Vishniac W. ***Extraterrestrial microbiology.*** **Aerosp Med 1960; 31:678–80.**

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This classic paper was presented by the legendary exobiologist, Wolf V. Vishniac, Ph.D., a great pioneer in astrobiology before this field was even recognized as a discipline. It set forth the principle that to find evidence of life on other planets, the focus must be on the detection of microorganisms. The success of culturing microorganisms depends upon selecting the correct culture media based upon the natural environment. For Mars, this means low O_2 (0.13%), a high concentration of CO_2, and energy capture by photosynthesis or chemoautotrophy. Dr. Vishniac postulated that hydrogen sulfide might provide an alternate source for oxidation. He suggested several possibilities for terminal respiration substrates including CO_2 and sulfate with the production of methane or nitrate and free nitrogen. Photosynthetic bacteria might also need to oxidize ammonium to complete the nitrogen cycle. Most importantly, he designed an apparatus to culture and detect microorganisms on other planets (Mars) which he called the "Wolf Trap." This apparatus would operate under photosynthetic anaerobic conditions with a high CO_2 atmosphere and would provide nutrients for the growth of microorganisms which would be indirectly detected by changes in the pH and culture media turbidity.

Dr. Vishniac joined 18 other scientists in December 1958 at the Massachusetts Institute of Technology to discuss the problems of detecting life on other planets. The group, called the Panel on Extraterrestrial Life (EASTEX) was jointly sponsored by the National Academy of Sciences-National Research Council and the Armed Forces Committee on Bioastronautics. A separate Stanford/Jet Propulsion Laboratory group led by Dr. Joshua Lederberg was called WESTEX. At that first meeting in December 1958, one of the basic questions addressed by the physicists and biologists was what kinds of life forms they might reasonably expect to find away from their own planet. They had to make some basic assumptions, and one of them was that life elsewhere would have a carbon base and suggested that there were five manifestations of life: growth, movement, irritability, reproduction, and metabolism.

Wolf Vishniac originally developed the device described in this paper, which he called the "Wolf Trap," in 1958–1960 under the very first NASA grant for biological science research to demonstrate the feasibility of automatic remote detection of the growth of microorganisms. At the heart of the instrument was a growth chamber with an acidity (pH) detector and light sensor to detect turbidity, two independent indirect indicators of growth and metabolism. By mid-1963, Vishniac had progressed from a simple feasibility model to a more complex design.

The Viking biology experiments (developed from 1970–1974 and flown in 1976) searched for the presence of Martian organisms by looking for metabolic products. Three distinct experiments, pyrolytic release (PR), labeled release (LR), and gas exchange (GEX), incubated samples of the Martian surface under a number of different environmental conditions. In some instances a sample was heat sterilized and reprocessed as a control. The PR, or carbon assimilation, instrument sought to detect the photosynthetic or chemical fixation of CO_2 or CO containing ^{14}C. The samples were incubated for several days in the presence of the radioactive gas mixture, some samples with simulated sunlight and some without. The soil was pyrolized at 650°C and any organic products were collected in an organic vapor trap. Finally, the trap was heated to combust the organic material to CO_2 and any evolved radioactive gas was measured. The LR experiment sought to detect metabolic processes through radiorespirometry. Liquid nutrients labeled with radioactive carbon were added to the samples and the atmosphere above was continuously monitored to detect any radioactive gases released from these nonvolatile nutrients. The GEX measured the production and/or uptake of CO_2, N_2, CH_4, H_2, and O_2 during incubation of a soil sample. After the addition of a selected quantity of a nutrient solution, the sample was incubated. At certain time intervals, samples of the atmosphere were removed and analyzed by a gas chromatograph mass spectrometer (GCMS). A fourth experiment (subsequently eliminated) was the Wolf Trap light-scattering experiment of Dr.Vishniac. This investigation provided the least Mars-like environment because it would suspend the sample in an aqueous solution and it also gave only indirect indications of growth and metabolism.

The Viking biology instrument was an extremely complex scientific package requiring a large amount of time, energy, and money (originally budgeted at $13.7 million but rose to $59.0 million) to develop. Devising a biology instrument that held three experiments inside a container less than 0.027 m^3 in volume and weighing 15.5 kg was more of a technical and scientific challenge than even the most pessimistic persons had believed. The instrument contained some 40,000 parts, half of them transistors. In addition to tiny ovens to heat the samples were ampules containing nutrients, which were to be broken on command; there were bottled radioactive gases, Geiger counters, over 50 valves, and a xenon lamp to duplicate the light of the sun. The Wolf Trap was eliminated from the payload in a controversial decision to save an additional 1.4 kg. Dr. Vishniac always believed that the decision was political and not scientific.

The principal investigator of the LR experiment, Dr. Gilbert Levin, believes his results are a definitive diagnostic for life on Mars. The Labeled Release experiment returned a positive result, showing increased $^{14}CO_2$ production on first exposure of soil to water and nutrients. However, this result is disputed by many scientists, who

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argue that superoxidant chemicals in the soil could have produced this effect without life being present. All scientists agree that radiolabeled $^{14}CO_2$ was evolved in the labeled release experiment, and that the GCMS detected no organic molecules. Several pyrolytic release experiments (particularly Chryse 1) are believed by many to have detected a positive result as well. This was dismissed by the principal investigator of that experiment, Dr. Norman Horowitz. There are recent claims that the GCMS flown on Viking was not a sensitive enough of an instrument. The results of the Viking mission concerning the detection of life are considered to be inconclusive, at best. If the Wolf Trap had not been eliminated, it would have helped to resolve this ongoing controversy since it was a very sensitive, although indirect, detector for the presence of life.

In retrospect, most of the issues and problems of the detection of life on Mars as outlined in this article by Dr. Vishniac were absolutely correct. Particularly important was his focusing on microorganisms and his understanding of how environments and organisms are interlinked. This strong interdependence is nowadays universally recognized. He realized that organisms in a very nutrient-poor environment such as Mars would have to be specially adapted to consume "mineral soup" (being chemoautotrophic, lithoautrophic) and would have a problem with a rich "chicken broth" (heterotrophic food source as used in the Viking experiments). He extrapolated that the basis of life on Mars would have to be photosynthesis, but not the aerobic type. This may or may not be correct as there are extremophiles in deep sea vents on Earth which utilize inorganic chemicals to produce energy and may provide another option for possible life on Mars. Photosynthesis, however, has again been recently suggested as a basis for Martian life, which could provide methane as a final metabolic by-product. The same organisms were speculated to use a hydrogen peroxide-water solvent as a special adaptation to Martian environmental conditions (1,2), an interpretation that would surely have spiked Wolf's interest. Wolf Vishniac discussed in some detail that methane would be a possible final product of microbial metabolism on Mars. The recent detections and localized variations of methane in the Martian atmosphere are likely the strongest hint for current microbial life on Mars (3,4,5), especially since methane has only a short-term residence time in the Martian atmosphere before it is oxidized to carbon dioxide. One problem for Martian life is available nitrogen and Wolf Vishniac correctly identified this as a problem in his article. The question of nitrogen and the nitrogen cycle is a problem that came up recently in context with the latest Mars mission. It has even been suggested to put the emphasis on the search for nitrogen when looking for life on Mars (6). Vishniac's emphasis was on anaerobic (oxygen-poor) pathways. In contrast, most scientists currently believe that the Martian surface is highly oxidizing due to the high influx of radiation and because of the results of the Viking experiments. However, none of the missions after Viking found any evidence of a strong oxidant existing on the surface of Mars, and anaerobic conditions may very well prevail a few millimeters below the surface of Mars. On this major issue we will still have to await clarification from upcoming missions such as the Mars Science Laboratory mission and the European ExoMars mission.

Early in 1972, Dr. Vishniac found microorganisms growing in what had previously been thought to be sterile soil in the dry Antarctic valleys. This discovery by Vishniac and his graduate student Stanley E. Mainzer, using a version of the Wolf Trap instrument, was a contradiction of the findings of Dr. Norman Horowitz, who in 5 yr of research had yet to detect any life forms in that barren land. In 1973, Vishniac was still pursuing his research into the origins of life and the possibility of life on other worlds when he fell 150 m to his death in Antarctica's Asgard Mountains. Searching for life in the dry valleys of that bitter cold and windswept region, Vishniac was attempting to prove that life forms could adapt to extremely hostile environments. He was successful and this concept is now widely accepted. As a fitting tribute, the crater Vishniac on Mars is named in his honor.

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