Antarctic Soil No. 726 and Implications for the Viking Labeled Release Experiment

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The failure of the Viking Molecular Analysis experiment (Biemann *et al.,* 1976) to detect organic matter on Mars is primarily responsible for the widespread belief (Klein, 1979; Young, 1979) that a chemical, rather than a biological, agent caused the response obtained by the Labeled Release (LR) life detection experiment (Levin & Straat, 1976*a,* 1977, 1979*a*).

In discussions concerning whether the relative sensitivities of the Molecular Analysis and the LR experiments could permit a biological interpretation of the Viking LR results, it was stated (Biemann *et al.,* 1976) that 106 typical prokaryotic cells contain the minimal amount of organic matter detectable by the gas chromatograph-mass spectrometer (GCMS) used in the Molecular Analysis experiment. However, it was pointed out (Biemann, 1976) that the mass of organic detritus from dead cells present in terrestrial soils containing living organisms exceeds the living biomass by ten to one hundred fold. The accumulated dead organic matter would, thus, produce a signal in the GCMS even though the living cells, by themselves, would not (Biemann, 1976). The sensitivity of the radiorespirometric method, its ability to detect small populations of microorganisms even prior to the onset of growth, and the biological nature of the response have been demonstrated (Levin, 1963, 1966; Levin & Heim, 1965; Levin & Straat, 1976*b*). Although the LR technique had detected (Levin & Straat, 1976*b*) as few as 50 colony-forming units in a terrestrial soil sample, it was doubted that LR-detectable levels of indigenous microorganisms could, in fact, exist in a soil in which the total organic content was below the GCMS limit of detection. We wish to report a new development that provides the first experimental evidence resolving this doubt.

A recent report (Biemann, 1979) on the sensitivity of the Molecular Analysis experiment gave results obtained when several sterile or sparsely populated Antarctic soils were tested in a flight-like GCMS instrument. One of the soils failed to produce a detectable response in that instrument. This soil, identified (Lavoie, 1979) as Antarctic soil No. 726, is a documented (Cameron, 1971) sample maintained by NASA, containing 0.03% organic carbon (which, interestingly, did not produce detectable organic vapors when pyrolyzed in the GCMS (Lavoie, 1979)), and failed to produce microbial colonies when inoculated in classical test media.

Prior to the landing of the Viking Spacecraft, we tested a variety of soils in the flight-like LR instrument, the Test Standards Module (TSM), to assemble a "library of responses" for comparison with the LR data to be obtained from Mars. The soils tested included sterile soils (e.g. lunar soil, Surtsey soil, soon after formation, sterile Antarctic soil No. 542 (Cameron, 1971)) and several low population Antarctic soils. Stringent aseptic techniques established for TSM work were rigorously followed. In some cases, we obtained small, but positive, labeled release responses in Antarctic soils not producing colonies when tested by classical methods. Growth is required by classical methods whereas metabolism in the absence of growth is sufficient for an LR response (Levin, 1966).

Upon learning of the new GCMS sensitivity test, we re-examined our response library. We found that one of the soils which gave a weak, but clearly positive, response in the TSM was the same Antarctic soil No. 726 reported negative by the GCMS. Figure 1 shows that response and its sterilized control (heated at 160°C for 3 hr according to the flight sterilization regime). For comparison, the Viking Lander 1 Cycle 1 LR response is shown to the same scale.

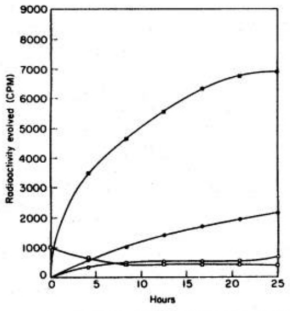

FIG. 1. LR response from Antarctic soil No. 726, active (●—●—●), control (○—○—○). The experiment, performed in the LR Test Standards Module under Mars flight regime, produced a weak, but definitely positive, result. The GCMS flight-like instrument of the Molecular Analysis experiment was unable to detect organic matter in this soil. The Viking 1 LR results are plotted for comparison: (■—■—■) = active, (□—□—□) = control.

Thus, the ability of the LR experiment to detect indigenous micro- organisms in soil containing levels of organic matter undetectable by the Viking Molecular Analysis experiment has been demonstrated. Although a variety of reports has been published claiming to have "simulated" the LR Mars results chemically or physically none, in fact, has published data simulating the peculiar, and life-like, thermal sensitivity shown in the LR Mars data. Taken together with the negative results of our three and a half year effort (Levin & Straat, 1979*b,* 1980,1981) to account satisfactorily for the LR Mars data by non-biological means, this finding supports our position that a biological interpretation of the LR experiment must still be considered.

While we were able to impose rigorous conditions to make hydrogen peroxide react with the LR nutrient in a manner approaching the kinetics and thermal sensitivity shown by the Mars agent, this required assumptions about constituents and properties still undetermined for Mars soil. Moreover, the reaction did not occur when it was attempted on the Mars analog soils developed by the Viking Inorganic Analysis Team from its Mars data. Nor were we able to sustain significant quantities of hydrogen peroxide on soils under simulated Mars ultraviolet light to make the formation and accumulation of that oxidant likely on Mars.

That suitable models exist for possible ecological niches on Mars is indicated in Fig. 2. The LR "getter" technique (Levin & Straat, 1976*b)* was used on 0.24 g of scrapings from an endolithic microorganism-bearing band 10 mm beneath the surface of a rock (Friedmann & Ocampo, 1976) brought to our laboratory from the dry valleys of Antarctic. As shown (Fig. 2), a positive response was obtained whereas a duplicate portion of the sample heated to 200°C for 1 hr did not respond. The results, plotted to a scale adjusting counting efficiency to correspond to that of the LR flight instrument, show that the magnitude of the response is comparable to that obtained on Mars. Other terrestrial models, discussed in detail elsewhere (Levin & Straat, *1979b),* include lichens which are capable of deriving water solely from atmospheric vapor (Lange, Schulze & Koch, 1980).

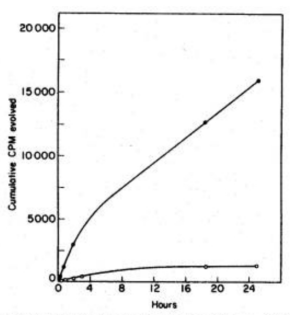

FIG. 2. LR responses from 0·24 g of material scraped from endolithic miroorganism populated band 10 mm beneath surface of Antarctic rock, (●—●—●) active, and (○—○—○) heat-sterilized control. Experiment was performed at room temperature by LR "getter" technique and results are normalized to counting efficiency of LR flight instrument.

We thank Bruce Connor for performing the LR TSM test on Antarctic soil No. 726. We are indebted to E. I. Friedmann and R. Ocampo for bringing the Antarctic rock containing endolithic organisms to our laboratory and for removing the portions tested. E. Merek kindly supplied Antarctic Soil No. 726 from the Ames Research Center Antarctic soil bank.

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