

Letters to the Editor

Bacterial Activity in South Pole Snow Is Questionable

Carpenter, Lin, and Capone (6) collected surface snow at South Pole Station, warmed it in the laboratory to temperatures of -17 to -12°C , injected solutions of radioactive thymidine and leucine, and found evidence for bacterial metabolism. They argued that the bacteria would also be metabolizing in situ and thus over a vast area of the Antarctic continent. Here we question that conclusion, by considering the availability of liquid water.

South Pole Station has a continuous record of weather observations since 1956, including temperatures registered by a thermometer 2 m above the snow surface. The two warmest months, December and January, both have average temperatures of -28°C (15). The maximum temperature has exceeded -17°C on only 8 days in 46 years, and the all-time record high is -13.6°C (15). At the South Pole there is no daily cycle, but at more northerly locations on the Antarctic Plateau, under direct sunlight at noon the snow surface can briefly be as much as 3 degrees warmer than the near-surface air. However, the highest temperatures are usually experienced under overcast cloud, when snow and air temperatures are equal.

Three processes might produce liquid water in such cold snow, as follows.

(i) A curved ice surface has a slightly lower melting temperature than a flat surface. The surface snow grains at South Pole have radii of 30 to 100 μm (11), for which the melting temperature is lowered by only 0.001 to 0.002 degrees (14), making this effect negligible.

(ii) At temperatures close to 0°C , a quasiliquid layer (QLL) exists on the surface of a snow grain, because the surface energy of an ice-vapor interface slightly exceeds the sum of ice-liquid and liquid-vapor surface energies. The thickness of the QLL has been measured by several methods (4, 8–10) and also computed theoretically (7, 16). It can be 10 to 100 nm thick at -0.1°C but shrinks rapidly with decreasing temperature, to 0.1 to 1 nm at -10°C . Carpenter et al. misquoted Anderson (1) by a factor of 100, saying that he found a QLL of 50 nm at -10°C . In fact, the thickness shown in Anderson's figure is only 5 \AA (i.e., 1 to 2 molecular layers). They also cited Yershov (18) for evidence of 0.5 to 3% unfrozen water in permafrost at -10°C . However, that water is in the form of monomolecular layers between the lamellae of expandable clay minerals (2, 18), so it would be inaccessible to bacteria. In any case, Antarctic snow is very different from permafrost; it contains only 15 ppb of mineral dust (12).

(iii) The one process that can produce nonnegligible quantities of liquid water is lowering of the freezing point by solutes. Solutes are rejected from the ice lattice, so they become concentrated on the surfaces of snow grains, where they may create a thin liquid layer. The major solutes in Antarctic snow are H_2SO_4 , HNO_3 , NaCl , and HCl (13). In their relative abundances at South Pole, a freezing-point depression of 13.6 degrees requires a concentration of 2.7 M. Their concentration in bulk snow, 4 μM (13), implies a mass ratio (liquid/ice) of 1.5×10^{-6} if all the ions are partitioned into the liquid phase, a generous assumption (3). In ice, this acidic brine ($\text{pH} \approx 0$) is located in veins at three-grain junctions (17). In snow, it would be located in an annular groove around the neck where two grains have joined by sintering. In South Polar snow, the radius

of this neck is approximately one-half the grain radius (see Fig. 1 of reference 11). Assuming each grain contacts four other grains with radii of 30 to 100 μm , the width of the brine channel is 30 to 100 nm at -13.6°C ; the channel is smaller and saltier at lower temperatures.

Thus, liquid water in Antarctic snow is hidden in narrow crevices much smaller than the 500-nm-diameter cells shown in the scanning electron microscopy images (6), so the water is unlikely to be accessible to them, even if they could tolerate its high acidity and salinity. In the laboratory incubations, by contrast, a minimum of 0.2 ml of low-salinity liquid water was present, at least until the injected solutions froze. This is 50,000 times the amount of liquid water naturally present in the snow. The mass of snow in the 7-ml test tube, at the average density of surface snow at the South Pole, 0.34 g cm^{-3} (5), would be 2.4 g. The heat capacity of ice is 0.5 $\text{cal g}^{-1} \text{deg}^{-1}$; the latent heat of freezing is 80 cal g^{-1} . So, injection of 0.2 ml of liquid at 0°C would (by freezing) warm the snow from -12°C to the melting point, resulting in a slush which would then eventually refreeze after returning to the cold room. We suggest that the metabolic activity shown for South Polar bacteria, in snow that was significantly altered in the laboratory, does not provide evidence for metabolism in situ.

Bacteria undoubtedly exist in South Polar snow; they can be carried by the wind, as are other atmospheric aerosols. But within 15 years they are buried to a depth of 3 m, where the temperature is close to -50°C year-round (5), and even during their brief sojourn at the surface, water is exceedingly scarce.

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Authors' Reply

We appreciate the thoughtful comments of Drs. Warren and Hudson regarding our AEM paper "Bacterial Activity in South Pole Snow." Warren and Hudson largely base their criticism of our research on their theoretical calculations of the physics of water and ice. Their main point concerns the predicted lack of sufficient liquid water in the snow to allow bacterial metabolism at the subzero temperatures at which our experiments were conducted. They also suggest that the injection of our isotopic tracer may have provided sufficient liquid water to cause melting of the snow core and to permit the observed uptake.

When we collected snow at the South Pole in 1999, the snow temperature in the upper 5 cm was -15°C , and this guided our incubation temperatures, which ranged from -12 to -17°C . We are aware of the relative scarcity of liquid water at these temperatures, and after noting measurable uptake of DNA and protein precursors using radioisotopes in the 1999 field season we were ourselves skeptical about the results and repeated our observations, using more precise techniques and more rigorous controls in the 2000 field season.

Warren and Hudson suggest that our injections resulted in a "slush which would then eventually refreeze." We can state that at no time was visible melting in the snow cores obvious or evident. If warming of the snow by the addition of 200 μl of

tracer, itself only slightly above 0°C , resulted in enhanced bacterial activity, one might have expected an initial spike in uptake followed by a flattening out of uptake as the snow refroze, within minutes in the cold room, particularly given the relatively small size of the sample.

Rather, in the majority of incubations we observed apparent linearity in uptake over relatively long time periods (16 to 24 h). Furthermore, no uptake was observed in either TCA or -70°C controls. The linearity in uptake over extended incubations plus the lack of uptake in controls strongly suggest bacterial metabolism.

In our paper, we cite several other observations of apparent metabolism at below 0°C . There have been several related recent observations as well. About the same time as our report came out, Rivkina et al. (3) reported that [^{14}C]acetate incorporation into lipids in permafrost soils occurred down to -20°C and could account for doubling times of 20 days at -10°C to 160 days at -20°C . More recently, Christner (1) reported that bacterial cells which he froze into distilled water and held for 50 to 100 days at -15°C incorporated DNA and protein precursors, thus indicating metabolic activity. No incorporation was seen at -70°C .

While we have provided empirical observations in support of our conclusions, we do not yet understand the biological or physical mechanisms that allow this phenomenon to occur. Warren and Hudson largely focus on the theoretical physics of liquid water at subzero temperatures. However, biological mechanisms may also be operative. Psychrophilic microorganisms may have developed physiological and/or biochemical adaptations which permit metabolism at subfreezing temperatures (2), for instance through modification of the lipid composition of their cell membrane, by production of cryoprotectant molecules, and, possibly, through their own metabolic heat production.

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