant and obvious, it would have been unambiguously seen. Could these experiments be a combination of chemistry and biology, could they be chemistry, or could they be biology? It is our goal and our duty to ask such questions. On Earth we can design possible mimics and test them, which we have done with the Fe(VI). The fact that the match was not perfect is acknowledged when biology experiments are repeated; even in the lab, they are seldom perfect matches. In our opinion, if the explanation for Viking is chemistry, then it will likely be a complex mixture of different oxidants acting together in ways that no single one could mimic. We are rather comforted by a qualitative agreement with some of the LR and GEx results.

As for the explanation that these results are due to biological activity of contaminating microbes, it was an oversight on our part not to include details of the measures of sterility and cleanliness that were taken. In fact, Fe(VI) is such a strong oxidant that it is now used for sterilization by our laboratory, and for the removal of contaminating nucleic acids, which are oxidized to CO₂ (Tsapin *et al.* 2000). While we acknowledge that this may be no excuse for leaving out the detailed sterilization procedure, we can only say emphatically that despite the page of calculations prepared by Dr. G. Levin, no biological activity accounted for our results.

In a final point, Dr. Levin states that "none of the 30 nonbiological explanations offered to date has been completely convincing" and infers that his conclusion that "the LR detected living microorganisms in the soil Mars" must thus be correct. We note here that the LR experiment was also extremely controversial and joins the nonbiological explanations as being less than completely convincing. The excitement of searching for life on Mars and elsewhere lives on, but we urge Dr. Levin not to dismiss other hypotheses, especially when they are eminently and rather easily testable.

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