

**A Habitat for Psychrophiles in Deep Antarctic Ice**

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**ABSTRACT**        Microbes, some of which may be viable, have been found in ice cores drilled at Vostok Station at depths down to ~3600 m, close to the surface of the huge subglacial Lake Vostok. Two types of ice have been found. The upper 3500 m comprises glacial ice containing traces of nutrients of aeolian origin including sulfuric, nitric, methanesulfonic, and formic acid, sea salts, and mineral grains. Ice below ~3500 m comprises refrozen water from Lake Vostok, accreted to the bottom of the glacial ice. Nutrients in the accretion ice include salts and dissolved organic carbon. There is great interest in searching for living microbes and especially for new species in deepest Antarctic ice. I propose a habitat consisting of interconnected liquid veins along three-grain boundaries in ice in which psychrophilic bacteria can move and obtain energy and carbon from ions in solution. In the accretion ice, with an age of a few  $10^4$  yr and a temperature a few degrees below freezing, the carbon and energy sources in the veins can maintain significant numbers of cells/cm<sup>3</sup> metabolizing but not multiplying. In the  $4 \times 10^5$  yr-old colder glacial ice, at least 1 cell/cm<sup>3</sup> in acid veins can be maintained. With fluorescence microscopy tuned to detect NADH in live organisms, motile bacteria could be detected by direct scanning of the veins in ice samples.

It seems to be a fundamental law that, wherever microbial life can survive, it will be found to exist (1). Microbes are amazingly hardy: viable specimens of a spore-forming bacillus and of an extremely halophilic bacterium have been found in an inclusion in a 250-million-year-old salt crystal (2). A bacterial spore has been revived, cultured, and identified from 40-million-year-old amber (3). Microbial life has been found at depths down to several km in the earth's crust (4), and viable bacterial populations have been discovered at Pacific Ocean sites to depths >500 m in sediments (5). Bacteria can grow and reproduce at temperatures  $\leq 0^{\circ}\text{C}$  in high-altitude cloud droplets (6). Microbial communities survive on wind-deposited sediment particles within liquid water inclusions in permanently ice-covered Antarctic lakes (7). Before Antarctica developed its permanent ice cap some 14 million years ago (8), microbes may well have existed in its continental crust, and their descendants may live in subglacial rock crevices, lakes and sediments, and perhaps even in the glacial ice itself. Far less is known about psychrophilic (cold-loving) bacteria than about thermophiles, and it is not even certain yet whether life on earth originated in a hot or cold environment (9). It seems worthwhile to devise an *in-situ* search for live microorganisms in ancient polar ice. In this paper we propose a habitat that will sustain a small population of psychrophilic bacteria in deep Antarctic ice in the absence of sunlight or oxygen, at pressures up to 400 bars, at temperatures well below  $0^{\circ}\text{C}$ , and in strongly acidic or saline solutions.

## MATERIALS AND METHODS

**Evidence for Microbial Life in Deep Antarctic Glacial Ice.** Consider first airborne sources of microbes blown onto snow and compacted into the polar ice. Working with filtered ( $0.2\ \mu\text{m}$ ), melted ice from depths down to 2750 m in the ice core from Vostok Station (east Antarctica), Abyzov *et al.* (10) studied the concentration and morphology of microbes stained with a fluorescent dye. Concentrations as a function of depth (and thus of age) ranged from  $\sim 10^3$  to  $\sim 10^4$  cells/cm<sup>3</sup> and correlated with dust concentration, which suggests that they were deposited in the snow preferentially during glacial periods when the flux of dust and the wind speed were greatest. In addition, they used consumption of a <sup>14</sup>C-labeled protein hydrolysate as a crude measure of cell viability. Superimposed on a general decline of radiocarbon uptake as a function of depth of origin of the sample (from 0.0044  $\mu\text{g/l/hr}$  at 1665 m to 0.0002  $\mu\text{g/l/hr}$  at 2750 m), they found a very low uptake in cells from depths corresponding to cold periods.

Willerslev *et al.* (11), using PCR amplification of fragments of the eukaryotic 18S rRNA gene, identified a diversity of fungi, plants, algae, and protists extracted from 2000- and 4000-year-old ice-core samples from North Greenland. They did not test them for viability.

The possibility of finding subglacial sources of microbes is now of great international interest. In 1974-75 airborne radar mapping (12) resulted in the discovery of a huge subglacial lake under nearly 4 km of ice near Vostok Station. Recently seismic sounding and radar mapping plus radar altimetry (13) showed that the lake water is  $\sim 650$  m deep at the deepest end and has a density consistent with fresh water. Workshops in St. Petersburg (14), Washington, D.C. (15), and Cambridge, England (16) were devoted to discussions of prospects for drilling into the lake. At the St. Petersburg workshop (17) Petit reported that the bottom  $\sim 100$  m of a 3623-m deep Vostok ice core that reached a depth  $\sim 120$  m above the lake consists of refrozen lake water, called "accretion ice", whose crystals range in size from  $\sim 0.1$  to 1 m. The accretion ice is believed to extend another 100 m from the bottom of the core to the lake surface. D.c. electrical conductivity measurements and ionic composition measurements (see Table 1) have since shown

that the accretion ice appears not to be acidic, presumably reflecting the composition of the lake water. At the Cambridge workshop Priscu *et al.* (17) reported detection in melted accretion ice of  $3 \times 10^3$  to  $4 \times 10^4$  bacterial cells/ml, which were not culturable and did not incorporate radiocarbon during 52 hr in air at 1 bar. Karl *et al.* (18) also reported evidence for microbial life in their sample of melted accretion ice: the presence of dissolved organic carbon (7  $\mu\text{M}$ ) including  $\sim 100$  pg/l of lipopolysaccharides, and sluggish uptake of  $^{14}\text{CO}_2$  into biomolecules. A small fraction of the microbes in the accretion ice is viable, but they are probably not capable of reproducing. In contrast to the atmospherically deposited microbial life found at depths down to 2750 m in the Vostok ice core (10), life in Lake Vostok might have emerged from sediments, from cracks in bedrock, or even from thermal vents, and have migrated upward into the accretion ice. Without direct measurements, this is only speculation. No one has yet observed living, motile organisms *in situ* in unmelted glacial freshwater ice.

**Liquid Veins in Vostok Ice.** Figure 1 shows a habitat that I argue can provide microbes in polar ice with the three ingredients essential for life – water, energy, and carbon. The high d.c. electrical conductivity of cold polar ice had long provided indirect evidence for aqueous acid solutions concentrated in veins (19). Aqueous veins at the linear junctions of three ice crystals in temperate glaciers are now known to form a continuous network, and laboratory experiments have clarified their geometry (20). Using an SEM equipped with a cold stage and an energy-dispersive X-ray microanalyser, Wolff *et al.* (21) showed that sulfur was concentrated in veins and was undetectable in the bulk of the ice. They estimated that in a region where a vein roughly  $1 \mu\text{m}^2$  in cross section intersected the surface of an ice sample, the concentration of sulfuric acid was about 2.5 M. From the mean crystal size of their sample, they estimated that the meltwater concentration of acid was  $\sim 7 \mu\text{M}$  and thus that most, and perhaps all, of the acid was concentrated in veins. They later showed that hydrochloric acid can also concentrate in veins (22). Recently, using a micron-size laser beam, Fukazawa *et al.* (23) inferred from Raman spectra that aqueous solutions of sulfuric and nitric acid are concentrated in veins in Antarctic ice as  $\text{HSO}_4^-$  and  $\text{NO}_3^-$  ions. The evidence is thus strong that all three of the major mineral acids do collect in veins.

The explanation for how foreign ions migrate into veins has to do with the fact that micron-size droplets of acids and sea salts deposited as aerosols are essentially insoluble in ice crystals. Coarsening and recrystallization of deep ice, in response to the shear stress induced by the weight of the overlying ice, take place by migration of grain (crystal) boundaries, which sweep through and scavenge the droplets. A droplet first encounters a two-grain boundary and then diffuses into veins at the edges of the boundary. The driving force for concentrating in a vein rather than in a planar boundary is the greater decrease in freezing point of a vein due to its curvature. The eutectic temperatures of acids such as sulfuric acid (-73 C), hydrochloric acid (-88 C), nitric acid (-43 C), methanesulfonic acid (-75 C) and formic acid (-49 C) are low enough to ensure that, in thermodynamic equilibrium, they will exist as liquids in veins within solid pure ice. It seems unlikely, however, that solid salt grains intersected by a grain boundary will end up in aqueous solution in veins, and certainly mineral dust grains will not.

To develop a quantitative model, consider ice at temperature  $T$  in which an aqueous solution of acids and other soluble impurities is entirely located in veins of uniform radius  $r_{\text{vein}}$ , where  $r_{\text{vein}}$  is the radius of an equivalent cylinder (not the radius of curvature of the vein interface, which is convex inwards). Following Frank (24), I model polycrystalline ice as consisting of grains of semi-regular truncated octahedra of diameter (between square faces)  $D$ .

The volume of a grain is  $D^3/6$  and it has 36 edges each of length  $\sqrt{2}D/4$ , each shared with three other grains. The total fractional volume in veins is

$$f = (6\pi\sqrt{2}) (r_{\text{vein}}/D)^2 \quad (1)$$

The molar concentration of an aqueous solution in veins is

$$C_{\text{vein}} = C_{\text{bulk}} \rho_{\text{ice}}/f = C_{\text{bulk}} \rho_{\text{ice}} (D/r_{\text{vein}})^2/(6\pi\sqrt{2}) \quad (2)$$

where  $C_{\text{bulk}}$  is measured in meltwater, and  $\rho_{\text{ice}} = 0.915 \text{ g/cm}^3$ .  $C_{\text{vein}}$  is determined by the free-energy requirement that in equilibrium the two-phase system, pure ice + aqueous solution, be on the freezing line of its phase diagram. Thus,  $C_{\text{vein}}$  is a function of ice temperature that can be found by judicious use of tables that give the depression of the freezing point as a function of concentration for various solutions (25). Once  $C_{\text{vein}}$  is known, vein diameter is found from

$$d_{\text{vein}} = 2D [(C_{\text{bulk}}/C_{\text{vein}})\rho_{\text{ice}}/6\pi\sqrt{2}]^{1/2} \quad (3)$$

**Ionic Composition.** Table 1 shows the data from which the molarity in veins and the vein diameter were calculated as a function of temperature of Vostok ice. The bulk concentrations of ions in Vostok ice at a depth  $\sim 3300$  m (corresponding to an age of  $\sim 4 \times 10^5$  yr) were taken from unpublished tables provided by M. Legrand (see also (26)), and the concentrations for accretion ice were provided by Priscu *et al.* (17) and Karl *et al.* (18). The ionic activities of each of the ion species contribute additively to depression of the freezing point (25,27). The balance of positive ions in the ancient ice is provided by  $\text{H}^+$  (28), whereas the composition of the accretion ice appears to be accounted for by salts and not to require  $\text{H}^+$  ions.

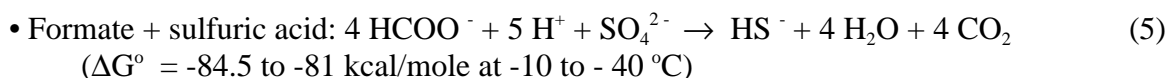
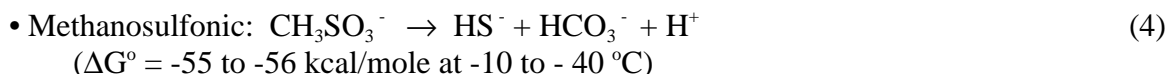
## RESULTS

**Composition and Size of Veins.** Figure 2 shows results for ancient Vostok ice and Fig. 3 shows results for accretion ice. The accretion ice extends from  $\sim 3538$  m to the lake surface at  $\sim 3750$  m, and the Vostok core retrieved ice down to a depth of 3623 m. From 3538 to 3608 m the ice contained numerous mud inclusions  $\sim 1$  mm in diameter, presumably incorporated from materials along the lake margin. Below 3608 m the ice is very clear and is thought to have accreted from freezing of lake water to the base of the ice sheet as it passed over the lake. The temperature gradient in the lowest few hundred meters of ice is taken to be  $0.02 \text{ }^\circ\text{C/m}$ . The abscissa in Figs. 2 and 3 gives freezing point depression relative to the pressure melting point, found from the relation  $dT/dP = 0.0074 \text{ }^\circ\text{C/bar}$  to be  $-2.8 \text{ }^\circ\text{C}$  for pure ice just above the lake surface. The mean grain size of the glacial ice was taken to be  $D = 2$  cm and that of accretion ice was taken to be  $D = 30$  cm (14). Since vein diameter scales linearly with  $D$ , it is easy to find  $d_{\text{vein}}$  for other choices of  $D$ . The main difference between the two environments, other than differences in impurity concentrations and differences in grain size, is that ancient ice scavenges only acids, not salts, into veins, whereas accretion ice freezes from lake water in which all the ions are dissolved. Figures 2 and 3 show that at temperatures down to a few degrees below the pressure melting point, vein diameter is easily large enough to accommodate motile micron-size bacteria. With decreasing depth the temperature decreases, reaching  $-56 \text{ }^\circ\text{C}$  at the surface, and

the vein diameter decreases to a few microns, with a concomitant increase in molarity of the acids.

**Energy Source.** For bacteria in veins, aqueous sulfuric acid and nitric acid are the main electron acceptors. Methanosulfonic acid (MSA,  $\text{HCH}_3\text{SO}_3$ ), formic acid ( $\text{HCOOH}$ ), and acetic acid ( $\text{CH}_3\text{COOH}$ ) will also be swept into veins and will provide energy and carbon for biosynthesis. Dissolved organic carbon is present in Vostok ice (Table 1), but its composition and location are not known.

We have shown that segregation of acids into veins raises the concentrations of nutrients and of dissolved carbon as much as a million-fold, depending on ice temperature and crystal size, thus providing channels of aqueous solution in which microorganisms can live and extract energy. For those microbes that find themselves in veins, maintenance of life seems likely, given the demonstrated adaptability of life to extreme environments. Equations 4 and 5 provide examples of reactions that provide both energy and carbon: decomposition of MSA at a concentration of  $\sim 0.1$  M in ancient ice, and formic acid at a lower composition together with sulfuric acid. The substantial changes in free energy are essentially independent of temperature and concentration in the ranges of interest. Formic + nitric acid would also work. Formate is interesting, because some bacteria are known to sense and move towards formate under anaerobic conditions.



Hyperacidophiles are known (29,30) that can exist at  $\text{pH} \approx 0$ . A fungus, *Acontium velatum*, has been cultured in 2.5 M  $\text{H}_2\text{SO}_4$  (31). *Thiobacillus*-like bacteria are able to grow (29) both at very low pH and at temperatures below 0° C in a mine in Greenland at 83° N. In order to survive in veins in the deep ice, the microbes depicted in Fig. 1 must be able to withstand temperatures below 0 °C, without oxygen, in the dark, and at a pressure up to 400 bars. In addition, those in strongly acidic veins must consume energy to operate a strong proton pump or have a low proton membrane permeability in order to maintain their interior at nearly neutral pH. Those in accretion ice at subfreezing temperature will have to tolerate salt at 1 to 2 M.

**Carbon Supply Limits Population Size.** The age of the glacial ice at  $\sim 3300$  m in the Vostok core is  $\sim 4 \times 10^5$  yr. A freezing rate of several mm to several cm per year (15) for the accretion ice implies that it is much younger, reaching only a few  $10^4$  yr at 100 m above Lake Vostok. Assuming that the source of energy and carbon is MSA, and taking a bulk molarity of 0.084  $\mu\text{M}$  for MSA in liquid veins, we use data from (32) and (6) to estimate the bacterial population size that could be maintained for times of  $4 \times 10^5$  yr in glacial ice and for  $10^4$  yr in accretion ice. We assume that microbes in veins in ice have a composition  $\text{CH}_{1.8}\text{O}_{0.5}\text{N}_{0.2}$ , a cell volume of 0.084  $\mu\text{m}^3$ , and a dry mass of 30 fg (6).

In a nutrient-poor soil, Morita (32) found that  $\sim 3 \times 10^{-5}$  g carbon per g biomass carbon per hour at 15 °C was needed to prevent microbial carbon loss during incubation. (This is about 1/1000 the amount typically consumed by biomass in laboratory cultures.) Then for  $4 \times 10^5$  yr

the MSA in glacial ice would maintain a population per  $\text{cm}^3$  of ice of  $\sim 7$  cells that are biologically functional but not multiplying. In  $10^4$ -year-old accretion ice, more than  $10^2$  cells/ $\text{cm}^3$  would be found. Since the ice temperature is at least  $20^\circ\text{C}$  colder than that of the biomass studied by Morita, the lower metabolism rate in ice would permit an even larger population to survive.

In their study of bacteria collected from supercooled cloud droplets, Sattler *et al.* (6) measured an uptake rate at  $0^\circ\text{C}$  of  $\sim 2 \times 10^{-21}$  mol thymidine  $\text{hr}^{-1}$   $\text{cell}^{-1}$ , a rough measure of cell growth. Equating MSA to thymidine as a source of carbon, the thymidine uptake rate leads to estimates of 10 and 400 cells/ $\text{cm}^3$  for the sustainable populations of bacteria in ice of ages  $4 \times 10^5$  and  $10^4$  yr respectively. These estimates are very similar to those based on Morita's data.

If the dissolved organic carbon listed in Table 1 can also be incorporated into cells, a still larger population could be maintained.

## DISCUSSION

**Searching for Living Bacteria in Veins.** By exploiting their confinement to liquid veins, one could easily search for bacteria *in situ*. In collecting an ice core from great depth where the ice is relatively warm, it would be desirable to preserve bacteria in the veins in an anaerobic and thermal environment similar to that to which they were acclimated. Since vein size in equilibrium depends on ice temperature, one would have to maintain the ice samples at a roughly constant temperature during shipping and study. In ancient ice with  $D = 2$  cm, the length of vein that would have to be scanned for bacterial cells would be only  $\sim 2$  cm per  $\text{cm}^3$  of ice. It would be easy to scan  $10 \text{ cm}^3$  or more in a low-power microscope, using phase contrast or fluorescence microscopy. In principle one could introduce a dye specific to certain molecules into liquid veins. Saito (33) has shown that 8-anilino-naphthalene 1-sulfonate will indicate the presence of cells; sulfofluorescein diacetate can be used to detect certain enzymes such as esterase; and ethidium bromide can identify nucleic acids. It would be easier, however, to avoid dyes by using 366-nm light to search for NADH, which marks the existence of a living cell by fluorescing at  $\sim 440$  nm (34,35). With  $10^3$  to  $10^6$  NADH molecules per living cell, the signal from a single cell would be strong. In a dead cell, NADH is oxidized to NAD, which does not fluoresce.

In accretion ice, with  $D = 30$  cm, veins would be very large in diameter, but the probability of finding a vein in a random sample would be very low. The best strategy would be to use a stereoscopic microscope to search for a portion of the core that contains a vein before preparing a sample for fluorescence microscopy. The risk of contamination by external organisms is far lower with the vein technique than with techniques that require melting and filtering of ice samples.

**A Subglacial Lake at the South Pole.** In their analysis of airborne radar surveys of Antarctica, Siegert *et al.* (36) found evidence for at least 77 subglacial lakes, one of which has subsequently proved to be only a few km from the South Pole Station (D. Blankenship and D. Morse, unpublished results). The idea of collecting water, ice, and sediments from the South Pole subglacial lake has both advantages and disadvantages compared with sampling Lake Vostok. Being only a few km in size, the South Pole lake and the surrounding bedrock would be easy to characterize by radar mapping and seismic sounding, and being close to the infrastructure of the South Pole Station, researchers would have easy access to modern laboratory facilities for study of samples to be recovered by sterile drilling techniques. One could, for example, use a

pressure-resistant waterproof fluorescent camera for assaying ice for living cells as a drill is being lowered toward the lake.

**Greenland Glacial Ice.** No subglacial lakes exist in Greenland. Two cores have been drilled to bedrock at Dome Summit, Greenland (37). The grain size reaches 2 to 3 cm in the bottom 80 m, at depths below 2950 m. The temperature and age near bedrock are  $\sim$ -9 °C and  $\sim$ 250,000 yr respectively. The impurities in Dome Summit ice are generally not acidic (unlike Vostok ice), and there is somewhat more formate and acetate than at Vostok. D. C. electrical measurements show that the conductivity (a measure of  $[H^+]$  and of conducting veins) is much lower than in Antarctic ice. It thus seems unlikely that there is a network of liquid veins hospitable to psychrophiles in these cores.

**Concluding Comments.** The liquid vein habitat provides microbes with intimate access to water, energy, and carbon. By contrast, metabolism of microbes encased in solid ice must overcome a seemingly insuperable barrier: diffusion of nutrients through a solid is orders of magnitude slower than through a liquid. The advantages of scanning liquid veins in solid ice with fluorescence microscopy are that contamination would be ruled out, that living microbes could be distinguished from dead ones, and that a concentration of as low as 1 cell per  $cm^3$  could be detected in veins despite a high background of dead matter not located in veins. We do not yet know of any species capable of tolerating *all* of the following conditions: no sunlight, no oxygen, pressure of  $\sim$ 400 bars, temperature below 0 °C, and a highly acidic or saline medium. It is thus likely that microbes living in liquid veins in ancient ice would be different from any yet known. Another interesting outcome of a search for living microbes in liquid veins in ice of known age would be to determine from the population density whether oligotrophic psychrophiles have developed an unusually high metabolic efficiency that permits long-term survival on very limited extracellular energy resources.

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Table 1. Major Ions in Vostok Glacial Ice at Depth ~3300 m and in Accretion Ice at ~3600 m

Ion or compound	Glacial ice ( $4 \times 10^5$ yr)		Accretion Ice (filtered)	
	ng/g	Bulk molarity	ng/g	Bulk molarity
SO <sub>4</sub> <sup>2-</sup>	122	1.24 μM	38	0.39 μM
NaCl in solid grains	58.2	1	--	--
NO <sub>3</sub> <sup>-</sup>	18.6	0.3	10	0.164
Na <sup>+</sup>	--	--	29	1.26
Cl <sup>-</sup>	5	0.14	37.6	1.06
CH <sub>3</sub> SO <sub>3</sub> <sup>-</sup>	7.99	0.084	--	--
HCOO <sup>-</sup>	0.95	0.02	--	--
CH <sub>3</sub> COO <sup>-</sup>	0.77	0.013	--	--
Mg <sup>2+</sup>	--	--	7.6	0.32
Ca <sup>2+</sup>	--	--	9.7	0.24
acidity in bulk	--	~2	--	~0
DOC* (18)	?	?	?	~7
<u>DOC* (17)</u>			~500	?

\* dissolved organic carbon of unknown composition

### Figure Legends

1. Microbial habitat consisting of solid ice grains (approximated by truncated semi-regular octahedra) bounded by liquid veins (not to scale). Two microbes are depicted as living in the vein of diameter  $d_{\text{vein}}$  surrounding a single grain of diameter  $D$ .
2. Relationships among molarity of impurities in aqueous solution, vein diameter, and depression of freezing point below pressure melting point, for the composition of impurities in glacial ice. Also shown is approximate height above Lake Vostok. For arbitrary grain size, vein diameter scales as grain size.
3. Relationships among molarity, vein diameter, and depression of freezing point for the composition of impurities in accretion ice, with  $D$  taken to be 30 cm.

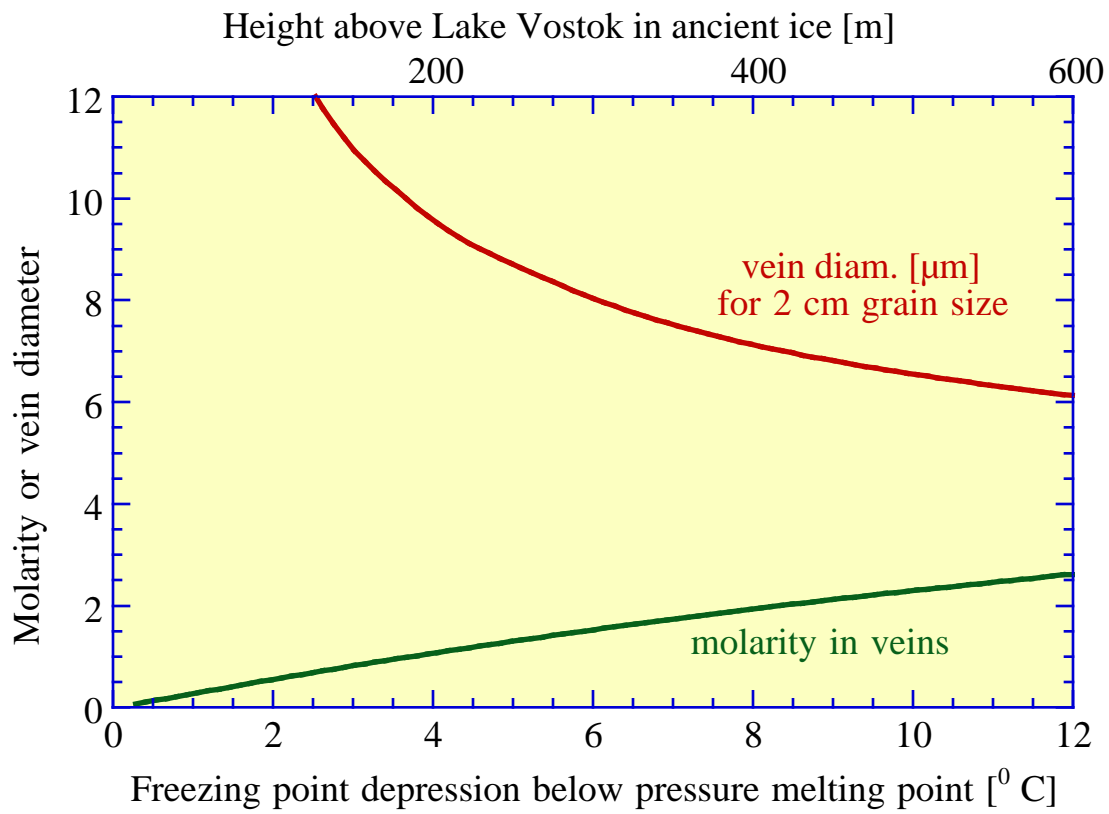


Figure 2

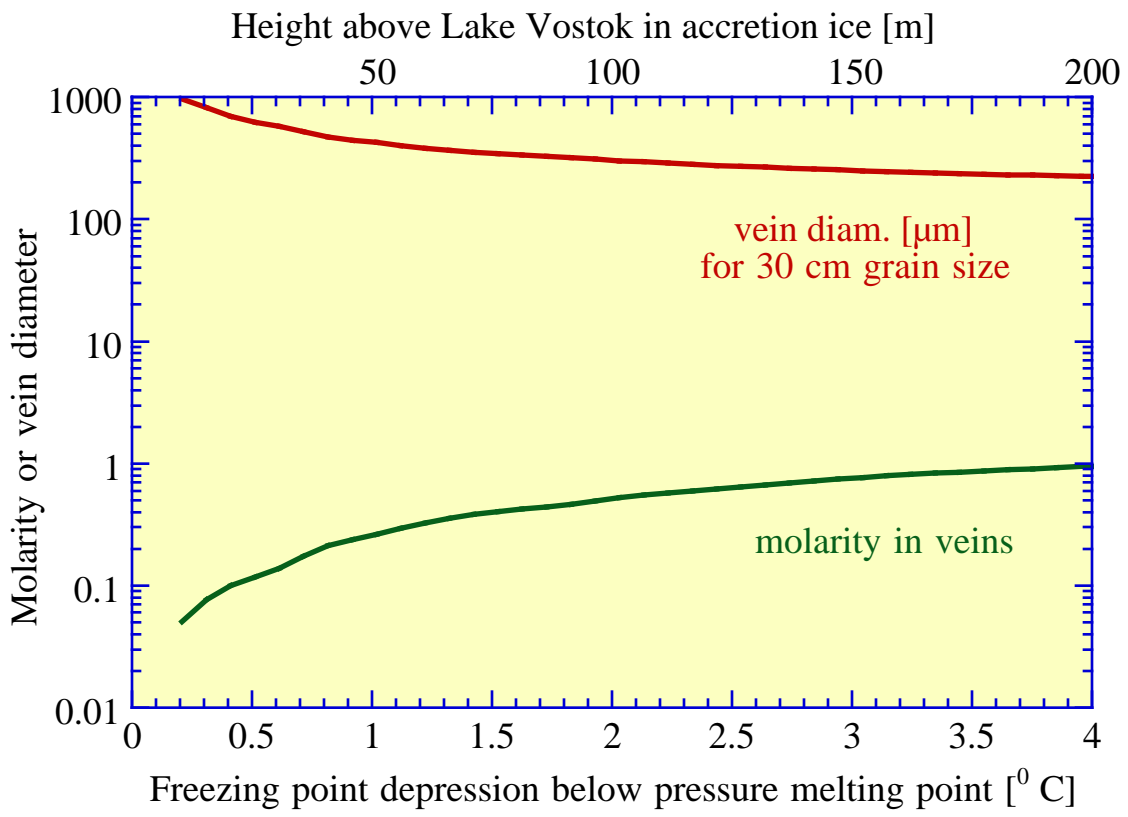


Figure 3