Microbiology of the Dry Valleys of Antarctica

Studies in the world's coldest and driest desert have implications for the Mars biological program.

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The dry valleys of South Victoria Land, Antarctica, together with a few other ice-free areas on the perimeter of the Antarctic continent, form what is generally considered to be the most extreme cold-desert region of the earth. During the past 5 years, we have been interested in the dry valleys as a model environment for investigating questions connected with the biological exploration of Mars. The extraordinary aridity of the region, its low temperature, and its geographical isolation give it a quasi-martian character, although it is to be understood that the actual martian environment is still more hostile than that of the valleys. With our associates, we have investigated the kinds, numbers, and distribution of soil microorganisms in the valleys in order to gain insight into the practical problems of searching for life in an extreme environment. Detailed results of these studies have been published by Cameron and co-workers (*1–5*). In this article, we review the major findings of our own and of other groups of investigators in this region, especially as they apply to martian exploration.

Dry Valleys

The dry-valley system of South Victoria Land comprises an area of several thousand square kilometers that is cut off by the Transantarctic Mountains from the flow of glaciers out of the Antarctic interior. Although the valleys are ice-free, their mean annual air temperature is −20° to −25°C. Even in the summer the air temperature does not rise much above 0°C, but the surface of the ground can reach 15°C or higher for short periods during the day. The ground is underlain by hard permafrost, which is found at depths from a few centimeters to over a meter, depending on elevation, proximity to lakes and glacial melt, valley orientation, and other factors determining the overall degree of desiccation of the site. Despite continual sunlight in the summer, diurnal cycles of freezing and thawing occur as the low sun moves around the sky and incoming radiation is blocked by the mountains surrounding the valleys.

Owing to the low temperature of the Antarctic atmosphere, its water vapor content is an order of magnitude less than that of the atmosphere in temperate latitudes (*6*), and precipitation is consequently sparse. In the dry-valley region, the mean annual precipitation is in the neighborhood of 15 grams per square centimeter per year, all of it in the form of snow. Because of the low humidity, the snow sublimes without visibly wetting the ground. The low relative humidity of the valleys is caused by katabatic winds that blow from the high Antarctic Plateau. Ventifacts and wind-drifted pebble ridges are common in the area and attest to the strength of the almost constant winds (*7*).

Considerable evidence indicates that the valleys have been drying out for thousands of years. Many saline lakes and ponds, fed by glacial meltwater, are found in the area. These bodies of water have no drainage, yet they are much smaller than their basins (*8*). The difference is due to loss by evaporation. Terraces around Lake Vanda in the Wright Valley show that its level was formerly 56 meters higher than it is at the present time (*8*). From dating of algal mats at the uppermost level, it is estimated that the lake was at its highest level some 3000 years ago (*9*). Similar evidence for Lake Vida in the Victoria Valley indicates a minimal age of the basin of 9300 years (*10*).

The lakes in the dry-valley region are generally frozen all year around, except for melting around the edges and at the inlets in the summer. One pond, the Don Juan in the upper Wright Valley, remains unfrozen because of its high salt content. Calcium chloride hexahydrate (antarcticite) is crystallizing out of the pond (*11*). The relative humidity of air in equilibrium with saturated $CaCl_2 \cdot 6H_2O$ is 39.8 percent at 5°C (*12*). The prevailing relative humidity of the region is thus estimated to be less than 45 percent (*9*), and field measurements in the valleys are in accord with this estimate (*1*).

The soil, as well as the lakes of the dry valleys, is saline and sometimes alkaline. Salt crusts are found throughout the area. Sodium, calcium, magnesium, chloride, sulfate, and nitrate are among the more usual ions. The presence of nitrate is of particular interest, since it indicates the virtual absence of leaching and of biological activity over a long period of time. Nitrate in the Antarctic may originate in the same way as that in the Atacama Desert of Chile—namely, by deposition from the atmosphere (*13*).

Biology of the Dry Valleys

Much of the foregoing description applies to Mars as it is known, or imagined, or hoped to be. The mean low temperature, the difference in temperature between surface and atmosphere, and the aridity are martian, except, of course, that Mars is colder and much drier than the Antarctic. Experiments mentioned below show that the shortage of water limits life in the dry valleys, just as we suppose it does on Mars. Permafrost is possible on Mars, although we have no evidence that it actually exists there. Saline deposits like that of the Don Juan Pond would permit liquid water to exist on Mars in the form of concentrated salt solutions (*14*), but it is questionable whether the leaching of rocks by running water—the process that creates salt deposits—has ever occurred on Mars.

Life in the dry valleys is almost entirely microbial. An occasional moss is found in protected niches, but no higher

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plants or animals live there. Dense populations of algae, especially blue-greens and coccoid greens, are found on the lakeshores. These support an ecology of bacteria, yeasts, molds, and protozoans; rotifers, tardigrades, and small nematodes are also found (*15*). As one leaves the lakeshores, the organism count in the soil declines rapidly; in the drier parts of the valleys, soils containing no detectable microorganisms are found. All workers in the field have reported finding such soils (*2, 16, 17*). Table 1 shows the frequency of sterile samples reported by Boyd and coworkers for the Wright and Taylor valleys (*16*) and by Cameron and coworkers for various locations in the dry valleys and in the Transantarctic Mountains (*1–3, 18*). Comparable statistics are not available for the studies carried out by Benoit and Hall, but these authors state that "at those soil sites which receive the least amount of water the surface inch of soil was often abiotic or had less than 10 bacteria per gram of soil" (*17*). Subsurface soils can also be sterile. Thus, Benoit and Hall have reported a site in Taylor Valley in which no bacteria were detected in an entire profile extending from the surface to a depth of 1 meter (*17*).

The discovery of sterile soils is surely one of the most interesting results of Antarctic research. It was so difficult for us to accept this result that our group at the Jet Propulsion Laboratory spent several months in an intensive investigation of one Victoria Valley subsurface soil. It was tested for growth on a variety of media under various conditions, with negative results. Its production of $^{14}CO_2$ when incubated with ^{14}C-labeled glucose and amino acids (*19*) was measured and found to be the same as that of sterilized controls. It was tested for toxicity and found to be nontoxic. Finally, its organic carbon, amounting to 0.11 percent of the soil, was isolated and identified on the chance that this might reveal the presence of an undetected population of microorganisms. It was found to be anthracite coal dust, probably originating from the Mount Bastion coal measures 25 kilometers away. We are now convinced that this soil is actually sterile (*3*). [We designate as sterile a soil which, in aliquots of up to 1 gram per test, fails to yield visible growth in any of the 5 to 15 media used in our laboratory for enumerating soil microorganisms (*1–3*). We use the term abiotic to refer to samples or localities where microorganisms are found, but where there is reason to believe they are not growing.]

Table 1. Sterile soils from Antarctica.

Bacterial content of soil (colonies per gram)	Number of samples	Percent
	255 samples (16)	
Sterile	66	26
	73 samples (1–3, 18)	
Sterile	10	14
< 10	13	18
< 100	18	25
< 1000	26	36

Those who have studied the dry valleys generally agree that lack of water is the life-limiting factor in this region. Although the soils typically contain little organic matter, experiments have shown that there is usually sufficient nutriment in them to permit population increases of 3 to 6 log units after the addition of water to the soil (*4*). Some of the dry-valley soils are weakly toxic, a soluble form of boron being suspected as the toxic agent (*5*). Even here, insufficient water to leach the soil is the ultimate cause of the toxicity.

Metabolic Activity of Dry-Valley Soils

Besides conventional plate counts and dilution-tube counts on a variety of media, we obtained an independent measure of the biological activity of the soils from the rate of $^{14}CO_2$ evolution during incubation with labeled glucose and amino acids, as mentioned above. Short incubation times and low concentrations of substrates of high specific activity were used in order to obtain initial rates without appreciable cell proliferation.

When applied to nine soils containing ten or fewer culturable organisms per gram, this assay showed no significant activity in the seven subsurface samples and barely significant activity in the two surface samples. In dry-valley soils in general, the rate of $^{14}CO_2$ production per hour per organism was higher in surface than in subsurface samples (Table 2). The lowest rate per culturable organism was found in the one soil tested from a temperate environment (JPL-mixed soil, from nine scattered sites on the grounds of the Jet Propulsion Laboratory). The rate of $^{14}CO_2$ evolution per organism in this soil differs from the rate in an average dry-valley surface soil containing fewer than 1000 organisms per gram by a factor of 600.

Several hypotheses can explain this anomaly. First is the possibility that the autochthonous microflora of Antarctic soils can utilize low concentrations of substrates, such as are provided in the experiment, with unusual efficiency. Estimates of the glucose concentration required for the half-maximal rate of CO_2 evolution in Antarctic surface and JPL-mixed soils, respectively, did not differ, however (*20*). Moreover, the metabolic activity of the predominant microorganism (a yeast) isolated from an Antarctic surface soil was comparable to that of the mixture of species in JPL-mixed soil (Table 2).

The second hypothesis is that the extra metabolism of Antarctic soils is due to an unculturable population of microorganisms. The readiness with which glucose and amino acids are metabolized by the soils in itself casts doubt on this explanation, but the following experiment was performed. Samples of a surface soil which contained no culturable organisms but which showed a slight oxidation of labeled

Table 2. Metabolic activity in Antarctic soils. The microbial counts are the highest values obtained with a variety of media (*2*). The evolution of $^{14}CO_2$ was measured by the procedure described by Horowitz *et al.* (*3*).

Sample	Viable micro-organisms (per gram)	Number of soils	$^{14}CO_2$ evolved (count/min per 1000 viable cells)
Antarctic surface soil	$10–10^3$	12	1574
	$10^3–10^4$	5	663
Antarctic subsurface soil	$10–10^3$	13	235
	$10^3–10^4$	4	24
	$10^4–1.7 \times 10^5$	4	14
JPL-mixed soil	1.2×10^7		2.6
Yeast isolated from Antarctic surface soil (see text)			4.4

substrates were moistened either with water or with diluted (1 percent) trypticase soy broth and incubated at 20°C. Samples assayed after 2, 5, 13, and 14 days showed no increase in activity such as would be expected if growth had occurred. We conclude that the oxidation is not carried out by viable cells.

The third possibility is that the extra production of CO_2 is due to inorganic catalysts in Antarctic soils. This appears unlikely in view of the fact that the evolution of $^{14}CO_2$ was accompanied by the formation of radioactive materials insoluble in hot trichloroacetic acid, as would be expected if part of the substrate were being converted into macromolecules. In addition, it was found that Antarctic surface soils assimilate $^{14}CO_2$ into organic compounds at a higher rate than would be expected from the small numbers of viable cells present (*21*).

By elimination, we are left with the hypothesis that the extra metabolic activity is due to the presence of dead but enzymatically active cells in the soils. A large literature exists on enzymatic activities in temperate-zone soils; many of these activities are believed to originate in extracellular enzymes or dead cells (*22*). It has been shown that soil sterilized by radiation continues to respire glucose, presumably resulting from the activity of dead cells (*23*). The aridity, high summertime insolation (with accompanying ultraviolet radiation), and low temperature of the Antarctic valleys would favor the production and preservation of such active but nonviable cells. The low relative humidity of the ground surface compared to that of the subsurface (*1*) may explain the greater abundance of such particles in surface samples.

Steady-State Model of the Dry-Valley Ecology

Most soil samples from even the drier regions of the valleys contain some culturable organisms, although the numbers are quite low by ordinary standards. The evidence suggests that these organisms are not indigenous to the places where they are found but are carried in, probably by winds, from more favorable environments, such as the lakeshores or the ocean. We note first that only heterotrophs—bacteria, yeasts, and actinomycetes—have been found in the more hostile environments of the valleys (*2*). The ecology of these regions is thus not a complete one, since it lacks any primary producers of organic matter. Lichens, which are popularly supposed to be among the hardiest of microorganisms, are not often found in the dry valleys, although they are abundant elsewhere in the Antarctic where more moisture is available. According to Wilson (*9*), lichens are confined to regions in the Antarctic where the mean relative humidity is at least 80 percent. It is worth noting that sporeformers are also uncommon in the dry valleys, among both bacteria and algae. In contrast to the usual presupposition, spore formation does not seem to confer an advantage in this harsh environment.

Table 3. Microbial counts in a saline soil from Taylor Dry Valley, Antarctica. The data are from Benoit and Hall (*17*) and Hall (*24*).

NaCl in medium (%)	Incubation temperature (°C)	Incubation time (weeks)	Colonies per gram of soil
0	15	2	7.2×10^4
	2	4	5.1×10^3
5	15	2	2×10^4
	2	4	1.2×10^3
15	15	2	1.4×10^3
	2	6	<40

The typical organisms of dry-valley soils are aerobic, heterotrophic, nonsporulating rods or cocci, often chromogenic in the upper 3 centimeters of the soil and usually colorless or less prominently pigmented below that level. They appear to be similar in all respects to bacteria found in more favorable Antarctic locales. Contrary to what would be expected of an indigenous population, the microorganisms of dry-valley soils are predominantly mesophilic, not psychrophilic (*17*). Psychrophils are abundant near favorable sites—such as pools of glacial meltwater—but become progressively less so as conditions become drier. Halotolerance, too, is less common than one would predict in view of the abundance of saline habitats. Table 3, from Benoit and Hall (*17*) and Hall (*24*), shows the growth of bacteria from a saline depression (possibly a dried-up pond) in Taylor Valley as a function of temperature and salinity. Halotolerance declines markedly as the temperature is lowered.

Microorganisms have been found in the Don Juan Pond (*25*), but they cannot grow at the calcium chloride concentration of the pond (*26*). It appears that microorganisms living around the freshwater inlets are periodically washed into the pond (*24*), but they are unable to grow there; the pond is consequently abiotic (*17*).

The evidence thus leads to the conclusion that no species able to cope with the drought and low temperature of the valleys has evolved there in the 10,000- to 60,000-year history (*9*) of the region. Apart from the lakes and the streams that feed them, the dry valleys are essentially abiotic areas where little, if any, microbial growth is occurring. The small microbial population of the region probably is in balance between influx from the atmosphere (with the possibility of a few cell divisions following a snowfall) and mortality on the ground. Similar conclusions have been reached independently by Benoit and Cameron (*27*) and Lacy (*28*).

This conclusion is consistent with what is known from other studies about the water requirements of microorganisms. The microbial species that are most tolerant of low water activities (equivalent to equilibrium relative humidities) are the extremely halophilic bacteria and certain osmophilic and xerophilic yeasts and molds (*29*). Halophilic bacteria can grow in saturated sodium chloride at a water activity of 0.75. Growth of fungi has been observed at water activities as low as 0.62, but this growth is extremely slow even under ideal conditions of temperature and nutrition. The water activity of 0.45 or less which, as was shown above, characterizes the dry valleys falls far short of meeting the water needs of even these xerophilic microorganisms.

Conclusions

These results have important implications for the Mars biological program. First, it is evident that the fear that terrestrial microorganisms carried to Mars could multiply and contaminate the planet is unfounded. The Antarctic desert is far more hospitable to terrestrial life than is Mars, particularly in regard to the abundance of water. In other respects, too—such as in the ultraviolet flux at the surface—Mars is decidedly more hostile than the Antarctic.

Second, martian life, if any, must have evolved special means for obtaining and retaining water, if it is assumed that that is the martian biological solvent. This has been known for some time. What is new in these findings is that even under severe selective pressure, microbial life in the Antarctic has been unable to discover a comparable mechanism. To some, this may suggest that life on Mars is an impossibility. In view of the very different histories of Mars and the dry valleys, including the recent age (10^4 to 10^5 years) of the latter, and the obvious uncertainties concerning the metabolic potentialities of extraterrestrial life forms, we believe that such a conclusion is not justified. The findings suggest, however, that martian life could not be built on a terrestrial model.

Finally, the Antarctic has provided us with a natural environment as much like Mars as any we are likely to find on Earth. In this environment, the capacity of life as we know it to adapt and survive is pushed to its limit. The concentration of living things around the sources of water in the dry valleys and their rapid thinning out in the most arid locales may be useful as a model of the distribution of the life we may, if we are lucky, find on Mars.

References and Notes

1. R. E. Cameron, in *Arid Lands in Perspective*, W. G. McGinnies and B. J. Goldman, Eds. (Univ. of Arizona Press, Tucson, 1969), p. 169.
2. ———, J. King, C. N. David, in *Antarctic Ecology*, M. W. Holdgate, Ed. (Academic Press, New York, 1970), p. 702; R. E. Cameron, in *Research in the Antarctic*, L. O. Quam and H. D. Porter, Eds. (AAAS, Washington, D.C., 1971), p. 137.
3. N. H. Horowitz, A. J. Bauman, R. E. Cameron, P. J. Geiger, J. S. Hubbard, G. P. Shulman, P. G. Simmonds, K. Westberg, *Science* **164**, 1054 (1969).
4. R. E. Cameron and H. P. Conrow, *Antarct. J.* **4**, 28 (1969); R. E. Cameron and E. L. Merek, *Technical Report 32-1522* (Jet Propulsion Laboratory, California Institute of Technology, Pasadena, 1971).
5. R. E. Cameron, C. N. David, J. King, *Antarct. J.* **3**, 164 (1968).
6. M. J. Rubin and W. S. Weyant, in *Antarctica*, T. Hatherton, Ed. (Praeger, New York, 1965), p. 375.
7. C. Bull, *Antarct. Res. Ser.* **9**, 177 (1966).
8. R. L. Nichols, *Amer. J. Sci.* **261**, 20 (1963).
9. A. T. Wilson, in *Antarctic Ecology*, M. W. Holdgate, Ed. (Academic Press, New York, 1970), p. 21.
10. P. E. Calkin, *Institute of Polar Studies Report No. 10* (Institute of Polar Studies, Ohio State University, Columbus, 1964).
11. T. Torii and J. Ossaka, *Science* **149**, 975 (1965).
12. *Handbook of Chemistry and Physics, 49th Edition* (Chemical Rubber Co., Cleveland, 1968), p. E-37.

→ G. G. C. Claridge and I. B. Campbell, *Nature* **217**, 428 (1968); G. Mueller, *ibid.* **219**, 1131 (1968).

14. A. P. Ingersoll, *Science* **168**, 972 (1970).
15. E. C. Dougherty and L. G. Harris, *Science* **140**, 497 (1963).
16. W. L. Boyd, J. T. Staley, J. W. Boyd, *Antarct. Res. Ser.* **8**, 125 (1966).
17. R. E. Benoit and C. E. Hall, Jr., in *Antarctic Ecology*, M. W. Holdgate, Ed. (Academic Press, New York, 1970), p. 697.
18. R. E. Cameron, unpublished data.
19. G. V. Levin, *Advan. Appl. Microbiol.* **10**, 55 (1968).
20. J. S. Hubbard, unpublished data.
21. ———, G. L. Hobby, N. H. Horowitz, P. J. Geiger, F. A. Morelli, *Appl. Microbiol.* **19**, 32 (1970).
22. J. J. Skujins, in *Soil Biochemistry*, A. D. McLaren and G. H. Peterson, Eds. (Dekker, New York, 1967), p. 371.
23. G. H. Peterson, *Soil Sci.* **94**, 71 (1962).
24. C. L. Hall, thesis, Virginia Polytechnic Institute (1968).
25. G. H. Meyer, M. B. Morrow, O. Wyss, T. E. Berg, J. L. Littlepage, *Science* **138**, 1103 (1962).
26. R. E. Cameron and J. S. Hubbard, unpublished data.
27. R. E. Benoit and R. E. Cameron, paper presented at the 67th annual meeting of the American Society for Microbiology in New York, 1967.
28. G. H. Lacy, personal communication.
29. W. J. Scott, *Advan. Food Res.* **7**, 83 (1957).
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Electron Spectroscopy with Monochromatized X-rays

This technique constitutes a second-generation approach for a new analytical method.

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In recent years electron spectroscopy for chemical analysis (ESCA) has been developed to the point where it promises to be of considerable importance to the chemist. In electron spectroscopy the sample to be studied is irradiated with x-ray or ultraviolet radiation. The sample emits electrons, which may be photoelectrons or Auger electrons. The energy spectra of these electrons are then analyzed in a high-resolution magnetic or electrostatic spectrometer. The nature of the resulting spectrum depends on the type of excitation used.

In the initial work in electron spectroscopy x-ray excitation was used (*1*, *2*). The energy of the photoelectrons is equal to the energy of the incident photons minus the binding energy. Hence, the electronic energy levels of elements present in the sample can be determined directly and with great precision. All elements except hydrogen have core electrons whose energy levels are characteristic of the element even when it is combined with other elements to form complex molecular compounds. Sufficient sensitivity can be attained so that elements can be detected and studied even if they constitute only a small fraction of the sample material. Measurement of the relative intensities of spectral lines from different elements in a compound yields quantitative information about its composition. With an appropriate calibration (taking care of the variation in photoelectric yield with atomic number and other factors), quantitative analysis can be carried out with an accuracy of a few percent.

The electronic states are modified by the molecular environment of the atom. The amount of structural information that can be obtained by measuring the chemical shift of electrons in the core levels is limited in many cases by the resolution of the available instrumentation. We describe here some recent developments that have resulted in improved resolution based on crystal monochromatization of the x-rays.

For a solid sample, the electrons which form the ESCA spectrum come from a very thin layer near the surface, typically less than 50 angstroms in depth, with the depth of the layer depending on the energy of the photoelectron and the material. The method is therefore useful for the study of surface phenomena. The sensitivity attainable is such that a spectrum can be

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