Series

Living in a physical world
IX.
Making and maintaining liquid water

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1. Introduction

Metabolically active organisms contain water in its liquid phase – I believe no exceptions are known. Life's domain consists of the intersection of the circumstances under which liquid water will persist and those at the earth's surface – except as we artificially maintain some bit of that domain elsewhere. No single phase of a single compound so characterizes the conditions necessary for life. Yes, a few ice crystals can often be tolerated, usually if extracellular. And water vapour can play some useful roles – in reducing evaporation, as a condensable resource, perhaps for producing the density variations that permit free convection. But liquid water is crucial to life as we know it. In a once well-known book, *The Fitness of the Environment*, Lawrence Henderson (1913) assigns a definitional rather than merely a facilitating role to the particular and peculiar physical and chemical properties of liquid water. The specifics of his argument still, one might say, hold water, even if their context now strikes the reader as offensively teleological and tautological and fails to persuade – at least as we contemporary biologists understand the idea of fitness.

Water abounds on earth, and most of that water will be in the liquid phase under conditions typical of its surface. But life perpetually pushes against environmental limits – in particular, against its abiotic barriers. Managing where water is minimally accessible can provide temporary refuges, open otherwise unexploited regions, and so forth. In all too many places, temperatures sometimes fall to levels at which water prefers its solid phase. Nor can life ignore its gaseous phase. While in few habitable places do temperatures exceed water's normal boiling point, vaporization occurs in virtually all terrestrial habitats.

So familiar is that last point that a subtle peculiarity of our immediate world can escape our notice. Enclose a dish of some volatile liquid in an air-filled container and keep the whole thing in a dark place at a constant temperature. Evaporation will proceed until the gas phase contains vapour at its saturation partial pressure, at 100% relative humidity for that substance. But atmospheric air, even over large bodies of fresh water, rarely reaches 100% humidity (or 97%, when equilibrated above seawater). Temperature variation, convection, and wind create innumerable opportunities for condensation. And thus we hang out our washing even on overcast days, confident that it will dry in the air. Nonetheless, equilibration can and does occur. For instance, the air within soil most often contains water at full saturation pressure, even in all but the few top centimeters of the soil of deserts (Schmidt-Nielsen and Schmidt-Nielsen 1950).

2. Coping with ice

Ice may be the most widespread toxic substance afflicting life. Not that one cannot imagine uses to which it might be put. Melting ice, even without raising its temperature, absorbs energy, which could be used for cooling elsewhere in a system – thus we can cool beverages, freeze ice cream, and so forth. It might provide an osmotically inert and minimally volatile store of liquefiable water. Fractional crystallization could concentrate desirable solutes. Its lower density suits it for use as a flotation device. Adjusting the ratio of liquid to solid water in a system at the freezing point could stabilize temperature, permitting a kind of 0ºC homeothermy. But as far as I know, all of these remain biologically hypothetical.

One can, though, point out a few instances where ice finds use. Ice has a low thermal conductivity, so it can provide insulation, especially in the flocculent form of snow, a cheap and disposable (but not easily portable) augmentation for fur or feathers. Water's high heat of fusion means that relative to deposited mass, frost formation will buffer a nighttime drop

in plant temperature from radiation to the sky better than will dew. A slight temperature increase will accompany ice formation within organisms. The reader might cast about for other possible cases – recognizing a possibility can provide a sieve, search image, or hypothesis.

Again, ice cannot be regarded as healthy for organisms. The phase change from liquid to solid presents a far greater challenge for active organisms than does low temperature *per se*. Perhaps the worst aspect of the problem of ice is that, unlike other environmental solids, it forms spontaneously from something that organisms absolutely require, at temperatures they commonly encounter. Pure water freezes at 0ºC; a 1 molal (M) solution freezes at –1.86ºC, and freezing point depression tracks solute concentration fairly linearly. Seawater of ordinary salinity, 35‰, freezes at about the same temperature, –1.85ºC. The bloods of teleost fishes freeze at warmer temperatures, –0.55º to –0.75ºC, with values for marine fishes a bit lower than those for freshwater forms. The cellular fluid of young leaves freezes at about – 0.56ºC (Nobel 1999).

What might be the options for a creature exposed to temperatures below the freezing point of its internal fluids? Of the possibilities, avoidance must be the most straightforward, and it is not at all uncommon. Soils have low thermal conductivities, so where year-round mean temperatures exceed 0ºC – where there is no permafrost – burrowing or moving into caves provides refuge. In addition, while water may be prone to solidification, the solid phase has a lower density than that of the liquid. As a result, freshwater lakes stratify as they cool toward the freezing point, with ice forming on top only after the whole body of water has cooled below the temperature of maximum density, 4ºC. Below that, contact with cold air above merely thickens the ice, and that only slowly due to the low thermal conductivity of ice. Nor need life hold itself in abeyance below. The lack of wind-driven stirring and the diffusion barrier at the top suggest that oxygen should be in short supply. But fortunately ice transmits light, so photosynthetic flora can support an active community – short daylength may limit activity as much as low temperature. In places, fishing through holes in lake ice is a major recreational activity.

A less obvious way to avoid ice formation consists of avoiding ice itself even while living at a temperature below one's own freezing point. The trick takes advantage, first, of water's propensity to supercool, and, then, of the way water rises just prior to freezing, so the depths of a body of water can be reliably ice-free. In practice, it requires that the freezing point of the surroundings be below the freezing point of the organism. Fish living deep in some polar estuaries meet the requirement. With no local ice to nucleate freezing (sometimes referred to as "inoculation"), they can spend entire winters at least slightly supercooled. Bringing one up to an icy surface or (in the laboratory) touching such

a supercooled fish with a bit of ice produces an immediate and lethal wave of solidification (Scholander and Maggert 1971).

Supercooling, in fact, turns out to be common; its practical lower limit, about –10ºC, imposes its main drawback – Lee and Costanzo (1998) give a good review of cases and mechanisms. Pure water, especially very small samples, can be supercooled much further, but organisms seem unable to achieve the requisite exclusion of ice-nucleating substances, inert particles, and microorganisms. Curiously, large animals can supercool less far than can small ones, whether compared intra- or interspecifically – apparently larger size brings a higher likelihood of harbouring ice-nucleating agents.

Cryoprotectants – substances that lower the freezing point of the water in organisms – provide a common alternative (or sometimes an addition) to supercooling. A variety of such compounds have been identified. Sugars and related compounds such as glycerol, glucose, sucrose, trehalose, and sorbitol increase the osmolarity of solutions without changing their ionic compositions. These occur in many plants as well as all kinds of terrestrial invertebrates and vertebrates. Overwintering insects may contain as much as 25% glycerol, which not only depresses their freezing points but permits supercooling well below that point – to almost –50ºC in a few species (Willmer *et al* 2000). As antifreezes, polar fishes and various other systems use glycopeptides and proteins; these work in some way other than by altering the bulk colligative properties of body fluids (DeVries 1983; Lee and Costanzo 1998).

Those proteinaceous antifreezes, extracted from fish, have received attention recently as possible protective devices for living biological material such as sperm for insemination and organs for transplantation (Fletcher *et al* 2001). As biosynthesized cheaply and in large quantities by cooperative bacteria, they hold promise for the production of stable ice cream and other frozen deserts without either the usual fat or sugar – and thus for 'diet' versions.

Many organisms simply freeze – or not so simply, as they control both the sizes and sites of ice crystals. In all but a few instances in both animals and plants, survival requires that solidification remain extracellular. The general lethality of intracellular ice has been recognized at least since a classic paper by Chambers and Hale (1932). But its presence in at least two very different kinds of animals, a nematode and an insect, shows that it is not inevitably fatal, as noted by Wharton (2002), who did the work on nematodes. While we can reasonably expect other cases to be uncovered, we still note their exceptional nature and wonder what makes the cells of some animals ice-tolerant.

At least two devices are needed to ensure that ice will only form outside cells. First, since water supercools readily, an organism must provide some device to nucleate extracellular freezing and to do so before the

situation becomes too metastable. To be tolerable, freezing should be slow. So-called ice-nucleating proteins, highly hydrophilic, commonly serve this purpose. In a very real sense, extracellular supercooling and freeze-toleration are antithetical solutions – the greater the degree of supercooling, the more rapidly the succeeding wave of solidification will travel. Unsurprisingly, the two only rarely appear in the same places within organisms.

In addition an organism must ensure that externally-initiated ice crystals do not penetrate its cells. Chambers and Hale (1932) looked quite specifically at the matter in onion epidermal cells, amoebae, and frog muscle fibers. In each case, cell membranes provided remarkably effective barriers to crystal propagation. Their manipulations leave no doubt that extracellular freezing and intracellular supercooling can occur simultaneously. We should remain impressed, especially in view of how easily ice penetrates most other biological structures, even macroscopic ones. We should note, as well, the remarkable extent of that entirely extracellular solidification. The water in intertidal periwinkle snails may be as much as 80% frozen (Kanwisher 1955; Murphy 1983), some frogs survive freezing of up to 65% of their water (Storey and Storey 1992), and ice in a wide variety of reptiles can reach 50% (Storey 2006). Neither antifreeze nor the ice-nucleation proteins so widespread among animals appear to have analogs in plants (Guy 1990).

Keeping ice outside one's cells does not render it entirely benign. Besides being mechanically disruptive and an impediment to movement – sand in the gears, so to speak – it must present a severe physiological challenge to cells and tissues. Unless frozen very rapidly ("flash-frozen" in the food industry), water excludes salts and most other solutes as it solidifies. Thus what liquid remains becomes ever more hypertonic. Hypertonic liquid outside cells draws water from inside. While that may lower the freezing point of intracellular liquid, it must affect a wide variety of cellular processes as well. Frozen snails may remain alive, but their metabolic rates drop to levels far below what one would extrapolate from ordinary temperature curves (Kanwisher 1959). Plant cells lose turgor, often with macroscopically obvious damage to the supportive systems of herbaceous parts or whole plants (Sakai and Larcher 1987).

3. Freeze-induced outgasing

Besides mechanical and osmotic problems, freezing of living material may make a further kind of trouble, one that draws less attention. As the temperature of liquid water drops, more and more gas can dissolve in it. Solidification not only stops the process but radically reverses it. Far less air can remain dissolved in ice than in liquid water, at least a thousand times less according to Scholander *et al* (1953). So water outgases as it freezes, and ice cubes, made from tap water in a

household freezer come out cloudy. (Icicles, though, may be clear since they freeze outward rather than inward and thus exclude air.) Thus freezing may involve the third state of matter as well. Gas presents the clearest problem in the most embolism-sensitive biological system, the xylem through which sap ascends in trees. Where winters are severe, xylem vessels normally freeze and embolize annually; they have to be refilled by positive pressure generated by osmolyte release in the roots each spring (Nobel 1999). Indeed, the death of most broad leaves each fall may represent an adaptive recognition of the incipient failure of their water supply. Even the much narrower tracheids of evergreen conifers may suffer embolisms, which must then be repaired each spring (Sparks *et al* 2001; Mayr *et al* 2003).

Air occupies volume, so the density of frozen water in nature may be significantly lower than the published values for pure ice. So that expansion itself may damage tissue. Nor will the air work its way out by diffusion at any reasonable rate. The diffusion coefficients making up air are even lower through ice than the low coefficients typical for diffusion through solids, something to which attention was also drawn by Scholander *et al* (1953).

Nor will the outgassing be limited to the extracellular areas where ice crystals form. Adding salt to a solution decreases the solubility in it of most non-polar substances, the so-called salting-out effect. (Edsall and Wyman 1958 give a good account of the phenomenon from a physiological perspective.) A commonly cited formula for the effect equates the logarithm of the ratio of solubilities (*S*) in adulterated and pure solvent with the product of minus one, the "salting constant" (K_s), and the molality of the solution (C_s):

$$\log\left(\frac{S_{solution}}{S_{solvent}}\right) = -K_s C_s. \tag{1}$$

Salting constants, in practice obtained from tabulations, range from about 0.1 to 0.4 l m^{-1}. The formula applies only to dilute solutions, so, since the effective molality of what remains after a cell loses much of its water is both high and far from certain, it provides only an approximate guide. But it corresponds at least roughly to the observation that about 20% less air dissolves in sea water than in freshwater (Krogh 1941). Based on a freezing point depression of 1.8ºC, seawater corresponds to about a 1 molal solution. A salting constant of 0.1 l m^{-1} produces about this value, fairly close to the value of 0.13 l m^{-1} cited by Edsall and Wyman (1958) for oxygen in a solution of NaCl. Seawater, of course, should be far less salty than what remains after half of the water in an organism has become ice.

So salting-out may be substantial. (However, nucleation of bubbles rather than salting out explains the sudden outgassing when one adds a pinch of salt to a carbonated beverage.) At least the gas it releases inside cells may in

part offset their osmotically-induced volumetric shrinkage. Still, Kruur *et al* (1985) and Lipp *et al* (1987) reported better recovery of mammalian cells from repeated cycles of freezing when suspended in degassed solutions.

A tissue may face yet another consequence of the exclusion of air from extracellular ice and of both intra- and extracellular salting out. The pressure within a tiny bubble of gas in a liquid depends on its size, a result of the way the squeeze of surface tension varies with the radius of curvature. The last essay (Vogel 2006) invoked the phenomenon in connection with the stability of gas bubbles in water and with the spacing of hairs in the plastrons of aquatic arthropods. To repeat, then, for a spherical shell with a radius of curvature of r and a corresponding diameter of d, the pressure developed by surface tension (γ) is

$$\Delta p = \frac{2\gamma}{r} = \frac{4\gamma}{C}. \tag{2}$$

So surface tension as well as atmospheric pressure squeezes bubbles, with a squeeze that depends on bubble size as described by eq. (2). Of particular concern here is the way coalescence of a group of gas bubbles yields a bubble of greater volume than the combined volumes of its contributors – simply by being bigger, it will be squeezed less. How much so? The problem becomes simple when one recognizes that the product of pressure and volume must be maintained in the coalescence. Volume (V) varies with the cube of radius, pressure (as above) inversely with radius, so, for coalescence of n bubbles, each of r_i into a final one of r_f,

$$r_f^{\,2} = n r_i^{\,2}. \tag{3a}$$

Recognizing that the volume of the product represents n bubbles,

$$V_f = n^{3/2} V_i. \tag{3b}$$

Combine 100 bubbles and the resulting one will have 10 times the sum of their original volumes.

Note the irrelevance of the specific value of surface tension – it drives the phenomenon, but any amount will do. The formula, though, ignores the atmospheric contribution to pressure and thus strictly applies only in cases in which this latter factor can be neglected; on earth that means infinitesimally small gas bubbles with their overwhelming pressure from surface tension.

Accounting as well for the squeeze of atmospheric pressure makes the value of surface tension a player once more, and it puts absolute size back into the picture:

$$r_f^{\,3}\left(A + \frac{2\gamma}{r_f}\right) = n\, r_i^{\,3}\left(A + \frac{2\gamma}{r_i}\right), \tag{4}$$

where A represents atmospheric pressure. For 100 bubbles, as earlier, the volumetric expansion factor expansion no longer reaches 10; for 1000 bubbles it no longer reaches 31.6. Figure 1 shows the results of eq. (4) for the coalescence of 10, 100, and 1000 equal-sized bubbles as a function of bubble diameter. For an initial diameter of 0.1 μm, the expansion when 1000 bubbles coalesce is 14-fold; for an initial diameter of 1 μm, it is 3.3-fold; for an initial diameter of 10 μm, it is only 1.3-fold. For an initial bubble at the threshold of direct visibility, 100 μm, the expansion factor drops to a trivial 1.03-fold.

In short, with our ordinary atmospheric pressure, the extra expansion during coalescence from reduced effectiveness of surface tension might matter at a cellular scale even if minimally relevant to everyday foams.

Is the parent phenomenon, outgassing, a significant problem? The existence of winter embolisms in the xylem of trees, mentioned earlier, is well established. A number of observations and passing comments suggest that other cases might bear further investigation. The clearest of these come from Scholander *et al* (1953), a paper with such statements as, "A principal difficulty in experimentally freezing animals in water is that gases are trapped in the ice and form bubbles which rupture the tissue." It reports that naturally frozen copepods, upon thawing, sometimes produce fatal internal gas bubbles. And exposure to seawater of higher salinity and lower oxygen tension – both of which mean less dissolved gas – improved survival of intertidal mollusks beyond what would be expected from the increase in tissue osmolarity (Murphy 1983). As mentioned earlier, Kruur *et al* (1985) and Lipp *et al* (1987) found that mammalian cells recovered better from repeated freeze-thaw cycles if the cycling was done in degassed solutions.

Evidence of troublesome coalescence during thawing is at this point less compelling. Slow thawing, which would permit better redissolution of gases, usually gives better survival than does rapid thawing. Murphy (1983) mentions that intertidal snails do better if kept cold following thawing, which also would permit better redissolution.

4. Minimizing evaporation

Water will not normally solidify unless the temperature drops below the freezing point of the particular solution. But it can vaporize at any temperature. Even ice can transform into the gaseous phase. Although slow, such sublimation can adversely affect the appearance and subsequent palatability of poorly packaged frozen food. Few terrestrial habitats that are exposed to the atmosphere can escape some evaporation of liquid water. And organisms that depend directly on atmospheric gases cannot readily seal themselves off from evaporation – whether breathing gaseous oxygen for respiration or absorbing carbon dioxide for photosynthesis.
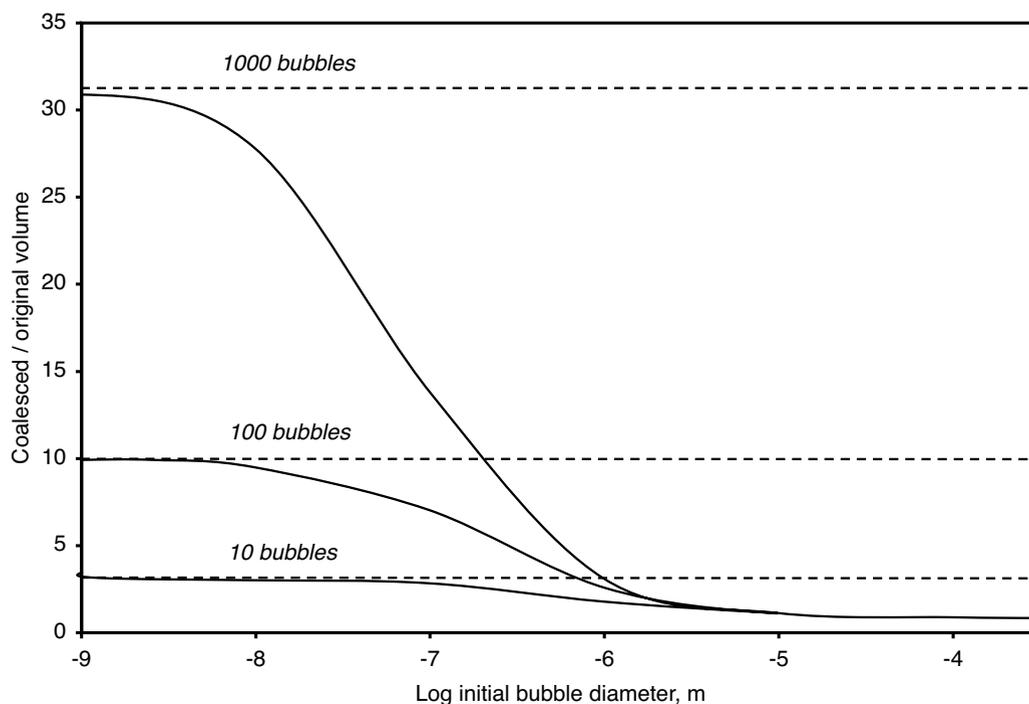
Figure 1.   The factor by which equal-sized bubbles of various sizes increase in volume when they coalesce into a single, larger bubble.

In biological terms, what differentiates evaporation from solidification is that evaporation can serve an important function. Water has an especially high heat of vaporization – 2.44 MJ kg⁻¹ at 25°C, greater, incidentally, than the commonly quoted 2.26 MJ kg⁻¹ at 100°C because of the additional hydrogen bonding at the lower temperature. For comparison, the heat of vaporization of propanol, with about the same boiling temperature, is 0.76 MJ kg⁻¹, over 3 times lower. That makes evaporation of water an especially good way to absorb heat, thus for disposing of excess heat either from atmospheric or solar input or from metabolic inefficiency. One can tolerate environmental temperatures well above that of one's body (as in a sauna), as long as a low enough humidity permits sufficient evaporative cooling. What limits the utility of evaporative cooling is supply. While the oxidation of food yields metabolic water, the subsequent vaporization of that water absorbs much less heat than that produced metabolically. Metabolic water thus cannot absorb sufficient heat to have much effect on body temperature, and so cooling by evaporating water requires other sources.

Only medium and large terrestrial animals, mainly mammals and a few large birds, limit body temperature through evaporation – by panting or sweating – almost certainly because for the process to be practical, an animal's surface-to-volume ratio cannot be too high. And those warm, open habitats where cooling may be critical tend to be ones with limited supplies of water.

More problematic are broad leaves, many of which get quite warm when in sunlight and which evaporate ('transpire') water at substantial rates. That evaporation can produce significant cooling, so its functional significance is undeniable, and energy budget accountings rarely ignore it. By contrast, its adaptive significance has long been controversial. Traditionally evaporative water loss has been regarded as an unavoidable evil, an unfortunate consequence of keeping leaves sufficiently permeable for inward diffusion of photosynthetically-critical carbon dioxide during daytime.

At least two facts argue against the generality of the case. For one thing, water use efficiency, the rate of CO_2 fixation relative to water loss, varies considerably, almost ten-fold from plant to plant, even among those for which the supply of water does not look especially copious. One might expect these latter to hug some upper physiological limit on water use efficiency. For another, many xerophytic (dry habitat) plants, representing several evolutionary lines, simply close their stomata during the daytime so they neither evaporate much liquid water nor absorb much CO_2. Instead they take in their CO_2 at night, when the air is at or near the dew point, and temporarily fix it as organic acids that can be decarboxylated during the day. They thereby raise water use efficiency as much as ten times further. Even so, one should note, a lot of water emerges from a plant relative to the amount of carbon dioxide fed into the photosynthetic process – or relative to the input of water to photosynthesis.

For mass of water relative to mass of CO_2 (the inverse of water use efficiency), ratios run between 25 and 1000-fold (Nobel 1999). Not only does water diffuse more readily than CO_2, but the past activities of the plants themselves have left the atmosphere with very little CO_2 – now a little over 0.03% but even less just a few years ago.

We terrestrial animals, seeking oxygen, face an analogous balancing act to that of plants, looking for carbon dioxide – an exchange surface that takes in oxygen will lose water. But we face a less unfavourable situation. While oxygen has a slightly higher diffusion coefficient than does CO_2, far more significant is oxygen's greater availability, hundreds of times, whether in terms of either relative mass or molecular concentration. The comparable ratio, mass of water to mass of oxygen, is 0.64 for a human, over an order of magnitude better than even the most efficient plants. Moreover, our metabolism yields rather than consumes water. Consuming pure starch gives an output of 0.47 mass units of water for each unit of oxygen used, so we can supply most – but not all – of our respiratory water loss from that metabolic yield.

So getting oxygen still requires that some precious liquid water disappear into thin air. We might envy the access of well-rooted plants to the interstitial liquid water in the soil. Alternatively, that access might be seen as a severe constraint on mobility, one we rarely consider. In other words, rootedness may be critical to the ability of plants to transcend what, by animal standards, are extremely low water use efficiencies.

But we humans win no prizes among animals for the efficiency with which we process water. Our kidneys cannot, for instance, produce urine even as salty as ocean water, and thus we dehydrate if we drink seawater. Besides having more effective excretory systems, many mammals and birds that are either small or lack reliable access to water have another way to minimize loss of precious liquid water. The nasal counter-current exchanger, first pointed out by Jackson and Schmidt Nielsen (1964) was described in essay 5 (Vogel 2005) in connection with heat conservation. Inhaling cold, dry air through intricate nasal passages causes evaporation of moisture from the walls of those warm, wet passages, so air arrives at the lungs at near saturation and body temperature. At the same time, it cools the nasal passages, so that moisture condenses and heat is removed from the subsequent exhalation. As a result, air can exit to the atmosphere only a little above atmospheric temperature, saturated with water at that temperature rather than at body temperature.

The arrangement conserves significant amounts of water. For a kangaroo rat, respiratory water loss over oxygen use is 0.41 rather than our 0.64 or a laboratory rat's 0.72. Significantly, 0.41 is less than the metabolic yield from metabolizing starch, 0.47. As a result, kangaroo rats and some other desert rodents need drink no liquid water at all – unless they eat a high protein diet and need extra water for disposing of excess nitrogen.

A greater difference between core body temperature and air temperature ought to increase the degree of conservation. Although no specific investigation appears to have been done, musk oxen (*Ovibos moschatus*), once common in the New World Arctic, may make exceptionally effective use of this temporal countercurrent arrangement. Their daily water turnover – loss relative to body mass – averages only 3.5%, only a little more than half that of a camel's 6.1%, and far less than the 13 to 20% of cattle and water buffalo (Prosser 1973); turnover during winter is at least five times less than in summer (Klein 2001). And musk oxen have unusually wide nasal openings and elaborate nasal turbinates – figure 2 provides a comparison with a domestic cow.
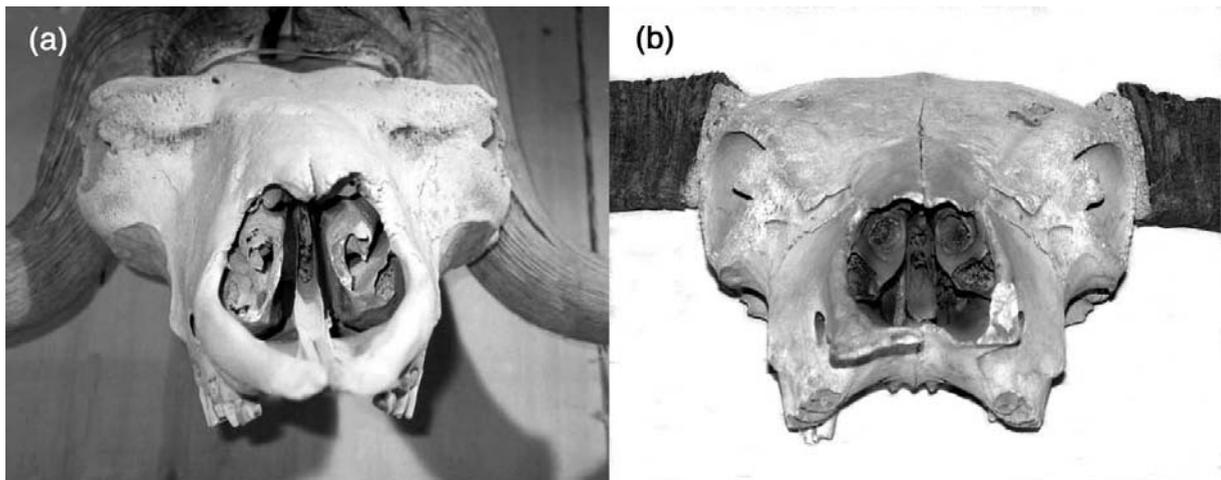

Figure 2. Front views of the skulls of **(a)** a musk ox (at the Musk Ox Farm, Palmer AK, USA) and **(b)** a domestic cow (at the Museum of Life and Science, Durham, NC, USA).

If musk oxen do use the device, one must wonder about its primary adaptive advantage. The extreme coldness of the air they breath during the long winter means that without a trapping system air arriving at their lungs will be almost devoid of water vapour and respiratory water loss will be high. They may be reliably surrounded by snow, but liquefying it takes energy – 6 to 14% of their overall expenditure (Soppela *et al* 1992). Nonetheless, heat trapping may be as important or perhaps more important than minimization of water loss, in sharp contrast with camels, for whom reduction in heat dissipation must be a drawback.

5.   Extracting liquid water from vapour

Since all active organisms require liquid water, and since gaining either oxygen or carbon dioxide from the atmosphere inevitably entails loss of water, all air-processing terrestrial organisms must be able to acquire it. In no habitat does the atmosphere entirely lack water vapour. So an organism merely needs to condense liquid from the limitless surrounding supply. But only a few, mainly arthropods and vascular plants, do just that – for some reason (or reasons) the task is daunting. Perhaps dissipating the heat yielded by the process presents some difficulty.

We can recognize several possible mechanisms for condensation – (i) exposure of a solution concentrated enough and thus with a sufficiently low vapour pressure to draw water from the local air, (ii) exposure of a hygroscopic surface followed by extraction of water from that surface, (iii) exposure of a negatively curved air-water interface (an 'antibubble') with a very small radius of curvature, and (iv) exposure of a surface with a temperature below the dew point of the air around it.

Organisms are adept at actively transporting ions as well as concentrating non-ionic osmolytes. The main difficulty in making solutions that condense water vapour comes from the unfavourable relationship between concentration and the relative humidity of the air at equilibrium, at least with ordinary salts as solutes. For instance, a 3 M solution of NaCl (16% by weight) will only condense water vapour from an atmosphere of at least 90% RH. For condensation, a 4 M solution requires a humidity above 86%. Further complicating things, few if any organisms can actively transport water, as opposed to ions or other solutes. So absorption of condensed water has to be done indirectly by such stratagems as pinocytosis of solution followed by extraction and ejection of solutes. Both the first, simple colligative condensation, and the second of our mechanisms, use of hygroscopic substances, will face the problem.

Nonetheless a variety of arthropods manage to condense water from substantially sub-saturated atmospheres, and they depend – although specific details remain obscure – on some mix of colligative and hygroscopic devices. And some do

so from remarkably dry air. Several kinds of fleas, booklice, and mites can get water from relative humidities below 80%, while some lice, a few silverfish, and a beetle larva can deal with humidities below 50%. In all cases investigated, absorption occurs at specialized surfaces, rectal, oral or Malpighian (Willmer *et al* 2000). Arthropods of deserts such as the Namib of Southwest Africa take advantage of the relatively moist air coming off the adjacent ocean; some use condensation of vapour, others collect liquid water from fogs (Hadley 1994). One case of condensation from an unsaturated atmosphere has been described in plants, a succulent shrub in the Atacama Desert of Chile (Mooney *et al* 1980).

Evaporating water requires a supply of energy equal to its heat of vaporization, hence its utility as a way to offload excess thermal energy. Condensation, its opposite, must yield energy, warming the condensate and anything in thermal contact with it. No animals have been reported to make use of that thermal energy, which is not unsurprising, given the low surface-to-volume ratios of animals compared with, say, leafy plants. Worse, water must be moved from whatever sludge in which it condensed to some higher concentration in the body fluids, and those severe osmotic gradients will impose a significant price – although, even so, the cost of condensation relative to an arthropod's other activities turns out to be fairly low (Hadley 1994).

For the third of our possible mechanisms, use of locally lowered pressure, we lack a specific case; and it should be considered quite unlikely. Willmer *et al* (2000) mention muscular pumping to produce pressure cycles, but only as a hypothetical. One might imagine using the opposite of the surface-tension induced compression of bubbles, mentioned earlier in connection with thawing. As noted in the last essay (Vogel 2006) when considering plastrons, pressure can be reduced by air-water interfaces with negative curvature, ones in which reduction of the area of the interface would decrease rather than increase pressure. For a plastron, pressure was lower on the gaseous side. In this terrestrial rather than aquatic system hydrophilic rather than hydrophobic pegs or grooves with water between might cover a surface and lower pressure on the liquid side. Atmospheric water vapour would then condense into the low-pressure liquid. But the scheme will not work. As long as the surface water remains liquid, its concentration will far exceed that in the adjacent atmosphere at any humidity. Indeed, if it did work, the highly negative pressures in their xylem would permit the leaves of tall trees to extract atmospheric water.

For the fourth mechanism, condensation at a locally subatmospheric temperature, we have no shortage of cases, at least among plants. We animals can achieve subambient body temperatures, but we inevitably do so by evaporative cooling, entirely inappropriate if gaining liquid water is the goal. Radiation to the night sky will work, though,
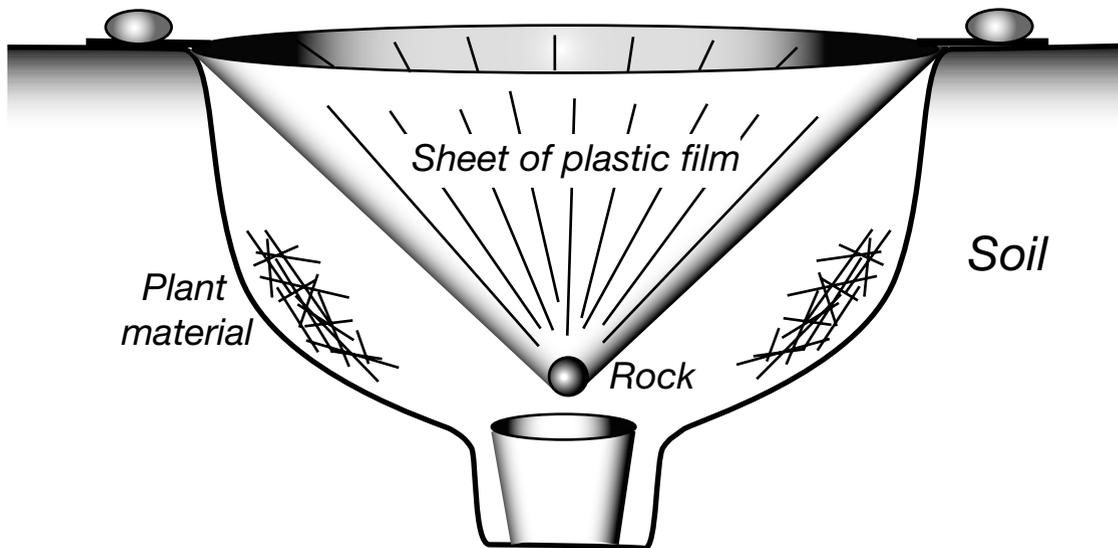
Figure 3. The solar still of Jackson and van Bavel (1965). The pit is about 1 m in diameter and 0.5 m deep.

as long as a few conditions can be met. The humidity (or dew point temperature) must be high, the sky must be nearly or entirely cloudless, the atmosphere near the ground must not be obscured by smoke or fog, the surface must be extensive enough to limit convective reheating by the surrounding air, and air movement must be minimal, for the same reason. We are, of course, defining the conditions for forming dew, conditions especially well met by low, broad-leaved or succulent vegetation in open areas or where trees surround but do not cover areas of low vegetation.

As Nobel (1999) points out, the requisite high humidity near the ground may come about as much from distillation from the soil as from the atmosphere itself. While wind can dry a surface as rapidly as condensation can occur, gaining liquid water from soil distillation or a humid atmosphere takes at least some slight air movement – in truly still air, diffusion of water vapour will be insufficient to offset local depletion. In practice air can be counted on to move at more than that minimal rate. The quantities of water condensed may be substantial, reaching 0.5 mm per night or 30 mm annually. Environmental structures occasionally provide the effective surfaces – Hadley (1994) mentions radiative cooling of, condensation on, and drips of water from rocks. Condensed water, whether on vegetation or other structures, may be imbibed by desert arthropods (Edney 1977), using radiative cooling indirectly where their own small sizes and bulky shapes preclude doing their own condensation.

We may at least occasionally condense moisture as a source of drinking water. Jackson and van Bavel (1965) described a lightweight, inexpensive survival device for use in arid places, shown in figure 3; with it they collected as

much as 2 l of water per day. It requires nothing more than a piece of reasonably hydrophilic, clear plastic film about 2 m in diameter and a wide-mouth container to catch the condensate that drips down the film – and a shovel to dig a pit. Sunlight passing through the film heats the soil (and any moisture-containing plant material that may be added to the pit), evaporating the water that then condenses on the film. A similar device, designed for use on life rafts, plays a central role in a recent novel (Martel 2001).

6. Condensation by location cycling

Lowering body temperature below the local dew point by radiative cooling may be an impractical way for animals to condense physiologically significant amounts of water. They have another option, though, one impractical for plants. An animal might shift back and forth between a hot and a cold location, chilling enough in the colder site to condense water on itself when it moves to the warmer one. The frequency at which it has to shift will be set by its size, since it has to stay in the cold site long enough to cool down and should not stay at the warm site until fully equilibrated lest the water evaporate again.

One thinks immediately about cycles of immersion in some cold ocean alternating with emersion. But that would be of use only to the relatively small numbers of animals (such as ourselves) that cannot obtain body water from the ocean or perhaps to the inhabitants of the edges of hypersaline lakes. Cycling between two terrestrial sites seems more likely, as long as the sites are sufficiently close to permit quick, cheap shifting. As far as I know, no specific case has been reported in the literature; if cases exist, they
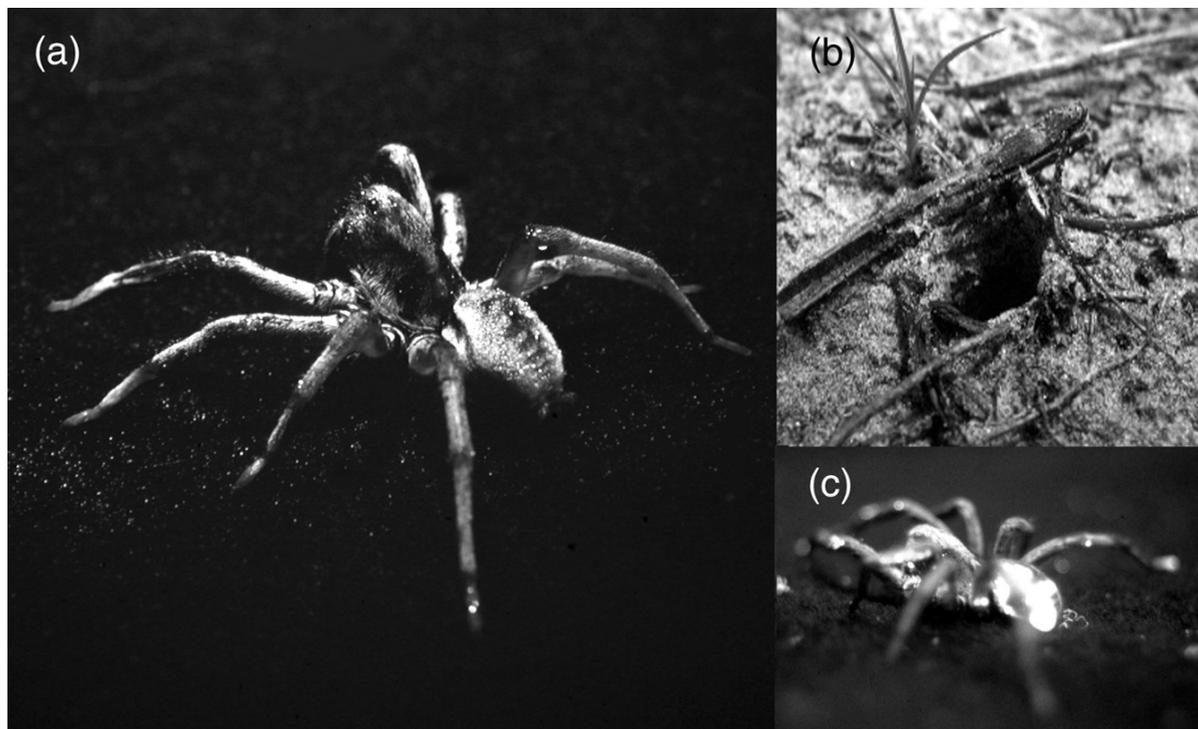
Figure 4. **(a)** A turret spider, probably *Geolycosa pikei*, from Bogue Bank, NC; **(b)** a burrow, with as minimal a turret as one finds; and **(c)** a spider that, when cool, has been exposed to warm, moist, moving air – unevenly dusted with uranine (sodium fluorescein) and illuminated with ultraviolet light.

will probably be uncommon. Still, I believe I have identified one, which I will describe below, prefaced by the candid admission of a serious gap in the chain of evidence.

Turret spiders (genus *Geolycosa*), relatively stout wolf spiders (figure 4a), live mainly in sandy soil amid low, sparse vegetation or among coastal sand dunes (Wallace 1942). Each spider constructs an almost perfectly vertical burrow 0.25 to 0.75 m deep and about 10 mm in diameter, preventing collapse with silk strands near the top where the soil is friable. It rests (reportedly) at the bottom during the day and straddles the top at night, feeding then on passing arthropods. The common name refers to the inevitable turret or crater at the top of the burrow, a construction of silk-stabilized sand and bits of vegetation that extends the burrow between a few millimeters and a centimeter above the ground (figure 4b). The turret has been presumed a barrier to flooding, but it appears too flimsy and porous to do that, or as a look-out perch, which is not borne out by my observation that the spider straddles it.

These spiders live in well-drained habitats that lack standing water for long periods. But the soils around their burrows contain substantial amounts of interstitial water with, as a result, very high interstitial humidity – as noted earlier, as do soils beneath the immediate surface, even in deserts. While some spiders can extract interstitial water

(Parry 1954), *Geolycosa* have not been reported to do so. Nor can they directly extract water from the air, even at 98% humidity (Humphreys 1975). In the warmer months, sunlight will ordinarily warm the surface well above the general air temperature, often to where we feel pain when walking barefoot. So during the day a burrow develops a severe temperature gradient, warm at the top and (relatively) cool deeper down, as in figure 5. The consequent stratification (an 'inversion') ought to minimize any internal mixing.

It would appear that a spider might obtain water by moving up and down within its burrow, climbing up near the top with a cool body and condensing water, and then retreating to the bottom to absorb the water and cool again. But no condensation was evident when slightly desiccated spiders were cycled between the conditions of temperature and humidity of the upper and lower portions of burrows in the laboratory. However, when the warmer air was made to flow slowly over the animals, droplets appeared almost immediately (figure 4c); when the animals were again in cooler air, they groomed themselves, the droplets disappeared, and the spiders regained some of their lost weight.

The necessity for some flow of air fits nicely with consideration of the Péclet number, speed (*v*) times length (*l*) over diffusion coefficient (*D*) (recall essay 1, Vogel 2004).
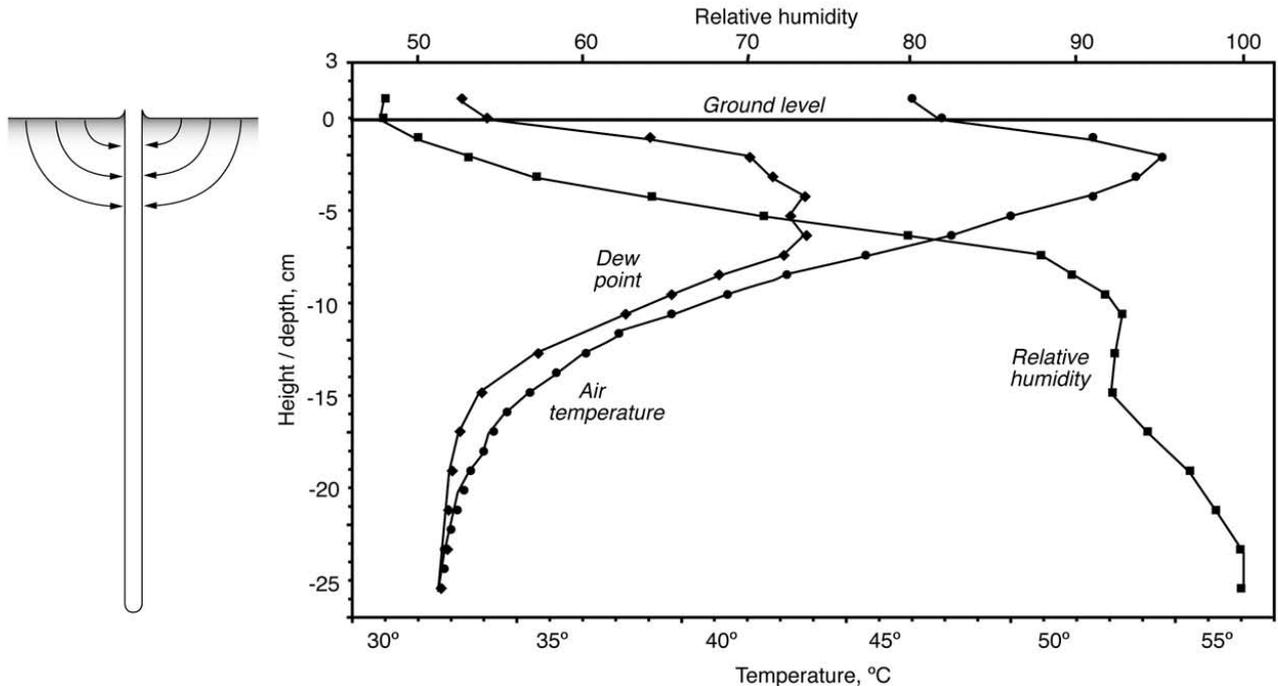
Figure 5. A typical mid-summer record of temperature, humidity, and dewpoint as a function of depth in a burrow such that of figure 4. In this particular burrow the maximum difference between bottom temperature and dewpoint is 11.1° C.

For $l = 0.01$ m and $D = 2.7 \times 10^{-5}$ m^{2}s^{-1} (water vapour at 40ºC), $Pé = 1$ will occur at a speed of 2.7 mm s^{-1}. Although very slow, that speed will be above that of spontaneous air motion in a small, closed container – or a severely stratified burrow. And at speeds significantly lower, the rate of condensation will be limited by diffusion. Since even warm, moist air contains relatively little water, a spider will thus warm up before much condensation can occur.

That may explain the role of the turret. Airflow across it will draw air up and out of the upper reaches of the burrow much more effectively than would flow across a flush opening (Vogel 1976), at least if the soil around it is sufficiently porous. That the porosity of the soil around these burrows is adequate was confirmed by measuring the pressure reduction needed to draw air out of burrows together with wind-induced pressure reduction in a model burrow. So enough air should pass through moist sandy soil and become humidified to maintain a supply of water vapour for condensation. As in figure 5, the dew point in the upper portion of the burrow will be well above the temperature near the bottom.

But despite several attempts, I did not observe spiders moving up and down during a dry spell in the summer. Spiders are strongly vibration-sensitive; these particular ones were, in addition, all too quick to abandon burrows that suffered any kind of intrusion, even that of a tiny bead

dangling from a thread. So the scenario remains conjectural until someone else pursues it. McQueen and Culik (1981) found that *Geolycosa* will go up and down around 20 times per hour, but in relatively disturbed burrows and under conditions of limited present relevance. Still, the rates they report are about right for getting water by condensation. An object of about the thermal mass of a spider, when subjected to a 20º temperature increase, warms by 10º in a little under 3 min; a longer stay would warm a spider above the local dew point, and it would then evaporate rather than condense water. In fact, 3 min will overestimate the time available for condensation, since the process itself generates heat. So high is the heat of vaporization of water that condensation equal to 1% of body mass will raise body temperature by almost 6ºC. So exposures of about a minute seems reasonable, with condensations limited to well less than 1% per cycle, and with ample time for internalizing the water at the bottom in between.

On the one hand, I think it more likely than not that these spiders do obtain liquid water by location cycling; on the other hand, the scenario illustrates the demanding requirements for using the device. Just this point was made by Lasiewski and Bartholomew (1969), reporting on work with models, (their italics), "*The temperature and humidity conditions in these experiments were chosen to demonstrate that condensation could occur in nature. We do not*

necessarily mean to imply that it does occur…" Still, many small desert animals build burrows in which they spend their daytimes (Wilmer *et al* 2000), so we do have candidates for use of the device.

The dependence of condensation on some air movement raises one further point. Measurements of water extraction from non-saturated atmospheres usually involve putting a small arthropod in a closed container at a fixed humidity (the latter maintained by an appropriate salt solution elsewhere in the container). If the container is too small or the temperature too close to constant for appreciable convection, then condensation will be abnormally slow. The minimum usable humidity may be overestimated in some reports (few give sufficient experimental detail to judge) – some of these animals may be doing even better than reported in the literature!

7.   Cryptobiosis – life without water

For most organisms, drying below some critical water content is fatal. A taxonomically diverse minority endure the loss of virtually their entire content of unbound water – they appear to live without water. But perhaps we should put 'live' in quotes, since they retain only one functional attribute of normal organisms, that of not being irreversibly dead. In almost every case, the addition of water restores normal activity after only a short period of physiological (and sometimes morphological) restoration. Such a state of suspended animation has been termed 'cryptobiosis', with this specific version sometimes labelled 'anhydrobiosis'. For most of these organisms, cryptobiosis provides a way to wait out unfavourable conditions; for a few it forms a normal aspect of the way they disperse. In the cryptobiotic state, organisms survive not only their ordinary stressful deprivations, but ones they would not normally encounter. For instance, drying of the habitat may induce the transition; but once cryptobiotic, the organisms can withstand moderately high or extremely low temperatures, high or low pressure, and even extraterrestrial vacuum. Whatever the role of cryptobiosis, its consistent feature remains that exclusion of liquid water.

The commonest example of a cryptobiotic creature is the brine shrimp, *Artemia salina*, normally an inhabitant of transient saline pools in deserts and sold as food for small fish. One can purchase jars of what look like coarsely ground grain and that have an almost indefinite shelf life. Add a bit to water and in a short time crustacean larvae swim off. Not only some crustaceans, but many rotifers, nematodes, tardigrades and collembola can enter cryptobiosis, as can vascular plants (as seeds), spores of many groups, and bacteria. We preserve many microorganisms by inducing cryptobiosis via lyophilization, that is, freezing-drying them in a vacuum – keeping a type collection of such microorganisms takes far less attention than maintaining, say, stocks of mutant fruit flies.

That non-adapted multicellular organisms cannot become cryptobiotic must be a result of the functional alterations it requires. Use of cryoprotectants is common, most often the same ones, such as glycerol and trehalose, that permitted non-fatal freezing. Surface area may be reduced by withdrawing appendages and rounding up, and extra surface material may reduce permeability, the latter most likely to slow loss while entering cryptobiosis (Wilmer *et al* 2000). How long can organisms remain cryptobiotic? Wharton (2002) cites a claim for bacteria of up to 250 million years, but with a proper caution about the problem of avoiding any contemporary contamination. Some invertebrates can apparently last over a century and seeds more than a millennium.

Other devices to hold life's functions in abeyance, such as aestivation and diapause, both represent less extreme shutdowns and require less extreme (if any) exclusion of liquid water. Cryptobiosis demonstrates that, while liquid water may be necessary for life, the absence of such water need not be lethal. In addition, it may tell us that the presence of liquid water is incompatible with anything approaching suspended animation. Put another way, life and death may be mutually exclusive, but together they are not fully inclusive of possible states.

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