

## LIFE WITHOUT WATER

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Anhydrobiosis, or "life without water" is commonly demonstrated by a number of plants and animals. Organisms that can become anhydrobiotic have been known and studied since Leewuenhoek first described them in 1702. These organisms have the capacity to lose all their body water, remain dry for various periods, and then be revived by rehydration. They can be divided into two distinct groups, those organisms which become anhydrobiotic at some early developmental stage such as fungal or bacterial spores, seeds, chironomid larvae, or encysted gastrulae of the brine shrimp *Artemia salina*; and those organisms which can be dried out as adults, such as soil or moss-dwelling rotifers, nematodes, and tardigrades. The latter category is useful for studying characteristics of anhydrobiotic organisms and the mechanisms of survival since the anhydrobiotic state is not complicated by developmental processes.

While in the anhydrobiotic state, these organisms become highly resistant to several environmental stresses such as extremely low temperatures (0.05 K), elevated temperatures (around 100° C), ionizing radiation, and high vacuum ( $10^{-6}$  to  $10^{-9}$  Torr). Their survival is increased by storage in the dark and the absence of O<sub>2</sub>, suggesting that adventitious chemistry may play a role in their eventual failure to revive after long periods of storage.

The question of whether anhydrobiotic organisms show metabolism has not been directly answered. One experiment has shown a very low level of O<sub>2</sub> uptake at a relative humidity of 25%. However, these organisms can survive temperatures as low as 0.05 K, at which temperature metabolic processes must be at a virtual standstill. Calculations on the hydration of proteins show that at the hydration levels commonly experienced by anhydrobiotic organisms, all the water is immobile and there is about one water molecule/polar group of the protein. At such hydrations, it is difficult to conceive how enzymatic processes might proceed. Thus, anhydrobiotic organisms most likely have suspended metabolism.

Since water is commonly thought to be essential for life, a major question is: How do anhydrobiotic organisms survive the almost total loss of water? Our laboratory has shown that during the slow drying essential to survival, nematodes manufacture large quantities of the disaccharide trehalose. More recently, other laboratories have shown that the ability of yeast to survive drying is strongly correlated with trehalose content, and that during repeated cycles of hydration and drying bacterial spore resistance to drying decreases as the trehalose content decreases. A search of the literature reveals that many anhydrobiotic organisms make large quantities of trehalose or other carbohydrates.

Further experiments in our laboratory have shown that trehalose is able to stabilize and preserve dry microsomes of sarcoplasmic reticulum and artificial liposomes. With a number of physical studies we have demonstrated that trehalose and other disaccharides can interact directly with phospholipid headgroups and maintain membranes in their native configuration by replacing water in the headgroup region. Our most recent studies show that trehalose is an effective stabilizer of proteins during drying and that it does so by direct interaction with groups on the protein.

If life that is able to withstand environmental extremes has ever developed on Mars, one would expect such life to have developed some protective compounds which can stabilize macromolecular

structure in the absence of water and at cold temperatures. On Earth, that role appears to be filled by carbohydrates that can stabilize both membrane and protein structures during freezing and drying. By analogy with terrestrial systems, such life forms might develop resistance either during some reproductive stage or at any time during their adult existence.

If the resistant form is a developmental stage, the life cycle of the organism must be completed within a reasonable time period relative to time when environmental conditions are favorable (on Mars or Earth) so that a new resistant developmental stage can be produced. This would suggest that simple organisms with a short life cycle might be most successful.

Anhydrobiotic organisms (either at some developmental stage or as adults) rapidly metabolize excess trehalose when they are rehydrated. Thus, adult organisms that undergo repeated cycles of rehydration and drying must spend sufficient time in the rehydrated and dehydrating periods to re-synthesize trehalose stores in order to survive another drying cycle.

Other environmental factors that favor prolonged survival in the anhydrobiotic state are the absence of oxygen and light.