

Probably some microorganisms divide if only because of the significant degree of microbial activity at temperatures below 0°C. But for the most part it appears unlikely. In any case, the microorganisms in permafrost are on the very edge of survival, and their ability to get enough water for division from thin layers of unfrozen water is uncertain. Accordingly this means that some live (without subdivision), if not forever (since accidents could befall them), at least for extraordinarily long times.

### Thousands of years of life?

Moss remains alive yet dormant while frozen for 40,000 years in the permafrost of the Kolyma Lowlands of northeastern Siberia according to Gilichinsky (1994). It is established that organisms resume metabolic activity upon thawing after being frozen for tens of thousands and maybe millions of years. The seeds of *Lupinus arcticus* found in permafrost 15,000 years old are able to grow (Gilichinsky, 1994), as well as microorganisms from Antarctic ice aged 10,000-13,000 years (Abyzov et al., 1979). According to Gilichinsky there are three types of life-forms found in permafrost: active ones that eke out a living in thin water layers between grains of soil and ice, viable but inactive forms that are frozen in suspended animation, and the frozen carcasses of microbes that gave up.

If microorganisms in permafrost occur that are alive but not able to divide, what mechanism keeps them alive? First, protein and DNA stability must be considered (Dean, 1978). The proteins of cells are frequently not stable; many proteins and enzymes have an extremely short (in minutes) period of life. At the same time, studies of spontaneous thermal disintegration of some enzymes have shown that maximum half-disintegration period is about 12,000 days (Segal et al., 1969). But that is orders of magnitude less than the survival times we seek to explain.

Decreasing water content inside a cell causes transfer to anabiosis, a condition in which it is believed (from experiments) that organisms may have no chemical and biological activity (Hinton, 1968; Clegg, 1973; Goldovsky, 1986). On the other hand, cell structures in anabiosis would be affected by temperature changes, radiation, and pressure. The cytoplasm of a cell is not completely frozen, very likely it is not frozen at all at the temperatures of -2 to -5°C commonly found in permafrost. In anabiosis the cytoplasm is not in a state of thermodynamic equilibrium. So tissue is probably slowly destroying itself even inside permafrost. There is also a spontaneous thermal disintegration

because the temperature is far from absolute zero. It must also be remembered that freezing soils, themselves, are rarely in a state of equilibrium. Virtually always there are gradients of temperature and potential, such that there is usually a very slow translocation of ice and water underway. Over a 'geological' time scale the microstructure of the materials of the permafrost and the distribution of ice goes through continuous modifications (Williams, 1988) because it is very close to its melting point.

The molecular basis of thermal stability of biological materials is a significant, as yet unsolved, problem (Baker and Agard, 1994; Jaenicke et al., 1996). In general, the stability of a protein is defined as the free energy change,  $G$ , for the reaction folded-unfolded under physiological conditions. Most proteins are characterized by values of  $G = 5-15$  kcal mol<sup>-1</sup>. In terms of thermodynamics, the fraction of residues in random coil regions divided by the fraction of residues in ordered regions ( $k$ ) would appear as the following:

$$k = \exp(-G/RT) \quad (2)$$

where  $G$  is the free energy change for the thermal transition of one residue from an ordered region to a random coil one, kcal mol<sup>-1</sup>;  $T$ —temperature, °K;  $R$ —gas constant, ~0.001989 kcal mol<sup>-1</sup> °K<sup>-1</sup>.

Another similar expression (Regel et al., 1974) could probably be written for an approximate estimation of time of existence  $t_e$  of proteins:

$$t_e = t^* \cdot \exp(G/RT) \quad (3)$$

where  $t^*$  - period of temperature fluctuations of molecules, normally about 10<sup>-12</sup> – 10<sup>-13</sup> s. Calculations on the basis of (3) show that even for  $G=30$  kcal mol<sup>-1</sup> the time of existence of molecular connections is less than 300 years. Also for the period of temperature fluctuations of molecules 10<sup>-8</sup> – 10<sup>-9</sup> sec and  $G=20$  kcal mol<sup>-1</sup> the time of existence of molecular connections is less than one year. Maximal value of energy of activation described is about 45 kcal mol<sup>-1</sup>; normally it is much less (Alexandrov, 1975). These calculations are very approximate and extreme, but they do show how unstable proteins and DNA are. Small, but specific changes of temperature, unpredictable influence of radiation, chemical reactions in the permafrost environment and unfrozen solution in the cell are certainly other processes leading to destruction of biological material.

Thus, live microorganisms in permafrost apparently have special mechanisms of repair of cell structures otherwise prone to collapse because of the