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Desiccation Tolerance of Prokaryotes

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INTRODUCTION

—they began to fit into one picture with the missing piece that small but plentiful entity—water.

Sydney J. Webb
Bound Water in Biological Integrity (403)

There is a theory which states that if ever anyone discovers exactly what the Universe is for and why it is here, it will instantly disappear and be replaced by something even more bizarre and inexplicable. There is another theory which states that this has already happened.

Douglas Adams
The Restaurant at the End of the Universe (3)

The removal of water from cells, the storage of cells in the air-dried state, and the rewetting of air-dried cells impose physiological constraints that few organisms can tolerate. No single group of organisms has monopolized the capacity to withstand air drying, and desiccation tolerance has been recorded for bacteria, higher and lower plants (including their seeds), insects, yeasts, fungi and their spores, and crustacea (67, 81, 83). Our present understanding of the anhydrobiotic cell derives, in large part, from studies with mycophagous nematodes, rotifers, tardigrades, the star moss *Tortula ruralis*, parasitic protozoa including microsporidia, the anostracan crustacean *Artemia salina*, plant seeds and pollen, the resurrection plants *Selaginella lepidophylla* and *Craterostigma plantagineum*, and the cyanobacterium *Nostoc commune* (74, 75, 106, 171, 239, 266, 267). Among the invertebrates, only the echinoderms appear to lack representatives, while none of the reports of desiccation tolerance in vertebrates has withstood critical scrutiny (68). The role of water in the structure-function relationships of anhydrobiotic cells has been inferred, in some cases, from biophysical studies with single purified proteins (53, 54, 56), while ecological studies, for the most part, include a good deal of phenomenological and anectodal observations that shed little light on the mechanisms of desiccation tolerance. The selective pressure of a water deficit is likely to have impinged upon prokaryotes at a very early stage of their evolution. More so than any other boundary/interface effect, the removal of cell-bound water through air drying and the addition of water to air-dried cells are the predominant forces that influence the distribution and activities of bacterial communities. Some bacteria can cope with these water problems, but many cannot. Why? The question has attracted surprisingly little attention. Considerations of the water relations of bacteria have been confined almost exclusively to osmotic systems where cells are immersed in solvent-solute mixtures (58, 85, 86). Curiously, despite its intrinsic importance and ecological significance, desiccation of bacteria as a major stress parameter seems to continually escape the critical attention of bacteriologists (e.g., see references 62, 150, and 330). As a consequence, there has been no review of the topic of desiccation tolerance in bacteria within the past quarter century—a period that has witnessed the formal recognition of the dichotomy between prokaryotes and eukaryotes (369) and the emergence of the archaebacteria (98). This review considers how and why air drying can induce water stress in prokaryotic cells and how and why some prokaryotic cells tolerate that stress. Vegetative cells are the primary focus of these considerations for reasons that will become apparent. The resilience of bacterial spores (111, 149) is referred to in comparative terms only. Osmotic stress in bacterial cells is the topic of recent reviews (85, 86) and is considered here only from the perspective that an osmotic stress is one consequence of the initial stages of the air drying of cells. To aid our understanding of the nature of dry bacterial cells, some reliance has been placed upon the data available for other anhydrobiotic cell models. If there is an emphasis in this present appraisal, it is with the cyanobacteria and with one form in particular, the terrestial nitrogen-fixing form *Nostoc commune*, which has become a very useful model with which to study the desiccation tolerance of prokaryotic cells (see reference 296 and references therein).

WATER RELATIONS AND THEORETICAL CONSIDERATIONS

At first glance, then, the topic of this review seems complex in view of the numerous biophysical and physiological components that contribute to, interact in, and require consideration with respect to desiccation tolerance. But the true complexity is this—everything must be explained from the perspective of one component, water, and the analysis of single-component systems is notoriously difficult. Water molecules are critical components of reaction mechanisms; they contribute to the stabilities of proteins, DNA, and lipids; and they confer a structural order upon cells. What properties of water make it so uniquely suited to the diverse roles it plays in cell processes? In large part, the properties of water reflect the dipole that results from the greater electronegativity of the single oxygen atom over the two hydrogen atoms in each molecule. Other molecules have electronic structures related to water, but water is singular as a liquid because of its ability to form three-dimensional networks of molecules that are mutually hydrogen bonded (420). Inelastic incoherent neutron-scattering spectra have provided evidence for the existence of two different kinds of hydrogen bond, of different strengths, in ice (226). The fact that electrons in sp^3 orbitals of oxygen atoms can easily be rehybridized to respond to the relative configurations of adjacent molecules may account for the two types of hydrogen bond. Spectra of thin films of water suggest that only hydrogen bonds of the strong variety are present, and these strongly hydrogen-bonded clusters should have all the properties reported for vicinal water (226). The formation and breakage of hydrogen bonds between different water molecules occurs on a timescale of approximately 1 ps—this is almost three times faster than the rotational relaxation time of the water molecule. As a consequence, molecules of water continually and rapidly shift positions through electrostatic and hydrogen bond interactions (214, 323). For model solutions of small sugars at room temperature, nuclear magnetic resonance and dielectric relaxation measurements have shown that the residence time of a given water molecule at a solvation site (e.g., a hydroxyl group on a sugar) is extremely short, i.e., 1 ns (see reference 357 and references therein). "Bound" water molecules are, in fact, highly mobile. Within the bulk phase, molecules separated by approximately 10 Å (1 nm) have no statistical interaction. Even at temperatures as low as −40°C (the homogeneous nucleation temperature for ice in pure water), a minimum of around 200 water molecules must associate within a domain of about 40 Å (4 nm) to form a critical nucleus that will grow spontaneously into an ice crystal. Thus, smaller numbers of water molecules would require temperatures much below −40°C (or heterogeneous catalysts) for ice crystals to grow (see reference 357 and references therein).

It has not escaped attention that water may have played a determinative role in the origin and evolution of the genetic code (425). The physical properties of water are discussed at length by Nobel (259) and elsewhere (159, 214, 323), and the implications of some of these properties for a number of cell processes have been considered critically by Wiggins (420). A brief discussion of some of these properties is provided here to give some perspective to the causes and consequences of a cell water deficit.

Properties of Water

The energy required to free a population of water molecules from a liquid phase and to move those molecules to an adjacent vapor (gas) phase, without a change in temperature, is the heat of vaporization (H_{vap}). Water has the highest H_{vap} value for any known liquid and, as such, is the only nonvolatile solvent found in cells in appreciable amounts (236). At 25°C, the evaporation of 1 mol of water requires 44.0 kJ. Most of this

energy is needed to disrupt hydrogen bonds—much of the remainder is needed to overcome van der Waals forces and to account for the relative expansion upon the transition from a liquid to a gas. For bacterial cells far removed from the air-water interface, the effects of vaporization are probably negligible. However, H_{vap} is of significance to cells or colonies in contact with films of water or in microdroplets, because a substantial heat loss accompanies the evaporation of water. Consider a single bacterial rod (2 by 1 μm) entrapped upon leaving an air-water interface in a 10-μm-diameter spherical droplet of pure water. The droplet contains approximately 30 μmol of free water. The amount of energy required to evaporate this water, over a physiological range of temperatures (20 to 50°C), is 1.27 J or approximately 0.3 cal. Clearly, the bacterium not only is dried upon movement of the droplet into the gas phase but also is cooled, and, what is more, these events take place extremely rapidly (see below). Aerophytic, epiphytic, and epidermal bacteria and those bacteria of communities in association with solid substrata are, to a greater or lesser degree, subject to these effects of H_{vap}.

As water molecules are brought from the interior of an aqueous phase to the air-water interface, energy is required to increase the surface area and to disrupt hydrogen bonds. The amount of energy required to expand a surface by unit area is the surface tension. The surface tension, or surface free energy σ_w, at an air-water interface is 0.0712 N m^{-1} or 7.12 × 10^{-8} MPa m (at 30°C). Surface tension is responsible for keeping liquid water (but not water vapor) out of bacterial intracellular protein gas vesicles (397), responds to the quantities of dissolved solutes, and therefore contributes to cell surface hydrophobicity. The surface tension of aqueous solutions is slightly influenced by the composition of the adjacent gas phase but is markedly affected by solutes. Certain solutes, such as sucrose or KCl, do not preferentially collect at air-liquid interfaces and consequently have little affect on σ_w. Fatty acids and lipids, however, may concentrate at interfaces and thus can markedly reduce σ_w. Salts of fatty acids (soaps) or denatured proteins with hydrophobic side chains may collect nearly exclusively at interfaces and reduce σ_w to less than one-third of the value for pure water. The biosurfactants of bacteria are usually complex lipids that participate in the solubilization of hydrophobic substances to aid in their assimilation (245).

Chemical Potential

The activity of water (a_w) is related to its concentration through an activity coefficient (γ_w), where $a_w = \gamma_w N_w$ (N_w is the mole fraction of water in the system). The chemical potential of water (μ_w) in a system is expressed according to the following equation:

$$\mu_w = \mu_w{}^* + RT \ln a_w + \overline{V}_w P + z_w FE + m_w gh \quad (1)$$

In equation 1, the term $RT \ln a_w$—the activity term (where R is the gas constant)—gives the water activity term the units of energy per mole. $\overline{V}_w$ is also the partial molal volume of water, i.e., in a bacterial cell, in contrast to μ_w, which is the partial molal Gibbs free energy ($\delta G/\delta n_w$). $\overline{V}_w$ is the differential increase or decrease in the volume of a bacterial cell when a differential amount of water is added or removed, respectively, and it is expressed as the volume per mole. Pure water, or a very dilute solution, has a value of $\overline{V}_w$ equal to 18 × 10^{-6} m^3 mol^{-1}. P is the hydrostatic pressure in excess of the atmospheric pressure, so that the $\overline{V}_w P$ term in equation 1 reflects the effects of pressure on the chemical potential of water and is expressed, therefore, in energy per mole. $z_w FE$ is the electrochemical potential, and because water is uncharged ($z_w = 0$), the electrical term $z_w FE$ can be ignored. The gravitational term, $m_w gh$, represents the work needed to move a given mass per mole of water, m_w (18.016 g mol^{-1}), to a given height (h) under gravitational acceleration (g). Only under circumstances when cells are distributed at high altitudes throughout a water vapor can the term contribute significantly to the overall μ_w of the cell. $\mu_w{}^*$ is an additive constant and represents the chemical potential of water in a standard (ideal) reference state where $RT \ln a_w = 0$, $\overline{V}_w P = 0$, $z_w FE = 0$, and $m_w gh = 0$. For practical purposes, one compares the chemical potentials of cells with different intermediate water contents, say those of a dried bacterial cell ($\mu_w{}^D$) and a cell at some stage of rehydration ($\mu_w{}^R$). During comparison of these two chemical potentials, the two $\mu_w{}^*$ terms cancel out.

Osmotic Pressure and Osmolarity

Bacterial cells contain finite amounts of dissolved solutes, with exceptions (the cytoplasm of *Halobacterium* spp. accumulates KCl to 5 M [327]), and any consideration of transitions in the water potentials of these cells requires an appreciation of osmotic pressure. The addition of a solute to a pool of water causes a net displacement of the water molecules. The decrease in the partial molal volume depends on the amounts of solute that go into solution and on the extent to which they do so. The lowering of μ_w causes a_w to decrease, and the $RT \ln a_w$ term in equation 1 becomes more negative. Concomitant with this decrease in μ_w, there is also an increase in the osmotic pressure (Π), which is attributable to the addition of a species of solute (j). As bacterial cells undergo changes in the amounts of water (and thus in the net concentrations of solutes) that they contain, the equilibrium is continuously shifted to one of higher or lower osmotic pressure with concomitant changes in a_w. Equation 2 relates water activity to osmotic pressure:

$$RT \ln a_w = \overline{V}_w \Pi \quad (2)$$

The osmotic potential, Ψ_Π, is one component that contributes to the overall water potential (Ψ; see below) of the system and is numerically equal to the prevailing osmotic pressure (Π) but has a negative sign (in units of bars, kilopascals, or megapascals; 1 bar = 100 kPa = 0.1 MPa). The two terms "osmotic pressure" and "osmotic potential" are often a source of some confusion, and the reader is directed to the more-detailed discussions and appraisals by Nobel (259) and Wiggins (420).

A more familiar relationship used to define osmotic pressure due to solutes (Π_s) combines equations 1 and 2 and is referred to as the Van't Hoff relation:

$$\Pi_s \approx RT \Sigma_j c_j \quad (3)$$

At low concentrations of solute (0.1 M) molarity and molality are virtually equivalent for a given solute, whereas at high solute concentrations the molarity is numerically less than the molality. Osmotic pressure increases linearly with increasing concentrations of a solute, but different slopes of Π_s versus dissociation are obtained for different electrolytes (e.g., NaCl) and different nonelectrolytes (e.g., sucrose). Osmolality refers to the mole fraction of active particles of the solute per kilogram of water and differs for given salts depending upon their degree of dissociation in water. The osmotic pressure of a 0.1 osmolal (i.e., 100 mmolal) solution can be calculated as follows: R = 8.3 × 10^{-6} m^3 MPa mol^{-1} K^{-1}, and T = temperature on the Kelvin scale, e.g., 37°C = 310 K; thus, RT at 37°C = 0.002573 m^3 MPa mol^{-1}. According to equation 3, Π_s = (0.002573)(100 mol m^{-3}) = + 0.26 MPa. Note that the

osmotic pressure (which results from the addition of species j) has a positive value. The osmotic potential of that same solution (Ψ_{Π_s}) = −0.26 MPa. The chemical potential of water can therefore be rewritten as

$$\mu_w = \mu_w{}^* - \overline{V}_w\Pi + \overline{V}_wP + m_wgh$$

and, by rearranging, we find

$$(\mu_w - \mu_w{}^*)/\overline{V}_w = P - \Pi + m_wgh = \Psi$$

Water Potential

The water potential of a system (Ψ) is proportional to $\mu_w - \mu_w{}^*$, so that the term $\mu_w - \mu_w{}^*$ has considerable utility when the water relations of bacterial cells are compared. The term represents the work involved in moving 1 mol of water from some point in a system (at constant pressure and temperature) to a pool of pure water at atmospheric pressure and at the same temperature as the system under consideration (the gravitational term is ignored for reasons described above). A difference between two locations in the value of $\mu_w - \mu_w{}^*$ indicates that water is not in equilibrium, so there will be a net tendency for water to flow toward a region where $\mu_w - \mu_w{}^*$ is lower.

Matric Water Potential

A term frequently included, and often ignored, when defining the chemical potential of water is $\overline{V}_w\tau$, where τ is the matric water potential, or matric water pressure (259). This term generally is applied to considerations of water interactions at surfaces and interfaces and is therefore of prime importance in the present review. When water molecules are associated with interfaces such as the surfaces of colloidal particles (solid particles that range from ~0.002 to 1 μm in diameter, e.g., proteins, ribosomes, some bacteria, and viruses) in an aqueous solution, they have less tendency to react chemically in bulk solution or to escape to the surrounding vapor phase. Interfaces thus lower the thermodynamic activity of the water (a_w), especially near the surface of the colloid. Wiggins (420) noted that water equilibrates by increasing its density where the concentration of solutes is high and decreases its density where the concentration of solutes is low. Solutes, of course, also lower the a_w (they also influence surface tension [see above]). As an approximation, it is possible to consider these two effects that lower water activity as being additive in solutions containing solutes and colloids.

Osmotic pressure (Π) depends on the activity of water regardless of the reason for the departure of a_w from unity. Therefore, we can consider that $\Pi = \Pi_s + \tau$; hence, P is strongly affected by proteins and other colloids present in the solution. Although Π and a_w may be the same throughout a system, both Π_s and τ may vary. For example, water activity in the bulk of the solution may be predominantly lowered by the pressure of the solutes, whereas at or near the surface of colloids the main factor decreasing the a_w from unity could be the interfacial attraction and binding of water. Such interfacial interactions do not change the mole fraction of water, but they do reduce its activity coefficient, γ_w.

Water Vapor

Water molecules in aqueous solution continually escape into the surrounding gas phase for reasons discussed above. Water vapor is the third (generally) most prevalent gas in air, although its mole fraction is relatively low compared with the levels of O_2 and N_2 (259). The partial pressure exerted by the water vapor (in equilibrium) is the saturation vapor pressure. Vapor pressure at equilibrium depends on the temperature and the amount of solutes in solution. The water vapor content of air is markedly dependent on temperature, and at saturation it decreases nearly exponentially with temperature. For example, the relative humidity (RH) of air saturated at 20°C (100% RH) drops to 50% when the air is heated to 32°C at constant pressure. Changes of temperature are significant because they often lead to a condensation of water. For example, early-morning mists often occur in desert localities, and their associated water may be sufficient to trigger nitrogen fixation by bacterial communities in the locality (291). Heating of air at constant pressure therefore causes the RH to drop dramatically. As solutes are added to the liquid phase, the activity of water is lowered; therefore, fewer water molecules have a tendency to leave the solution to the vapor phase. Concentrated solutions of solutes are therefore useful for controlling the vapor pressure of the air with which they are in contact. Raoult's law states that for dilute solutions, the actual partial pressure of water vapor (P_{wv}) at equilibrium depends linearly on the mole fraction of water (N_w) in the liquid phase. For pure water $N_w = 1$ and P_{wv} has its maximum value $P_{wv}{}^*$, which is the saturation vapor pressure. The chemical potential of water vapor is μ_{wv}. In consideration of equation 1, then,

$$\mu_{wv} = \mu_{wv}{}^* + RT\ln(P_{wv}/P_{wv}{}^*) + m_{wv}gh \qquad (4)$$

where $P_{wv}{}^*$ is the saturation vapor pressure in equilibrium with pure liquid water at atmospheric pressure and at the same temperature as the system under consideration and m_{wv} is the mass per mole of water vapor, which is the same as the mass per mole of water, m_w. Therefore, the water potential of water vapor in a gas phase such as air is Ψ_{wv}, which is expressed as

$$\Psi_{wv} = (RT/\overline{V}_w)\ln(\%\mathrm{RH}/100) + \rho_wgh \qquad (5)$$

where ρ_w is the density of water. A convenient relationship used for the estimation of matric water potentials in such systems can be derived from equation 4:

$$\Psi_m = 1{,}065T\log P/P_0$$

where P/P_0 is simply a_w (%RH/100) and 1,065 is a derived constant (301).

Glasses

In intermediate-moisture systems, such as bacterial cells, most physical and chemical processes, perhaps with the exception of free radical-induced reactions, are under kinetic control; i.e., they are diffusion limited. As a consequence, the system, or the cell, within which these processes occur may be in a stationary state but not in equilibrium. To appreciate the inherent complexity of the aqueous cytoplasm in a bacterial cell, it is necessary to begin by considering "simple" systems. A solute, in water, can achieve a continuum of hydration states that range from the anhydrous solute to a solution of infinite dilution (pure water). Each of these hydration states has a characteristic temperature that defines the point of a kinetic (time- or frequency-dependent) material-specific change in physical state, from a glassy mechanical solid that is capable of supporting its own weight against flow due to gravity to a rubbery viscous fluid that can flow in real time (357). At temperatures below this glass transition temperature, $T < T_g$, diffusion-limited processes are inhibited by the extremely high local melt viscosity (η) and elastic modulus so that water is, in essence, immobilized and unavailable. In such a glass, molecular diffusion periods are greater than 10^5 s for rotation or

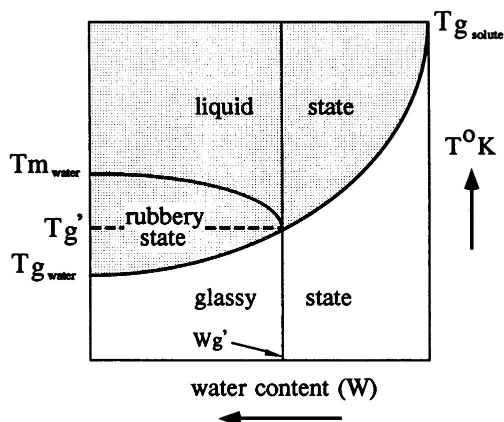


FIG. 1. Idealized phase diagram for a polymeric solute. See the text for details. Reproduced from reference 357 with the permission of authors and publisher.

translation through one molecular distance, i.e. 10^6 s nm^{-1} or around 300,000 years cm^{-1} (see reference 44 and references therein). Unlike a crystalline solid, however, a glass has surface characteristics that may lead to much lower water vapor pressures. The singular importance of water is that it acts as a mobility enhancer and serves to both increase the free volume and decrease the viscosity of a given solute system. The net effect of increasing the moisture content, W, at a constant temperature is equivalent to the net effect of increasing the temperature at a constant value of W. In each case there is a lowering of T_g (Fig. 1). The significance of this point is discussed further below. Different solutes and polymers have comparatively high values of T_g in their undiluted, anhydrous state. For example, starch and gelatin have T_g values around 200°C, while the T_g of anhydrous sucrose is 67°C. In contrast, the T_g of water is −137°C (44, 233), and this temperature marks the lower limit of state diagrams that describe the characteristics of aqueous glasses (Fig. 1). One further qualification required is that solute systems, including those encompassed by a cell membrane, typically are heterogeneous and may contain both crystalline and amorphous solid phases. If the crystalline phase is anhydrous, the water is physically confined to the amorphous domains. When the crystalline phase is also hydrated, the water may be distributed, in a nonuniform manner, between each of the different domains. The ease of migration of water through such a multiphase material depends in part on the properties of the crystalline-amorphous interface (357). At atmospheric pressure, those regions of the system which are in equilibrium can be described solely with respect to the two dimensions of temperature and composition. The description of regions which are not in equilibrium requires the consideration of a third dimension, time, expressed as t/τ, where τ is a relaxation time.

T_g' is an invariant point on the continuum (curve) of T_g values and represents the state-specific subzero T_g of the maximally freeze-concentrated, amorphous solute/unfrozen water matrix surrounding the ice crystals in a frozen solution. T_g' corresponds to, and is determined by, the point of intersection of the glass curve and the nonequilibrium extension of the equilibrium liquidus curve for the T_m of ice (T_m is the crystal-melting temperature). This solute-specific location defines the composition of the glass that contains the maximum practical amount of plasticizing moisture (W_g', with units of grams of unfrozen H_2O per gram of solute). The term "collapse" refers to the microscopic and macroscopic consequences that are manifest at around 20°C (note the difference between 20°C [a fixed temperature] and 20C° [a temperature range]) above the glass transition T_g, i.e., at T_c, the collapse transition temperature.

For nonequilibrium glassy and rubbery systems, mobility transitions can be described in terms of a dynamics map with axes of temperature, pressure, concentration, and time (Fig. 1). The glass transition is a second-order transition and is characterized by a change in the slope of the volume of expansion (the first-order derivative of free energy), a discontinuity in the thermal expansion coefficient, and a discontinuity in the heat capacity (a second-order derivative of the free energy [357]). In the low-viscosity highly fluid region, the coefficient of temperature dependence (activation energy) is a constant and a plot of log relaxation rate (viscosity) versus $1/T$ is a straight line. If the relaxation rate is viewed as the velocity of a reaction, this relationship is described by Arrhenius kinetics:

$$v = Se^{-\Delta E^*/RT} \quad (6)$$

where v is velocity (in this case relaxation rate or change in viscosity, η), S is a constant (collision or frequency factor [A] when chemical reactions are considered), E^* is the activation energy, R is the gas constant, and T is temperature in Kelvin. Taking the logarithm,

$$\ln v = (-\Delta E^*/R)(1/T) + \text{constant}$$

The logarithm of the velocity of a chemical reaction is a linear function of the reciprocal of the absolute temperature. In the glassy state, rates are, of course, much lower but Arrhenius kinetics nevertheless still apply. A quite different situation applies for the region between T_g to a point approximately 100C° higher than T_g or the crystalline melting temperature (T_m) for partially crystalline polymers. In this region, the dependence of viscoelastic properties on temperature is described by Williams-Landen-Ferry theory (WLF kinetics [357]):

$$\log_{10}[(\eta/\rho T)/(\eta_g/\rho_g T_g)] = -C_1(T - T_g)/[C_2 + (T - T_g)]$$

where η is the viscosity or another diffusion-limited relaxation process, ρ is the density, and C_1 and C_2 are coefficients that describe the temperature dependence of the relaxation process at temperatures above the reference temperature, T_g. C_1 is proportional to the inverse of the free volume of the system at T_g, while C_2 is proportional to the ratio of the free volume at T_g over the increase in free volume due to thermal expansion above T_g (i.e., a ratio of free volume at T_g to the difference between the volumes of the rubbery liquid and glassy solid state as a function of temperature above T_g). The significance of the WLF expression is that activation energy is, itself, temperature dependent such that a plot of log relaxation rate (or velocity) versus $1/T$ is curvilinear. Simply stated, this means that as ΔT increases, the faster the system is able to move, the greater is its mobility, and the shorter is the relaxation time. One consequence of this is that there is a change of 5 orders of magnitude in the rates of relaxation processes (such as viscosity) over a 20C° interval near T_g. A factor of 10 change in the rate of a diffusion-limited process above T_m, where $Q_{10} = 2$ (Arrhenius behavior), would require a 33C° change in temperature, in comparison with only a 3C° change for WLF behavior near T_g of a partially crystalline polymer (where $T_g/T_m = 0.67$). It is important to note that both synthetic and naturally occurring solute systems have been shown to follow WLF kinetics.

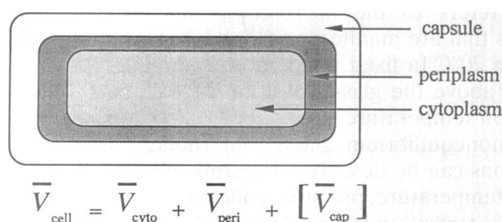


FIG. 2. Physical compartments of a prokaryotic cell. The capsule (or EPS) is a source of interstitial water that can be considered an additional compartment. Methods to calculate the volumes ($\overline{V}$) of the different compartments are described in the text.

Glasses are expected to have lower water vapor pressures than the corresponding crystalline solid, and therefore they may add resistance to further dehydration of the system. The consequences of glass theory and WLF kinetics in desiccation kinetics will be discussed in a later section.

WATER INSIDE AND OUTSIDE CELLS

Living cells achieve a dynamic state that is characterized by temporal and transitory shifts in the net concentrations of intracellular water, salts, lipids, macromolecules, trace metals, and cofactors. These changes may be so subtle that they are virtually immeasurable or so extreme as to cloud any considerations of their ultimate cause. From a biophysical perspective it can be argued that bacterial cells, while not bags of enzymes, are membrane-bound bags of water. These bags of water also happen to contain on the average some 2,000 different proteins (some of which are present in up to 100,000 copies) (285), one or more chromosomes each of the order of several millimeters in length when uncoiled (104), around 600 different mRNAs, and 10×10^3 to 20×10^3 ribosomes (255). We have no notion how these components are dispersed throughout the aqueous solvent that represents some 70% of the wet weight of the cell. The question whether there is an inherent organization, an underlying structure, or a chaos inside cells has caught the attention of a number of groups with quite opposing views (65, 66, 235, 420). The topic is by no means resolved. In an attempt to visualize the interior of an *Escherichia coli* cell, "water (was) omitted, to clarify the distribution of macromolecules" (145)!

Water in Different Cell Compartments

How much water is in a bacterial cell? Independent measurements made since the 1950s on cells of heterotrophic and phototrophic forms have all provided rather similar values (255, 398, 403). One widely quoted assumption is that the protoplasm of *E. coli* contains 70% water and 30% solids (255), and recent studies by Cayley et al. (57) lend biophysical data to support this assumption. The water-accessible volume of the cytoplasm of *E. coli* K-12 strain MG 1655 ($\overline{V}_{cell}$) grown at 37°C in MBM medium was measured as approximately 2.5 μl of H_2O mg (dry wt)$^{-1}$ (72% water and 28% dry weight) (57). Intracellular water in the cyanobacterium *Anabena flos-aquae* was measured as some 88% of the cell mass (12% solids) (398).

Cell water is distributed among several structurally distinct cell compartments: the cytoplasm, the periplasm, and, if present, the capsule or extracellular investments (Fig. 2). The term "structurally distinct" is emphasized here, as there is now some indication of additional physiological compartments such as the one speculated to house the 10,000 or so copies of thioredoxin in *E. coli* cells (258). It is unknown whether the properties of water may cause discrete subpartitioning of physiological zones within the cell compartment. There are few data available for the turgor pressures generated in bacterial cells—largely because of technical difficulties—and all measurements have been made with cells from liquid cultures. A value of 187 kPa was determined in *Ancyclobacter aquaticus*. The turgor pressure varied considerably from cell to cell but nevertheless was independent of cell size (207, 286). A similar value of 2 to 4.5 bars (200 to 450 kPa) was determined in *Anabaena flos-aquae* (398).

Interstitial Water

Measurements of cell volume and the amounts of water in cells are more complex than they would otherwise seem, in large part because of the variable amounts of interstitial (extracellular) water. Substantial amounts of interstitial water may be associated with bacterial cells. Fibrils and polymers may form strong links between the cell and a solid surface and encourage film development. A film provides the largest surface area available for rewetting, and a film with a clay envelope, especially monmorillonite, may protect bacteria from excessive desiccation (390). The presence of a water-retaining structure poses problems if that structure is physically attached to the cell wall, in view of the torsional and elastic forces that may be conducted to the cell as the structure changes its water content. If the structure is also stress bearing, any addition of new material to the structure must take place as far from the cell surface as possible (364). Measurements involving extracellular investments from some cells suggest that the cells hold in excess of 95% of their own weight as water (381). Most of that water, of course, may be removed upon air drying.

Water in Enzymatic Reactions

Water formally acts as any allosteric ligand, but, unlike any other ligand, it is the only one that is always active (70). It is now being recognized that water and solvation play crucial roles in membrane function and protein regulation (231, 284, 315, 316, 318, 433). When a protein undergoes a conformational change, the partial specific volume of the structure changes. Often this change, ΔV, is small and cannot be easily detected; sometimes the change is large, the equilibrium between the forms is in a reasonable range, and ΔV can readily be evaluated at pressures below 300 MPa. In recent work, Kornblatt and Hoa (211) have determined that some 10 molecules of water are directly involved in the reaction mechanism of cytochrome *c* oxidase and enter and exit the protein during every turnover (Fig. 3). The role of these water molecules is not clear. One suggestion is that the water movements may be part of the channel-gating processes of the oxidase and that these movement can lead to the pumping of protons across the membrane. It is clear that perturbations of the water structure at and around the oxidase, especially $\overline{V}_b$, during dehydration can be expected to significantly influence not only the structure but also the function of the protein. Water plays a critical role during the catalytic cycle of cytochrome P-450$_{cam}$, in which at one level, movements of water molecules in and out of the active site regulate enzymatic activity, and at the second, during dissociation of putidaredoxin with cytochrome P-450$_{cam}$, roughly 28 molecules of water are involved in the catalytic cycle (91). Some 50 to 70 solute-excluding water molecules become part of the hemoglobin tetramer in its transition from the full deoxgenated (tense) T state to the fully oxygenated R (relaxed) state. The osmotic work required for the binding of these 60 water molecules in

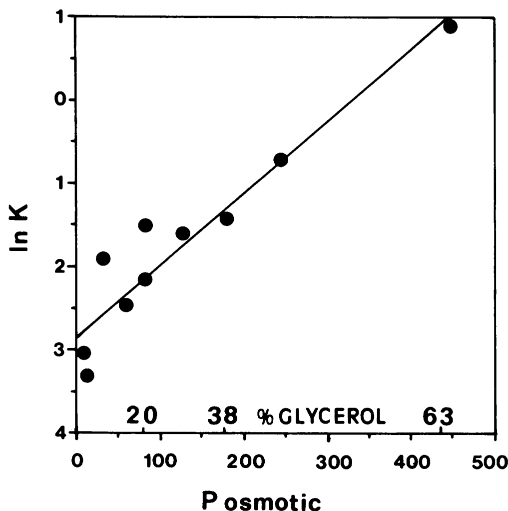


FIG. 3. Relationship between ln K and osmotic pressure (P). By treating values of the ratio of reduced cytochrome a to pulsed oxidase as equilibrium constants (K)—each indicating the extent of inhibition of electron transfer between the cyt a Cu_A pair and the cyt a_3 Cu_B pair of the oxidase, at a given water potential—ln K scales as a linear function of the osmotic pressure of the system at a hydrostatic pressure of 1 bar (0.1 MPa). The slope of the line extrapolates the magnitude of the ΔV as 190 = 22 ml mol^{-1}. At 90% glycerol, the cytochrome oxidase maintains the majority of its spectral character but there are noticeable shifts of the protein toward low-spin forms. Both oxidized and reduced proteins show an invariant spectrum up to 60% glycerol. It is postulated that cytochrome c oxidase contains an osmotically active compartment when the protein is turning over, which is not detectable when the protein is in either the fully oxidized or fully reduced static state. Reproduced from reference 211 with permission of the authors and publisher.

the change from the T to the R state is about 0.2 kcal mol^{-1} (0.84 kJ mol^{-1}) at 0.28 osmol ($\psi_{\pi s}$ of −0.73 MPa [70]). Both binding and kinetic studies show that approximately 65 water molecules are released when hexokinase binds glucose (315). Dehydration and rehydration reactions contribute far more to the energetics of protein and membrane conformation than was previously thought. Considerable progress has been made in understanding the structure of water and its role in physiological processes. These studies emphasize the important role of water in the structure and function of macromolecules, but they also seem to only emphasize the complexity of the whole cell system. Are there discrete fluxes in the cycling of water molecules across membranes? How many water molecules are required to sustain the translational efficiency of a ribosome, the fidelity of RNA polymerase, or the secretion of a protein as it traverses a membrane? More importantly, how are these waters influenced as a cell is dried and then rewetted?

How Much Water Is in a Desiccated Cell, and Where Is It?

Webb's measurements were in agreement with those from earlier studies on a range of desiccation-sensitive and -tolerant bacteria and viruses (see reference 403 and references therein). When dried at RH 40 and 30%, bacterial cells contain around 10 g of H_2O (per 100 g of solids) and 3 g of H_2O, respectively. The lower value, 0.03 g g (dry weight)$^{-1}$, is comparable to those measured for other anhydrobiotic cell types, such as *Artemia* cysts, plant seeds, etc., that have water contents of about 0.02 g g^{-1} under extreme desiccation (64, 392). The amount of water removed through freeze-drying of desiccated colonies of *N. commune* CHEN was equivalent to that which was lost after heating them, i.e., about 0.06 g g (dry weight)$^{-1}$ (170). All these examples clearly involve very low water contents, much lower than the level (0.3 to 0.4 g g^{-1}) at which there is monolayer coverage of proteins by water, for example. For proteins, water contents at and below 0.05 g of H_2O g of protein^{-1} are the minimum needed to hydrate charged and polar groups and are the minimum needed to form clusters of water. Acids are not saturated below values of 0.1 g g^{-1}, nor are polar side chains or peptide -NH bonds. The low water contents of desiccated cells imply that the cell proteins have been subject to a transition in proton distribution. For purified proteins, such transitions lead to a reordering of disulfides, side-chain and backbone conformational shifts are prevented, and the proteins are "tense" as opposed to being "loose."

The water contents of bacterial spores are considered to be difficult to measure but appear to be lower than those of their corresponding vegetative cells. The spore, spore coat, cortex, core, and protoplasm of *Bacillus stearothermophilus* had values in the range of 0.21 to 0.58 g of H_2O g (dry weight)$^{-1}$, and the a_w of the cortex was 0.83 (5). Akinetes (Dauerzellen) are resting stages (differentiated cells) that are produced by certain species of heterocystous cyanobacteria (136). Calculations made with optical measurements derived from analysis by light microscopy indicated that in *Anabaena variabilis* the akinetes, heterocysts, and vegetative cells contained 2.06 × 10^{-10} g of solids and 63% water, 0.46 × 10^{-10} g of solids and 85% water, and 0.31 × 10^{-10} g of solids and 77% water, respectively. Akinetes thus had more dry matter and the lowest water content, and it was also demonstrated that both their formation and their germination were associated with changes in their hydration level (36). But what is the significance of the numbers presented above? It seems that anhydrobiotic cells have such low water contents that their major constituents must lack a monolayer of water molecules. However, one other very curious fact emerges after considering these values. If we can believe the values quoted for bacterial spores (5; also see reference 149 for a review) and cyanobacterial akinetes (36), these structures do not belong in the class of anhydrobiotic cells—they contain too much water! Spores and akinetes must belong to the physiological group of cells that respond to water deficit through osmotic adjustment; i.e., the mechanism of tolerance is the use of compatible solutes (see below).

REMOVAL OF WATER FROM CELLS

The removal of almost all or some of the water from a cell can occur slowly or rapidly. The removal of that water can, depending on the quantity removed, cause mild, moderate, severe, or extreme water deficit. The early literature has described bacterial distribution in terms of the prescribed limits of water activity within which the cells function. Although this view is now the subject of considerable criticism (357), it is clear that bacteria may respond to water deficits through a number of different physiological responses.

Methods To Remove Cell Water

The addition of variable amounts of a solute to growth media has become the method of choice in studies concerned with the subjection of microorganisms to water stress. The advantages of this approach are that physiological processes such as the uptake of radioisotopes and secretion of metabolites are easy to measure and, more significantly, that the microorganisms can be readily harvested and the cell mass can

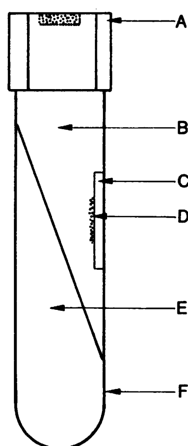


FIG. 4. Isopiestic control of water potential. Cells (D) are immobilized on an inert support such as a filter (C) that is curled around the inner surface of a glass tube (F). The tube contains salt-amended agar (E) and is sealed with a cap (A) that can permit sampling of, or additions to, the gas phase of controlled water potential (B). See reference 301.

be readily recovered. The limitations of this approach are that concentrated solutions tend to retard gas exchange and that high ion concentrations may impair membrane function and prove toxic to cells. Furthermore, this approach can never reproduce the extreme water deficit characteristic of anhydrobiotic cells. As a result, only a comparatively restricted range of water potentials can be achieved without compromising cell viability. The desiccation of microorganisms and the exposure of cells to various degrees of matric water stress require different approaches. One method for controlling the water status of a solid microbial substrate (community) is to bring the substrate into water vapor equilibrium with a solution of known water potential. The exposed surface of a bacterial colony growing on an agar surface is a good example of a community subjected to such isopiestic control of water potential. An experimentally convenient system can be constructed by placing the cells in an enclosure in close proximity to agar that has been amended with an appropriate concentration of a solute (160). Comprehensive lists of saturated solutions and their vapor pressures and humidities at different temperatures are provided by Winston and Bates (423). One isopiestic system, designed to measure $^{14}CO_2$ uptake by chasmoendolithic cyanobacteria (301), is shown in Fig. 4. A more elaborate system that achieved isopiestic equilibration through the control of temperature differentials has been described by Palmer et al. (272). The immobilization of cells in the form of pastes, slurries, and thick suspensions on various inert supports such as sterile sand, paper and nylon filters, cotton gauze, and even curtain material also affords the means to achieve comparatively rapid drying (and storage) of microorganisms (92, 93, 294, 300–302).

The studies of Webb focused on many aspects of the desiccation tolerance of bacteria (401–409). Using a system based on one devised originally by L. Goldberg, Webb built a drum system that, when operated at 28 lb/in^2, generated a collision spray of bacteria with liquid droplets with a mean diameter of 10 μm. Air was cleaned by passage through spin filters and charcoal and was dried in a column of silica gel. Samples were removed from the aerosol by means of a critical orifice with a liquid impinger operating at 12.5 liters min^{-1}. With this system, it was possible to study the survival of airborne bacteria atomized from various media. This latter approach is being used extensively in studies directed at understanding the dispersal of bacterial aerosols.

Preferential Exclusion Hypothesis

Consider a bacterial cell as it undergoes a transition between two intermediate-moisture states, x and y (with y at a lower water activity than x), in response to a change in the prevailing matric water potential.

$$\underset{\text{(maximum } E \text{ derived)}}{\overset{\substack{k_{\text{matric}} \\ \text{(minimum } E \text{ required)}}}{\text{State } x \rightleftharpoons \text{State } y}}$$

In such a system it is the change in the chemical potential of water ($\mu_w{}^x$ to $\mu_w{}^y$) that shifts the equilibrium. The most extreme case would be for a change when $\mu_w{}^x = \mu_w{}^R$ (the rehydrated state) and $\mu_w{}^y = \mu_w{}^D$ (the desiccated state) (see equation 1 and the accompanying discussion). These changes in the chemical potential of water involve net free energy changes, and the magnitude of these changes will differ depending on factors such as the time of drying and the temperature changes.

The following discussion considers the removal of water from a bacterial cell with a somewhat rigid wall and an elastic cytoplasmic membrane appressed to the cell wall and encompassing the cell compartment. At this point, let us assume that the cell has no sheath or outer investments. There are several ways in which such a population of cells may be subjected to a water deficit ($\mu_w{}^x$ to $\mu_w{}^y$) with respect to the environment. The one most often considered is an "osmotic stress" in which the cells are suspended in an aqueous solution of some solute that cannot enter them (Fig. 5a). In accordance with Gibbs-Donnan equilibria, there is a net efflux of water molecules until a state is reached at which there is a balance between the water activities of the two compartments. Such osmotic adjustment, which may occur rapidly, can be viewed as being a passive alteration of cell volume (Fig. 5a). A similar balance in water activity can be achieved if the cells accumulate compatible solutes or osmoprotectants (Fig. 5a). The latter include K^+ ions, glutamate, glutamine, proline, quaternary amines (glycine betaine), and the sugars trehalose and glucosylglycerol (58, 85, 86). An accumulation of one or more intracellular solutes can be achieved either by transport from the environment or through de novo synthesis or by both means.

What do these "compatible" solutes do, or, rather, why are they compatible? The value $\Delta G_{N \rightarrow U}$ (N = native, U = unfolded) defines the equilibrium that determines the conformational state of a protein, and it is a tenuous one. The half-life of a protein in an aqueous environment may be increased or decreased by a range of different chemical agents. Agents that stabilize proteins include sugars, amino acids, glycerol, polyols, amines, and salts (13). There is considerable experimental evidence that in a three-component system composed of a protein, water, and a cosolvent (solute), the solute may have a stabilizing effect on the protein. The mechanism is, in principle, a simple one—a stabilizing solute is excluded from the immediate vicinity of the protein. The solute is held at bay, albeit at some finite distance from the surface of the protein, such that the protein is, in effect, preferentially hydrated in time and space. Such preferential exclusion is thermodynamically unfavorable (entropically unfavorable) but would become even more unfavorable if the protein unfolded to provide yet more exclusion domains. As a consequence, preferential exclusion drives the equilibrium between the native and unfolded

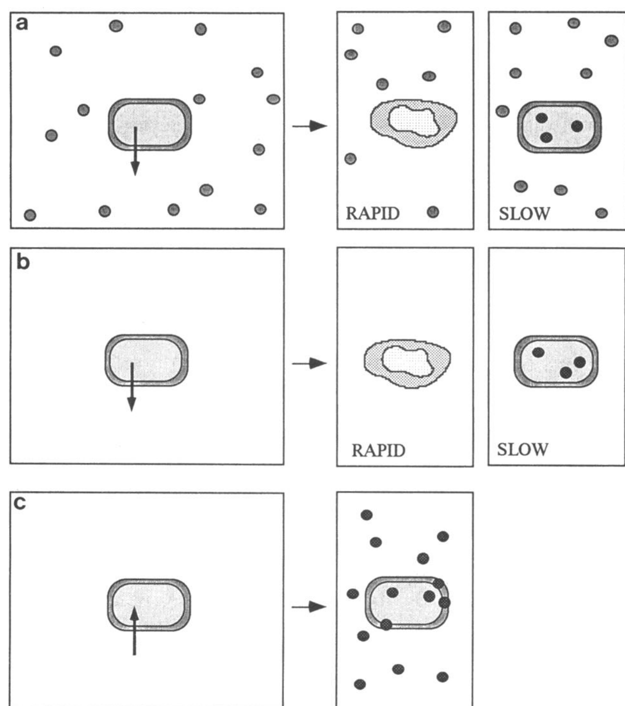


FIG. 5. Consequences of the removal of cell water. (a) Hyperosmotic stress. (b) Matric stress (air drying). (c) Hypoosmotic stress. The outer and inner membranes of a hypothetical cell are shown. Movement of water (vertical arrows) is either rapid or slow. Hatched circles represent a cosolvent (e.g., a salt). Cross-hatched circles represent a compatible solute (e.g., a carbohydrate).

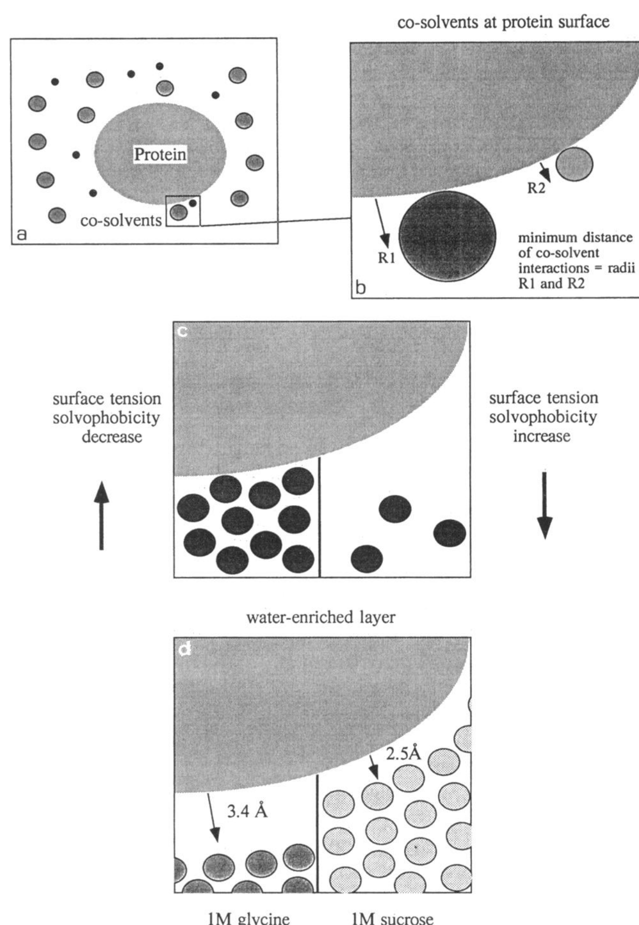


FIG. 6. Principles of water and solute exclusion by proteins. (a) A protein in water that contains different cosolvents (circles). (b) The minimum distance of cosolvent interaction is determined by the effective radius of the cosolvent molecule; in the steric mechanism, the cosolvent cannot penetrate the outer shell of the protein but water can. Thus, the solvent is enriched in water in the immediate vicinity of the protein. (c) Influence of cosolvents on surface tension and solvophobicity, and the effect on the water layer at the protein surface; sugars, nonhydrophobic amino acids, and most salts increase the surface tension of water. Therefore, their concentration in the surface layer must be reduced relative to that of the bulk solvent and they must raise the chemical potential of the protein, leading to its stabilization. (d) The size of the zone of exclusion at the protein surface differs with different cosolvents. See reference 383 for more details.

state toward that of protein stabilization. In contrast, destabilizing agents, which include ethylene glycol, polyethylene glycol, 2-methyl-2,4-pentanediol, dimethyl sulfoxide, urea, and guanidine HCl, bind preferentially to proteins. Here, there is an excess of solute in the immediate vicinity of the protein relative to the bulk solution, the equilibrium is thus shifted towards denaturation, and the unfolded form of the protein is favored.

Solutes that interact with proteins belong to two classes: the kosmotropes (water structure builders) and the chaotropes (water structure breakers). The former can be characterized through Hofmeister series considerations. Compatible solutes, for the most part, are kosmotropes and include sugars, other polyols, amino acids and amino acid derivatives, methylamines, and urea (a chaotrope), frequently in combination (13, 383, 384). All are electrically neutral, and all, except urea, are compatible solutes. Potassium, the exception, is used as a compatible solute by some bacteria (56). The charged amino acids Arg and Lys are not used, and neither are amino acids with large hydrophobic side chains.

Timasheff has described how, in accordance with the preferential exclusion hypothesis, there may be three consequences of the immersion of a protein in an aqueous solution of a given solute. There may be a preferential hydration of the protein, a preferential binding of the solute to the protein, or no preferential interaction (383, 384) (Fig. 6). In reality, however, preferential exclusion and binding are not all-or-nothing events. Some salts that are considered to be stabilizers will, at sufficiently low concentration, bind to charged regions of a protein. The preferential exclusion mechanism represents the additive properties of a given solute, and these must reflect a continuum of specific, and in some cases opposed and quite different, interactions with the protein.

The binding of cosolvent by the protein can be described in simple Scatchard notation (ligand binding) with designations where 1 is water, 2 is protein, 3 is cosolvent, m is the molal concentration, μ is the chemical potential, g is gram of component per gram of water, and T is the temperature in Kelvin:

$$\nu_3 = [\delta m_3/\delta m_2]_{T,\mu_1,\mu_3}$$

When ν_3 is positive, there is preferential binding of component 3 (the cosolvent); when ν_3 is negative, there is preferential hydration of the protein (preferential exclusion of the ligand). Stabilizing compounds all have negative binding values. As the

chemical potential increases, the derivative is positive and the measured binding is therefore negative. Equilibrium constants for the native and unfolded states can be presented as follows (note that I have introduced a hypothetical constant K_2 here to emphasize that an unfolded protein is not necessarily a denatured [dead] protein; Finney (117) made the wry point, "dry proteins are dead, or at best asleep."):

It is seen that these K values will vary with the concentration of the cosolvent:

$$[\delta \ln K/\delta \ln a_3]_{T,P} = \nu_3^D - \nu_3^N = \Delta\nu_3$$

were $\Delta\nu_3$ is the difference between the preferential binding of component 3 (cosolvent) in the denatured and native states. If the preferential exclusion is greater in the denatured than the native state, $\Delta\nu_3$ is negative, the equilibrium is shifted to the left, and there is protein stabilization.

The mechanisms of stabilization include steric exclusion, increase of the surface tension of water by the cosolvent, and the so-called solvophobic effect (Fig. 6). Solvophobicity is a consequence of the reinforcement of the hydrophobic effect by a solvent additive as a result of water structure enhancement by the additive. Contact between nonpolar residues on the protein surface and water molecules which are constantly fluctuating in and out of structure clusters is thermodynamically unfavorable. A redistribution of solvent molecules is thought to occur at the protein surface, but current opinion does not favor the idea that there is an impenetrable hydration layer consisting of water molecules ordered on the protein surface ("iceberg" hypothesis). The range in osmotic pressure gradients that can derive from different concentrations of different cosolvents leads to different extents of cosolvent exclusion from protein surfaces. As a consequence, the extent of cosolvent exclusion, and hence the degree of stabilization, varies (Fig. 6). A positive deviation of the osmotic pressure from ideal reduces the redistribution of solvent components in a cellular compartment.

Not every protein will have the same zone of exclusion for a given cosolvent. Many solutes that stabilize or destabilize proteins in solution by preferential exclusion or binding may also be excluded from, or be bound to, membranes. However, such effects are hard to assess, although many, such as the destabilization of the phospholipid bilayer, the formation of the H_{II} phase at low temperature, and leakage from phosphoethanolamine/phosphatidylcholine liposomes, are clearly deleterious (78). Many molecules that stabilize proteins in solution will prevent this dissociation when excess water is present, but when the hydration shell of the protein is removed, as occurs in the desiccated cell, the specificity for the stabilization becomes very high (383, 384).

Drying versus Salting (or Sugaring)—What Is the Difference?

Water efflux occurs when bacterial cells are exposed to a gas phase with a water activity that is lower than the cell compartment. If there is a considerable difference between the water activities of the two compartments, exposure of the cells for a limited time may lead to rapid shrinkage of the cytoplasm (Fig. 5b). However, if the water activity of the gas phase is sufficient to permit growth, albeit slow growth, the cells may achieve a water balance through de novo synthesis of a compatible solute as described above. These drying stresses are often termed matric water stresses. The removal of a substantial fraction of the bulk water from cells through a matric water stress is termed desiccation, and such desiccation can be achieved through either rapid or slow drying. There is one distinction between matric and osmotic systems and one that is evident from a comparison of Fig. 5a and b. The immediate environment of a cell under matric stress is the atmosphere; i.e., the surfaces of their cell walls are exposed to a gas phase, while cells under osmotic stress are bathed in an aqueous solution, albeit it one of diminished water activity. As such, considerations of osmotic stress (even when that stress is administered with 5 M KCl) deal with restricted, and high, water activities. What this really means, and it is the crux of an understanding of desiccation, is that a cell that has been subjected to an extreme of air drying, say incubation at −400 MPa (see, e.g., reference 92), has no capacity to rely on preferential exclusion mechanisms for its protection—virtually all water is gone. And if the water is gone, what of our single-component system—how can emphasis be placed upon the properties of water in a discussion about desiccation tolerance and the anhydrobiotic cell? This is a misleading argument, of course, but it has lead to the development by John and Lois Crowe, their students, and colleagues of a water replacement hypothesis to account for the preservation of biological integrity in the absence of liquid water (67, 81). The essential elements of this hypothesis and its consequences for understanding mechanisms of desiccation tolerance are reviewed in detail later in this review.

To account for either survival under more moderate matric stress or the attenuation of the effects of rapid drying, the discussion presented above requires certain qualifications. First, our cell was assumed to have no sheath or outer investments that may contribute a substantial fraction of interstitial water (Fig. 2). Second, although a balance in the water activities of different compartments is implied by osmoregulation, it should be noted that water is never at equilibrium throughout a given system "at balance" as a consequence of local changes in density (420). Third, osmotic and matric water stresses are generally regarded as being quite distinct and are treated as such in discussions of water stress. Such distinctions are rarely easy to make in microbial ecology. For example, an intertidal microbial mat may be submerged by seawater for part of the tidal cycle (Fig. 5a), yet, for many days of the year, the mat may remain "high and dry"—desiccated and salted (Fig. 5b). In this dry state the mat community is prone to rehydration stresses imposed by transitory rainfall and/or hypersaline tidal waters.

The different methods available to remove water from cells have been used with a wide range of bacteria. Some of the general consequences of drying cells are summarized in Table 1. Are the initial consequences of water removal under matric (drying) and osmotic conditions really similar? The cost of dehydrating or the benefit of fully rehydrating any hydrophilic surface is remarkably uniform and high at 1.5 to 15 kcal mol^{-1} (6.3 to 63 kJ mol^{-1}) per 100 $Å^2$ (1 nm^2) of surface area (316). Theoretically the energy gain or loss upon rehydration or drying of a bacterial cell of 1 by 2 μm (6.28 μm^2) would be on the order of 10^8 kcal mol^{-1} (4.2×10^8 kJ mol^{-1})! The removal of water itself should result in a positive entropy change (403). Upon drying bacterial cells, the magnitude of the activation energy (ΔH) was found to be small in comparison with that required to denature protein, and ΔS was negative (403). The studies of Webb (401–409) provide comprehensive measurements on the responses of bacterial cells to the stress of air drying. In these experiments cells were dispersed in aerosols at different relative humidities or were immobilized and dried by

TABLE 1. Gross responses of cells to air drying

Level of effect	Response
Community	Change (usually increase) in surface area Shrinkage Salt precipitation Change in texture Change in shape Change in color (oxidation of pigments)
Cell	Shrinkage of capsular layers Increase in intracellular salt levels Crowding of macromolecules Changes in volumes of cell compartments Changes in biophysical properties (e.g., surface tension) Reduced fluidity (increased viscosity) Damage to external layers (e.g., pili, membranes) Change in physiological processes (e.g., growth arrest)

isopiestic equilibration. These studies indicated that all evaporation, as far as free water ($\overline{V}_f$) is concerned, takes place almost instantaneously upon aerosolization, and an equilibrium between cell-bound water ($\overline{V}_b$) and the environmental water vapor pressure is reached very quickly. Under these conditions, then, the principal consideration is cell survival—the drying event is too rapid to permit either growth adjustment or de novo regulated synthesis of osmoprotectants. Webb calculated death rate constants (*K*) for bacterial strains from the plots of ln N_t/N_0 against time for the period up to 1 h and then from 1 to 5 h, following aerosolization. Highly reproducible death curves were obtained for sensitive bacteria such as *E. coli* and for tolerant bacteria such as *Staphylococcus* spp. Typical data obtained in these experiments are presented in Fig. 7. Webb (403) estimated that sensitive bacteria such as *E. coli* and *Serratia marcescens* had a $\overline{V}_{cell}$ of ~4 (80% water, 20% solids), and cell death upon drying occurred only at or below $\overline{V}_{cell}$ = 0.3. This value was reached when the cells were dried at or below a relative humidity of 80% (a_w = 0.80; equivalent to a Ψ_{Π_s} of some −31 MPa). Arrhenius plots (ln *K* versus 1/*T*) indicated that the activation energy (slope) associated with cell death appeared to increase as the time of storage in air lengthened (see equation 6). The calculated *E* values were thought to represent the strengths and/or numbers of the new inter- or intramolecular hydrogen bonds that formed either when the remaining water molecules reorientated themselves or as dehydrated groups interacted. To examine these events further, Webb calculated the entropy (ΔS) changes associated with time of storage of cells in air, taking into consideration the Boltzmann energy distribution:

$$K = (K_b/h)e^{\Delta S/R}e^{-\Delta H/RT}$$

where *K* is the death factor, K_b is the Boltzmann constant, *h* is Planck's constant, and ΔH is the heat of activation. Although the removal of water itself should result in a positive entropy change, comparisons for various viruses and bacteria indicated that the values of ΔS were negative when microorganisms were inactivated in the dry state, with energy values of 9 to 12 kcal mol^{-1} (37.7 to 50.2 kJ mol^{-1}) for *S. marcescens* (ΔS = −40 to −18) and 8.5 to 10 (35.6 to 41.8 kJ mol^{-1}) (ΔS = −38 to −19) for *E. coli*. The ΔS and ΔH values which Webb estimated and which were thought to be responsible for the reactions leading to cell death were small, suggesting very subtle changes. As the entropy change was not thought to be related to the size of the organism, it was concluded that inactivation due to drying takes place in relatively small regions of the cell. Webb concluded that only "bound" water was involved in the mechanism by which cells died when they were desiccated, and the negative entropies of aerosol deaths were interpreted to reflect a "tightening" (crowding) of molecules (note the previous discussion referring to tight and loose states of proteins). The two-phase death rate was explained in terms of the removal of water molecules first from —N, =N-H, or —OH groups and then, upon further drying of cells, from =C=O or =P=O groups.

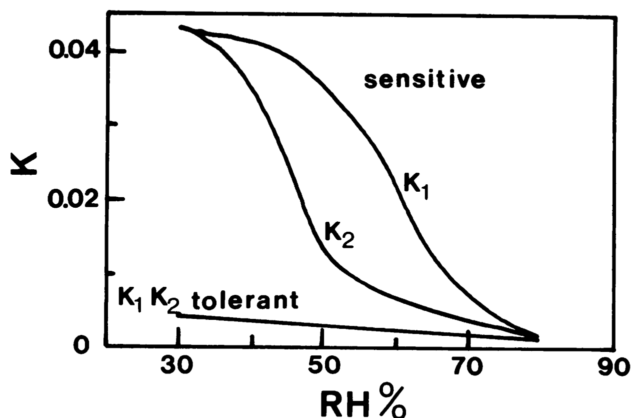


FIG. 7. Relationship of estimated death constants (*K*) to RH. Constants were measured during the first 1 h (K_1) and subsequent 1 to 5 h of drying, for sensitive bacteria (e.g., *Serratia marcescens*) and drying-tolerant bacteria (e.g., *Staphylococcus aureus*). Adapted and redrawn from reference 403.

There has been virtually no appraisal of Webb's findings within the past 30 years. Webb considered much about why bacterial cells are sensitive to drying and desiccation but less so to why some cells are tolerant. The air drying of cells clearly leads to a very rapid curtailment of cell growth. What of osmotic stress? If the osmolarity of the suspending medium is increased, cells of *E. coli* respond by decreasing their growth rate in a linear fashion (57). The cells also show changes in behavioral responses (225). Growth of the cells was found to effectively cease at an extrapolated value (not determined experimentally) of 1.8 osm (Ψ_{Π_s} = −4.64 MPa). The final arrest of growth due to this osmotic shock was thought not to be due to energy limitations (178). One must question, therefore, what the cells respond to in this situation if water removal does not compromise their survival but simply modulates their growth rate. While the steady-state water-accessible volumes for the cytoplasm ($\overline{V}_{cyto}$) and the cell ($\overline{V}_{cell}$) decreased linearly with increasing osmolarity, $\overline{V}_{peri}$ (equal to $\overline{V}_{cell} - \overline{V}_{cyto}$) increased linearly with increasing external osmolarity. Extrapolation of the data suggested that growth ceased when the steady state value for $\overline{V}_{cyto}$ = 0.5 ± 0.2 μl mg (cell dry weight)$^{-1}$ or, more significantly, when $\overline{V}_{cyto} = \overline{V}_b$, the quantity of "bound" water ($\overline{V}_{cyto} = \overline{V}_f + \overline{V}_b$, where *f* is free water and *b* is bound water). This limiting value of cytoplasmic water is very similar to that calculated to cause loss of viability of *Serratia marcescens* and *E. coli* when these bacteria were air dried (see above) and is equivalent to the value for the amount of bound water associated with protein. In a three-component system containing water, lysozyme, and 1 M glycine as a cosolvent, the value of bound water is around 0.54 g of H_2O g of protein^{-1} (384). In the absence of cosolvent, the value is closer to around 0.2 g g^{-1}. What do these measurements tell

us? For *E. coli*, the mean cell volume is related to growth rate such that, at 37°C,

$$\overline{V}_{\mu} = 0.4 \times 2^{\mu}\ \mu m^3$$

where $\overline{V}_{\mu}$ is the average volume per cell at a growth rate of μ. More generally, $\overline{V} = \overline{V}_u \times 2^{\mu}$ where $\overline{V}_u$ is a constant and is equal to the average cell volume in a population growing at a rate μ approaching 0 doubling per h (96). In these respects, one can argue that the reduced growth rate of *E. coli* at higher osmolarities and the linear dependence of the growth rate on external osmolarity are responses to a change in the space available ($\overline{V}_{cell}$) for cell functions (growth) and not a lack of water per se. Parallel observations that the relationship of $\overline{V}_{cyto}$ and growth rate at a given osmolarity changes when the carbon source is changed are consistent with this conclusion. If only a small amount of $\overline{V}_f$ is required for growth, then the growth of cells and different growth rates can be accommodated within a wide range of $\overline{V}_f$ values. The real water stress therefore is the perturbation of $\overline{V}_{cyto}$ at levels at which $\overline{V}_{cyto}$ becomes equivalent to or less than $\overline{V}_b$. The factors that lead to perturbation of $\overline{V}_b$, and the mechanisms used to restrict that perturbation, may be of relevance to considerations of the consequences of the initial stages of desiccation. An understanding of desiccation tolerance requires an appreciation of the function of $\overline{V}_b$, the means by which $\overline{V}_b$ is perturbed, and the mechanisms used to prevent and/or survive that perturbation. One conclusion of the study of Cayley et al. (57) was that the mechanism by which osmotic stress controls growth rate is fundamentally different from the means by which nutrient limitation controls it, even though the patterns of gene expression under stationary-phase growth and under osmotic upshift are regarded, superficially, as being rather similar (62, 354). Should we assume that the mechanism by which the initial stages of desiccation control growth rate is also different?

The results from drying and salting (and sugaring) of cells emphasize the critical role of bound water. Only a small amount of free cytoplasmic water is required for cell growth, or, simply stated, growth depends on the presence (or absence) of only a small fraction of $\overline{V}_{cyto}$. For enzymatic activity, only a monolayer coverage of a protein with water molecules is required for activity. In the air-dried cell, however, even monolayer aggregations of water molecules have been perturbed and diminished.

Hypertonicity and Hypotonicity

As cells generally have elastic cell walls, the removal of water may lead to plasmolysis but not necessarily to cell death. Even though cells of *E. coli* are relatively sensitive to osmotic stress, their suspension in 3 M NaCl for 60 min does not lead to a decrease in viability (57). One bacterial cell that does appear to plasmolyze readily, however, is the cyanobacterial heterocyst (398). The heterocyst is a structurally and biochemically modified cell that accommodates active nitrogenase (426). Heterocysts tend to be larger than vegetative cells, they have an inelastic cell wall, and, through physical connections with adjacent vegetative cells, they receive supplies of carbohydrate that sustain their high respiratory rate (426). Evidence suggests that plasmolysis of the heterocyst in hypertonic solution is due to the separation of the cell wall from the enclosing envelope. In one study, heterocysts of *Nostoc muscorum* were found to collapse in 0.3 M mannitol ($\Psi_0 = -0.46$ MPa) (29) and the authors concluded that heterocysts are unable to perform osmoregulation. This is an interesting proposal—particularly in view of the fluxes in reductant (carbohydrate) supply that take place between the vegetative cell and heterocysts—but is the proposal true? The heterocysts of *N. commune* UTEX 584, but not the vegetative cells, collapsed when cells were dried, desiccated, and then rehydrated in nonionic osmotica (1% [wt/vol] glucose; $\Psi_0 = -0.14$ MPa) but not when they were rehydrated in a minimal-salts medium of equivalent osmotic strength ($\Psi_0 = -0.18$ MPa) (298). Heterocystous cyanobacteria are a conspicuous feature of the marine shore (290, 293, 308), an environment where the rates of evaporation, the rainfall, and repeated inundation with salt waters would seem to guarantee a rather miserable life for a nonosmoregulating heterocyst. On the other hand, a survey of a range of hypersaline environments along the coast of the Sinai, Israel, that support highly abundant communities of nitrogen-fixing cyanobacteria failed to identify one heterocystous form (292, 293), and heterocysts of laboratory-grown strains (in logarithmic growth) do appear to be particularly prone to drying damage, as judged from analyses at the ultrastructural level (278). Curiously, a nonheterocystous form, *Trichodesmium*, is the dominant nitrogen-fixing cyanobacterial genus in the open ocean (118). Why? No one has looked at the volume water relationships of heterocysts as thoroughly as the water relationships of vegetative cells (398). Perhaps such studies would be very informative.

Paradoxically, cells may also be placed under a water stress through dispersal under hypoosmotic conditions, and in this case the bacteria are faced with the prospect of continually bailing out their water (57). To counteract this hypoosmotic stress, the cells may secrete osmotically active macromolecules such as glucans (Fig. 5c). In a MOPS (morpholinepropanesulfonate)-buffered glucose minimal medium supplemented with various amounts of NaCl (nonpenetrating), optimum growth of *E. coli* K-12 strain MG 1655 occurred at 0.28 osm (57). From equation 3, this value is equivalent to an osmotic pressure (Π_s, at 37°C) of +0.72 MPa ($\Psi_{\Pi_s} = -0.72$ MPa). These data suggest that the cells were hypotonically stressed in this growth medium and that the 0.28-osm value must represent an equilibrium value. Under these hypoosmotic conditions, *E. coli* cells accumulate oligosaccharides (membrane derived [see below]) in the periplasm (201) and replace cytoplasmic K^+ with putrescine (154).

Although it is ultimately the singular removal of water that is to blame, the exposure of a cell to an extreme osmotic stress and the exposure of the same cell to dry air result in fundamentally different changes that affect growth potential and physiology.

Sensitivities of Prokaryotes to Air Drying

Priestley summarized much of what is published on the longevity of plant seeds, including the results of a survey conducted by the British Association for the Advancement of Science in the 19th century (310). While the data suggest that seeds may survive many tens of years, perhaps even centuries, of desiccation, Priestley dealt with many of the reports of extreme longevity in a balanced and critical manner. It appears that the best-substantiated incident of longevity, a record of six centuries, is for a seed of *Canna compacta* (Cannaceae) found within a walnut shell rattle, at a pre-Hispanic archeological site in northwestern Argentina. Critical summaries of the longevities of desiccated bacteria are harder to come by, for several reasons. A plant seed represents the culmination of a prescribed and readily observed series of developmental events, while the history of dried bacterial cells, certainly those in nature and often those dried under laboratory conditions, is cryptic if not virtually impossible to assess. It is difficult to compare the sensitivities of different groups or genera of

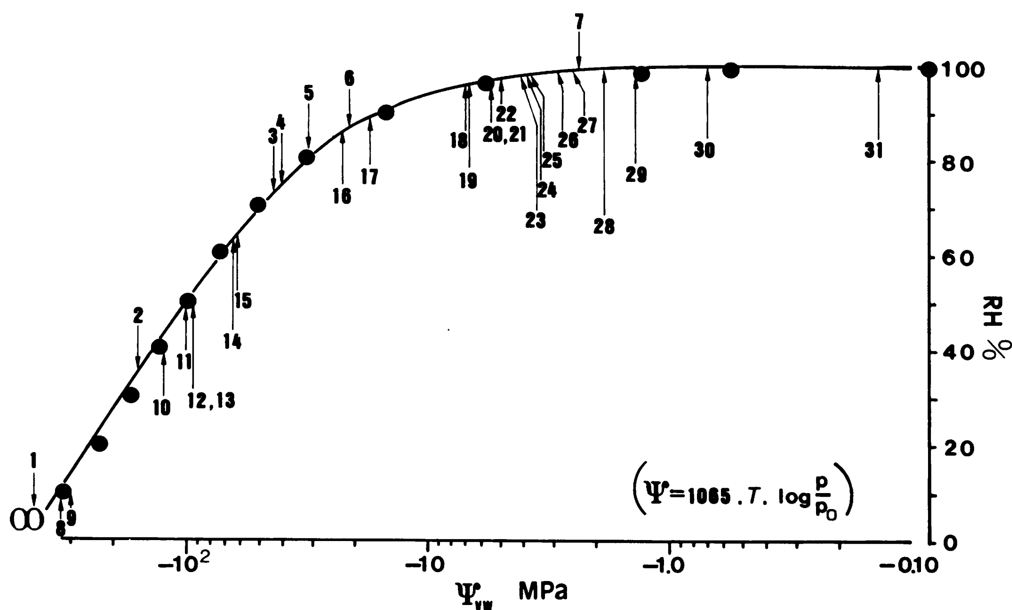


FIG. 8. Relationship between water potential (Ψ_{wv}) and relative humidity (RH %). The scale on the x axis is logarithmic. Solid circles represent the values of Ψ calculated with the equation shown (301) from values of RH/100. Numbers refer to data presented in Table 2. Numbers above the curve relate to physical constants, and those below the curve relate to physiological processes (see Table 2).

bacteria in view of the many different techniques used to grow cells and to dry cells (see, e.g., reference 339) and the fact that some cells have an inherent ability to form resting stages, cysts, and spores (see below). Nevertheless, many studies have attempted to describe the values of a_w at which cells maintain or lose viability and grow or cease growing or the a_w values at which particular physiological properties or activities are modulated (61, 159, 180, 222, 277, 289) (Fig. 8; Table 2). However, the utility of such ranges of water activity for the assessment of bacterial growth relationships has come under question (357).

The rate at which cells are dried in air is critical to cell survival. The survival rates of *Arthrobacter*, *Pseudomonas*, *Mycobacterium*, *Escherichia*, *Micrococcus*, and *Saccharomyces* cells were rather similar when the cells were counted immediately after fast or slow drying (20 min and 24 h, respectively). After prolonged storage, viabilities were higher for slow-dried, as opposed to fast-dried, cells (11, 12). These observations were true for *E. coli* only when more than about 8 mg of water per 10^8 cells was present on paper filters. To obtain optimum resistance to water loss, it was estimated that the drying period for a 25-μl aliquot of such a cell suspension of *E. coli* should be greater than 13 h. The times of survival following air drying of representatives from the major groups of prokaryotes are summarized in Fig. 9. To achieve some degree of consistency, only data relating to the immobilization and drying of suspensions of bacteria on a hydrophobic surface, i.e., glass, are included. Even so, the times summarized in Fig. 8 and 9 should be viewed as being very approximate, even artificial, given that in many instances the descriptions of the physiological status of the cells were lacking and experimental conditions were variable (see, e.g., reference 358). However, these data do indicate that a capacity for a certain degree of tolerance to drying is associated with all bacteria, to greater or lesser extents, and the range of time during which bacteria may remain viable in the air-dried state is extreme. There is no obvious trend here, other than the seeming resistance of spore-forming bacteria. For perspective, the estimated ages of what may be the oldest bacterial populations on Earth, those in Antarctic rocks and Siberian permafrost, are included.

When distributed in air, in association with mucus or slimes, cells may settle out and adhere to a surface or otherwise remain in liquid droplets for finite periods (Fig. 10). Evaporation of water in aerosols is essentially instantaneous (403). Following desiccation, the same cells may also remain suspended in air as components of dust. These different stages of drying may influence the viability of cells considerably. Cells of *Mycobacterium tuberculosis* remain viable for around 1 week when they dried as an aerosol on glass in physiological saline. In dust, tubercle bacilli remain viable for up to 120 days, and if they are stored under various vegetable oils the viability is extended to around 2 years (241). Solid media are generally used to sample the populations of bacteria present in air. It is thought that lengthy periods of sampling cause desiccation of the growth media, which leads to loss of viability of the airborne bacteria. In fact, it appears that bacteria commonly found in room air are little affected (less than a 10% drop in viability) by substantial (13%) reductions in the water content of agar. Therefore, exposure times can be extended considerably to permit accurate sampling, for example to over 1 h in a 30-liter min^{-1} bacterial slit sampler (419). Viabilities increase if cells are dried in the presence of certain sugars, blood, serum, or complex media, and survival times increase if these dried cells are kept in the dark, under an inert-gas phase (291, 299). Optimum growth of *E. coli*, generally assumed to be a desiccation-sensitive bacterium, occurs at -0.72 MPa, cessation of growth occurs at -4.6 MPa, and cell death occurs at or below relative humidities of around 70% (-51 MPa). How do the responses of other bacteria respond to drying? The survival rates of *Clostridium mangenoti* (spores), *Halobacterium halobium*, *Bacillus subtilis* (spores), and *E. coli* were tested by applying droplets of cell suspensions to aluminum foil and then drying them at 10^{-8} torr (1.3×10^{-6} Pa) for 24 h at 77 K (209). Only spores survived under these conditions (55 and 75% survival of *Bacillus* and *Clostridium* spores, respectively). Fol-

TABLE 2. Water deficits that limit growth or physiological activity

No.[a]	Pressure (MPa)	Comment	Reference
1	0	Atmosphere over fuming P_2O_5	423
8	−400	*Crinalium epipsammum* and *Tychonema* spp. survive	92
9	−300	*Enterobacter cloacae* and *Alcaligenes eutrophus* become nonculturable	281
2	−168	Saturated solution of $CaCl_2$	423
10	−129	Limit for survival of *Rhizobium meliloti* in alginate beads	212
11	−100	Typical exposure of *Nostoc* colonies in situ	290
12	−99.5	Nitrogenase activity lost in 30 min in *Nostoc* strain UTEX 584	300
13	−99.5	Polysomes of *Nostoc* strain UTEX 584 intact after 2 h	7
14	−66	Mean lowest value in Antarctic rocks	256
15	−62.5	Ambient values above marine *Scytonema* mats	290
3	−44	Saturated solution of urea	423
4	−41	Saturated solution of NaCl	423
5	−31	Nucleic acids and proteins fully hydrated	405
16	−26	Cortex and core of *Bacillus* spores	5
6	−22	Saturated solution of sucrose	423
17	−17	Lower limit for growth of *Arthrobacter* spp.	61
18	−7	Lower limit for growth of *Bacillus subtilis*	422
19	−6.9	Minimum required for photosynthesis by *Chroococcidiopsis* in hot-desert rocks	271
20	−5.6	Nitrogenase active for at least 3 h	298
21	−5.6	Minimum for growth of *Flavobacterium*, *Pseudomonas*, and *Rhizobium* spp.	61
22	−5	Bacterial respiration ceases	422
23	−4.6	Growth of *E. coli* ceases	57
24	−4.4	Competitive bacterial growth ceases	159
25	−4.2	Nitrification and sulfur oxidation cease	159
26	−2.8	Inhibition of photosynthesis in desert crusts of *Microcoleus* spp.	41
27	−2.7	35‰ seawater at 34°C	301
28	−1.8	Inhibition of growth of *Microcoleus* spp.	41
29	−1.5	*E. coli* MM294(pEMR1) nonviable	317
30	−0.7	Mean of minimum value that supports bacterial growth (see no. 21)	61
31	−0.14	Motility of bacteria ceases	159

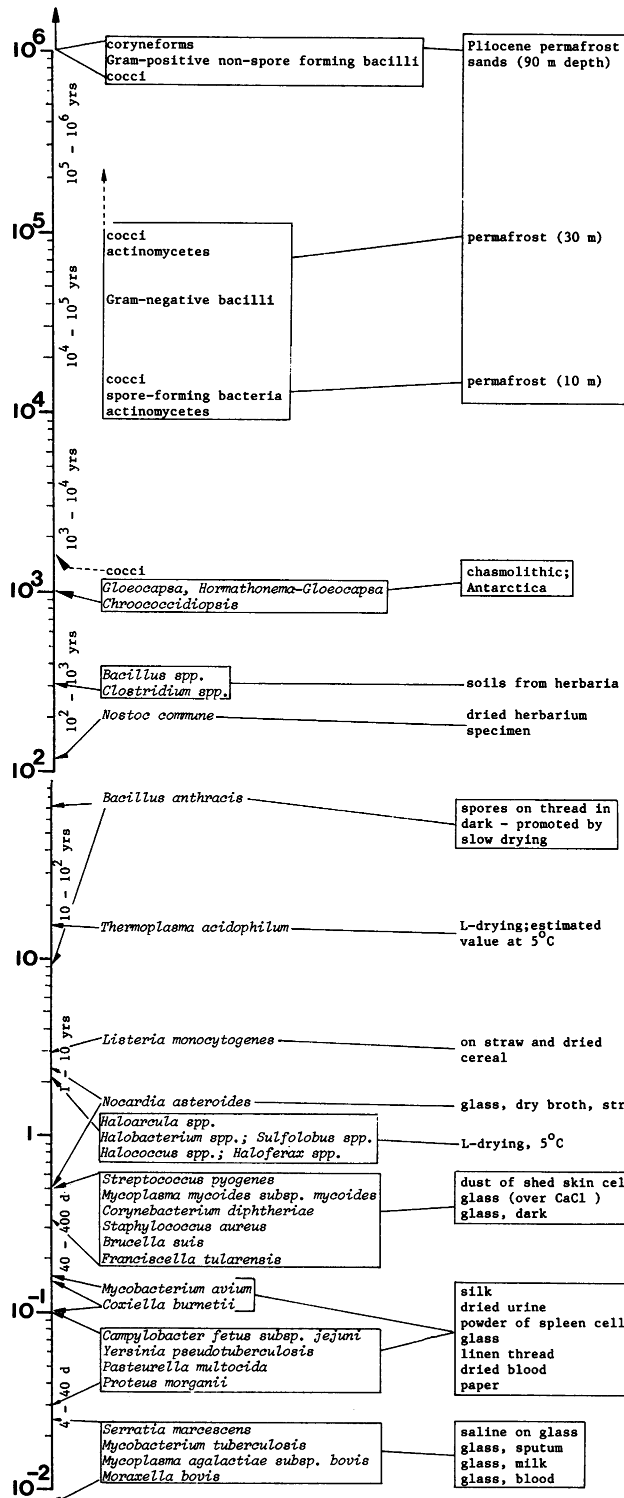
[a] For the relationship between RH and water potential, see Fig. 8. Note that examples are presented from most to least extreme water deficit.

lowing the exposure of dried cells to 200 mC (a barrage corresponding to about 250 years of irradiation in near-Earth orbit), the survival rates for *Bacillus* and *Clostridium* spores were 45 and 25%, respectively.

The vegetative cells of many different species of bacteria can undergo some degree of differentiation into structurally and physiologically distinct forms classified variously as endospores, exospores, myxospores, cysts, akinetes, and resting stages (24, 116, 261, 370, 389). While highly diverse, common features of these growth forms are that they possess thickened, structurally characteristic extracellular cell wall layers and are desiccation resistant. More significant, however, is the fact that these forms develop during the time the parent vegetative cell is growing in an aqueous environment, i.e., desiccation and drying are not cues for the synthesis of spores, etc.

Drying rarely occurs without being superimposed upon some other stress. In fact, the combined effects of desiccation, or osmotic stress, and pH stress (2% acetic acid) provide a useful sanitization procedure for *Salmonella typhimurium* and *Listeria monocytogenes* (95). The factors that appear to influence the upper and lower limits of the range of longevity are the additive effects of cold, enhancing survival to perhaps millions of years, and oxygen and radiation (sunlight), decreasing survival times to seconds. It is unclear from the data summarized in Fig. 8 and 9 whether other factors, such as the possession of capsules and sheaths, influenced the reported times of survival. One characteristic of desiccation-tolerant cells is that while they are tolerant of desiccation, they tend to be physiologically active only at comparatively high water potentials. A *Microcoleus* sp. in desert crusts was partially inactive at −7 bars (−0.7 MPa) and completely inactive at −18 bars (−1.8 MPa) (41), and isolates of coccoid cyanobacteria from hot-desert rocks cease to photosynthesize below a matric water potential of approximately −7 MPa (205, 234) (Fig. 8; Table 2).

Desiccation plays a determinative role in the ecophysiology of bacterial communities that are found in aerophytic environments, on and inside rocks, on and in soils (276), in crusts and accretions, in soils and sediments, in the phyllosphere, in dusts and aerosols, and on the skins of animals and humans. At high matric potentials, water retention in soils and sediments is dependent on capilliary effects and is therefore strongly influenced by soil structure (274). At lower potentials, the effects of structure are much less pronounced and the texture and specific surface are of greater consequence. In terms of the distribution of water in soils, a soil water potential, Ψ_m, of 0.1 bar (−10 kPa) is normally associated with water saturation of soil capilliaries less than 30 μm in diameter; a Ψ_m of −0.3 bar (−30 kPa) is associated with saturation of capilliaries less than 4 μm in diameter; and at a Ψ_m of less than −5 bars (less than −0.5 MPa), the soil water is thought to be distributed as a film only a few water molecules thick. Matric water potential can also markedly affect gas diffusion characteristics of sediments. The drying of soils is clearly a problem if water is required for locomotion. Such effects are expected to influence the types of communities that colonize the surfaces of sediments where water loss is greatest, including the surface sands that support a Sandkörnerflora (epipsammic bacteria on sand grains [136]), the surfaces of sand dunes (290, 415, 416), and the surface sediments of Farbstreifen-SandWatt—laminated and colored sands and muds in intertidal localities that support a varied community of photosynthetic and nonphotosynthetic bacteria



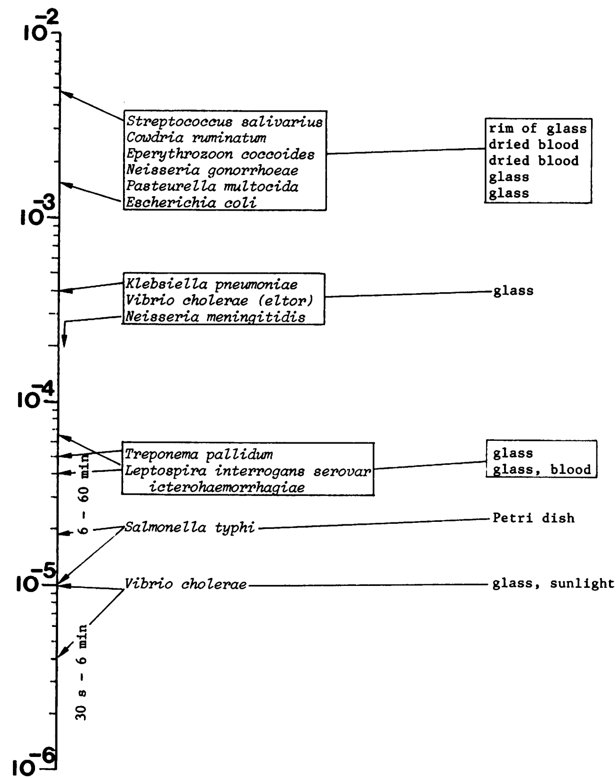


FIG. 9. Survival times of representative prokaryotic species in the air-dried state. The scale is logarithmic (seconds to years). Arrows indicate the single time measured or the range. In most cases the bacteria were air dried on a glass surface. Data were obtained from references 2, 138, 197, 291, 339, and 360. Note that the times for some archaea (339) were extrapolated (not measured directly).

(307) (Fig. 8; Table 2). The importance of soil structure and water retention characteristics can be emphasized by noting the quite different results obtained from drying cells under identical conditions on different immobilization supports (92, 93). Here, rehydration enabled the recovery of photosynthesis of desiccated cyanobacteria only on filters with good water retention. In an *Oscillatoria* strain isolated from sand dunes, the recovery of photosynthesis was instantaneous (100% viability) after storage of cells for 16 h in the dark at −400 MPa above silica gel in a desiccator (92). Loss of viability in such habitats may also be ecologically significant, because water potential increases associated with the rewetting of dry soil may be a major catalyst for soil C turnover (202). At least for cowpea rhizobia, no relationship was apparent between the capacity of the bacteria for desiccation tolerance and the aridity of the soil where sampling occurred (269).

The sensitivities of soil bacteria to relatively small negative water potentials have been attributed to the restriction of movement as the soil water drained (422). *Pseudomonas aeruginosa* requires water-filled pores 1 to 1.5 μm in diameter or larger to move readily in soil, so it is easy to understand how a matric deficit could influence viability if the latter is dependent on motility. Because the drainage characteristics of sediments differ, the optimum matric water potentials for movement and motility must vary between different sediments (274). Strains of cyanobacteria were found to have quite different survival times when liquid-grown cultures were used to inoculate dry soils (385). In this case, the different sensitivities were thought to be due to the ability of some strains to form akinetes and/or extracellular polysaccharide sheaths.

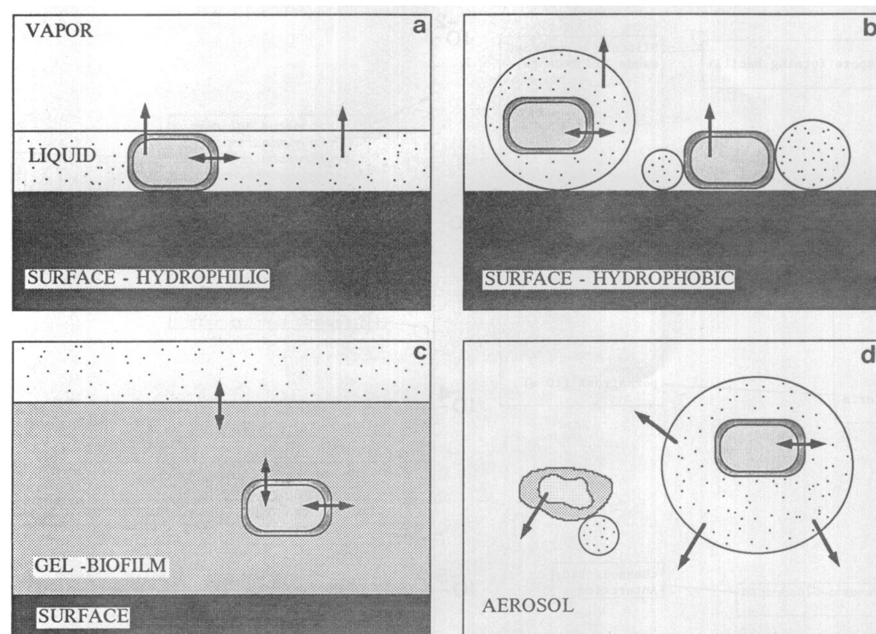


FIG. 10. Interactions of bacterial cells, water, and surfaces. (a) Bacteria in a thin film of water; (b) hydrophobic interactions at the air/surface interface; (c) bacteria in an extracellular biopolymer; (d) bacteria in an aerosol. Arrows indicate the direction of potential water movement.

Matric water potential in soils and sediments has become a topic of greater interest with the increasing attempts to introduce genetically engineered bacteria into the environment (212, 280, 282). *Enterobacter cloacae* and *Alcaligenes eutrophus* AE106(pR0101)—an engineered strain that degrades 2,4-dichlorophenoxyacetic acid—remain as intact DNA-carrying units but lose viability within 2 h of inoculation into air-dried soils (−300 MPa) (281). Light output from cells of *E. coli* MM294(pEMR1) (Lux^+) following soil inoculation decreased with time of exposure at different matric water potentials, whereas substrate-amended respiration remained constant (317). Relatively recent studies have measured the survival in aerosols of *E. coli* strains which contain Tn*5* derivatives of ColE1 (234). The plasmid-containing strains showed enhanced survival relative to the wild type, and survival was markedly dependent on the surfaces colonized.

The most extreme xerophytic environments are found in the Antarctic. Bacteria have been isolated from ice cores taken from sediments between 10,000 and 13,000 years old at Vostok station (2). Here, the bacteria can be considered to be freeze-dried. There was a general correlation between the drop in the number of viable bacteria and the depth of the sediment (below 114 m, i.e., 3,000 years old), with *Streptomyces* and *Nocardia* species being found in these deeper cores. A non-spore-forming *Pseudomonas* strain represented 7% of the biomass, and *a* and *b* variants, with two distinct morphologies, arose during culture of this bacterium. Such dissociation is known to occur when bacteria are kept for long periods under laboratory conditions, and increased storage under anaerobiosis also promotes dissociation. In deeper ice (2,450 m) only a few samples were able to generate growths in liquid culture, and all contained spore formers (*Bacillus* spp.). Moisture limitation caused by rapidly changing low temperature is thought to be the main cause of death of these cells in situ. The additive effects of drying and freezing contribute to the survival times of bacteria in ice sediments (Fig. 9). In the comparatively warmer regions of the Antarctic continent, the soils are frigic, ahumic, and are largely aerobic (2, 393). Seasonal changes in the numbers of bacteria found in these soils, including chromogenic forms, generally correlate with water availability, but in general it seems that there is a poor understanding of the distribution and activities of bacteria in Antarctic soils. Low temperatures make free water extremely scarce, and the rapid wetting of dried soil may cause cell lysis and release of a substantial fraction (17 to 70%) of the biomass carbon. In the colder regions of the continent the water potential of soils is lowered by a high mineral salt burden characteristic of soils in which evaporation exceeds leaching. Matric forces are usually unimportant, because the sediments typically consist of sandy gravel. Here, the soils are effectively sterile and the scattered populations of bacteria sustain their existence inside rocks. The rock-dwelling prokaryotes of the Dry Valleys of the Ross Desert provide a unique model for the study of desiccation tolerance (see below).

Since (i) desiccation in nature involves air drying, (ii) one consequence of desiccation is damage caused by reactive oxygen species, and (iii) the drying of bacteria is accomplished under laboratory conditions in the presence of oxygen, it is not surprising that studies of desiccation tolerance in bacteria have focused on aerobes. Do anaerobic bacteria show a greater susceptibility to air drying than aerobic forms? Probably not. Oxygen may lead to a cessation of growth of strict anaerobes, but their spore-forming representatives appear to include some of the most desiccation tolerant of bacteria (Fig. 9). No spore formers have been identified among the *Archaebacteria*, yet some archaeal strains withstand air drying and comparatively long-term storage under aerobic conditions (Fig. 9).

Freeze-Thawing, Freeze-Drying, and Air Drying—the Differences

For many research laboratories, the method of choice for storage of bacterial cultures, such as those of *E. coli*, is to add glycerol to a final concentration of anywhere between 10 and

30% (vol/vol) and to freeze the mixture, usually rapidly in liquid nitrogen, for storage at −70°C. Upon thawing, the slush can be used to inoculate fresh media. Such "permanents" can be stored for many years, and they provide a useful means to recover cell lines. Culture collections, such as the American Type Culture Collection, and industrial firms that specialize in the distribution of cultures, for example starter cultures for use in the manufacture of yoghurt and cheese, rely on freeze-drying of bacteria, fungi, algae, and viruses. Here, the culture may be mixed with some cryoprotectant, such as double-strength skim milk or growth media supplemented with 12% (vol/vol) sucrose, before being subjected to a programmed rate of cooling and drying under vacuum (45). For long-term storage, the freeze-dried pellets can be sealed in borosilicate glass vials that contain a plug of desiccant or in metal cans that are sealed under vacuum. In each case the cells are reconstituted through the addition of fresh media. To dry cells in air, cultures may be applied to some inert support which can be held, until dry, under a vapor phase of controlled humidity or a stream of air. Immobilization of cells on dry sand or dry paper has been used to preserve the viability of beta-hemolytic streptococci and other bacteria for more than 4 years (12, 213). As the research community is aware, post offices worldwide are unwittingly in the business of distributing samples of bacteria spotted on paper filters and enclosed within envelopes after preparation using either the first or the last of the three methods described above.

The same types of compounds that act as compatible solutes for osmotically stressed bacteria offer protection during freeze-thawing of proteins (13, 55). For freeze-thawing, the cryprotection afforded to isolated proteins can be accounted for by the same mechanism, the preferential exclusion hypothesis, that is thought to stabilize proteins in nonfrozen systems (53). In this respect, we can view osmotic stress and freeze-thawing as being similar in that they impinge upon systems where there is still plenty of water around. In contrast, anhydrobiotic cells appear to rely upon only disaccharides, either trehalose or sucrose, to achieve their stabilization and that of their components through a mechanism accounted for by the water replacement hypothesis (81; see reference 64 and references therein). At subzero temperatures, the increase in solute concentration due to ice formation, alterations in pH, and other changes that may occur are solution-related phenomena. Despite ice formation, then, interactions in the aqueous phase permit the exclusion mechanism to occur (55). So what about freeze-drying? Evidence shows that a compatible solute, polyethylene glycol, can protect enzymes during freeze-thawing but cannot do so when the same enzymes are freeze-dried. The same enzymes can be stabilized during freeze-drying, however, if a sugar is added together with the polyethylene glycol. Under these conditions, it was observed that the polyethylene glycol was crystalline while the sugar remained amorphous. In instances when the sugar underwent devitrification, the proteins degraded. The conclusions from these studies were that the carbohydrate forms hydrogen bonds with the protein in the dry state and serves as a water substitute and that to do so it must remain in the amorphous state. Freeze-thawing (cryprotection) and freeze-drying are fundamentally different for both intact cells and purified macromolecules (53).

There is an extensive literature on the freeze-drying of microorganisms including bacteria (249). Freeze-drying and desiccation of *E. coli* both lead to the induction of mutations (16). Recent work has focused on the survival differences among freeze-dried genetically engineered and wild-type bacteria (185). Early stationary-phase cultures of wild-type and engineered strains of *Pseudomonas syringae* and *E. coli* were flash-frozen at −80°C and then dried in a freeze-drier under vacuum. The cultures were then exposed to air of 60% RH for different time periods under other experimental variables. The engineered strains of *P. syringae* (cit7del1b [Ice$^-$] and cit#17 [Ice$^-$]) showed more sensitivity to air exposure than did the wild-type cit7 wild-type (Ice$^+$). The latter contains the IceC product in its membrane. All of these *Pseudomonas* strains showed survival during 4 h of exposure, while all *E. coli* strains tested showed survival of less than 0.01% within the first 30 min. Survival rates with a second-order decay pattern increased with dark exposure and were greater at 60% rather than at 56% RH. In the light, comparable survival rates were obtained for cells but the survival curves generated were first order. The conclusions of the study were that (i) plasmid constructs and expressed recombinant gene products represent an energy demand that can induce higher death rates upon drying and (ii) an open-air factor, identified as cyclohexane, may enhance death rates outside of the laboratory setting. This study did not provide details of the growth characteristics of the strains in liquid culture that may have permitted a more critical appraisal of the "DNA load" suggestion. The data obtained by these workers were consistent with predictions of oxygen-induced death for *Serratia marcescens* and other dehydrated bacteria (72).

TARGETS OF DESICCATION DAMAGE

At the level of the bacterial community, air drying may lead to a change in the surface area of a community, its shrinkage, a change in texture, precipitation of salts, and color changes as pigments are oxidized (Table 1). In the cells themselves, the changes may be more subtle. Discoloration may be due to condensation reactions between lysine and methionine residues of proteins and reducing sugars—the Maillard (browning) reaction. It seems that the energy changes involved in the loss of cell viability upon drying are small and that even desiccation-sensitive cells can cope with the removal of a substantial fraction of their $\overline{V}_f$ but the perturbation of a restricted number of cell water molecules leads to growth rate adjustment and/or cessation of growth. The fact that reliable viability measurements can be made at all on air-dried bacteria tells us that not all cells in a population respond in a similar fashion to a water deficit. The physiological status of the cell at the time of drying, and the time of the drying event, seem to be potential sources of this variation in sensitivity (Fig. 11). In these respects, the numbers and activities of cell macromolecules must markedly influence the physiological status of cells, and, in the long-term, macromolecules must represent the ultimate targets for desiccation-induced damage.

Proteins

Proteins may be present in cells in a few copies (e.g., regulatory proteins such as LacI) or in as many as 100,000 or so copies (e.g., ribosomal translation factors such as Ef-Tu [40]). These proteins are distributed throughout the cell compartment in an aqueous environment, whose properties remain poorly understood. Again, it must be emphasized that the organization and function of water in protein crystals and in polymers and the concept of "bound" water are topics of some controversy (117, 264). A global model of the protein-solvent interface has recently been provided (232). The essential features of the model are that there is a persistence of water structure beyond the primary layer of hydration. Second, the three-dimensional network distribution of water is strongly correlated to the protein surface topography on both local and

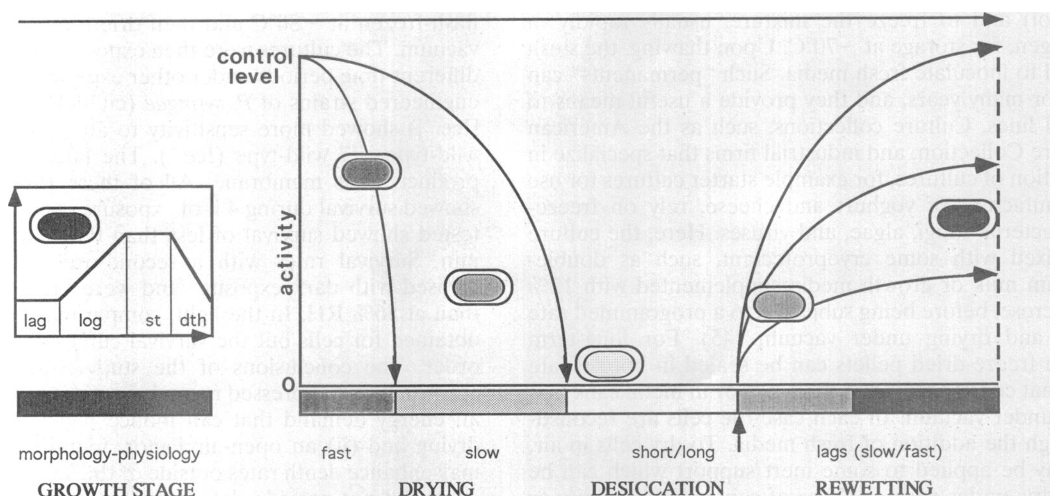
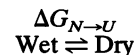


FIG. 11. Schematic time course of events during the change in water status of a prokaryotic cell following its removal from a liquid culture at a given stage of growth (extreme left) (st, stationary phase; dth, death phase). Straight horizontal arrows indicate time. A small vertical arrow indicates the time of rewetting. Shading indicates relative water content, and curved arrows indicate changes in cell activities. The *y* axis represents any measurable activity—the control level is the activity measured at the time the cell is removed from liquid culture and immobilized.

global scales. Specifically, the local solvent mobility is greatly enhanced for certain locations at the protein surface and in its interior. The immediate environment of each molecule in a cell must be vastly different. Are these differences maintained after the cells are dried, or is the state of the dried cell more homogeneous? As a cell is completely dried, are proteins left in an inanimate state ready to resume activity simply at the onset of rehydration, or do they change their state? These are important considerations because microenvironments will influence the equilibria between the different intermediates of folding pathways for each protein. For actively growing bacterial cells in liquid culture, the turnover of individual proteins can be characterized in terms of k^{cat} values which have magnitudes of seconds or minutes such that aberrant or otherwise damaged proteins do not accumulate. As cells are dried and then desiccated, these k^{cat} values tend to have less meaning as cells enter a period of quiescence that may last for years. At the time a cell is rehydrated, a considerable fraction of its protein pool may be represented by modified (damaged) proteins. Fractions of this pool may be degraded rapidly and turned over upon cell rehydration (295). How do rehydrating cells discriminate between damaged and undamaged proteins?

The normal degree of hydration of proteins (bound water) is around 0.25 g of H_2O g of protein^{-1} (189), and the ordering of these water molecules contributes directly to the properties of the proteins by influencing their interactions with ligands (24, 314). There appear to be two qualitatively different types of hydration sites. A well-defined, small number of water molecules in the interior of the protein are in identical locations in the crystal structure and in solution, and their residence times are in the range from about 10^{-2} to 10^{-8} s. Hydration of the protein surface in solution is carried out by water molecules with residence times in the subnanosecond range (270). Anhydrous lysozyme, exposed to atmospheres of different RH, has been used to obtain information on the effects of water on protein structure (see reference 357 and references therein). Franks has summarized how the protein responds to a sequential stripping of its water (120, 121). These studies have used measurements of infrared amide bonds (peptide bonds), infrared COO- bands (to monitor acidic residues; D, E), infrared -OD stretch (to monitor perturbations of water molecules), specific heat (to monitor internal degrees of freedom of macromolecules), electron paramagnetic resonance (or nuclear magnetic resonance) that can estimate rotational correlation time (freedom of rotational diffusion of macromolecules), and enzyme activity.

Both osmotic and matric methods show that cessation of bacterial growth and the onset of cell deaths occur after $\overline{V}_f$ is removed, i.e., at some compensation point when $\overline{V}_{cyto}$ approaches $\overline{V}_b$ (34, 322). When cells are dried, this value is reached at a comparatively high RH (80%). Cells that express desiccation tolerance undergo drying at much lower water potentials, and it would appear that they must withstand the most extensive perturbations of $\overline{V}_b$ for their various cellular constituents over the long term—the absolute lower limit beyond which no reactivation can occur is unknown—and/or ensure that such perturbations do not occur or at least minimize them. There is a major problem when attempting to consider these alternatives. The free energy of stabilization of globular proteins in aqueous solution is marginal, and $\Delta G_{N\rightarrow U}$ is no more than 24 kJ mol^{-1} (189). $\Delta G_{N\rightarrow U}$ is equivalent to the energy required to break a maximum of five hydrogen bonds, corresponding to about 1% of the total number of bonds in the folded structures of proteins taken from crystallographic data (189). This means that a marginal difference in hydrogen bond strengths in contacts between water-water and water-protein will lead to an energy change which may exceed $\Delta G_{N\rightarrow U}$ by an order of magnitude.



$$\text{Wet} \overset{\Delta G_{N\rightarrow U}}{\rightleftharpoons} \text{Dry}$$

During drying, will there be a tendency for the equilibrium to shift to the right? This equilibrium is controlled directly by the degree of solvation of polypeptides and has a specific effect on protein folding (107). The relationship between amino acid sequence, folding pathways and kinetics, and the functional spatial arrangement of the polypeptide chain, is presently the least well understood step in the "central dogma" that relates storage of genetic information with its expression by protein functions (187–191, 270). Faced with these facts, the prospects for explaining how a single cell with a complement of some

3,000 proteins can have the bulk of its water removed, remain desiccated for perhaps tens of years at water potentials (assuming cell equilibrium) where $\overline{V}_b$ has been drastically perturbed, and then resume coordinated metabolic activities within seconds of rehydration is daunting at best!

In considerations of the adaptation of microorganism to extremes of temperature, pH, and pressure, it is generally assumed that evolution of protein structure is driven toward the achievement of optimum function rather than maximum stability (191). Adaptation to desiccation can be viewed as being quite different for one important reason, namely, a desiccated cell does not grow, and the time the cell remains desiccated may represent the greater part of the life (the time the cell remains viable) of that cell and its component proteins. Unless desiccation-tolerant cells accumulate proteins that serve some structural or protective role (and no evidence for this has been forthcoming), the consideration of protein "function" during desiccation is irrelevant. This fact places the question of function versus stability in a quite different context. However, there may be many ways to accumulate the ΔG_{stab} required for stabilization of a protein during desiccation and, the potential for optimized function may be of some consequence at the critical time the cells emerge from desiccation upon rehydration. If the proteins of desiccation-tolerant cells do differ from those of desiccation-sensitive cells (again, no evidence for this has been forthcoming), the features that contribute to increased protein stability are unlikely to be apparent from an inspection of the primary sequence. The proteins of desiccation tolerant cells may be either (i) inherently more stable than their counterparts in other sensitive cells, (ii) no different from those of sensitive cells but able to remain in a stable state because of one or more extrinsic factors (including other cellular components) exclusive to desiccation-tolerant cells, or (iii) no different from those of sensitive cells and equally sensitive to inactivation during desiccation (i.e., protein stability is not a feature of desiccated cells). What is known? Studies with field materials of the desiccation-tolerant cyanobacterium *N. commune* and a clonal axenic culture of *Nostoc* strain UTEX 584 have provided clues which hint that some, but not all, proteins remain stable despite extended desiccation (168, 345). The commencement of de novo lipid biosynthesis upon the rewetting of desiccated cells is instantaneous (379). With the experimental conditions used to measure this biosynthesis, it must be concluded that the lipid biosynthetic machinery, as well as the proteins required for the uptake and phosphorylation of glycerol (presumably GlpF- and GlpK-like proteins, respectively [412]) and the uptake of sulfate (presumably a permease complex [217]) and phosphate (399), remains functional during desiccation. Although the drying of *Nostoc* cells leads to a rapid cessation of nitrogenase activity (298, 300), no evidence was obtained for hydrolysis of at least one structural component of nitrogenase, Fe protein, which was present in cells following 10 years of desiccation (279). The intracellular ATP pool, as well as the protein-biosynthetic machinery, of desiccated cells remained unperturbed during 30 min and 2 h, respectively, after rapid drying at −99.5 MPa (7, 298, 300). In contrast, even short-term drying leads to structural changes in the pigment antenna complexes of cyanobacteria, the phycobilisomes. In the light, the phycobiliproteins are degraded, and even in the dark, short-term drying leads to subtle changes in the polydispersity of the complexes when analyzed in sucrose gradients (294, 345). Long-term desiccation leads to destruction of phycobiliproteins (345). In view of the short half-lives of mRNA and the susceptibility of nucleic acids to desiccation-induced damage (see below), the need to protect RNA-binding proteins (ribosomal proteins, transcription factors, DNA-dependent RNA polymerase) may be crucial. The immobilization and rapid drying of *Nostoc* strain UTEX 584 cells lead to a rapid loss of the *rpoC1C2* transcripts that encode two subunits of the RNA polymerase (γ and β′). These transcripts accumulated to control levels within 60 min of rewetting of cells that had been kept dry for 24 h, at −99.5 MPa. Immunoblotting confirmed the presence of Rpo proteins in the same dried cells (see above). De novo transcription in rehydrated cells of *Nostoc* strain UTEX 584 is therefore directed by extant RNA polymerase holoenzyme that maintains its stability during desiccation and at least during the time of rehydration when some transcripts accumulate to control (predrying) levels (430). Are DNA- or RNA-binding proteins any more stable than other cellular proteins, or does the stability of RNA polymerase in the example mentioned here simply reflect the fact that there are numerous copies per cell and a finite pool escapes damage? The work needed to overcome frictional drag under turgid conditions in *E. coli* can be calculated as roughly 0.2 kcal (0.84 kJ) (432). One consequence of drying cells must be that it imposes some restriction upon the efficiency of enzymes, such as RNA and DNA polymerases, by increasing the work needed to overcome the frictional drag against the viscous solvent—remember the discussion on glasses and WLF kinetics. Over the long term (millions of years), catalase is another enzyme that has the capacity to maintain its stability (138). Yet, although it seems clear that many proteins remain stable in air-dried cells of desiccation-tolerant bacteria, it should be noted that even in desiccation-sensitive cells of *E. coli* the enzymes required for productive T2 infection remain active in dried, nonviable (dead) cells (403). The anhydrobiotic cell is more than simply a collection of dried components.

Nucleic Acids

Nucleic acids represent prime targets for desiccation-induced damage. In large part the damage reflects the accumulation of mutations during the time when there is no cell growth (during desiccation). It is unlikely that repair mechanisms operate in air-dried cells, and this damage will become manifest only upon rehydration (351). Mutation of an arginine auxotroph to the prototroph was induced in *E. coli* strains dried to an a_w of 0.53 and below but not when dried to an a_w of 0.75 and above (15). Because significant mutation on drying occurred in wild-type strains and in strains carrying *uvrA* and *polA*, but not in *recA* strains, it was concluded that the mutation is caused by errors in *rec*-dependent repair of the drying-induced breakage of the DNA. Damage to DNA may arise through chemical modifications (alkylation or oxidation), cross-linking, base removal such as depurination, or ionizing and nonionizing radiation. For bacterial spores, it is thought that the reduced water content retards and/or alters reactions that affect DNA; however, it is quite clear that even with reduced water contents, damage sufficient to compromise viability over even short periods occurs. For example, the measurement of more than 10 breaks per single-stranded genome in cells of *E. coli* K-12 strain AB 1157 (*uvrA*$^+$ *recA*$^+$), caused by drying them for only 12 min, emphasises the sensitivity of DNA to drying (28). The genome of a *Bacillus* spore suffers around 50 strand breaks during exposure to vacuum for 3 weeks (28) and becomes progressively altered upon more prolonged storage. One feature of the alteration may be an increase in the number of cross-links between protein and DNA that accumulate continuously during dry storage. Even the DNA of radiation-resistant *Deinococcus* strains suffers single-strand breaks during a few days of vacuum

exposure (28). Single-strand breaks and other DNA lesions are readily repaired during the germination phase of *B. subtilis* spores (101). In a study that compared the survival of different mutants, the survival of one mutant (*ssp uvr*) was about 20% less than that of other strains. The conclusion from this study was that single-strand breaks are probably repaired by the same ligases that are required for DNA replication and therefore that special "repair-ligase"-deficient mutants may not exist (101). A substance has been identified that degrades both DNA and RNA in cells of the anaerobe *Roseburia cecicola* when they are exposed to air (263). The substance has an M_r of 2,800 and requires a reducing agent for activity. The spectrum of factors that can contribute to cell death upon drying is clearly very broad.

An important factor in the accumulation of damage must be the number of chromosome copies present per cell, and this in fact may be one mechanism of desiccation tolerance. Fifty strand breaks may be critical for a sensitive cell that carries one chromosome equivalent, such as *E. coli*, and for other bacteria that may partition different sets of genes on different chromosomal elements but much less so for bacteria, such as cyanobacteria, which may have multiple copies of their genome present in each cell (355).

For *Nostoc* DNA, a rate of depurination at 37°C of ~20 per genome per day can be estimated from the median genome size measured for several *Nostoc* spp. (5×10^3 MDa = 7.6×10^6 bp [166]), assuming an internal pH of 7.4 for the cytoplasm (note that the thylakoid space may have a different pH and that the pHs of the two compartments vary with light intensity [398]), and an in vitro rate of depurination of $k = 2.58 \times 10^{-6}$ day^{-1} (227). The rate of depurination of a desiccated *Nostoc* cell would achieve a 1% depurination of the genome after storage of the cells at 37°C for 10 years. However, desiccated crusts in situ are exposed to temperatures far in excess of 37°C, and depurination rates would be expected to increase; furthermore, the genomes of *N. commune* contain significant amounts of 6-methyladenine (193) and these residues are lost from DNA at a rate approximately two to three times higher than for other purines. Yet herbarium specimens remain viable after more than a century in the desiccated state (49)! These considerations again lead back to the question of the state of dried cytoplasm. The DNA in laboratory-grown cells of *Nostoc* strain UTEX 584 undergoes appreciable light-dependent nicking after only short periods when the cells are dried in air (375); however, it lacks the conspicuous glycan of field material. The mRNAs isolated from desiccated *N. commune* supported low rates of translation in vitro in either homologous or heterologous translation systems, suggesting that they may be modified (192). It is interesting that the activation energy for depurination (31 ± 2 kcal mol^{-1} [130 kJ mol^{-1}]) is around sixfold greater than that needed to unfold a protein. Superficially, then, it seems that DNA damage would be expected to follow, rather than precede, protein damage if only the removal of water is considered and no extrinsic factors are taken into account. Mechanisms must be present to retard the rate of depurination and other DNA damage in cells growing in situ.

It is expected that lesions in DNA may be accentuated in organisms that are dried in situ where incident UV fluxes may be appreciable, yet such communities may have a range of induced systems, not present in laboratory-grown strains, to prevent damage. Studies suggest that induction of DNA damage by UV in dried cells is a multistep process (186). Photon energy is initially absorbed in a sugar-phosphate group, and destruction of the sugar follows, accompanied by breakage of the ester bond between the 3′ C of the sugar and the phosphate, leaving a 5′ phosphate terminal. A base attached to the destroyed sugar moiety may then be released. The other end of the DNA strand may have a sugar or a 3′ OH residue (P_i may also be released during this process), and in polynucleotides 5′ deoxynucleoside triphosphate is lost at the site of the strand break. Drying of cells certainly leads to an accumulation of free radical and reactive species, and these may participate in further DNA damage, as indicated in Fig. 10. The kind of UV damage to DNA appears to depend on the secondary structure of the double helix—which is further influenced by the amount of bound water (228)—and apparently the availability of water (238). In mature spores of *B. subtilis*, the A form of DNA prevails, and one must presume that it does so also in desiccated vegetative cells of other bacterial species. Irradiation with UV results in the formation of 5-thyminyl-5,6-dihydrothymine, the so-called spore photoproduct. Only when cells are irradiated in vacuo (a_w = 0) do the *cis,syn* and *trans,syn* isomers of the thymine dimer accumulate, and these appear to be responsible for the lethal effects of acute drying. The degree of hydration around forespore DNA is reduced by the presence of dipicolinic acid, which influences photoproduct formation without causing a change in the conformational state of DNA (229). Desiccated field materials of *N. commune* contain two discrete fractions of genomic DNA, which are readily separated by cesium chloride density ultracentrifugation (193). One fraction is hypermethylated, and the other is hypomethylated. The two fractions associate with different types of carbohydrate complexes, a feature that probably permits the separation of the DNA fractions in gradients. The functional significance of these two fractions remains unknown.

Lipids and Membranes

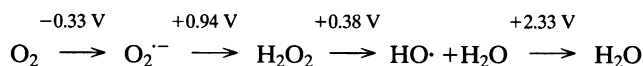
The concept of lipid fluidity in membranes has its origin in the studies of Chapman and colleagues (59). The surface tension of water holds membranes together. To deform (stretch) a membrane, or to make a hole in it, requires an increase in the water/membrane interface. The latter requires that hydrogen bonds be broken and that the water molecules reorientate; these requirements contribute positive enthalpy, and negative entropy, respectively, to the large surface free energy of water (424). However, this does not mean that if a membrane is dried it will simply fall apart. Because of a decrease of the lateral spacing of the polar head groups and the subsequent lining up of the hydrocarbon chains, the transition temperature (T_m) of membranes increases considerably when they are dried (48, 172). As such, during drying T_m surpasses ambient temperature and the gel and liquid-crystal phases transiently coexist. As the permeability is very high in such two-phase systems, this event will be catastrophic for a cell if free water is available for solute transport (76, 77). Desiccation-tolerant cells must have mechanisms to suppress the dehydration-induced rise in T_m in order to postpone phase changes that take place during drying to moisture contents at which no free water is left for solute transport and leakage. The polar head groups of phospholipids, by influencing Fe^{2+} autooxidation, generate dangerous oxygen species which may play a role in the oxidation of the hydrophobic components of the lipids (246). Considerations of the effects of drying on membranes are extensive (76–80, 82, 84, 172, 173, 175). For *Rhizobium* spp., studies indicate that death of these bacteria in response to desiccation and rehydration is caused by changes in membrane permeability (47). Differences in the polar/nonpolar nature of the outer membrane may account for the variabilities in susceptibilities to desiccation of different strains.

The freeze-drying of *E. coli* and exposure of the dried cells

to oxygen cause damage to the bacterial cytoplasmic membrane so as to make it leaky to potassium. The damage is most extensive, and becomes irreversible, if the cells are not held under vacuum (when dried) prior to rehydration (183). The damage is localized in the cell membrane, seems to involve the DNA initiation complex, and appears to occur in two stages. The primary damage is due to freeze-drying, but this damage can be repaired upon subsequent rehydration of the cells and following a period of incubation that requires protein synthesis. Second, after exposure of the freeze-dried cells to oxygen, the injury becomes irreversible and the cells die. The lethal effects can be countered by engaging the DNA initiation sites, for example with colicin E1, or by arresting the activity of the initiation complex. Such arrest occurs in temperature-sensitive mutants at the nonpermissive temperature or in auxotrophic mutants starved of amino acids or thymine (184).

Mechanisms of Damage

Damage to both DNA and proteins is mediated through reactive oxygen species (37). Although molecular oxygen is strongly oxidative with respect to its fully reduced form, water, its oxidative potential is normally held in check by kinetic restrictions imposed by its two unpaired spin-parallel electrons (179). Consecutive univalent reductions of oxygen produce superoxide ($O_2^{\cdot -}$), H_2O_2, and hydroxyl radical ($HO\cdot$), with the following reduction potentials (158):



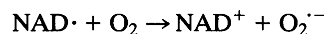
$$O_2 \xrightarrow{-0.33\text{ V}} O_2^{\cdot -} \xrightarrow{+0.94\text{ V}} H_2O_2 \xrightarrow{+0.38\text{ V}} HO\cdot + H_2O \xrightarrow{+2.33\text{ V}} H_2O$$

DNA breaks accumulate during exposure of bacterial cells to $O_2^{\cdot -}$ and H_2O_2. It is expected that free radicals will accumulate during drying, especially in the light, when cells are subjected to high incident solar radiation. Electron spin resonance spectra suggest that desiccated cells of cyanobacteria do, in fact, contain appreciable amounts of free radicals (291). Oxidative damage will be manifest in protein damage and lipid peroxidation, leading to a loss of a diffusion barrier to membrane-impermeable markers and ultimately to cell lysis. Some indications of the type of damage that may occur come from studies in which bacterial cells have been exposed to H_2O_2. Such exposure of *E. coli* cells leads to two types of cell killing. Mode 1 killing is pronounced at low concentrations of H_2O_2, and mode 2 killing is pronounced at high concentrations. It seems likely that mode 1 is of most relevance to desiccated cells, and the acute sensitivity of *Treponema pallidum* (Fig. 9) to air drying may be a reflection of its acute sensitivity to H_2O_2 (371). One proposed mechanism for single-strand nicking of DNA catalyzed by reactive hydroxyl groups is shown in Fig. 12. Single-strand DNA breaks that contribute to mode 1 killing in DNA repair-deficient strains result from the collapse of the deoxyribose ring after abstraction of a hydrogen atom (179). Abstraction of the hydrogen proceeds through the Fenton reaction and is catalyzed by transition metals, such as cellular iron, and affected by the ferryl radical. To maintain an ongoing Fenton reaction, an electron source must be available to regenerate the reduced metal. Evidence suggests that the Fenton-active metal is reduced on the surface of DNA—where it is probably chelated to the phosphodiester backbone—and the metal reductant is a small diffusible molecule such as NAD(P)H. Lines of defense against such Fenton reactions include DNA repair mechanisms, synthesis of scavenging enzymes such as catalase, and the depletion of intracellular NADH. As such, these reactions would represent three potential responses to drying-induced damage. Thiols such as glutathione (H donation) may either prevent or enhance damage, depending on the prevailing physiological conditions (216).

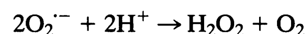
Conformational changes in DNA can be influenced by hydrophobic interactions in the major groove of the DNA as a result of base methylation (196). It is not clear to what extent methylated and nonmethylated DNAs are subject to damage. Damage may be prevented by lowering the intracellular pool of reduced pyridine dinucleotide. It is interesting that the cyanobacterial heterocyst has a very high level of NAD(P)H (161) and this may contribute to the sensitivity of heterocysts to drying, despite their capacity to synthesize superoxide dismutase (SOD) and catalase (51, 153, 426). Small, α/β-type acid-soluble proteins influence the structure and photoreactivity of the spore DNA in *Bacillus* and *Clostridium* species (113). The binding of the proteins to DNA prevents formation of cyclobutane-type thymine dimers upon UV irradiation and promotes formation of the spore photoproduct. These DNA-binding proteins require that the DNA be able to form A-type DNA, and they prevent depurination of more than 20-fold (112, 351). Although the affinity of the proteins for DNA is not great, they are present in such large amounts that virtually all of the DNA is likely to be coated. The repair systems in spores include an excision repair system which can repair spore photoproduct and cyclobutane dimers and an error-free spore photoproduct-specific system (characterized by the *spe* locus) that requires energy but not light (351). Because depurination is water catalyzed at neutral pH, the low a_w of desiccated cells may slow strand scission but will not prevent a certain proportion from occurring. The low a_w will cause conformational changes in DNA, converting it from the B form to the A form and, as shown for dried spore DNA, may decrease the accumulation of T<>T. No T<>T is formed in dormant spores. Curiously, the drying of spores in a vacuum, where the atmospheric a_w is effectively at infinity, does lead to the accumulation of T<>T dimers (228).

Oxygen itself can act as a free-radical scavenger! When oxygen is present, it rapidly forms $O_2^{\cdot -}$ from Fe^{2+}, and the following reactions become physiologically relevant:

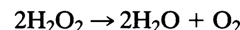
$$Fe^{2+}\text{ (chelated)} + O_2 \rightleftharpoons Fe^{3+} + O_2^{\cdot -}$$



$$NAD\cdot + O_2 \rightarrow NAD^+ + O_2^{\cdot -}$$



$$2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$$



$$2H_2O_2 \rightarrow 2H_2O + O_2$$

These reactions suggest how SOD could prevent the Fenton reaction-catalyzed damage to DNA. Interestingly, there is some argument whether inactivation of bacteria by singlet oxygen occurs in a lipid or an aqueous environment (275).

Dehydration of proteins induces significant and measurable conformational changes, as deduced from analyses by Fourier transform infrared spectroscopy (309; see above). The changes observed are protein dependent, the removal of water has no effect on the amide I vibration, and the observed spectral changes upon dehydration are conformational. These changes may be prevented in large part through the addition of sugars and polyols (see below). The very fact that water is removed at all may be the reason why some proteins remain stable when they are dry (asleep).

Metal-catalyzed oxidation of proteins is an additional likely consequence of drying cells. The oxygen-dependent reaction proceeds in a manner that is rather similar to the one described

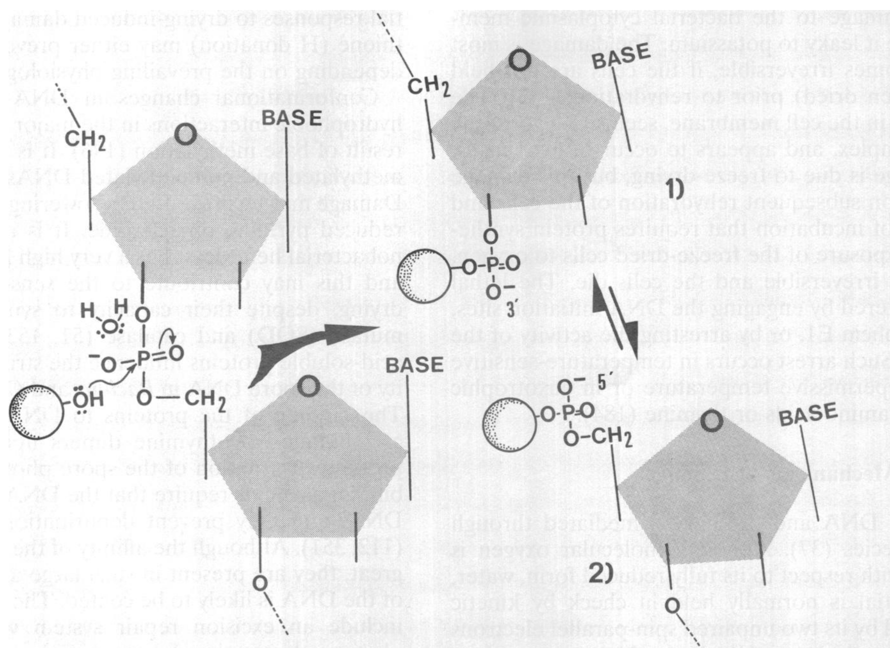


FIG. 12. Possible mechanism for the introduction of single-strand breaks into desiccated DNA. Shown is a molecule (sphere) with a reactive hydroxyl group that nucleophilically attacks the P atom of the phosphodiester backbone. The reaction depends on the availability of water molecules. The formation of DNA strands with molecules covalently attached at their 3′ or 5′ positions is in equilibrium.

above that leads to DNA damage, in that the reaction is mediated through [Fe^{3+} OH·]. Reduction of oxygen yields H_2O_2 directly or yields superoxide as an intermediate, which reacts with Fe^{3+} to give Fe^{2+} and oxygen. The Fe^{2+} binds a metal-binding site in the protein, and the Fe^{2+} complex reacts with the H_2O_2 to generate ferryl ion, leading to reactions with protein side chains. After oxidative modification, the proteins become sensitive to proteolysis and/or may be inactivated, or they may show reduced activity. Histidyl, prolyl, lysyl, and methionyl residues are converted to aspariginyl, pyroglutamyl, aldehyde, and methionyl sulfate residues, respectively (367). Some residues are converted to carbonyl derivatives. Carbonyl derivatives accumulate in proteins as they age (4, 361), and it seems that they may accumulate in desiccated cells, although, again, it is unclear to what extent the very low a_w will diminish the effects of these reactions. Protein damage is clearly of some consequence to cells that remain desiccated for long periods with very low turnover rates, such as the Antarctic chasmolithic communities described below.

Cells stored for long periods also may become subject to the effects of ionizing radiation (360). Apart from any intentional exposure, only the natural abundance of ^{40}K in cells requires consideration. The intracellular K content of *E. coli* grown between 148 and 938 mosm varies between 150 and 495 mmol liter of cell water^{-1} and is 0.6 M when cells are grown at 1.2 osm (181). Similar values have been measured for *Anacystis nidulans* (398). Assuming that a bacterial cell contains approximately 6.7×10^{-16} liter of water (255), at 1.2 osm a single cell will contain 4×10^{17} atoms of K, of which 4×10^{3} atoms are ^{40}K, each with a half-life of 10^9 years and a single decay energy of 1.3 to 1.5 MeV. It seems that the contribution of ^{40}K can be considered negligible on the scale of desiccation discussed here, although it is true that the form of this decay is not well understood and short-term effects may still occur.

RESPONSES TO DESICCATION—MECHANISMS OF TOLERANCE

Fulton (128) has drawn an analogy between the contents of a cell (cytoplasm) and a protein crystal. In the latter, the solvent component represents between 20 and 90% of the total weight (an average ratio of 40:60 of H_2O to protein; or 0.66 g of H_2O g of protein^{-1}). Using this analogy, Fulton considered that "either size variations in the bulk phase are accompanied by exquisitely balanced reciprocal variations in enzyme activities with changes in concentration or, the precise volume of the bulk water phase is of minor importance because the major site of regulation is the water of hydration." Certain of the discussions presented so far would seem to favor the latter opinion.

Certain advantages may be gained from a juxtaposition (chaneling) of groups of enzymes that catalyze sequential reactions—so-called metabolons (235)—and one advantage may be to protect the solvation capacity of cell water. One feature of the recovery of desiccated cells upon rehydration is a very rapid onset of cellular metabolism in conjunction with a stepwise and stringent recovery of metabolic processes (379). Perhaps desiccated cells have evolved such that they make good use of global metabolon organization with a limited amount of water. The inherent structural organization inside air-dried cells, the most critical consideration, is the least understood feature of desiccation tolerance.

The Anhydrobiotic Cell and a Water Replacement Hypothesis

The anhydrobiotic cell is characterized by its singular deficiency in water. The water deficit is far greater than can arise if a cell is immersed in a concentrated solution of solute (osmotic stress) and far greater than can arise in freeze-tolerant cells in the presence of extracellular ice. There is also

another important distinction. Some desiccation-tolerant cells accumulate large amounts (sometimes in excess of 20% of their dry weight) of either one or both of the disaccharides trehalose and sucrose (81). Such observations have led to the realization that such disaccharides are effective at protecting enzymes during both freeze-drying and air drying. The important point is that the preferential exclusion mechanism described for systems of intermediate water content cannot be applied to the desiccated cell in which the proteins have been stripped of their solvent monolayer. That is, trehalose and sucrose are not acting as compatible solutes here. How can we reconcile the functions of oligosaccharides such as trehalose and cell survival without water? John and Lois Crowe and colleagues have developed a water replacement hypothesis to explain how components may be protected during extreme drying (64). Essentially, the hypothesis is that polyhydroxyl compounds, such as trehalose, replace the shell of water around macromolecules, circumventing damaging effects during drying. Webb alluded to a similar effect, due to polyhydroxyl compounds such as inositol (but see below), in dried bacterial cells (403, 404). Experimental evidence for a water replacement hypothesis has, indeed, been forthcoming (see, e.g., reference 388). When carbohydrates are dried in the presence of proteins, the capacity for the carbohydrate molecules to form intermolecular hydrogen bonds between themselves is diminished. Significantly, not only does hydrogen bonding occur between stabilizing carbohydrate and protein but also the binding of the solute is requisite for preservation of labile proteins (54). Dehydration-induced alterations in the vibrational spectrum of lysozyme were reversed, in part, when the protein was dried in the presence of trehalose and lactose but much less so when it was dried with *myo*-inositol. The data indicate that carbohydrates appear to be more effective at reversing changes in the infrared spectrum of lysozyme that derive directly from from removal of water (shift of amide II to a lower frequency and loss of the carboxylate band at 1,583 cm^{-1}). Recent evidence for this includes the observation that the P═O stretch of the phospholipid increases in frequency by about 30 cm^{-1} when the protein was dried without trehalose but is decreased to or below the frequency of hydrated P═O when the protein was dried with trehalose. Europium, which binds phosphate, competitively inhibits the stabilizing effect of trehalose, and molecular modeling shows that trehalose can fit between the phosphates of adjacent phospholipids (334). Dextran is a noncompetitive inhibitor for stabilization by trehalose. At low trehalose/lipid ratios, the trehalose is not available to bind water; this is taken as evidence for a direct interaction between the sugar and lipid. Stabilization of liposomes is achieved in the region where the water content remains low, suggesting that residual water in dry samples is not involved in stabilization (78, 82, 84).

As far as we know, the properties of water are immutable. With a leap of faith, we could assume that every type of anhydrobiotic cell must conform to the same constraints imposed by the removal of water. The state of one particular anhydrobiotic cell may therefore be representative of other types of dried cells. In this context, Clegg has provided a most lucid and illuminating discussion of the state and content of water in *Artemia* cysts and the nature of the cytoplasm in an air-dried cell (64, 67). The most significant finding is that if cysts contain any water that exhibits the dielectric properties of water in aqueous solutions, it can only be a tiny fraction of that present. Only a fraction of the water detected at low water contents had the properties ascribable to "bound" water as deduced through measurements of quasielastic neutron scattering, and, in the initial stages of rehydration (unlike anhydrous protein), cysts exhibited no initial independence of e' (dielectric permittivity). Such an independence is taken as a measure of "bound" water. At this point the reader may review, again, recent discussions on some of the controversy surrounding the nature and existence of "bound" water (357). An additional feature of *Artemia* cysts was, perhaps as expected, the conclusion that their internal viscosity must be enormous. In short, Clegg's study of whole cells provided data that supported, and in some cases were consistent with, the water replacement hypothesis. This work provided some hints as to how dried cell components remain viable. What this study and others cannot explain fully is how these components can be redirected, instantaneously and in perhaps an ordered and stringent fashion, to resume integrated metabolism (and all that it means) upon rehydration of the dried cell.

Physiological Mechanisms

In isolated membranes, free radicals cause fatty acid deesterification from phospholipid. Free fatty acids typically accumulate in desiccation-sensitive cells during aging and are a cause of reduced membrane integrity. The respiratory rate prior to desiccation correlates well with the number of free radicals in the dry state, which suggests that the curtailment of respiratory metabolism prior to rehydration may be essential for the retention of membrane integrity and desiccation tolerance in general (172). Of course, cells will not know when they are due to be dried—or will they? There is recent evidence for a circadian rhythm in a prokaryotic cell, a cyanobacterium (210), and there are many cases in which the desiccation of bacterial cells is subject to an environmental rhythm, e.g., communities in the upper intertidal of maritime zones.

Imbibition of viable, dry cells may result in extensive leakage and death, particularly when it occurs at low temperature, because rehydration may involve a reverse phase change of membrane lipids from the gel to the liquid-crystal phase—this phase change occurs in the presence of ample water (82). Two methods can circumvent this effect: humidifying with water vapor prior to imbibition, and heating at imbibition (172).

Studies suggest that cells in the stationary phase are structurally, physiologically, and functionally distinct from those in the log phase. Stationary-phase cells of *E. coli* show a marked enhancement of resistance to air drying (Fig. 13), and the genes expressed in response to osmotic shock also appear to be involved in the adaptation to stationary-phase growth (354). Some of the properties of stationary-phase cells may therefore contribute to this enhanced resistance and deserve consideration here (Fig. 11). Table 3 summarizes the major events that occur when bacterial cells enter stationary-phase growth (354). The decrease in overall translation activity occurring with the transition from exponential to stationary-phase growth is accompanied by the appearance of 100S (dimers) ribosomes. A protein (protein E) is thought to be a ribosome modulation factor responsible for the dimerization. Interestingly, the gene encoding protein E, *rmf*, maps next to *fabA* (395). FabA is required for the introduction of double bonds into growing fatty acid chains of membrane phospholipids (73). The role of the double bond is to decrease the phase transition temperature of the phospholipid that contains the fatty acid (see below). As cells enter the stationary phase, however, cyclopropane fatty acid synthase modifies the phospholipids of the inner and outer membranes to cyclopropane fatty acids, a modification of *cis* double bonds with CH_2 (using CH_3 from *S*-adenosylmethionine). Cyclopropane fatty acids thus accumulate in stationary-phase cells. Although the function of this modification seems unclear, it has been observed that "they

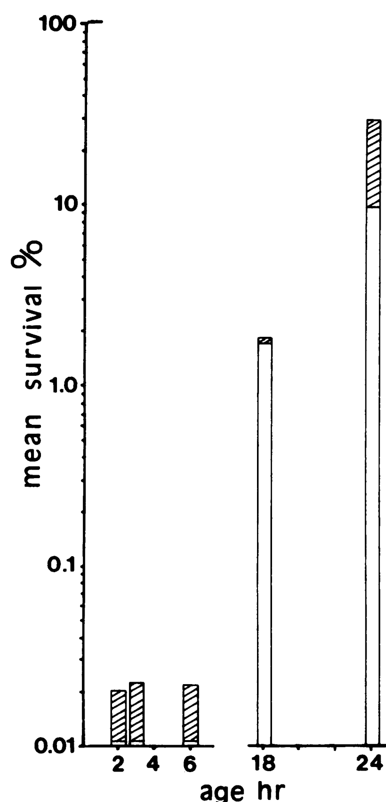


FIG. 13. Mean survival of air-dried *E. coli* cells according to the age of the culture at the time of immobilization (hours). Viability was measured after either 2 h (▨) or 24 h (□) of drying. The scale on the *y* axis is logarithmic. Data from reference 241.

play a vital role in the natural environment that has not been duplicated in the laboratory (73)." No accumulations of cyclopropane fatty acids could be detected in desiccated samples of *N. commune* collected from a range of different geographical locations (304).

Drying of cells may lead to a change in ribosome structure, because water is involved in the maintenance of the integrity of the ribosome (88). The translational machinery of *Nostoc* strain UTEX 584 was maintained for 2 h following drying of cells at −99.5 MPa, but longer periods of drying led to a loss of polysomes (7).

Bacterial Glasses

What do "glass theory" and WLF kinetics really mean in the context of desiccation tolerance? Three questions must be considered here. First, do bacterial glasses exist? Second, if bacterial glasses do exist, could they represent a mechanism for the desiccation tolerance of the cell in question? And third, but not least, how could a bacterial cell enter (and leave!) the glassy state through the corridor controlled by WLF kinetics?

Bacterial cells may accumulate significant amounts of a diverse collection of solutes, including sucrose and trehalose, that behave as glasses, as well as other polymers such as polyglucosyl granules that may also have the capacity to do so. Some sugars that replace bound water, e.g., trehalose, sorbitol, fructose, sucrose, and glucose, all can form aqueous glasses, and some of these are stable at 90°C. The glass transition temperatures and phase relations for several saccharide-water systems have been reported (151). The trehalose-water system is distinguished from the others by a significantly higher T_g at all water contents, with a particularly large advantage near the stoichiometry of 1 water molecule per glucose ring. The significance of glasses is that, in principle, the complete dehydration of bacterial cells may be avoided at temperatures below the melting point of the glass. The thermal transition characteristics of glasses is such that they could provide protection between 0 K and 90°C (44). However, the temperature range over which glasses form is unclear for complex biological systems such as bacterial cells. Yet, some bacterial cells elaborate conspicuous amounts of complex extracellular polysaccharides (EPS) (biopolymers) that are so varied in their gel and sol properties to suggest that they may have properties that mirror those described for other natural and synthetic glass-forming polymers. The characterization of naturally occurring bacterial EPS may in fact shed light on the development of new synthetic polymers. The extracellular glycan of *N. commune* has been characterized in detail. When dry, colonies are brittle. In the initial stages of wetting, the colonies become leathery and then achieve the consistency of a semirigid, malleable gel. Liquid cultures derived from these colonies secrete a viscous polysaccharide (169). How could we identify bacterial glasses? The interpretation of differential scanning calorimetry isotherms of purified components has become a controversial subject (see reference 1 and references therein). The interpretation of transitions in cell systems would be even more complex.

In the glassy state, reactions are slowed to periods that are more than sufficient for the times some bacterial cells may remain viable in the desiccated state. All water in a bacterial glass would, in effect, be immobilized, and the properties of this extremely crowded cytoplasmic glass would appear to be consistent with the requirements for long-term stability. Now the problem. If a bacterial cell enters into a glassy state upon the removal of a large fraction of its intracellular water (Fig. 1), it must pass through the glass transition state. An equivalent transition (different direction) would occur upon the arrival of water (rehydration = plasticization). These transitions may be accompanied by a change in T (ΔT), and the cells during these transtions will then be subject to the effects of WLF kinetics. The special case when a transition occurs at constant temperature in response to ΔW is considered further below. What could be the effects of WLF kinetics on the rehydrating or drying cell? The rates of biological reactions, like those of chemical reactions, are limited by temperature, but they are also limited by the rates at which diffusion can bring the reactants together (394). The maximum rate is thus set by the encounter probabilities of the components:

$$k_{\text{encounter}} = 4\pi(D_A + D_B)(r_A + r_B)N_0/1{,}000$$

the so-called Smoluchowski limit where D_A and D_B are the diffusion constants and r_A and r_B are the hydronamic radii of molecules A and B, respectively. N_0 is Avogadro's number, and the 1,000 factor normalizes units of $k_{\text{encounter}}$ to reciprocal molar seconds. The net rate at which A and B (assumed to be spheres) diffuse together depends on their sizes, the temperature (T), and the solvent viscosity (η), as defined by the Stokes-Einstein relation:

$$D_A = kT/6\pi\eta r_A \text{ and } D_B = kT/6\pi\eta r_B$$

It is important to emphasize that the problem under consideration here is the consequence, say during rehydration, of the rapid and abrupt change in the rate of a reaction within a prescribed range of temperature. The substrate and its catalyst

TABLE 3. Characteristics of the transition from log phase to stationary phase[a]

Physiological target	Consequences
Cell morphology	Cells become smaller (almost spherical); cytoplasm condenses; volume of periplasm increases
Surface properties	More hydrophobic, adhesive, may make fimbriae
Fatty acid composition	All unsaturated fatty acids converted to cyclopropane derivatives
DNA	Remains stable but the nucleoid(s) condenses
Metabolic rate	Rate decreases, and ΔpH and ΔΨ contribute to a change in the membrane proton motive force; trehalose synthesis increases in response to C starvation (osmotic challenge); starvation-specific thermotolerance and osmoprotection induced
Gene regulation	*katF* expression increased
Proteins	Turnover increases fivefold, enhanced proteolysis; 30–50 new proteins are synthesized; expression of catalase, HPII
RNA	Stability diminishes; 20–40% of total RNA lost during first several hours of starvation; tRNAs may be hypermodified
Ribosomes	Dimerization
Spontaneous mutation rate	Rate increases

[a] Data are for cells of *E. coli*; data are taken from reference 354.

are, however, also under mutual constraints with respect to reaction specificity. In the crowded cytoplasm of a desiccated cell, reactants will certainly be in high concentration initially. Upon rehydration, there will be an instantaneous appearance of moisture gradients, a rapid dilution of reactants, and an activation of enzymes. The speed at which these processes occur and the time during which WLF kinetics may apply appear to be very important considerations, yet they are hard to gauge. While chemical reactions can be interpreted in terms of Arrhenius kinetics, the sum of a collection of reactions, in the context of bacterial growth, shows a somewhat different response. In the midrange of growth, Arrhenius kinetics do appear to operate. Above and below this range, however, the growth rate is less than would be predicted through extrapolation (181). Furthermore, the binding of *E. coli lac* repressor to its target (operator) DNA site occurs at a rate that is almost 3 orders of magnitude greater than the upper limit estimated for a diffusion-controlled process involving macromolecules of equivalent size (see reference 394 and references therein). We really have little idea of how desiccation and rehydration affect the whole cell, and it is clearly more complex than simply a sum of a collection of reactions in, or out of, equilibrium. It is interesting that for anhydrous (0.01 g of H_2O g of protein^{-1} up to 0.1 g g^{-1}) samples of lysozyme, hemoglobin, and myoglobin, their heat capacity slowly increases with increasing temperature without showing an abrupt increase characteristic of glass-liquid transitions (342).

WLF theory predicts the viscoelastic properties of solutes and polymers in terms of ΔT above T_g. Can an analogous equation be used to predict those same properties on the basis of water content changes such as those associated with desiccation and rehydration, i.e., ΔH_2O? This is not a trivial question, because glass transitions in biological systems, if they occur, may occur in response to large fluctuations in W at or close to constant temperature. Consider the following hypothetical example. Cell$_x$ of intermediate water content (W_{g_x}) is in the fluid low-viscosity state at temperature $T_{ambient}$. As shown in the hypothesized state diagram in Fig. 14, $T_{ambient}$ is above T_{g_x}. If the cell is now dried, such that it achieves a lower water content, W_{g_y}, a glass transition occurs. Now, because of the dependence of T_g on the mass fraction of water, T_{g_y} is above $T_{ambient}$ (the latter is assumed to have remained constant). If cell$_y$ is now rehydrated, such that W_{g_y} returns to the intermediate value W_{g_x}, a glass transition occurs again as T_{g_y} falls below $T_{ambient}$, and cell$_y$ enters the fluid phase and returns to the state of cell$_x$. The question of interest here is whether, under this set of conditions where $T_{ambient}$ remains constant, cell$_x$ and cell$_y$ undergo their respective transitions under the influence of WLF kinetics. It seems that the transformation from ΔT to ΔH_2O might be possible but only under quite restricted conditions that appear to have limited biological significance. One such condition includes the plasticization of a linear polymer with different organic solvents. In any event, the conditions are not met when water is the plasticizer, not even with small sugars or starch as the solute. Furthermore, it seems that it would be essentially impossible to verify this under experimental conditions (357). Of course, a number of different permutations are possible with the system described in Fig. 14. For example, another way for cell$_x$ to enter the glassy state would be for $T_{ambient}$ to be lowered, at a constant value of W_{g_x}, to $T < T_{g_x}$. Note also that dried cell$_y$ in the glassy state could, in principle, enter the active fluid state at $T > T_{g_y}$ without an external input of water. Any interpretation of the events that could occur in situ becomes difficult, largely because the water content and temperature will fluctuate simultaneously. This simple example does, however, indicate why any consideration of desiccation must involve a consideration of thermal stress, be it heating or freezing. As such, it seems that if bacterial glasses do exist, and the case for one good candidate is presented in the latter part of the review, it is likely that some sets of environmental conditions will guarantee that the bacterial cytoplasm must march to the tune of WLF kinetics.

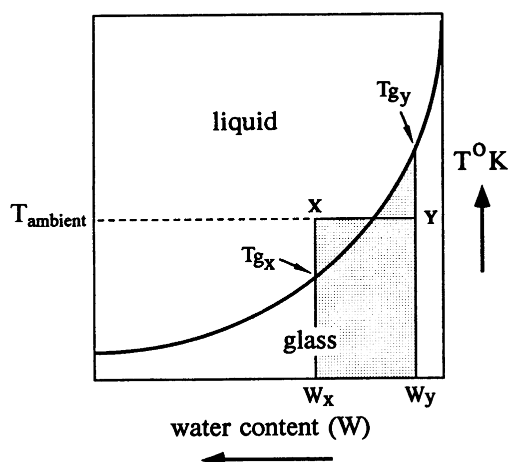


FIG. 14. Phase diagram showing the consequences of a water deficit (ΔW) and the subsequent transitions in a bacterial glass.

Protein Modification and Synthesis

Evolution may have conserved structural features (of proteins) that might determine the abilities of proteins to associate with one another, i.e., through channeling (235). It is important to understand the environment of proteins in a dried cell. For example, it has been suggested that sulfhydryls may play a part in the stabilization of desiccated cyanobacteria (386), so what is known about the redox potential inside a dried cell? In exposed aerobic habitats, endolithic bacteria may be subject to redox potentials of around −700 mV (Eh_{Pt} [348]), presumably as a consequence of nitrogenase-catalyzed hydrogen evolution. Disulfide bridges stabilize the native state of proteins rather than determine the spatial arrangement of the polypeptide backbone (187). Approximately 70% of the coenzyme A in dormant spores of *Bacillus megaterium* is in disulfide linkage to protein (352). The rapid cleavage of coenzyme A protein disulfides upon germination is thought to be a simple mechanism for activation of metabolism and loss of heat resistance. It seems doubtful whether the use of disulfides can be viewed as a general mechanism to stabilize proteins in dried bacterial cells, because bacteria, as a group, do not synthesize disulfides under physiological redox conditions (89, 288, 320, 414). Premature disulfide formation in the cytoplasm may block secretion and is thought to occur only in the periplasm of gram-negative bacteria and in the extracellular media of gram-positive bacteria (258). One protein involved in disulfide formation is DsbA, a strong oxidant with an intrinsic redox potential (E_0') of −0.089 V (428).

The Hsp70/DnaK and Hsp60/GroEL chaperones function in the folding of polypeptides. It appears that what is generally a poorly understood process may involve the sequestration of an aqueous environment, shielded from most if not all nonnative hydrophobic interactions (258). The effects of chaperones would seem to be particularly relevant when desiccated cells are rehydrated, and even a role for chaperonins as compatible solutes has been suggested (163).

With respect to protein stability, extracellular proteins or cell surface-associated proteins must be faced with more drastic perturbations upon drying than their cytoplasmic counterparts. The cell surface proteins of halobacteria represent the only convincing examples of prokaryotic glycoproteins—there are no examples of intracellular prokaryotic glycoproteins (219). The significance of protein-associated sugars is discussed further below.

Proteins exhibit marginal stability, equivalent to only a few weak interactions (350). Expressed in ΔG_{stab} per residue, the free energy is below the level of thermal energy. Molecular adaptation to extreme conditions may be accomplished by the accumulation of minute local structural changes. In addition, "extrinsic factors" (not encoded in the amino acid sequence) may be of importance (191). However, no general strategies of stabilization of proteins have been established for any of the stress parameters in nature (189). Are there environmental conditions in a given biotope required to generate or maintain the functional state of proteins? What determines the limits of growth and reproduction in extreme biotopes? Is there a hierarchy of stress parameters regarding selective pressure (189, 362)? In recent years there has been a flurry of reports of so-called water stress proteins in a wide range of taxa, particularly in plants (356). Identifications of the roles of these proteins—including the Lea proteins, osmotins, and dehydrins—and particularly the uncovering of evidence for their interaction in the water relations of cells have not been forthcoming (21, 32, 43, 69, 356). Interestingly, a eukaryotic histone-like protein has been reported to respond to cell shrinkage or swelling through changes in its phosphorylation state (340), and salt-dependent protein phosphorylation in the cyanobacterium *Synechococcus* sp. strain PCC 6803 has recently been described (157). However, the prospects for finding proteins that are induced specifically in response to cell drying in order to provide a direct measure of protection, both to themselves and to other cell components by effecting measurable biophysical interactions, would seem to be remote. The idea is very attractive, of course, from the perspective of genetic manipulation of desiccation tolerance in sensitive cells. Proteins, such as those induced in response to oxidative stress, may exert indirect effects on cell stability (242). The water stress proteins (Wsp) of the cyanobacterium *N. commune* (Fig. 15) were initially thought to confer a stabilizing effect on desiccated cells in view of (i) their abundance (they accumulate to around 70% or greater of the total soluble protein) and (ii) their great stability (immunoblotting has revealed that Wsp proteins are stable in desiccated cells stored for decades) (168, 345). Our recent data tend to discount a structural role for Wsp, but they do point to a very subtle and critical role in desiccation tolerance (see below).

Membrane Modification

Alteration of the lipid content of membranes is of major importance in response to environmental stresses (265, 324). There is a comprehensive literature on the role of membrane fluidity and lipid composition on survival of bacteria at extremes of temperature and salinity, but equivalent data for changes associated with the drying of cells are few (208, 336, 337). Maintenance of membrane integrity in anhydrobiotic organisms represents a central mechanism of desiccation tolerance (54, 77). In addition to its ability to preserve the integrity of proteins and enzyme activity, trehalose can stabilize membranes (76, 77, 80, 83, 224). Membranes dried without trehalose undergo vesicle fusion, change in morphology, and loss of Ca transport activity upon subsequent rehydration (81). It appears that the dry vesicles, as well as liposomes, may be stored for at least 6 months without loss of stability, providing that they are protected from oxygen (82). A similar enhancement of stability can be achieved with dried cells if they are stored under an inert gas phase (64, 291).

What is the basis for the protection of membranes with

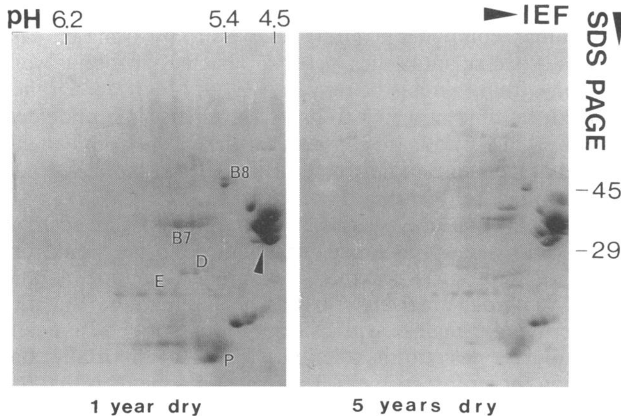


FIG. 15. Wsp proteins are the most abundant soluble proteins in desiccated *N. commune*. Shown are two-dimensional gels of protein extracts from cells desiccated for 1 and 5 years. P, phycobiliproteins. The arrowhead indicates the cluster of Wsp isoforms. Other letters refer to marker protein constellations (295). Markers to the right are molecular masses in kilodaltons. Note that Wsp is the most abundant soluble protein. Recent work has shown that Wsp is secreted (168); the true abundance of Wsp may be greater than that shown in the gels, since no attempt was made, in this experiment, to retain the secreted portion for gel analysis. IEF, isoelectric focusing; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Reproduced from reference 345 with permission of the publisher.

trehalose? The phospholipids in membranes are hydrated; in the case of phosphatidylcholine, some 10 to 12 water molecules are hydrogen bonded around each phosphate (around 20% of the water in the membrane). When that water is removed, the packing of the head groups increases, which leads to increased van der Waals interactions among the hydrocarbon chains (81). As a result, the phase transition temperature, T_m, increases enormously. For experimental systems the T_m may be elevated from around −10 to around 60°C. As a consequence, dry lipids would be in a gel phase at room temperature—a temperature that, under a normal state of hydration, would permit the liquid-crystal phase of the membrane to be achieved. As a result, when such dry lipids are placed in water, they would be expected to undergo a phase transition and become “leaky.” Trehalose appears to depress the phase transition temperature of the dry lipids after water has been removed and maintains them in the liquid-crystal state (81, 224). Trehalose can depress the first-order phase transition of dry phospholipids to a temperature close to that of hydrated bilayers, leading to a transition that is referred to as the L_k-to-L_l transition (221). In dry mixtures of trehalose and 1,2-dipalmitoyl-*sn*-phosphatidylcholine (DPPC), nuclear magnetic resonance spectroscopy measurements indicate that the sugar is in close proximity to the hydrophilic region from the phosphate head group to the interfacial regions (221). The eight hydroxyls on each trehalose are all available for hydrogen bonding to the phosphate and carbonyl groups of the lipids. Furthermore, the sugar is thought to occupy some space between lipid molecules. The lowering of the transition temperature of DPPC in this model system may involve spacing of the acyl chains to permit their disordering (221).

An understanding of the role of sugars in the stabilizations of membranes in dried cells has been derived from studies with pollen. The T_m for dry pollen is very low, considerably lower than the T_m for dry phospholipids isolated from the same pollen (81). For *Typha* pollen, 97% of the total soluble sugars present is sucrose (25% of the dry weight). Hoekstra and colleagues have provided evidence that it is this endogenous sucrose in dry, intact pollen grains that is responsible for the depression of the T_m relative to that seen in isolated membranes (172–176). Sucrose was able to depress the T_m for purified and dried microsomal membranes from 58 to 31°C. The sucrose content at which minimal T_m was achieved was 3 g g of membranes^{-1}, and the calculated mass ratio of sucrose to phospholipid that achieved maximal reduction of the T_m was 6 or less.

A depression of T_m in pollen grains can also be achieved through an increase in the degree of unsaturation of membrane phospholipids (81, 175). In this case, there is an inverse correlation between the amount of sucrose present and the degree of unsaturation. Cells utilizing unsaturation, however, tend to have a shorter lifetime in the dry state, presumably because of increased susceptibility to oxidation of their lipid components.

An accumulation of a nonreducing disaccharide, trehalose or sucrose, and the synthesis of lipids with an elevated degree of unsaturation are two strategies that permit pollen phospholipids to exist in the liquid-crystal phase at room temperature when dry. As such, rehydration does not lead to a phase transition, and damage following rehydration, through leakage, is circumvented (224). Both of these strategies would seem to be within the capabilities of bacterial cells. In fact, almost 60% of the total phospholipids in the purified cytoplasmic membrane of *Nostoc* UTEX 584 was found to be 20:3ω3 fatty acid (265).

Trehalose is a substrate for trehalase, and the activity of the latter is likely to play an important role in the kinetics of stabilization of whole cells. Recently, it has been shown that the binding of yeast trehalase to membranes requires that the enzyme be in its active (phosphorylated) state (89). The significance and basis of this reversible binding are unknown; however, phosphorylation and dephosphorylation reactions are crucial signals that are known to induce diverse metabolic cascades in both prokaryotic and eukaryotic cells.

Trehalose—a Panacea for Water Stress?

Two features of the water relations of cells have taken on significance in recent years. The first is the realization that water molecules play a critical role in structure-function relationships of proteins, and the second is the documentation that certain sugars contribute to the stabilities of proteins, membranes, and whole cells when water is removed from them. Under conditions of moderate water deficit, compatible solutes appear to be important components of mechanisms that contribute to the maintenance of viability. Under the most extreme water deficit, however, only the disaccharides trehalose and sucrose seem to afford protection. Disaccharides appear to be the most effective stabilizers of dried enzymes in vitro, and the protective effects of trehalose, in particular, have attracted most attention (53, 224, 309). In assessing the effects of trehalose, it is necessary to consider the following: what are the properties of trehalose, what is the proposed mechanism of protection, and is trehalose an all-purpose protectant?

Many chemical properties of sugars, such as their reducing properties, depend on the free hydroxyl group of the hemiacetal (at C-1 of the aldoses or C-2 of the ketoses). Disaccharides in which both hemiacetal hydroxyls have reacted with one another possess entirely different properties. Both sugar components exist in their full acetal form. Such oligosaccharides are not reducing, show no mutarotation, and form no osazones. The simplest natural representative is trehalose [1-α-

glucosido-1-α-glucoside; *O*-α-glucosyl-(1,1)-α-D-glucoside]. Trehalose is the only nonreducing oligosaccharide of glucose. A second representative is sucrose (α-glucopyranosido-β-fructofuranoside). In sucrose, fructose is present in the furanose (less-stable) ring form. Fructose is closely related to D-glucose, and free fructose is most stable in its pyranose form (six-membered ring). Only in oligosaccharides, polysacacharides, and several phosphate esters is the furanose five-membered ring system realized—as in sucrose. Why anhydrobiotic plants should rely upon sucrose and not trehalose is not known.

Because trehalose and sucrose seem to be accumulated by many different cell types in response to water stress, attention initially focused on the reducing property of these sugars as the basis for their protective effect. The property of being nonreducing, however, does not, in itself, constitute a property for protecting proteins (81, 83). Glycerol, without any reducing groups, fails to show particularly enhanced protective effects, although it is used extensively as a cryoprotectant of bacterial cultures. Largely on the basis of these facts, it is generally believed that trehalose is physiologically more relevant than sucrose in terms of its efficiency and the stoichiometric amounts required for protection. Other properties of trehalose that may be of importance include the inability to participate in browning reactions that lead to insoluble, discolored protein products after long-term storage (76). Glycerol, for example, cannot prevent browning reactions. It has been speculated that the ability of sugars to hydrogen bond to water could reflect the spacing of OH groups on the hydrocarbon backbone of the sugar and that equatorial groups on pyranose and cyclitol rings could fit precisely into the water lattice of bulk water; the experimental data fail to support this proposition (76). What is important in the mechanisms of stabilization by trehalose if the reducing property is not?

The interaction of trehalose with membranes has received more critical attention that its interaction with proteins (see, e.g., reference 220). The mechanism for the latter may include exclusion phenomena, although trehalose also seems to interact directly with the dry protein probably by hydrogen bonding of -OH groups to polar residues in the protein, but it is not yet clear how this interaction leads to stabilization (53). Crowe and colleagues have provided evidence for a direct interaction between trehalose and lipids. The sugar is thought to replace water molecules around the polar head groups of the phospholipid in the dry state (129). This "water replacement hypothesis" has been discussed at length (64, 67, 74–84). Direct evidence for trehalose binding to phospholipid membranes has been obtained for dry phospholipid vesicles. Apparently, hydrated membranes do not undergo interactions with trehalose, sucrose, or glucose (18). Trehalose does not react with proteins at elevated temperatures, as reducing sugars do, and its solutions are more readily dehydrated. The glass transition of trehalose solutions is also atypical with respect to other sugars. Another important observation is that transition metals enhance the effects of protection—a feature that is thought to reflect the general inhibitory effect of zinc, for example, on enzymes in vitro (56). Curiously, if phosphofructokinase is partially dried it is inactivated; addition of proline, which stabilizes phosphofructokinase in solution, leads to stabilization during partial dehydration, but activity is lost at lower water contents. In contrast, trehalose maintains activity over the range of water deficit, including most extreme desiccation.

In desiccated *N. commune*, the amounts of trehalose and sucrose were comparatively small at around 0.1 mg of disaccharide g (dry weight) of colony^{-1}, but the extracellular glycan constitutes the bulk of the colony dry weight, and the sugar concentrations may, in fact, be sufficient for the protection of intracellular components (170). However, by using *E. coli* trehalase-specific antibodies, two cyanobacterial proteins were detected within minutes of rehydration, and their appearance matched the time at which trehalose levels became undetectable (169). These data are consistent with reports from Panek's group that trehalose in desiccated yeast cells is rapidly turned over upon cell rehydration.

It is clear that the synthesis of a disaccharide such as trehalose or sucrose is sufficient to afford protection from drying for liposomes, enzymes, membrane components, and dried cells. Not surprisingly, it has been suggested that the engineering of trehalose synthesis in cells will provide a means to manipulate desiccation tolerance (81). An important consideration for a protective effect on cell components is that the effect must be mediated through, and incorporated within, the stringently regulated physiological tension of the bacterial cell. For such cells, an accumulation of trehalose may protect intracellular components, but what of the outer leaflet of the cytoplasmic membrane and the different faces of the outer membrane of gram-negative bacteria? A significant recent finding of the group of Panek is the role of the trehalose carrier in the dehydration resistance of *Saccharomyces cerevisiae* (108). As expected, trehalose must be present on either side of the bilayer to stabilize dry membranes. A specific trehalose carrier translocates trehalose from the cytosol to the extracellular environment. Mutants that lacked the transporter accumulated trehalose but did not survive dehydration. Mixing those same mutants with trehalose increased their resistance.

A clear correlation between trehalose or sucrose accumulation and the maintenance of integrity in anhydrobiotic cells has been established (see above). In contrast, there are numerous reports in the literature on the accumulation and turnover of trehalose in bacterial cells, when the physiological status of the cells and their fate after drying are very hard to assess (374). Cells of *Rhizobium leguminosarum* bv. trifoli TA-1 cells can accumulate 0.13 g of trehalose g of protein^{-1}, yet the cells lose viability beyond 0.35 M NaCl (38). Surveys of the solutes accumulated in response to water stress in bacteria growing in situ indicate that there are clear preferences for which solute should be accumulated. For example, of 22 strains of cyanobacteria shown to accumulate low-molecular-mass carbohydrates in response to salt stress, only 4 accumulated trehalose (368). In rhizobia that do make trehalose, trehalose may accumulate until stationary phase is reached, and then it is rapidly metabolized. Trehalose accumulates in response to anaerobiosis (1% [vol/vol] oxygen) in rhizobia and decreases at ambient O_2 levels, when oxidative effects would appear to be at their most acute (177). Trehalose synthesis also appears to be correlated with a number of cell processes that do not involve water stress (177). In a filamentous cyanobacterium, *Phormidium autumnale*, trehalose accumulated up to 6.2 μg μg chlorophyll a^{-1} in response to matric water stress to −5 MPa. At lower activities (up to −25 MPa; a_w = 0.83), the concentration of trehalose dropped to approximately half of this level (167). A similar response was achieved for another cyanobacterium that accumulated sucrose in response to matric water stress.

Sheaths, Capsules, Slimes, and MDOs—a Mechanism beyond Trehalose?

Do bacterial cells that show resistance to air drying secrete trehalose? Perhaps they do. One thing that many certainly do is to secrete conspicuous amounts of EPS. Here, I first review what is known about these EPS layers and then attempt to

sketch the outlines of what could be a general mechanism of protection in anhydrobiotic bacterial cells.

Extracellular investments are a conspicuous feature of many bacterial cells. These investments may have the appearance of diffuse slimes or of rigid layers with a defined and complex ultrastructure (303). Many bacteria live within an EPS in environments that range from soil to the human lungs (325). These EPS layers are formed by the accumulation of various types of polymeric substances of high viscosity around bacterial cell walls; they tend to be hygroscopic, often contain more water than the bulk environment, and may decrease the rate of water loss from the cells. It is widely believed, and generally stated, that EPS provide bacterial cells with a means to survive drying, yet studies on specific responses of polysaccharide synthesis to drying are few and the mechanisms of sensing, induction, and regulation remain little studied (110). Protective roles for EPS have been confirmed in some studies (204), yet others failed to show any obvious correlation between the ability to produce EPS and a capacity for desiccation tolerance (269). Functions attributed to EPS, in addition to protection against desiccation, include anchorage to the substrate, protection against phagocytic predation, the masking of antibody recognition, and prevention of lysis by other bacteria and viruses (381). The EPS of *Beijerinckia* spp. may also protect the cells against oxygen damage (19). The presence of proteins, uronic acids, pyruvic acids, and *O*-methyl, *O*-acetyl, and sulfate groups in these layers emphasizes their complexity and also suggests that a number of enzymes would be required to degrade (and synthesize) the polysaccharide. The occurrence of carbonyl, carboxyl, hydroxyl, and sulfate groups provides a means to attach cations. In the latter respect, these sheaths may scavenge metals that may be used either in physiological processes, such as nitrogen fixation, or as toxins against predators.

EPS, such as capsules, tend to have a very low affinity for various dyes, and electron microscopy studies have established that in general they are less electron dense than the cell wall and the cytoplasm (331). Nontuberculosis mycobacterial species contain a variety of trehalose-containing lipopolysaccharides. Isolates of the tubercle bacillus are generally devoid of these but have simple acyltrehaloses (27, 134, 223). There are no indications that these structures provide any measure of membrane stabilization with respect to the drying tolerance of members of the family *Mycobacteriaceae*; however, it has been proposed that because of their structure and amphipathic nature, they may represent a "pseudo-outer membrane" (50). Eubacteria and archaebacteria have other surface layers that constitute an interface between the cell and its environment. Although the functions of these surface layers are not readily apparent, it has been suggested that they may serve a protective role by modulating environmental stress (363). The proteinaceous sheath of *Methanospirillum hungatei* GP1 is a resilient, proteinaceous, paracrystalline bilayer structure (364). The sheath is rigid, presumably because of covalent bonding in combination with weaker bonds (ionic, hydrophobic), and it contains disulfides. An important feature of these layers is that because they may be stress-bearing structures, new material must be inserted without loss of cell turgor. Surface structures of *Desulfurococcus mobilis*, another archaebacterium, have an unusual protein lattice at the surface, providing almost an exoskeleton, that has been speculated to afford protection from water stress (421). One component of the cell surface components of *Halobacterium* spp., bacteria that are subjected to salt stress in situ, provides the only convincing example of a prokaryotic glycoprotein (219). The cell surface glycoprotein has a mass of 120 kDa and is extremely acidic, with 20% Asp or Glu, 1 mol of cell surface glycoprotein per 40 to 50 mol of uronic acids, and 40 to 50 mol of sulfate per mol of glycoprotein in ester linkages.

The EPS of bacteria represent an additional cell compartment, which may contain the bulk of the water associated with a single cell (Fig. 2). Studies with a soil *Pseudomonas* strain—yellow pigmented and oxidase positive—showed that when dried at -1.5 MPa, the cells remained embedded within the EPS and were less distinct at the electron-microscopic level (325). At -1.5 MPa the EPS held 5 times its weight in water, and at -0.5 MPa it held 10 times its weight in water. The water content of the acidic heteropolysaccharide of *Gloeothece* sp. strain ATCC 27152 was 98.8% when cells were grown under N_2 and 99.5% when they were grown under $NaNO_3$ (381). These structures are engorged with water. Why does this water not simply flow out of the sheath, especially if many of the carbohydrate components appear to be water insoluble? It seems that these sheaths must represent well-mixed gels. Liquid water is restrained by the small amount of gel material because of the force of mixing. To separate water from a gel, work must be applied against this force (see reference 420 for a more extensive discussion). The sheaths of *N. commune* UTEX 584 have distinctive gel-sol transition zones, implying that the underlying structure of the sheath and its water-bearing properties can be modulated during the growth of the cells (23, 169). *N. commune* grows as spherical, resilient cartilaginous colonies under matric conditions at low humidity (76); however, the derived strain, *N. commune* DRH1, exudes polysaccharides that have much lower viscosity in liquid media (169). In view of the copious amounts of water trapped in these types of extracellular gels, their structural analysis has proved to be difficult (331). It is also widely stated, largely on the basis of what seems to be anectodal evidence, that certain structures observed in the electron microscope are artifacts and are the result of the "collapse" of cellular material during embedding for electron microscopy. As a consequence, while analytical data are available, structural analysis of extracellular structures and elucidation of structure-function relationships remain to be performed. Particular controversy has surrounded the interpretation of the capsular polysaccharide (M antigen; colanic acid) of *E. coli* (331). The data of Schmid (347), however, clearly document that in dehydrated and fixed cells the capsule is readily observable with the light microscope but with the same fixed material the capsule cannot be imaged by the electron microscope—clearly, electron-microscopic analyses cannot be used as the basis to describe these structures as dehydration artifacts.

The EPS of cyanobacteria provide some of the most complex examples of bacterial sheath structures, and they have been well documented in early and contemporary literature (136, 410). Many of these have an intricate ultrastructure (311), and, especially in communities growing in situ, they tend to be pigmented (see below). For the most part, these external layers of polysaccharide are reminiscent of the glycocalyxes, slimes, and capsules of other eubacteria (248, 381). For example, in gross morphology, the appearance of packets of cells of the cyanobacterium *Myxosarcina* spp. and those of the archaean *Methanosarcina* spp. are virtually indistinguishable. In one desiccation-tolerant cyanobacterium, *Crinalium epipsammum*, isolated from sand dunes of the Dutch coast, the cell wall was found to be hydrophilic and thickened and contained poly-β-(1,4)-glucan (cellulose, known to retain water efficiently [94]). The cells were also elliptical. The presence of this polymer and the shape of the cells are two properties that have been suggested to aid survival of this organism, although the significance of the latter property seems obscure.

Early studies on the biosynthesis of EPS in the cyanobacterium *Anabaena flos-aquae* indicated that they are derived from intracellular polysaccharides of the same composition (244). Synthesis appeared to be intracellular, and the products were thought to diffuse through the cell wall. At least for the sheath of *Gloeobacter violaceus* (349), no phosphodiester bonds appeared to be involved in the binding of the sheath to the peptidoglycan layer. Of the different substrates tested with *Anabaena flos-aquae*, only D-fructose was able to substitute efficiently for CO_2 in sheath synthesis (244). Similarly, fructose was the most effective carbohydrate tested that induced floc formation in the eubacterium *Azospirillum brasilense Sp7* (ATCC 29145) (338). It was concluded that overproduction of exocellular polymers induced the flocculent growths, which consisted of nonmotile, refractive, highly desiccation-resistant forms entangled within a fibrillar matrix.

EPS may be produced under both hypoosmotic and hyperosmotic conditions. What could be the nature of the regulation? In members of the family *Rhizobiaceae*, periplasmic cyclic β-(1,2)-glucans are involved in osmoregulation in media with low ionic strength (39). The glucans are synthesized to obtain an increased osmolarity in the periplasm in order to minimize differences in osmotic pressure across the inner membrane (240). In media of high osmotic strength (0.5 M NaCl), the production of cyclic glucans is strongly repressed in *Agrobacterium tumefaciens* and *R. meliloti*, glycogen synthesis is inhibited, and trehalose is accumulated. In contrast, other cells, e.g., *R. leguminosarum*, excrete up to 1,600 mg of glucan liter^{-1} in the presence of 200 mM NaCl. It has been postulated that the outer membrane of this strain becomes more permeable when exposed to salt, and constant loss of glucans prevents end product inhibition (39). Cyclic oligosaccharides and membrane-derived oligosaccharides have similar compositions and are regulated in a similar fashion. Membrane-derived oligosaccharides of *E. coli* are periplasmic glucans variously substituted with *sn*-1-phosphoglycerol, phosphoethanolamine, and *O*-succinyl ester residues (365, 382). The synthesis of membrane-derived oligosaccharides and of analagous periplasmic glucans in other gram-negative bacteria is subject to strict osmotic regulation. Adaptation to growth at low osmolarity appears to involve the signaling functions of the periplasmic glucans, which are themselves regulated osmotically (335). They may represent a focal point in the hierarchy of an osmotic signaling system and must be considered in the context of cell drying. Also, in this context it is important to note that osmotic regulation occurs principally at the level of modulation of enzyme activity rather than at the level of gene expression. A general role for periplasmic oligosaccharides in osmotic adaptation of gram-negative bacteria as ecologically diverse as enteric bacteria and soil bacteria has been suggested (240, 287). Studies with *mdoA* mutants indicate that a certain minimal ionic strength in the periplasm is crucial for normal porin regulation that is mediated through EnvZ, the proposed sensor of external osmolarity (135). Additional evidence that pressure or stretch is a signal for behavioral response to osmotic upshift is the identification of mechanosensitive ion channels which are stretch activated (225).

It is now becoming clear that the synthesis of EPS in bacteria not only is complex but also requires the coordinated expression of sets of genes that respond to changes in the water potential of the cell and its environment. The regulation of EPS synthesis is even more complex than it first appears, because it may also occur together with the coordinated expression of other systems such as those under oxygen control (148). Several different EPS systems are under active investigation. The M antigen (colanic acid; capsule) is distributed widely in enteric bacteria. The capsule is made only under high osmolarity, at low temperature, and at low humidity; in fact, its production is favored under matric as opposed to osmotic conditions (200, 257). Although the function of the capsule is unknown, it has been suggested that it may protect cells from desiccation when they are outside of the host. Synthesis of this capsule involves assembly from nucleotide sugars of a repeating polysaccharide containing glucose, galactose, glucuronic acid, and fucose (147). The genes necessary for this synthesis are scattered around the *E. coli* map and include *galE* (UDP galactose 4-epimerase) at 17 min, *galU* (glucose-1-phosphate uridylyltransferase) at 27 min, *manA* (mannose phosphate isomerase) at 36 min, and the *cpsA–E* cluster mapping near *rfb* (rough phenotype; TDP-glucose oxidoreductase) at 44 min. Synthesis of the capsule is complex. Transcription of the *cps* gene cluster is regulated by three positive regulators, RcsA (373), RcsB, and RcsF, and two negative regulators, RcsC and Lon. RcsC and RcsB may be sensor and effector, respectively, of a two-component regulatory system. RcsB interacts with *ftsZ*, a cell division gene, implying that the *rcs* system may be part of a global regulon. In view of the importance of cell volume changes in the responses of cells to water stress, it would be of interest to see how *rcsB* expression is influenced during the slow drying of *E. coli* cells.

Genes encoding the *E. coli* K4 capsular polysaccharide, a fructose-substituted chondroitin, have been cloned and expressed (103). Expression of the K4 capsular polysaccharide is also complex and requires coordinated expression of protein products for transport through the periplasm and outer membrane, translocation across the cytoplasmic membrane, and polymerization (42). Some analyses of the genes have been carried out. The *exoYFQ* operon is involved in the transport of the succinoglycan cell surface polysaccharide in *R. meliloti* (139).

Alginate is an EPS in *P. aeruginosa*, and its presence permits encapsulation of the cells and protects them from phagocytosis in the cystic fibrosis environment (127). Activation of synthesis occurs in response to growth in the lungs of patients with cystic fibrosis but also under high osmolarity, suggesting that alginate excretion could be the relict of an adaptation to drying. The expression of the genes involved in alginate production and secretion have been well characterized. The *alg* operons are activated at two promoters upstream of *algD* and *algC*. Signaling, like that of capsular polysaccharide synthesis, is mediated by a two-component system. An ATP/GTP-dependent protein kinase, AlgR2, undergoes autophosphorylation and transfers a PO_4^{3-} group to a DNA-binding response regulator, AlgR1. The phosphorylated form of AlgR1 has a high affinity for binding at upstream sequences in the *algC* and *algD* promoters. Evidence that mobile genetic elements are involved in EPS synthesis of a marine strain, *P. atlantica*, has also been obtained (22). The element that inserts and excises from the *eps* locus has the properties of an IS element.

Bacteria respond to desiccation by channeling energy and nutrients into polysaccharide production. For example, *Pseudomonas* sp. contains more EPS than protein when desiccated (325). It seems necessary that EPS production be coordinated within the overall carbon metabolism of the bacterial cell. In this regard, the regulation of glucan biosynthesis is likely to be important, and relatively facile methods are now available to clone the structural and regulatory genes involved in glucan biosynthesis from any bacterium of interest (328).

So, what of the role, if any, that these EPS components play in the stabilization of air-dried cells? Recent studies by Goodrich and colleagues have provided possible clues. Three synthetic carbohydrate derivatives (glycolipids), triethoxycholes-

terol, maltosyltriethoxycholesterol, and galactosyltriethoxycholesterol, were shown to have subtle effects on the physical properties of membranes (142, 143). The carbohydrate portion of each derivative appeared to mimic the effects of water. First, there was an expansion of the lipid lattice normally associated with lipids when they pass from the pretransition to a liquid-crystal state. Second, the carbohydrate at the membrane surface altered the organization of the acyl chains and carbonyl groups. The lipid linker functioned to position the carbohydrate group between the lipid molecules of the membrane, in the region of the hydrophilic portion of the lipid head group. These derivatives were shown to offer protection following freezing and thawing, and the effects were maximized at a carbohydrate-to-lipid molar ratio of 0.4:1. The data were consistent with a water replacement hypothesis, yet the amount of derivative required to induce cryoprotection was on the order of 100 times smaller than that recorded for the amount of trehalose and sucrose required to reduce probe intermixing to comparable levels. Even more important was the observation that there did not appear to be any significant differences in samples containing derivatives with galactose or maltose as the terminal sugar. This latter result suggests that the cryprotective effect is a characteristic of saccharides in general and is not specific to mono- or disaccharides. What these studies seem to be hinting at is that carbohydrates in general and EPS in particular may function as very efficient protective agents if they have an appropriate orientation with respect to the target. Application of drying in the presence of compatible solutes is being used to successfully store erythrocytes (140, 141, 144). It seems reasonable to question whether similar compounds and similar effects may play a role in anhydrobiotic cells. Is it possible that the nature of the mechanism described for synthetic glycolipids is applicable to dried bacterial cells that elaborate carbohydrate sheath layers? A feature of the latter is an intimate association between the EPS and the outer membrane layer—an association that is accentuated when the extracellular layers dry and shrink. Bacteria elaborate a wide range of carbohydrate derivatives, including many glycolipids, and there are plenty of unusual examples to consider in regard to the mechanism described above. Gliding bacteria produce carotenoids that include sugar-linked acyl chains, and their lipopolysaccharides also contain the unusual sugar 3-*O*-methyl-D-xylose (321). One bacterial compound appears to be an excellent candidate. Cord factor or (α-trehalose 6,6′-dimycolate) is a cell wall glycolipid of *Mycobacterium*, *Nocardia*, *Rhodococcus*, and *Corynebacterium* species and is thought to play a role in the pathogenesis of some of these bacteria through prevention of fusion of bacterium-containing phagosomes with primary or secondary lysosomes (366). Cord factor was found to be more effective than free trehalose in preventing membrane fusion. This effect was thought to reflect immobilization of the trehalose component at the membrane surface through its hydrophobic anchor. In this way, the glycolipid could increase hydration force; alternatively, it could act as steric block to fusion. In view of the results of Goodrich et al., it seems possible that cord factor could, in principle, provide a measure of protection to dry membranes.

The most obvious components of the cell walls of many types of bacteria are their EPS layers. Figure 16 shows a section through a portion of a desiccated colony of *N. commune*. The chemical and physical features of the EPS layer, a complex glycan, have recently been described in detail (169). The prominent features shown in Fig. 16 include the spherical, intact cell of a filament that permeates the dense, copious extracellular glycan and an unstained, electron-translucent layer that immediately surrounds the cell and separates it from the glycan. This translucent layer is not some artifact of drying; in fact, an electron-translucent layer of comparable dimensions and location is a diagnostic feature of the akinetes (spores) and heterocysts of many cyanobacteria (see Fig. 2a and 3b in reference 25 and Fig. 10 in reference 377). The structure is also reminiscent of the translucent spore cortex layer of *Bacillus* spores (see Fig. 1 in reference 261). The singular distinction here, however, is that every vegetative cell in a filament of *N. commune*, in the desiccated state, has such a translucent layer. To place this fact in some perspective, it should be noted that under appropriate culture conditions all of the vegetative cells in filaments of some cyanobacteria may differentiate synchronously into akinetes (see Fig. 3 in reference 35). These akinetes of cyanobacteria are generally regarded as being freeze tolerant and desiccation tolerant, although critical studies to support these assumptions, as well as explanations of the mechanisms that provide the basis for these properties, are lacking (252, 377). Curiously, akinetes of one strain when frozen in liquid nitrogen in the presence of dimethyl sulfoxide—a known chaotropic agent—were reported to show no loss in viability upon storage (60). Are desiccated filaments of *N. commune* simply chains of akinetes? This would be a simple explanation to account for the desiccation tolerance of this cyanobacterium, but there are a number of reasons why it is both unsatisfactory and untenable, not the least of which is the obvious one that we conveniently remove a consideration of desiccation tolerance from a vegetative cell of *N. commune* to the equally misunderstood akinete. The peptidoglycan layers of *N. commune* cells lack the thickness that is characteristic of akinetes (252; see Fig. 10 in reference 377). More importantly, rehydration of desiccated *N. commune* cells leads to marked changes in the appearance of the extracellular glycan (Fig. 17) (169) and to the resumption of metabolic activities, but it does not lead to any outgrowth or germination of the cells. In contrast, a developmental cue leads each akinete, upon revival, to divide into a short trichome that grows from, and breaks out of, the mother akinete (see Fig. 5 to 9 in reference 35). Perhaps extant akinetes, elaborated by several different genera of cyanobacteria, and the vegetative cells *N. commune* share certain features that provide them with an enhanced capacity to tolerate desiccation. Are the translucent layers of the different cell types comparable? Is the outer layer of an akinete the remnant of a more extensive carbohydrate layer such as the bulk glycan retained by colonies of forms such as *N. commune*? The translucent layer of *N. commune* cells is appressed to, and in intimate contact with, the cell membrane and the outer sheath layer. What could be in this layer? In *Anabaena cylindrica*, the homogeneous translucent layer of heterocysts and akinetes contains largely polysaccharide (426). The lack of post-staining suggests that lipids are absent, although at least for heterocysts this homogeneous layer may also contain phosphoglycolipid intermediates (426). Analysis of desiccated material of *N. commune* prepared with specific stains for analysis by light microscopy suggests that sugar-containing compounds are present in this layer and that they are antigenically distinct from the bulk glycan (169). Critical-point drying leads to removal, or possibly rearrangement, of the contents of this translucent layer, suggesting that they may be volatile (169). As cells are rehydrated, however, there are marked changes in the structure of the translucent layer. Could the translucent layer be a glass, which interacts with the outer membrane in the way that the glycolipids described by Goodrich et al. (142, 143) interact with membranes? The extracellular sheath layer is composed of a complex, high-molecular-mass polysaccharide. Biopolymers and synthetic polymers

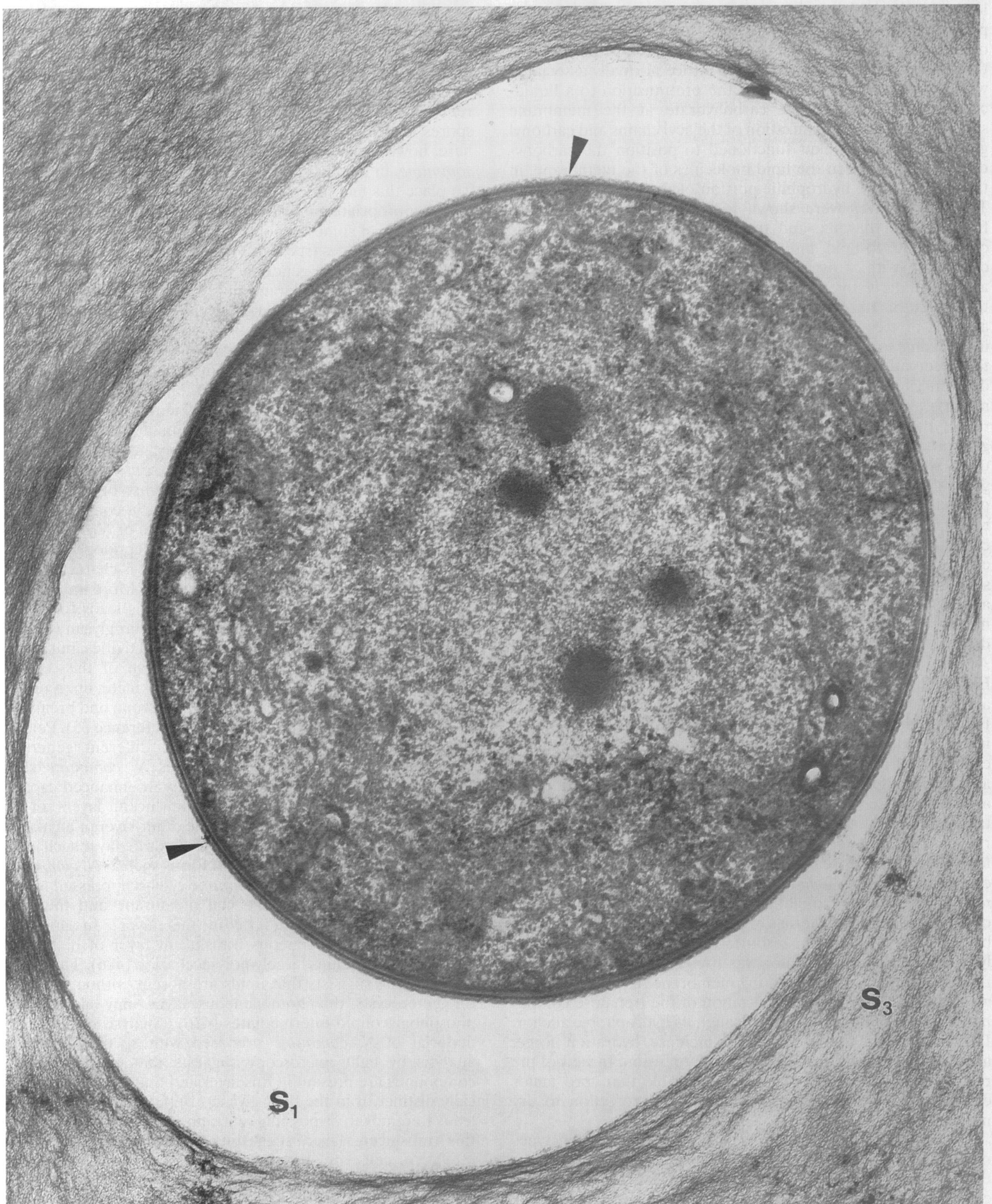


FIG. 16. Transverse cross-section through a desiccated colony (12 years dry) of *N. commune* CHEN (169). The section includes a single desiccated cell of a filament with an intact cell envelope (arrows), the electron-translucent layer S_1, and fibrous extracellular glycan (169). Magnification, ×37,500. Micrograph 10016 by Donna R. Hill.

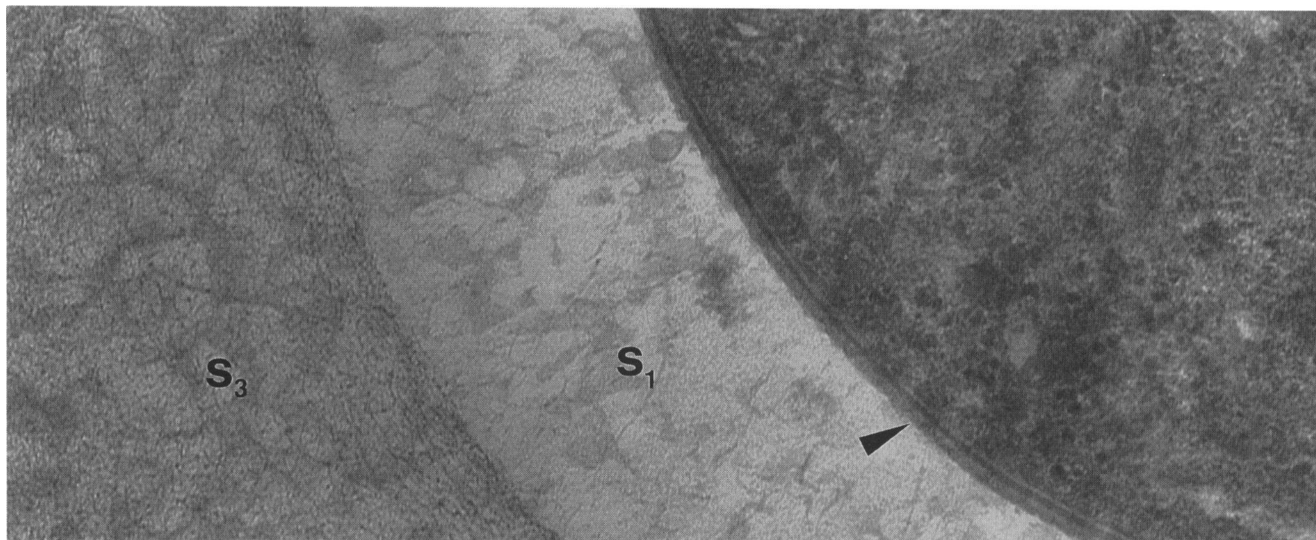


FIG. 17. Influence of rehydration on the extracellular sheath of *N. commune* CHEN. Same material as Fig. 16, but rewetted for 15 min prior to fixation. Note the marked changes in structure and staining characteristics of the S_1 and S_3 layers and the intact cell membrane (arrowhead). Magnification ×60,000. Micrograph 10040 by Donna R. Hill.

form glasses, so that it seems not unreasonable to suggest that a considerable portion of a dried *Nostoc* colony may be in the glassy state. These dried colonies have the consistency of dried bacon rinds: they are friable and easily crushed to a powder. They can be heated and frozen without a loss of cell viability. Wetting leads to an instantaneous change in physical properties, including considerable swelling, and to the consistency of a stiff yet malleable gel. However, the colonies never form slimes or diffuse aqueous gels under matric conditions. It is expected that the structural analysis of the glycan will provide much information on the mechanisms for desiccation tolerance in this organism.

In summary, it seems likely that EPS synthesis represents a focal point of the ability of some bacteria to express desiccation tolerance. Table 4 summarizes those properties of EPS that may be important in this regard.

Photoprotective Pigments

General aspects of UV radiation and a discussion of the role of UV pigments in desiccation tolerance has been reviewed by Whitton (415, 416). Communities of terrestial cyanobacteria often appear black or brown (Fig. 18), and many other communities have highly pigmented sheaths upon close inspection. The colors of many of these pigments change readily with pH, a feature that is apparent when cells are removed from rocks or sediments by using mild acids. One pigment that is unique to, and common in, terrestial cyanobacteria is the sheath pigment scytonemin. Scytonemin is a yellow-brown lipid-soluble pigment that can undergo reduction to a red form, and it has a broad absorption spectrum centered around 400 nm depending on the conditions of pH and solvent (130). In *N. commune* communities, the pigment is easily extracted by mild abrasion of the colonies in the presence of 1% Nonidet P-40 and 6 M urea (169). Scytonemin appears to be present predominantly in nonplanktonic species, and its structure has recently been solved (312). The pigment is dimeric with a molecular mass of 554 Da, it is optically inactive, and it is composed of a skeleton of indolic and phenolic subunits (Fig. 19A). It has been suggested that the pigment is formed from

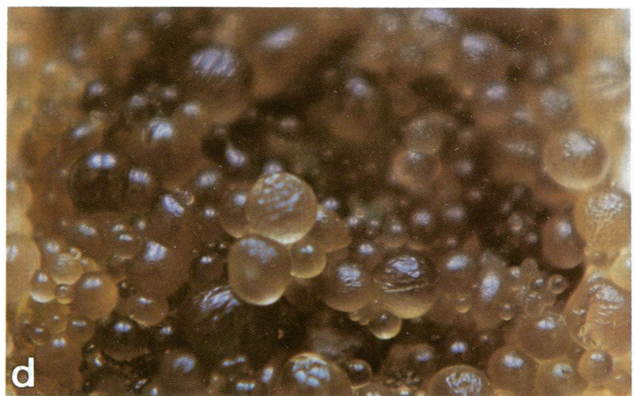
TABLE 4. Essential features of a protective extracellular biopolymer

Feature
(i) High water retention, probably with an ordered structure and intricate network of fibers
(ii) Complex repeating structure that requires several enzymes for dissolution and therefore presents a poor substrate for utilization by competitors
(iii) Ideally should be toxic to prevent grazing by eukaryotes, or should provide immunoglobulin G masking if the cell is a pathogen
(iv) Should have absorption properties that provide scavenging of cations, metals [see (iii)], etc.
(v) Attachment to cell surface should permit new growth without loss of turgor
(vi) Regulation—inducible/constitutive, CO_2 transport problem
(vii) Manipulative biophysical properties (gel-to-sol transition to permit motility of cells within sheath); viscosity/polymerization should be controlled by cell-encoded and secreted enzymes
(viii) The skew, twist, and shear properties upon drying should not be disadvantageous
(ix) Low matter transport
(x) Because of (vii), the cell should have a means to scavenge and take up short-chain-length sugars
(xi) Induction, synthesis, and secretion of the polymer should be stringently regulated, as should (x)
(xii) A CO_2-concentrating mechanism may be advantageous [see (vi)]
(xiii) Capacity to form a glass
(xiv) Intimate contact with the cell surface [leaflet of the outer membrane; see (v) and (viii)]

a

b

c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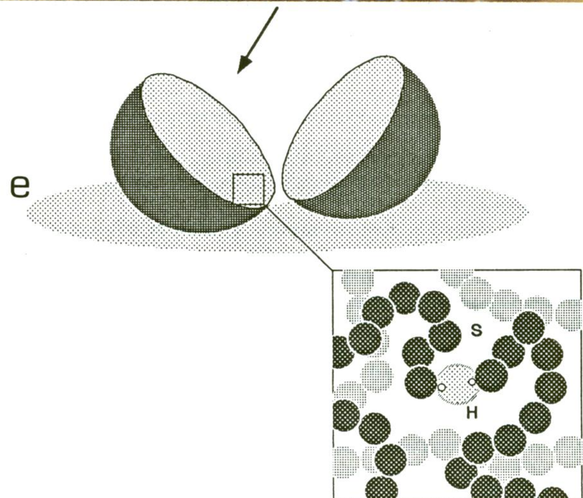

e
S
H

the condensation of tryptophan and phenylpropanoid-derived subunits (312).

Another class of UV-absorbing pigments present in many strains of cyanobacteria is the mycosporin-like amino acids (MAAs). MAA synthesis is not restricted to nonplanktonic species as seems to be the case for the sheath pigment scytonemin (131). Chemically, MAAs contain a substituted cyclohexenone linked to an amino acid (or its imino alcohol). These compounds have maximal absorbance from 310 to 360 nm and are water soluble. The UV-A/B-absorbing mycosporin of *N. commune* is colorless, water soluble, and secreted (168, 344). This pigment absorbs at 312 and 330 nm and contains a number of chromophores bound to a polysaccharide core (Fig. 19B). The structure of one of these chromophores, E_{335}, has recently been solved, and it has been shown to contain serine, threonine, and xylose (30). This MAA is unique because of its covalent attachment to carbohydrates. It is unknown whether the pigment is secured to the extracellular glycan of *N. commune* (Fig. 16 and 17). A potential role for Wsp polypeptides (Fig. 15) in the synthesis and/or modification of these pigments is described below (168). A survey of different laboratory-grown strains of cyanobacteria showed no significant absorption in the spectral region where these water-soluble pigments pigments absorb (344). In contrast, cultures of *N. commune* CHEN and *N. commune* DRH1 showed induction in response to UV irradiation.

The responses to UV radiation of liquid-grown cultures of cyanobacteria have been measured. The presence of MAAs provided only a modest increase in UV resistance when cells of *Gloeocapsa* were desiccated, and it was assumed that inoperative physiological and photorepair mechanisms were the cause (132). With other strains there appeared to be some overlap, as deduced from the two-dimensional protein indices, in the responses to UV irradiation and nalidixic acid. One protein produced under UV irradiation was a 33-kDa polypeptide with a pI of approximately 4.2 (253, 254); these properties are consistent with those of the Wsp polypeptides of *N. commune* (345) (Fig. 15). The conditions of desiccation are clearly important for consideration of the roles of pigments when studied in the laboratory situation; however, measurements suggest that MAAs probably contribute to protection of cells during active growth and desiccation in situ (132).

Other types of bacterial pigments have been implicated in protection of communities from radiation. However, the production of a gold-orange pigment in a methicillin-resistant strain of *Staphylococcus aureus* was not correlated with resistance of this strain to drying (114). Chromogenic bacteria from the Antarctic are frequently reported. All *Pseudomonas* cultures isolated from ice cores in Antarctic sediments produce an exogenous, water-soluble, dark-brown melanin-like pigment in tyrosine-containing media (2). This "pyomelanin" has features that suggest that it may be a MAA.

The thick and dense carbohydrate coats of *Deinococcus* cells have been implicated in the UV resistance of this bacterium, although the generalized comments as they pertain to both the desiccation resistance and radiation resistance of *Deinococcus* species are unclear (359).

Colony Structure

A conspicuous feature of many bacterial colonies that grow exposed to air is that they tend to be spherical. For a given volume, a sphere presents the minimal surface area to the vapor phase, thus retarding the net rate of evaporation. The water present in the colony—usually the interstitial component present in the extracellular wall layers—reduces the net diffusion of gases by 4 orders of magnitude relative to air (274). Bacterial colonies therefore undergo changes in water content, shape, and diffusion characteristics as they are dried. The shapes and forms of bacterial colonies may play an important role in determining the extent to which cells evade damage from drying, oxygen, and other perturbants. The intrinsic capacitance of soybean nodules containing *Bradyrhizobium japonicum* was measured as 0.29 MPa^{-1}, indicating that the nodules can release relatively large amounts of water from the symplast with only small changes in total nodule water potential. Estimates of the bulk modulus of elasticity of the nodules ranged from 0.91 to 2.60 MPa, indicating a high degree of elasticity (313), and the modulation of water volume of the nodule was thought to influence nodule permeability and thus the amount of oxygen entering the nodule. Oxygen exclusion is accomplished in *Frankia* vesicles by an envelope that contains two hopanoid lipids (26). The extracellular glycan of *N. commune* DRH1 imparts a spherical structure to the colonies, and the centers of these tend to be reducing (169, 170) (Fig. 17). Some strains of *N. commune* also synthesize an oxygen-binding hemoprotein (cyanoglobin) when they are starved of oxygen (297). Perhaps the need to scavenge oxygen is one consequence of abundant sheath biosynthesis.

The greater susceptibility to desiccation of the *R. leguminosarum* group of rhizobia compared with the slow-growing rhizobia is correlated with the different amounts of water retained by the bacteria at any relative vapor pressure, as opposed to the rates of water movement into or out of the bacteria (46). The higher retention of water by the former group appears to be related to a greater availability of adsorptive surface area and to higher surface energies rather than to differences in internal solute concentrations.

Genetic Mechanisms

E. coli has been subjected to all manner of insults, including extremes of heat and cold, osmotic shock, starvation, and high pressure, in order to understand the plasticity of its gene expression (255, 413). Comparable studies on the drying of *E. coli* are lacking. Even though this bacterium is not considered to be especially tolerant of drying, such studies could identify whether desiccation overlaps with other stresses and may help reveal some of the fundamental aspects of desiccation sensitivity.

Genes that are likely to be involved in the responses of cells to desiccation can be inferred from a study of the targets of desiccation damage. One gene that may be important is the putative sigma factor *rpoS* (*nur*, *katF*), which appears to be of major importance for regulating switches in responses to

FIG. 18. Life in the dry lane. (a) Karst scenery near Malham Tarn, Ingleborough, England. (b) Small depressions (ca. 0.3 m in diameter) in limestone support populations of *N. commune* whose colonies appear black and cartilaginous when rehydrated. (c) Desiccated colonies of *N. commune* collected in situ (approximately actual size); the dark-brown color of the colonies is due to the UV-absorbing pigment scytonemin (see the text and Fig. 19). (d) Spherical colonies of *N. commune* DRH1 (ca. 5 mm in diameter) grown under isopiestic water equilibration. Note the brown pigmentation due to scytonemin. Each colony contains numerous filaments of the cyanobacterium. (e) Schematic representation of the interior of a single sphere (shown in panel d). S, sheath; H, heterocyst.

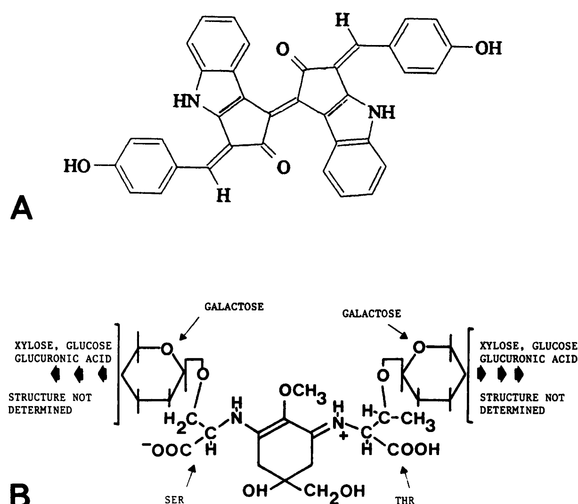


FIG. 19. Solved structures of cyanobacterial UV-absorbing pigments. (A) The reduced (red) form of lipid-soluble scytonemin (312). (B) The E_{335} chromophore of the water-soluble UV-A/B-absorbing pigment (UVP) from *N. commune* (30).

environmental fluxes such as stationary-phase growth, regulation of catalase activity, exonuclease III activity, acid phosphatase activities, and near-UV resistance (165). The *rpoS* (*katF*) gene product (σ^{38}) is a second principal σ factor of RNA polymerase in stationary-phase *E. coli* cells. This sigma factor is involved in the transcription of type III ("gearbox") promoters such as the one upstream of the *fic-pabA* operon (378). In contrast to the detailed studies of spore outgrowth, there are no data available for the promoter specificity of the RNA polymerases present inside drying or rehydrating bacterial cells. Of 20 proteins induced under osmotic shock, 5 were also starvation proteins (195). Interestingly, although starvation, growth rate modulation, and osmoregulation seem to involve similar proteins, neither σ^s nor trehalose is required for adaptive thermotolerance (164). A number of osmotically inducible genes (*osmA* to *osmK*) are also growth phase dependent (156, 164). Such dual control was found for *osmB*, a6.9-kDa polypeptide modified with a lipid moiety, that may be an outer membrane protein. The function of *osmB* was speculated to cross-link the outer membrane and peptidoglycan in a manner that restricts growth rate damage during water removal (199).

In view of the destructive effects of oxygen during desiccation, the genes involved in oxygen-scavenging mechanisms are likely to be important in the tolerance of bacterial cells to air drying. *E. coli* has three isozymic forms of SOD:MnSOD (*sodA*, 88 min), FeSOD (*sodB*, 38 min), and a hybrid enzyme that contains one subunit each of Fe and MnSOD (162). The regulation of *sodA* by Fur (ferric uptake regulation protein), Arc (aerobic respiratory control protein), and Fnr (fumarate nitrate reduction/regulation of anaerobic respiration) is independent of the superoxide response regulon *soxRS* (162). Products of *soxRS* and *soxQ* activate oxidative stress proteins such as SodA as part of the global response (71). SoxR is a regulatory protein that is part of a global response mechanism responding to the presence of $O_2^{\cdot -}$ (152, 387). In addition, it appears that SoxR can respond to aspects of the redox status of bacterial cells other than $O_2^{\cdot -}$ (230). The *soxRS* regulon is controlled by a two-stage system in which SoxR protein is a redox-sensing transcriptional activator of *soxS*, whose product subsequently activates the various *soxRS* regulon genes (262). A FeSOD has been found in heterocysts of *Anabaena cylindrica* Lemm. (51), and a parallel increase in the levels of this enzyme and nitrogenase was noted in cells grown in the light. However, as mentioned elsewhere, despite the presence of these enzymes, heterocysts of laboratory-grown cells are prone to damage through air drying (278). The protective effect of *oxyR* expression is due to the induction of enzymes capable of scavenging active oxygen species e.g., catalase (*katG*), Mn-containing SOD (*sodA*), glutathione reductase, and alkyl hydroperoxide reductase (*ahp*) (372). The cascade of effects is induced when OxyR becomes oxidized (380). A survey of a range of cyanobacteria indicated that there are two distinct physiological groups with respect to oxygen-protective mechanisms—those that synthesize ascorbate peroxidase to scavenge H_2O_2 by using a photoreductant as electron donor, and those that scavenge H_2O_2 only with catalase (243).

UV-induced photoproducts can be recognized and repaired by several systems in *E. coli*, including photoreactivation (*phrB*), excision repair (*uvrABCD*), and postreplication repair (*recA*) (215). Apurinic and apyrimidinic sites left in DNA as by-products of DNA glycosylases are worked on by AP endonucleases that cleave at either the 5′ or 3′ side of the AP site.

Some sugars offer a degree of protection for enzymes, membranes, and cells against short-term drying. It is also clear that for effective desiccation tolerance by bacterial cells, constitutive synthesis must be necessary and synthesis of sugars can represent only one component of a multicomponent system directed at cell stabilization. Trehalose and sucrose appear to be the principal stabilizing solutes in anhydrobiotes; however, the role of other compounds known to be produced by bacteria in response to water deficit must be appraised (86). *E. coli*, which is not desiccation tolerant, can utilize trehalose as both a carbon source and an osmoprotectant (164). It is synthesized from UDP-glucose and glucose-6-phosphate via trehalose-6-phosphate. Accumulation is regulated on two levels: synthesis of trehalose-6-phosphate synthase is induced by osmotic stress (and apparently other conditions [see above]), and the synthase is activated by potassium glutamate (137, 376). Trehalose synthesis is dependent on *otsA* and *otsB*, encoding trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase, respectively, both of which are induced at high osmolarity. Stressed *E. coli* cells regulate the cytoplasmic level of trehalose by a futile cycle involving overproduction, excretion, and degradation to glucose, which is reutilized (376). Degradation is mediated by a periplasmic enzyme, trehalase, encoded by *treA* (33, 156). The latter permits cells to utilize trehalose at high osmolarity! The K_m for trehalose of this trehalase is high and may represent the evolution of a catabolic enzyme that uses a compatible solute as a carbon source. *rpoS* directs expression of *otsA*, *otsB*, *treA*, and *osmB* (164), which are not only stationary-phase induced but also osmotically induced. As discussed above, it has been suggested that the engineering of trehalose overproduction in cells would provide a measure of tolerance toward drying (77). Irrespective of whether the overproduction would provide tolerance, it is clear that it would cause a number of problems with respect to carbon source regulation, utilization, coordination with glycogen metabolism, etc.

ECOLOGICAL CONSIDERATIONS

Desiccation is likely to play a determinative role in the ecophysiology of bacterial communities that grow in aerophytic environments; on and inside of rocks; on and in soils and sediments; in the phyllosphere; in crusts, accretions, dusts, and

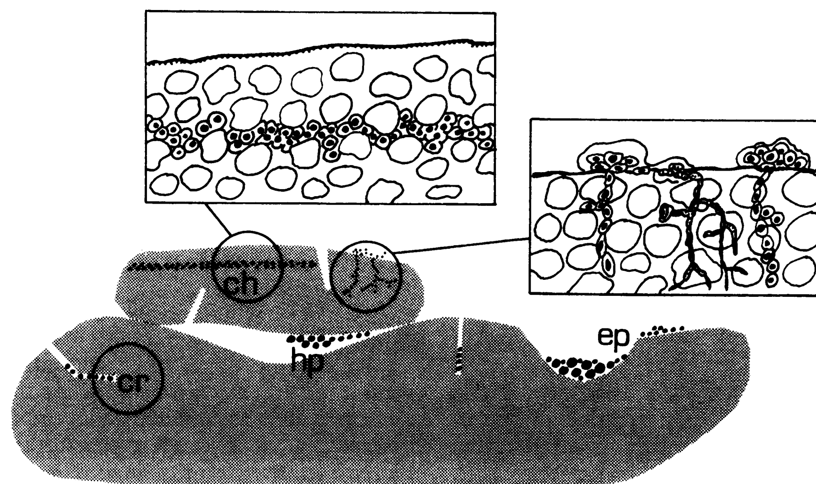


FIG. 20. Interactions of prokaryotic cells with rocks. ch, chasmoendolith; cr, cryptoendolith; ep, epilith. A rock-boring endolith is shown (right inset). The chasmoendolithic communities of cyanobacteria in Antarctic rocks (123–126, 197, 256) are discussed further in the text. A euendolith is shown in Fig. 22.

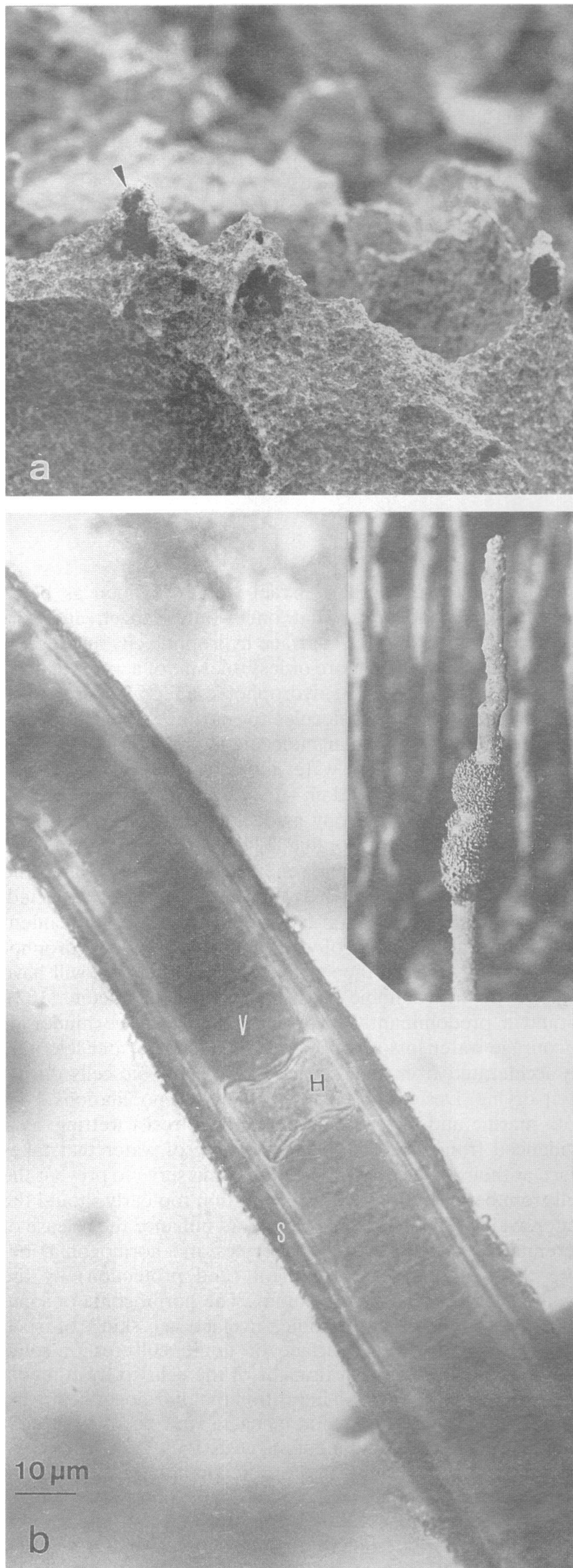
aerosols; and on human and animal skins (Fig. 20). An important factor to consider in desiccation-tolerant communities is their overall growth rate and longevity. Terrestial limestones in regions with extended dry spells require years for recolonization, despite a ready immediate source of inoculum. In contrast, marine rocks, as freshly exposed substrates, can be colonized within days (290). Here, wetting frequency is of importance, as is position in the tidal zone (218, 290).

Bacterium-Air Interface

In a consideration of the available data on the influences of interfaces on microbial activity, changes in water activity were thought to have an indirect effect (390). Water equilibrium across the bacterium/air interface or colony/air interface occurs when the water potential in bacterial cells equals that of the surrounding atmosphere (water vapor). At 20°C the value of $RT/\bar{V}_w$ is 135 MPa, which, in consideration of equation 5, indicates that extremely large and negative values of Ψ_{wv} (Ψ_m) are possible (Fig. 8). When the water potential of the population is in equilibrium with air, the temperatures of each may be quite different (290). The actual value of $\Pi_{\text{bacterium}}$ will depend on the its physiological status and the ambient conditions.

Hydrophobicity

Cell surface hydrophobicity (or its antonymn, hydrophilicity) is thought to play a major role in determining the distribution and activities of microbial populations (87, 102). An assessment of whether particular cells have a hydrophobic character relies on the results of a number of tests (97, 341). One test, for example, relies on whether the cells partition to the organic phase in an aqueous mixture that contains *n*-hexadecane (102). Details of these tests and some of their drawbacks are discussed in reference 102. Implicit in the consideration of cell surface hydrophobicity is that it occurs at an interface—one that separates a cell engorged with water (!) from its environment (Fig. 10). Ironically, a detailed study of the distribution of the hydrophobic character of cyanobacteria has focused solely on strains isolated from benthic (submerged) environments (115); all strains were shown to have a hydrophobic character. In contrast, cyanobacterial mats covering extensive areas of the sandy soil in arid southern Israel were described as being hydrophilic (20). Is this what one would expect, and is a predisposition to show cell surface hydrophobicity any indication of a tendency to be more or less tolerant of a water deficit? Consider the origin of the hydrophobic effect. It arises from the tendency of water molecules to maximize their hydrogen bonding around a nonpolar molecule (so decreasing enthalpy [105]). In doing so, these water molecules lose some of their rotational degrees of freedom—they become more ordered—and the decrease in entropy associated with this ordering is thermodynamically unfavorable. The hydrophobic interaction is spontaneous ($-\Delta G$) and endothermic ($+\Delta H$), and the reaction is favored entropically ($+T\Delta S$). As may be expected, the hydrophobic interaction is highly temperature dependent and leads to a structuring of water molecules at the hydrophobic interface. Overall, the surface of a bacterial cell will have regions of hydrophobic and hydrophilic character (182). Would a predominantly hydrophobic cell surface hinder or encourage water loss upon drying of the cell? Water loss may be accelerated from the surface of hydrophobic cells during their drying. Desiccated crusts of microbial populations from both marine and terrestial origins initially resist wetting, as is evidenced from the pronounced beading of water that takes place at their surfaces. Such beading could serve to prevent the cell from being committed to rehydration too early should the water availability be limited, or it could enhance the release of perennating structures (e.g., baeocytes and hormogonia) before their subsequent entrapment (and protection) by the swelling gel-like mass of the colony. The hormogonia of some cyanobacteria are hydrophobic; in fact, a "skin" of such hormogonia covers the surface of liquid cultures of some *Nostoc* spp. within 24 h of transfer of the cells to fresh media (291). However, there is no indication that hormogonia are any more tolerant of drying than are vegetative cells; the fact that they lack a sheath component suggests they may, in fact, be more sensitive to drying. Photoprotective pigments found in some bacterial colonies include those which are water soluble as well as those which are lipid soluble (Fig. 19). The former, but not the latter, are released upon rehydration of desiccated colonies (2, 169).



Model Systems—the Cyanobacteria

Cyanobacteria dominate the bacterial populations of extreme environments such as deserts (90, 271), thermal springs (400), hot brines (100), frigid lakes (268), soda lakes (63), and the nutrient-poor open ocean (118). Marine mats are dominated invariably by *Microcoleus* species (283, 306, 308). In terrestial localities, growths of *Tolypothrix*, *Scytonema* (Fig. 21), and *Nostoc* spp. form visually conspicuous mats and crusts in exposed habitats from the Tropics to the polar regions (290, 416, 417) (Fig. 18).

The deserts of hot and cold regions support a range of different bacterial communities. Where hot brines accumulate, and along the shores of coastal sabkha and lagoons, communities of *Halobacterium* spp., photosynthetic bacteria, and cyanobacteria accumulate under the surface of, and within, salt crusts (34, 99, 293). Here the surface crusts are often populated by intensely pigmented diatoms that offer protection to underlying prokaryotic populations (293). Where water is more scarce, the populations are restricted to rocks in which the bacteria accumulate below the surface. Bacteria may colonize the rock surface (epiliths); may actively bore into the rock substrate (euendoliths [218] [Fig. 20 and 22]); may seek out cracks, fissures (chasmoendoliths), and the microspaces of porous rocks (generally under a crust) (cryptoendoliths); or may remain at the stone-sediment interface (hypoliths) (Fig. 20). Endolithic organisms in hot deserts are subject to much more severe environmental stress than those in cold deserts because of the sudden changes between warm-humid and hot-dry conditions that can occur. This apparently is the reason for the exclusion of eukaryotic organisms in the endolithic microbial communities of hot desert rocks which are dominated by cyanobacteria and heterotrophic eubacteria (122, 256, 271).

The work of Imre Friedmann and his colleagues has provided comprehensive data on the physiological ecology of the bacterial communities that populate rocks in the Dry Valleys of the Ross Desert, Antarctica, which is considered, perhaps justifiably, the most extreme environment on Earth (122–126, 256, 429). Here the nanoclimate is the primary factor that influences growth of the bacteria. When rocks are saturated with water, the light flux is increased 40-fold, to almost 0.2% of the incident radiation. Rocks receive between 425 and 1,050 h of light per year depending on their orientation. Prolonged periods of evaporation can be measured in rocks following a fall of the very restricted amounts of snow, and this evaporation may continue for days or even weeks. Biological nitrogen fixation—a process that seems to be sensitive to short-term drying of cells grown under laboratory conditions—is rare here. Cyanobacteria dominate three of the five cryptoendolithic communities found in the sandstone rocks. A green zone of lichen-dominated community is formed by an association of *Hemichloris antarctica* (a green alga) and cyanobacteria. In the

FIG. 21. Extreme xerophytic habitats colonized by the desiccation-tolerant cyanobacterium *Scytonema* sp. (a) Upper intertidal zone along south shore of Aldabra Atoll, Indian Ocean (290, 308). Actual size; arrow indicates tufts of filaments. (b) Individual filaments are calcified, and the laminated sheath (S) contains the dark-brown UV-absorbing pigment scytonemin. H, heterocyst; V, vegetative cell. Colonies colonizing pneumatophores of the mangrove *Avicennia* sp., upper intertidal along the coast of the Sinai desert, Gulf of Elat, Israel (292, 293) (inset); the filaments form collars around prop roots and are exposed at low tide. The collars may retain water for some time after exposure (99) but ultimately are completely desiccated for parts of the tidal cycle (292).

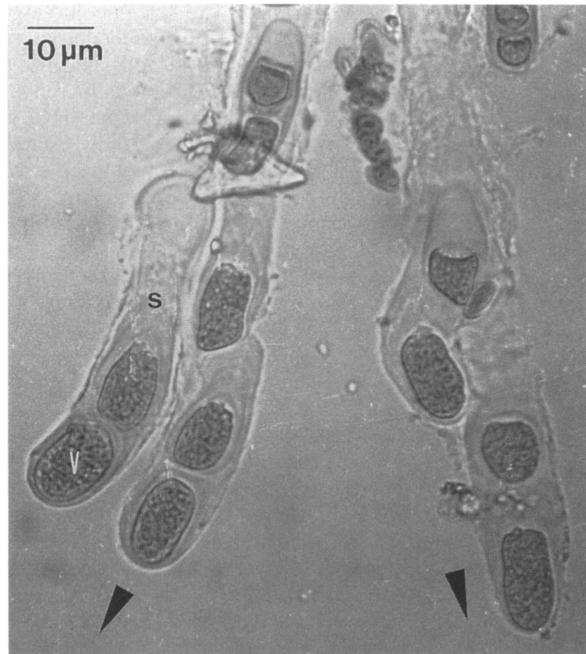


FIG. 22. A rock-boring marine cyanobacterium, *Solentia stratosa*, Pseudofilaments formed by single cells in a communal mucilage bore vertically into limestone rock substrata (arrows).

lower zone of the communities, *Micrococcus roseus*, *Deinococcus radiopugnans*, *Brevibacterium* sp., and *Arthrobacter* sp. are present. It is noteworthy that the hot-desert and cold-desert *Chroococcidiopsis* (cyanobacteria) strains appear to belong to a single species—the most xerotolerant cyanobacterium (see below)? Growth of a community dominated by a red-pigmented *Gloeocapsa* sp. is favored in continuously wetted boulders where liquid water is available; with decreasing moisture, this cyanobacterium is replaced by a lichen-dominated community. The different cyanobacterial communities colonize rocks according to strict preferences and requirements of pH and relative humidity. A *Gloeocapsa-Hormathonema* community colonizes rocks, between pH 7.3 and 8.2, that contain one-third the amount of Fe_2O_3 of rocks where lichens predominate (125). Moisture gradients are responsible for the distribution of microorganisms and occur largely in response to snow melts.

These cyanobacterium-dominated communities appear to be the oldest on Earth. Preliminary radiocarbon dating of cryptoendolithic microbial communities of the Ross Desert (McMurdo Dry Valleys) indicated a ^{14}C deficiency corresponding to approximately 1,000 years (31). Productivity measurements of cyanobacterium-dominated cryptoendolithic communities at Battleship Promontory, Ross Desert, were 4 μg of C m^{-2} $year^{-1}$, with a net turnover of lipid carbon of 19,000 years (197). These latter measurements require some adjustment (reduction), because temperatures below 5°C were not considered in the calculations. Even so, the adjusted numbers still are of the order of 10^4 years. The disintegration of rock-inhabiting communities in Antarctica is induced by salt weathering; grain-by-grain abrasion through frost, salt, or wind action; and polishing by eolian weathering (126). It would seem that successful colonization of rocks by these organisms, over some distance, can be achieved only if they have a tendency to withstand the most extreme cold and air-drying.

Blackened and desiccated colonies of *N. commune* are a characteristic feature of karst regions, where the brittle growths appear scattered over the exposed limestone (343, 346) (Fig. 18). Here, the colonies are subjected to repeated cycles of wetting and drying interspersed with short or extended periods of desiccation (Fig. 18). The mechanisms involved in the desiccation tolerance of this form appear to be varied. Compound 20:3ω3 constitutes 58% of the total fatty acids present in the purified cytoplasmic membrane of *Nostoc* strain UTEX 584 (265)—a feature that would be expected to contribute to membrane fluidity. Cells contain sucrose and trehalose (169). Field materials of *N. commune* elaborate a complex extracellular glycan (Fig. 16 to 18), which is secreted in copious amounts by liquid cultures of *N. commune* DRH1 and which lends a spherical appearance to colonies grown on solid media (Fig. 18) (168). When they are rehydrated, colonies secrete water-soluble UV-A/B-absorbing pigments that constitute up to 10% of the dry weight of the desiccated cell mass. The dark-brown appearance of the colonies is due to scytonemin (Fig. 18 and 19). Analyses of the two-dimensional protein index of cells of laboratory-grown cultures of *Nostoc* strain UTEX 584, following their exposure to different water stresses, failed to identify any novel classes of protein synthesized in response to drying (294, 295). In fact, rehydrated cells underwent a rapid turnover of protein within a short period of rehydration (295). Similar studies with colonies of *N. commune* collected in situ provided quite different results. A group of acidic proteins with molecular masses of 32 to 39 kDa constitute the bulk of the soluble protein (345). The proteins are very stable, and their synthesis was induced in laboratory-grown cultures in response to drying (Fig. 15). These "water stress proteins" (Wsp) were initially thought to have a structural role in cell stability in view of their abundance and their high content of hydroxylated amino acids (serine, threonine, and tyrosine). Recent studies suggest a related but more subtle role. The three Wsp polypeptides (of 32, 37, and 39 kDa) appear to be isoforms; they are secreted, accumulate in the extracellular glycan, and show homologies with carbohydrate-modifying enzymes (168). The polypeptides copurify with an associated 1,4-β-D-xylanxylanohydrolase (EC 3.2.1.8) activity that is inhibited by Wsp-specific antibodies—a property that suggests some role for Wsp in the modification of the extracellular glycan. This appeared puzzling at first, because the purified extracellular glycan of *N. commune* contains no, or very little, xylose (169). Xylose is found, however, in one of the chromophores of the secreted UV-A/B-absorbing pigment (Fig. 19B) (30). Wsp polypeptides and UV-absorbing pigments form complexes with each other in the absence of salt but appear to exist in a monomeric state in the presence of salt (168). These ionic interactions are expected to be attenuated in situ through drying and wetting of colonies and the resultant changes in salt concentration. The secretion of Wsp polypeptides, water-soluble UV-absorbing pigments, and extracellular glycan represents considerable metabolic investments by the cells—it remains to be determined if and how these processes are related and coordinated. One additional feature of the water relations of *N. commune* deserves mention. Upon rehydration, cells accumulate massive amounts of a singular polypeptide, cyanophycin, which quickly disappear (Fig. 23). Cyanophycin, first characterized by Simon (see reference 6 and references therein) is found only in cyanobacteria, it is non-ribosomally synthesized, and it contains only arginine and aspartic acid, in a 1:1 molar ratio (multi-L-arginyl-poly-L-aspartate) (5). The amounts of cyanophycin that accumulate in rehydrated cells are dramatic. Inspection of Fig. 23 suggests that these inclusions exclude at least 50% of the volume of the

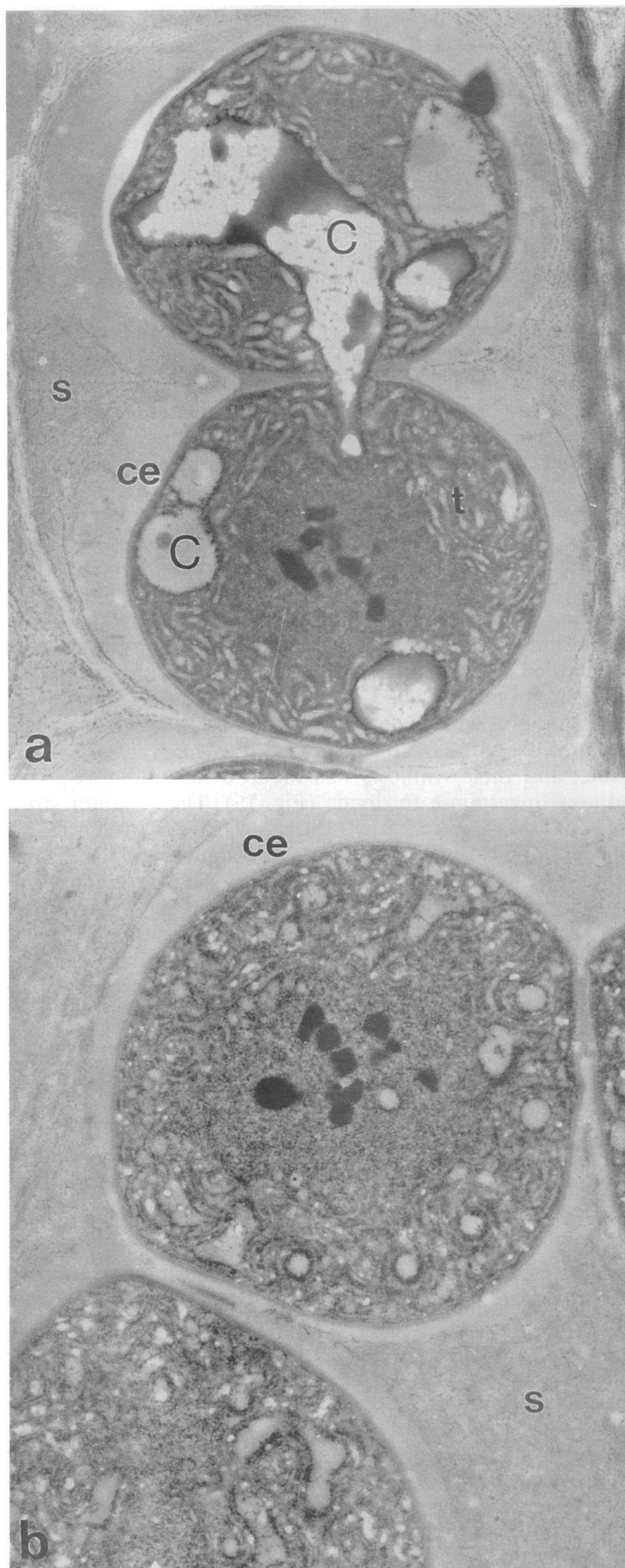


FIG. 23. Ultrastructure of desiccated cells of *N. commune* HUN following 24 and 72 h of rehydration. C, cyanophycin granules; s, extracellular glycan (note the fibrous ultrastructure); ce = capsule-like envelope; t, thylakoid membranes. Note that the cells are structurally intact despite long-term desiccation (years). Reproduced from reference 279 with permission of the publisher.

cell compartment during rehydration. The arginine and aspartic acid used for cyanophycin synthesis presumably derive from rapid protein turnover upon rewetting of the cells, because the onset of nitrogen fixation requires longer periods of rehydration (298, 326, 343). It is interesting that arginine · HCl is a noncompatible solute and, while it is preferentially excluded from globular proteins in the native state, it may interact with the denatured state through its guanidinium group and thus induce structure destabilization (384). Although lysine and to some extent valine are also noncompatible in this sense, it is possible that the scavenging of argine by multi-L-arginyl-poly-L-aspartate synthase contributes to some stabilization of proteins. Can or does cyanophycin function as a compatible protein solute?

Many bacteria have the capacity to withstand a certain degree of air drying under laboratory conditions, and this is also true of many cyanobacteria. The indications from our studies with strains of *N. commune* are that desiccation tolerance represents a complex array of interactions at many different levels in the cell, and there appears to be a battery of mechanisms to diminish the effects of air drying. It is difficult, therefore, to gauge how the tolerance of the garden variety ("weeds") of cyanobacteria has been modified by long-term culture (decades) in liquid media. One feature readily lost from some cyanobacterial strains following repeated subculture is the production of a conspicuous extracellular sheath (291)—often convenient, as this makes manipulation under laboratory conditions more facile. Not surprisingly, immobilization of such strains in alginate beads (43), or simply allowing them to dry in solid media (agar) in a petri dish, can improve their long-term stability.

Here is a story that returns to the question of bacterial glasses, alluded to above, and it includes an account of perhaps the most desiccation tolerant type of cyanobacterium. The normally attractive appearance of asphalt shingle roofs, on buildings from Canada to the southeastern region of the United States, frequently becomes an eyesore as a result of the development of dark stains commonly referred to as "black fungus" and "algae" (8–10). The growth is most pronounced on light-colored shingles and, not surprisingly, has generated considerable investments of time and money from roofing manufacturers in view of consumer dissatisfaction. The growth is caused by a form of the genus *Chroococcidiopsis* (291), a coccoid cyanobacterium that dominates the rock-dwelling communities of hot and cold deserts (122, 123). On clear summer days the dried, dark-pigmented microscopic spherical colonies of this organism are baked on the roof at temperatures that exceed 85°C (291). In winter, freezing and sublimation exact equivalent water stresses. In response to wetting, which could be a long-term downpour or, worse, a transitory shower, the gelatinous colonies become visible within seconds. Here, as for terrestial communities and intertidal communities that become desiccated, the cells are subject to an array of changes in temperature, water deficits, convection, etc. Observations that the growth was absent or markedly reduced in areas immediately below and around metal structures such as galvanized roof vents and copper or lead flashing led to the development of shingles in which heavy metals, particularly zinc, had been incorporated (260). While it became clear that the incorporation of calcium carbonate in the fillings of the shingle promoted the "algal" growth, the use of shingles free of these fillers and containing zinc granules has been less than successful. There are three especially ironical facets to this story. The first is the very existence of this unusual, virtually monospecific, anhydrobiotic "life on the roof"—a prime candidate in the search for bacterial glasses. The second is that

trial experiments (see exhibit 9 in reference 9) by 3M Corporation probably represent the largest single outdoor experiments ever conducted on the stress responses of bacteria! The third is that the incorporation of zinc in shingles should have been attempted as a control measure. Cyanobacteria are tolerant of heavy metals in general and of zinc in particular, a metal that appears to enhance the stability of dried proteins (17, 80, 155, 353)!

TECHNOLOGIES FOR DRIED CELLS AND ENZYMES

Immobilized Cells, Enzymes, and Biopolymers

Desiccated cells are immobilized cells and their enzymes are immobilized enzymes. It has been recognized that the carbohydrate investments of cells may contribute substantially to their resistance to air drying, and the entrapment of cells and enzymes in polysaccharide gels is an immobilization technique that has been applied extensively in different systems (203, 427). Compared with free-living cyanobacterial cells, matrix-immobilized cells exhibit superior temperature tolerance and storage longevity of photoinduced electron transport, and desiccation-sensitive proteins such as phycobiliproteins are more stable (273). Owing to the desiccation resistance and radiation resistance of *Deinococcus* (*Micrococcus*) *radiodurans*, it has been suggested that packets of dried cells could be used as a dosimeter for sterilizing doses of radiation (359). Studies of the skew and uniaxial deformation of immobilized photosynthetic bacterial cells in polymer matrices can provide important information on the positioning of natural chromophores or artificially introduced dyes in their membranes (119). The introduction of a drying event to such immobilized cells and their components—effectively mimicking the lifestyle of desiccation-tolerant cells—has been used to achieve further stabilization (247). These studies suggest that not all polymers may afford the same degree of protection. The study of the characteristics of desiccation-tolerant cells, such as their extracellular biopolymers and the genes involved in their mode of synthesis, can provide important information for use in methods to stabilize cells, inoculants (including seeds), and proteins such as liposome-encapsulated hemoglobin for extended periods (141, 194, 198, 205, 206, 247, 332, 333, 427). A knowledge of desiccation tolerance is providing the means to develop desiccated-synthetic-seed technologies, encapsulation technologies that rely heavily upon polymer mass transfer characteristics, etc. (146, 319). Desiccation-tolerant cells can be used effectively in biosensors (427), and they may have utility in long-term space travel. Novel techniques have been developed to characterize the nucleic acids of dried cells for use in forensics (52). The use of compatible solutes such as trehalose has already made it possible to more efficiently retain enzymatic activity and to prevent desiccation damage when cells and cell components are dried (53; see below).

The sol-gel process is a technique that can be used to prepare transparent oxide glasses by hydrolysis and polycondensation of alkoxides (109). These gels can be supplemented with macromolecules that become entrapped in the growing covalent gel network. Xerogels (glasses) can be formed from the aged gels by slow evaporation of the solvents (methanol and water). The fine-pore networks in dried gels (<100 Å [<10 nm]) do not scatter visible radiation, and they allow the diffusion of small molecules. In many respects, including their formation (solution, gelation, ageing, and drying), these xerogels are comparable to the extracellular gels of bacteria (also the cytoplasm of a desiccated cell?), and as such they may offer considerable potential for the analysis of aspects of the biochemistry of the desiccation of bacteria. The characterization of new biopolymers from desiccation-tolerant bacteria will provide data of use to the modeling of liquid transport processes (391).

It has been suggested that dried films of bacteriorhodopsin from *Halobacterium halobium* can be repeatedly used as a real-time holographic medium with characteristics that outperform other existing films of similar type. Bacteriorhodopsin from *Halobacterium halobium* 96N was successfully entrapped in a sol-gel glass and was found to retain its light-sensitive and spectroscopic properties (411). Such a product may have implications in molecular computing, holography, and the general area of molecular electronics.

Damp Enzymes

Enzymes catalyze their reactions in either direction depending on the equilibrium position, which can be attenuated by water availability. Enzyme catalysis in water-restricted environments has attracted considerable attention in recent years (250, 251, 396). Two experimental approaches have been used to study "damp" enzymes. The first makes use of the fact that certain surfactants aggregate in organic solvents and form thermodynamically stable micelles in the presence of limiting amounts of water (396). The "reverse" micelles have their polar head groups in contact with the encapsulated water and their hydrophobic tails in contact with the organic solvent, and they can solubilize enzymes and proteins. Because the micelles are optically transparent, it is possible to make direct measurements of enzyme kinetics. The second approach uses mixtures of enzyme catalysts and organic solvents (133, 322). By introducing organic solvents to enzyme-catalyzed reactions, hydrophobic substrates may be used, and it is possible to reverse hydrolytic reactions. Since enzymes are insoluble in most organic solvents, only simple immobilization techniques are needed (322). The cell surface enzymes of desiccation-tolerant cells which must function, or at least remain functional, under "damp" conditions may be particularly useful in industrial processes that rely on catalysis in organic solvents. Such enzymes may also be useful in enzyme assays that require

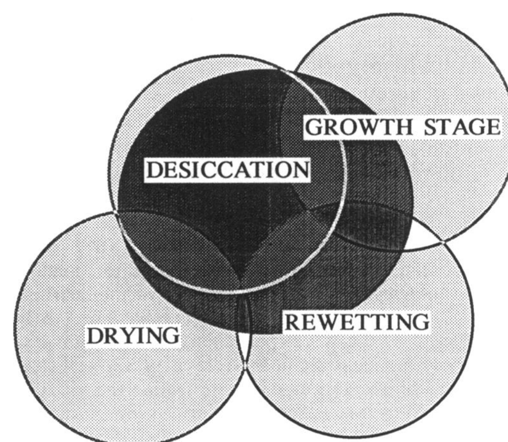


FIG. 24. The interactions between the different events that occur during the removal and addition of water to a prokaryotic cell. The darkened oval is a subjective indication of the relative extents to which these different events influence, or contribute to, the overall tolerance or sensitivity of the cell. Note that the interactions between certain events (unshaded) indicate that they play no direct part in desiccation tolerance.

immobilization to membranes. A secreted phosphomonoesterase of *Nostoc* strain UTEX 584 was found to be stable during desiccation and rapidly activated upon rehydration, and the recombinant protein has since been shown to be amenable to immobilization (237, 305, 418, 431). A test kit for methanol based upon lyophilized methanol dehydrogenase (stabilized with trehalose) from *Hyphomicrobium* X has been devised (14).

Water Replacement

Roser has suggested that the drying of proteins, cells, viruses, pharmaceuticals, etc., in the presence of trehalose can replace freeze-drying as the method of choice for their preservation (329). The ability to dispense with dry ice and bulky packaging is one example of a method that would clearly be of interest to companies that supply DNA modification enzymes, for example, to the scientific research community. Techniques that involve trehalose drying are now described in several patents filed with the International Patent Corporation Treaty (329). The University of California has a patent on liposome stabilization (81), and methods to dry erythrocytes have also been placed under patent law (140, 141, 144).

CONCLUDING COMMENTS

Figure 24 illustrates a subjective impression of the degree to which the different phases of water deficit, and their associated physiological consequences, contribute to desiccation tolerance, compare with Fig. 11. What is remarkable about desiccation tolerance is not what is known but what is not known. The real barrier to our understanding seems to have been, and continues to be, an inability to fathom the complexity of the state of dried (and wet!) cytoplasm—desiccation tolerance is a manifestation of the unique properties of water, and the basis for many of those properties remains obscure. Only very recently has there been a revival of the realization that water plays the pivotal role in biological integrity and cellular function. While the important role of trehalose in the stabilization of air-dried cells appears to be beyond question, there are many questions left unanswered with regard to life without water. More so than many other problems in contemporary cell biology, the study of desiccation tolerance requires, and will benefit from, the application of a judicious mix of biophysics, structural biochemistry, and molecular ecology to the study of whole cells and their purified components. Whether trehalose drying can indeed encompass all of the problems in anhydrobiotechnology remains to be seen. And what of the future for desiccation-tolerant bacteria? To satirize a hypothesis of water biophysics, now out of favor, this is the tip of the "iceberg."

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REFERENCES

1. **Ablett, S., M. J. Izzard, P. J. Lillford, I. Arvanitoyannis, and J. M. V. Blanshard.** 1993. Calorimetric study of the glass transition occurring in fructose solutions. Carbohydr. Res. **246:**13–22.
2. **Abyzov, S. S.** 1993. Microorganisms in Antarctic ice, p. 265–295. *In* E. I. Friedmann (ed.), Antarctic microbiology. John Wiley & Sons, Inc., New York.
3. **Adams, D.** 1980. The restaurant at the end of the Universe. Pan Books, London.
4. **Agarwal, S., and R. S. Sohal.** 1993. Relationship between aging and susceptibility to protein oxidative damage. Biochem. Biophys. Res. Commun. **194:**1203–1206.
5. **Algie, J. E., and I. C. Wyatt.** 1984. Calculation of mass and water content between the core, cortex, and coat of *Bacillus stearothermophilus* spores. Curr. Microbiol. **10:**249–254.
6. **Allen, M. M.** 1984. Cyanobacterial cell inclusions. Annu. Rev. Microbiol. **38:**1–25.
7. **Angeloni, S. V., and M. Potts.** 1986. Polysome turnover in immobilized cells of *Nostoc commune* (cyanobacteria) exposed to water stress. J. Bacteriol. **168:**1036–1039.
8. **Anonymous.** 1973. Algae discoloration of roofs. Technical bulletin 102-R-73. Asphalt Roofing Manufacturers Association, Rockville, Md.
9. **Anonymous.** 1987. Study of algal discoloration of asphalt roofing shingles. Executive summary, p. 1–20. 3M Industrial Mineral Products division, St. Paul, Minn.
10. **Anonymous.** 1989. Algae discoloration of roofs. Technical bulletin 102-RR-89. Asphalt Roofing Manufacturers Association, Rockville, Md.
11. **Antheunisse, J., and L. Arkesteijn-Dijksman.** 1979. Rate of drying and the survival of microorganisms. Antonie Leeuwenhoek **45:**177–184.
12. **Antheunisse, J., J. W. de Bruin-Tol, and M. E. van der Pol-van Soest.** 1981. Survival of microorganisms after drying and storage. Antonie Leeuwenhoek **47:**539–545.
13. **Arakawa, T., J. F. Carpenter, Y. A. Kita, and J. H. Crowe.** 1990. The basis for toxicity of certain cryprotectants: a hypothesis. Cryobiology **27:**401–415.
14. **Argall, M. E., and G. D. Smith.** 1993. The use of trehalose-stabilized lyophilized methanol dehydrogenase from *Hyphomicrobium X* for the detection of methanol. Biochem. Mol. Biol. Int. **30:**491–497.
15. **Asada, S., M. Takano, and I. Shibasaki.** 1980. Mutation induced by drying of *Escherichia coli* on a hydrophobic filter membrane. Appl. Environ. Microbiol. **40:**274–281.
16. **Ashwood-Smith, J.** 1979. Mutation induction in bacteria *Escherichia coli* produced by freeze drying and by desiccation. Cryobiology **16:**6.
17. **Avery, S. A., G. A. Codd, and G. M. Gadd.** 1991. Caesium accumulation and interactions with other monovalent cations in the cyanobacterium *Synechocystis* PCC 6803. J. Gen. Microbiol. **137:**405–413.
18. **Bakaltcheva, I., W. P. Williams, J. M. Schmitt, and D. K. Hincha.** 1994. The solute permeability of thylakoid membranes is reduced by low concentrations of trehalose as a co-solute. Biochim. Biophys. Acta **1189:**38–44.
19. **Barbosa, H. R., and A. Alterthum.** 1992. The role of extracellular polysaccharide in cell viability and nitrogenase activity of *Beijerinckia derxii*. Can. J. Microbiol. **38:**986–988.
20. **Bar-Or, Y.** 1990. Hydrophobicity in the aquatic environment, p. 211–228. *In* R. J. Doyle and M. Rosenberg (ed.), Microbial cell surface hydrophobicity. American Society for Microbiology, Washington, D.C.
21. **Bartels, D., and D. Nelson.** 1994. Approaches to improve stress tolerance using molecular genetics. Plant Cell Environ. **17:**659–667.
22. **Bartlett, D. H., M. E. Wright, and M. Silverman.** 1988. Variable expression of extracellular polysaccharide in the marine bacterium *Pseudomonas atlantica* is controlled by genome rearrangement. Proc. Natl. Acad. Sci. USA **85:**3923–3927.
23. **Bazzichelli, G., N. Abdelahad, and F. Ventola.** 1989. Structural modifications in the extracellular investment of *Nostoc commune*

Vauch. during the life cycle. III. Macrocolonies. Arch. Hydrobiol. **82**:365–370.

24. **Becking, J. H.** 1981. The family Azotobacteriaceae, p. 795–817. *In* M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes, vol. 1, 1st ed. Springer-Verlag KG, Heidelberg, Germany.
25. **Bergman, B., P. Lindblad, A. Pettersson, E. Renström, and E. Tiberg.** 1985. Immuno-gold localization of glutamine synthetase in a nitrogen-fixing cyanobacterium (*Anabaena cylindrica*). Planta **166**:329–334.
26. **Berry, A. M., O. T. Harriot, R. A. Moreau, S. F. Osman, D. R. Benson, and A. D. Jones.** 1993. Hopanoid lipids compose the *Frankia* vesicle envelope, presumptive barrier of oxygen diffusion to nitrogenase. Proc. Natl. Acad. Sci. USA **90**:6091–6094.
27. **Besra, G. S., R. C. Bolton, M. R. McNeil, M. Ridell, K. E. Simpson, J. Glushka, H. van Halbeek, P. J. Brennan, and D. E. Minnikin.** 1992. Structural elucidation of a novel family of acyltrehaloses from *Mycobacterium tuberculosis*. Biochemistry **31**: 9832–9837.
28. **Bieger-Dose, A., K. Dose, R. Meffert, M. Mehler, and S. Risi.** 1992. Extreme dryness and DNA-protein cross-links. Adv. Space Res. **12**:265–270.
29. **Blumwald, E., and E. Tel-Or.** 1982. Osmoregulation and cell composition in salt-adaptation of *Nostoc muscorum*. Arch. Microbiol. **132**:168–172.
30. **Böhm, G.** 1993. Isolierung und Strukturaufklärung eines UV-A/B-absorbierenden pigmentes aus dem Cyanobakterium *Nostoc commune*. Ph.D. thesis. Universität Konstanz, Konstanz, Germany.
31. **Bonani, G., E. I. Friedmann, R. Ocampo-Friedmann, C. P. McKay, and W. Woelfli.** 1988. 2.11 Preliminary report on radiocarbon dating of cryptoendolithic microorganisms. Polarforschung **58**:199–200.
32. **Bongianni, J., S. Mori, D. McLaughlin, T. J. Close, and P. J. Lammers.** 1994. Dehydrin-like proteins in cyanobacteria are expressed in response to osmotic stress, salt stress, and matric water stress. Abstr. 94th Annu. Meet. Am. Soc. Plant Physiol. **105**:25.
33. **Boos, W., U. Ehmann, E. Bremer, A. Middendorf, and P. Postma.** 1987. Trehalase of *Escherichia coli*. Mapping and cloning of its structural gene and identification of the enzyme as a periplasmic protein induced under high osmolarity growth conditions. J. Biol. Chem. **262**:13212–13218.
34. **Braithwaite, C. J. R., and B. A. Whitton.** 1987. Gypsum and halite associated with the cyanobacterium *Entophysalis*. Geomicrobiol. J. **5**:43–55.
35. **Braune, W.** 1980. Structural aspects of akinete germination in the cyanobacterium *Anabaena variabilis*. Arch. Microbiol. **126**:257–261.
36. **Braune, W., and H. Sanke.** 1979. Interferometrische untersuchungen zur dynamik von hydratur und trockenmassegehalt während der lichtabhängigen keimung der akineten von *Anabaena variabilis*. Z. Allg. Mikrobiol. **19**:535–546.
37. **Brawn, K., and I. Fridovich.** 1981. DNA strand scission by enzymatically generated oxygen radicals. Arch. Biochem. Biophys. **206**:414–419.
38. **Breedveld, M. W., L. P. T. M. Zevenhuizen, and A. J. B. Zehnder.** 1991. Osmotically-regulated trehalose accumulation and cyclic β-(1,2)-glucan excretion by *Rhizobium leguminosarum* biovar trifolii TA-1. Arch. Microbiol. **156**:501–506.
39. **Breedveld, M. W., L. P. T. M. Zevenhuizen, and A. J. B. Zehnder.** 1992. Synthesis of cyclic β-(1,2)-glucans by *Rhizobium leguminosarum* biovar trifolii TA-1: factors influencing excretion. J. Bacteriol. **174**:6336–6342.
40. **Bremer, H., and P. P. Dennis.** 1987. Modulation of chemical composition and other parameters of the cell by growth rate, p. 1527–1542. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
41. **Brock, T. D.** 1975. Effect of water potential on a *Microcoleus* (Cyanophyceae) from a desert crust. J. Phycol. **11**:316–320.
42. **Bronner, D., V. Sieberth, C. Pazzani, I. S. Roberts, G. J. Boulnois, B. Jann, and K. Jann.** 1993. Expression of the capsular K5 polysaccharide of *Escherichia coli*: biochemical and electron microscopic analyses of mutants with defects in region 1 of the K5 gene cluster. J. Bacteriol. **175**:5984–5992.
43. **Brouers, M., and D. O. Hall.** 1985. Drying enhances the photosynthetic stability of alginate immobilised *Phormidium laminosum*. Biotechnol. Lett. **7**:567–572.
44. **Burke, M. J.** 1986. The glassy state and survival of anhydrous biological systems, p. 358–363. *In* A. C. Leopold (ed.), Membranes, metabolism and dry organisms, 1st ed. Cornell University Press, Ithaca, N.Y.
45. **Burlacu, E., C. David, and T. Vasilescu.** 1989. Comparative evaluation of three in vacuo desiccation procedures on bacterial cultures. Arch. Roum Pathol. Exp. Microbiol. **48**:65–78.
46. **Bushby, H. V. A., and K. C. Marshall.** 1977. Water status of *Rhizobia* in relation to their susceptibility to desiccation and to their protection by montmorillonite. J. Gen. Microbiol. **99**:19–28.
47. **Bushby, H. V. A., and K. C. Marshall.** 1977. Desiccation induced damage to the cell envelope of root nodule bacteria. Soil Biol. Biochem. **9**:149–152.
48. **Caffrey, M.** 1986. The influence of water on the phase properties of membrane lipids: relevance to anhydrobiosis, p. 242–258. *In* A. C. Leopold (ed.), Membranes, metabolism and dry organisms, 1st ed. Cornell University Press, Ithaca, N.Y.
49. **Cameron, R. E.** 1962. Species of *Nostoc* Vaucher occurring in the Sonoran Desert in Arizona. Trans. Am. Microsc. Soc. **81**:379–384.
50. **Camphausen, R. T., M. McNeil, I. Jardine, and P. J. Brennan.** 1987. Location of acyl groups of trehalose-containing lipooligosaccharides of mycobacteria. J. Bacteriol. **169**:5473–5480.
51. **Canini, A., P. Civitareale, S. Marini, M. Grilli Caiola, and G. Rotilo.** 1992. Purification of iron superoxide dismutase from the cyanobacterium *Anabaena cylindrica* Lemm. and localization of the enzyme in heterocysts by immunogold labelling. Planta **187**: 438–444.
52. **Carducci, C., L. Ellul, I. Antonozzi, and A. Pontecorvi.** 1992. DNA elution and amplification by polymerase chain reaction from dried blood spots. BioTechniques **13**:735–737.
53. **Carpenter, J. F.** 1993. Stabilization of proteins during freezing and dehydration:application of lessons from nature. Cryobiology **30**:220–221.
54. **Carpenter, J. F., and J. H. Crowe.** 1989. An infrared spectroscopic study of the interactions of carbohydrates with dried proteins. Biochemistry **28**:3916–3922.
55. **Carpenter, J. F., J. H. Crowe, and T. Arakawa.** 1990. Comparison of solute-induced protein stabilization in aqueous solution and in the frozen and dried states. J. Dairy Sci. **73**:3627–3636.
56. **Carpenter, J. F., B. Martin, L. M. Crowe, and J. H. Crowe.** 1987. Stabilization of phosphofructokinase during air-drying with sugars and sugar/transition metal mixtures. Cryobiology **24**:455–464.
57. **Cayley, S., B. A. Lewis, H. J. Guttman, and M. T. Record, Jr.** 1991. Characterization of the cytoplasm of *Escherichia coli* K-12 as a function of external osmolarity. Implications for protein-DNA interactions *in vivo*. J. Mol. Biol. **222**:281–300.
58. **Cayley, S., B. A. Lewis, and M. T. Record, Jr.** 1992. Origins of the osmoprotective properties of betaine and proline in *Escherichia coli* K-12. J. Bacteriol. **174**:1586–1595.
59. **Chapman, D.** 1975. Fluidity and phase transitions of cell membranes, p. 1–9. *In* H. Eisenberg, E. Katchalski-Katzir, and L. A. Manson (ed.), Biomembranes. Plenum Press, New York.
60. **Chauvat, F., and F. Joset-Espardellier.** 1981. A freezing method for purification and storage of akinete suspensions of the cyanobacterium *Nostoc* PCC 7524. FEMS Microbiol. Lett. **10**:319–321.
61. **Chen, M., and M. Alexander.** 1973. Survival of soil bacteria during prolonged desiccation. Soil Biol. Biochem. **5**:213–221.
62. **Chuang, S.-E., D. L. Daniels, and F. R. Blattner.** 1993. Global regulation of gene expression in *Escherichia coli*. J. Bacteriol. **175**:2026–2036.
63. **Ciferri, O.** 1983. *Spirulina*, the edible microorganism. Microbiol. Rev. **47**:551–578.
64. **Clegg, J. S.** 1978. Hydration-dependent metabolic transitions and the state of cellular water in *Artemia* cysts, p. 117–153. *In* J. H.

Crowe and J. S. Clegg (ed.), Dry biological systems, 1st ed. Academic Press, Inc., New York.

65. **Clegg, J. S.** 1984. Properties and metabolism of the aqueous cytoplasm and its boundaries. Am. J. Physiol. **246:**R133–R151.
66. **Clegg, J. S.** 1984. Intracellular water and the cytomatrix: some methods of study and current views. J. Cell Biol. **99:**167s–171s.
67. **Clegg, J. S.** 1986. The physical properties and metabolic status of *Artemia* cysts at low water contents: the "water replacement hypothesis," p. 169–187. *In* A. C. Leopold (ed.), Membranes, metabolism and dry organisms, 1st ed. Cornell University Press, Ithaca.
68. **Clegg, J. S. (University of California, Davis).** 1993. Personal communication.
69. **Close, T. J., and P. J. Lammers.** 1993. An osmotic stress protein of cyanobacteria is immunologically related to plant dehydrins. Plant Physiol. (Bethesda) **101:**773–779.
70. **Columbo, M. F., D. C. Rau, and V. A. Parsegian.** 1992. Protein solvation in allosteric regulation: a water effect on hemoglobin. Science **256:**655–659.
71. **Compan, I., and D. Touati.** 1993. Interaction of six global transcription regulators in expression of manganese superoxide dismutase in *Escherichia coli* K-12. J. Bacteriol. **175:**1687–1696.
72. **Cox, C. S., J. Baxter, and B. J. Maidment.** 1973. A mathematical expression for oxygen-induced death in dehydrated bacteria. J. Gen. Microbiol. **75:**179–185.
73. **Cronan, J. E., Jr., and C. O. Rock.** 1987. Biosynthesis of membrane lipids, p. 474–497. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
74. **Crowe, J. H., and J. S. Clegg.** 1973. Anhydrobiosis. Dowden, Hutchison & Ross, Inc., Stroudsberg, Pa.
75. **Crowe, J. H., and J. S. Clegg.** 1978. Dry biological systems. Academic Press, Inc., New York.
76. **Crowe, J. H., and L. M. Crowe.** 1986. Stabilization of membranes in anhydrobiotic organisms, p. 188–209. *In* A. C. Leopold (ed.), Membranes, metabolism and dry organisms, 1st ed. Cornell University Press, Ithaca.
77. **Crowe, J. H., and L. M. Crowe.** 1992. Membrane integrity in anhydrobiotic organisms: toward a mechanism for stabilizing dry cells, p. 87–103. *In* G. N. Somero, C. B. Osmond, and C. L. Bolis (ed.), Water and life, 1st ed. Springer-Verlag KG, Berlin.
78. **Crowe, J. H., and L. M. Crowe.** 1993. Evidence for direct interaction between disaccharides and dry phospholipids. Cryobiology **30:**226–227.
79. **Crowe, J. H., L. M. Crowe, J. F. Carpenter, A. S. Rudolph, C. A. Wistrom, B. J. Spargo, and T. J. Anchordoguy.** 1988. Interactions of sugars with membranes. Biochim. Biophys. Acta **947:**367–384.
80. **Crowe, J. H., L. M. Crowe, J. F. Carpenter, and C. A. Wistrom.** 1987. Stabilization of dry phospholipid bilayers and proteins by sugars. Biochem. J. **242:**1–10.
81. **Crowe, J. H., F. A. Hoekstra, and L. M. Crowe.** 1992. Anhydrobiosis. Annu. Rev. Physiol. **54:**579–599.
82. **Crowe, J. H., B. J. Spargo, and L. M. Crowe.** 1987. Preservation of dry liposomes does not require retention of residual water. Proc. Natl. Acad. Sci. USA **84:**157–1540.
83. **Crowe, L. M., and J. H. Crowe.** 1992. Anhydrobiosis: a strategy for survival. Adv. Space Res. **12:**239–247.
84. **Crowe, L. M., C. A. Wistrom, and J. H. Crowe.** 1993. Does the preferential exclusion hypothesis apply to hydrated phospholipid bilayers? Cryobiology **30:**224–225.
85. **Csonka, L. N.** 1989. Physiological and genetic responses of bacteria to osmotic stress. Microbiol. Rev. **53:**121–147.
86. **Csonka, L. N., and A. D. Hanson.** 1991. Prokaryotic osmoregulation: genetics and physiology. Annu. Rev. Microbiol. **45:**569–606.
87. **Dahlbäck, B., M. Hermansson, S. Kjelleberg, and B. Norkrans.** 1981. The hydrophobicity of bacteria—an important factor in their initial adhesion at the air-water interface. Arch. Microbiol. **128:**267–270.
88. **Damodaran, S.** 1985. Possible role of water on the structural stability of ribosomes. Int. J. Pept. Protein Res. **26:**598–604.
89. **de Araujo, P. S., and A. D. Panek.** 1993. The interaction of *Saccharomyces cerevisiae* trehalase with membranes. Biochim. Biophys. Acta **1148:**303–307.
90. **de Chazal, N. M., S. Smaglinski, and G. D. Smith.** 1992. Methods involving light variation for isolation of cyanobacteria: characterization of isolates from central Australia. Appl. Environ. Microbiol. **58:**3561–3566.
91. **de Primo, C., S. G. Sligar, G. H. B. Hoa, and P. Douzou.** 1992. A critical role of protein-bound water in the catalytic cycle of cytochrome P-450 camphor. FEBS Lett. **312:**252–254.
92. **de Winder, B.** 1990. Ph.D. thesis. University of Amsterdam, Amsterdam, The Netherlands.
93. **de Winder, B., H. C. P. Matthijs, and L. R. Mur.** 1989. The role of water retaining substrata on the photosynthetic response of three drought tolerant phototrophic micro-organisms isolated from a terrestial habitat. Arch. Microbiol. **152:**458–462.
94. **de Winder, B., L. J. Stahl, and L. R. Mur.** 1990. *Crinalium epipsammum* sp. nov.: a filamentous cyanobacterium with trichomes composed of elliptical cells and containing poly-β-(1,4) glucan (cellulose). J. Gen. Microbiol. **136:**1645–1653.
95. **Dickson, J. S.** 1990. Surface moisture and osmotic stress as factors that affect the sanitizing of beef tissue surfaces. J. Food Prot. **53:**674–679.
96. **Donachie, W. D., and A. C. Robinson.** 1987. Cell division: parameter values and the process, p. 1578–1593. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
97. **Donlon, B., and E. Colleran.** 1993. A comparison of different methods to determine the hydrophobicity of acetogenic bacteria. J. Microbiol. Methods **17:**27–37.
98. **Doolittle, W. F.** 1992. What are the archaebacteria and why are they important? p. 1–6. *In* M. J. Danson, D. W. Hough, and G. G. Lunt (ed.), The archaebacteria: biochemistry and biotechnology, 1st ed. The Biochemical Society, London.
99. **Dor, I.** 1984. Epiphytic blue-green algae (cyanobacteria) of the Sinai mangal: considerations on vertical zonation and morphological adaptations, p. 35–54. *In* F. D. Por and I. Dor (ed.), Hydrobiology of the mangal, 1st ed. Dr. W. Junk Publishers, The Hague, The Netherlands.
100. **Dor, I., and N. Paz.** 1989. Temporal and spatial distribution of mat microalgae in the experimental solar ponds, Dead Sea area, Israel, p. 114–122. *In* Y. Cohen and E. Rosenberg (ed.), Microbial mats: physiological ecology of benthic microbial communities. American Society for Microbiology, Washington, D.C.
101. **Dose, K., A. Bieger-Dose, M. Labusch, and M. Gill.** 1992. Survival in extreme dryness and DNA-single-strand breaks. Adv. Space Res. **12:**221–229.
102. **Doyle, R. J., and M. Rosenberg.** 1990. Microbial cell surface hydrophobicity. American Society for Microbiology, Washington, D.C.
103. **Drake, C. R.** 1990. Molecular cloning and expression of the genes encoding the *Escherichia coli* K4 capsular polysaccharide, a fructose-substituted chondroitin. FEMS Microbiol. Lett. **66:**227–230.
104. **Drlica, K.** 1987. The nucleoid, p. 91–103. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
105. **Duncan-Hewitt, W. C.** 1990. Nature of the hydrophobic effect, p. 39–73. *In* R. J. Doyle and M. Rosenberg (ed.), Microbial cell surface hydrophobicity. American Society for Microbiology, Washington, D.C.
106. **Eickmeier, W. G., C. Casper, and C. B. Osmond.** 1993. Chlorophyll fluorescence in the resurrection plant *Selaginella lepidophylla* (Hook. & Grev.) Spring during high-light and desiccation stress, and evidence for zeaxanthin-associated photoprotection. Planta **189:**30–38.
107. **Eisenberg, D., and A. D. McLachlan.** 1986. Solvation energy in protein folding and binding. Nature (London) **319:**199–203.
108. **Eleutherio, E. C. A., P. S. de Araujo, and A. D. Panek.** 1993. Role

of the trehalose carrier in dehydration resistance of *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **1156**:263–266.

109. **Ellerby, L. M., C. R. Nishida, F. Nishida, S. A. Yamanaka, B. Dunn, J. S. Valentine, and J. I. Zink.** 1992. Encapsulation of proteins in transparent porous silicate glasses prepared by the sol-gel method. Science **255**:1041–1180.
110. **Ernst, A., T.-W. Chen, and P. Böger.** 1987. Carbohydrate formation in rewetted terrestial cyanobacteria. Oecologia (Berlin) **72**: 574–576.
111. **Errington, J.** 1993. *Bacillus subtilis* sporulation: regulation of gene expression and control of morphogenesis. Microbiol. Rev. **57**:1–33.
112. **Fairhead, H., B. Setlow, and P. Setlow.** 1993. Prevention of DNA damage in spores and in vitro by small, acid-soluble proteins from *Bacillus* species. J. Bacteriol. **175**:1367–1374.
113. **Fairhead, H., and P. Setlow.** 1992. Binding of DNA to α/β-type small, acid-soluble proteins from spores of *Bacillus* or *Clostridium* species prevents formation of cytosine dimers, cytosine-thymine dimers, and bipyrimidine photoadducts after UV irradiation. J. Bacteriol. **174**:2874–2880.
114. **Farrington, M., N. Brenwald, D. Haines, and E. Walpole.** 1992. Resistance to desiccation and skin fatty acids in outbreak strains of methicillin-resistant *Staphylococcus aureus*. J. Med. Microbiol. **36**:56–60.
115. **Fattom, A., and M. Shilo.** 1984. Hydrophobicity as an adhesion mechanism of benthic cyanobacteria. Appl. Environ. Microbiol. **47**:135–143.
116. **Favinger, J., R. Stadwald, and H. Gest.** 1989. *Rhodospirillum centenum*, sp. nov., a thermotolerant cyst-forming anoxygenic photosynthetic bacterium. Antonie Leeuwenhoek **55**:291–296.
117. **Finney, J. L.** 1977. The organization and function of water in protein crystals. Proc. R. Soc. London Ser. B **278**:3–32.
118. **Fogg, G. E.** 1982. Marine plankton, p. 491–513. *In* N. G. Carr and B. A. Whitton (ed.), The biology of cyanobacteria, 1st ed. Blackwell Scientific Publications Ltd., Oxford.
119. **Frackowiak, D., A. Skibinski, B. Zelent, and R. M. Leblanc.** 1992. Skew orientation of biological samples *Anacystis nidulans* cyanobacteria and their fragments in a polymer matrix. Biochem. Biophys. Res. Commun. **184**:441–447.
120. **Franks, F.** 1977. Solvation interactions of proteins in solution. Proc. R. Soc. London Ser. B **278**:89–96.
121. **Franks, F.** 1988. Characterization of proteins. Humana Press, Clifton, N.J.
122. **Friedmann, E. I.** 1971. Light and scanning electron microscopy of the endolithic desert algal habitat. Phycologia **10**:411–428.
123. **Friedmann, E. I.** 1982. Endolithic microorganisms in the Antarctic cold desert. Science **215**:1045–1053.
124. **Friedmann, E. I., L. Kappen, M. A. Meyer, and J. A. Nienow.** 1993. Long-term productivity in the cryptoendolithic microbial community of the Ross Desert, Antarctica. Microb. Ecol. **25**:51–69.
125. **Friedmann, E. I., M. Hua, and R. Ocampo-Friedmann.** 1988. 3.6 cryptoendolithic lichen and cyanobacterial communities of the Ross Desert, Antarctica. Polarforschung **58**:251–159.
126. **Friedmann, E. I., and R. Weed.** 1987. Microbial trace-fossil formation, biogenous, and abiotic weathering in the Antarctic Cold Desert. Science **236**:703–705.
127. **Fujiwara, S., N. A. Zielinski, and A. M. Chakrabarty.** 1993. Enhancer-like activity of AlgR1-binding site in alginate gene activation: positional, orientational, and sequence specificity. J. Bacteriol. **175**:5452–5459.
128. **Fulton, A. B.** 1982. How crowded is the cytoplasm? Cell **30**:345–347.
129. **Gaber, B. P., I. Chandrasekhar, and N. Pattabiraman.** 1986. The interaction of trehalose with the phospholipid bilayer: a molecular modeling study, p. 231–241. *In* A. C. Leopold (ed.), Membranes, metabolism and dry organisms, 1st ed. Cornell University Press, Ithaca.
130. **Garcia-Pichel, F., and R. W. Castenholz.** 1991. Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. J. Phycol. **27**:395–409.
131. **Garcia-Pichel, F., and R. W. Castenholz.** 1993. Occurrence of UV-absorbing, mycosporine-like compounds among cyanobacterial isolates and an estimate of their screening capacity. Appl. Environ. Microbiol. **59**:163–169.
132. **Garcia-Pichel, F., C. E. Wingard, and R. W. Castenholz.** 1993. Evidence regarding the UV sunscreen role of a mycosporine-like compound in the cyanobacterium *Gloeocapsa* sp. Appl. Environ. Microbiol. **59**:170–176.
133. **Garza-Ramos, G., A. Darszon, M. Tuena de Gomez-Puyou, and A. Gomez-Poyou.** 1990. Enzyme catalysis in organic solvents with low water content at high temperatures. The adenosinetriphosphatase of submitochondrial particles. Biochemistry **29**:751–757.
134. **Gautier, N., L. M. L. Marin, M.-A. Laneelle, and M. Daffe.** 1992. Structure of mycoside F, a family of trehalose-containing glycolipids of *Mycobacterium fortuitum*. FEMS Microbiol. Lett. **98**:81–88.
135. **Geiger, O., F. D. Russo, T. J. Silhavy, and E. P. Kennedy.** 1992. Membrane-derived oligosaccharides affect porin osmoregulation only in media of low ionic strength. J. Bacteriol. **174**:1410–1413.
136. **Geitler, L.** 1932. Cyanophyceae, p. 1–1196. *In* L. Rabenhorst (ed.), Kryptogamenflora von Deutschland, Österreich und der Schweiz. Akademische Verlagsgesellschaft m.b. H., Leipzig, Germany.
137. **Giaever, H. M., O. B. Styrvold, I. Kaasen, and A. R. Strom.** 1988. Biochemical and genetic characterization of osmoregulatory trehalose synthesis in *Escherichia coli*. J. Bacteriol. **170**:2841–2849.
138. **Gilichinsky, D. A., E. A. Vorobyova, L. G. Erokhina, D. G. Fyordorov-Dayvdov, and N. R. Chaikovskaya.** 1992. Long-term preservation of microbial ecosystems in permafrost. Adv. Space Res. **12**:255–263.
139. **Glucksman, M. A., T. L. Reuber, and G. C. Walker.** 1993. Family of glycosyl transferases needed for the synthesis of succinoglycan by *Rhizobium meliloti*. J. Bacteriol. **175**:7033–7034.
140. **Goodrich, R. P.** August 1989. U.S. patent 4,874,690.
141. **Goodrich, R. P.** 1993. Lyophilization of human red blood cells: applications and implications for transfusion medicine. Cryobiology **30**:240–241.
142. **Goodrich, R. P., and J. D. Baldeschwieler.** 1991. The cryoprotective action of synthetic glycolipids. Cryobiology **28**:327–334.
143. **Goodrich, R. P., J. H. Crowe, L. M. Crowe, and J. D. Baldeschwieler.** 1991. Alterations in membrane surfaces induced by attachment of carbohydrates. Biochemistry **30**:5313–5318.
144. **Goodrich, R. P., S. O. Sowemimo-Coker, C. R. Zerez, and K. R. Tanaka.** 1992. Preservation of metabolic activity in lyophilized human erythrocytes. Proc. Natl. Acad. Sci. USA **89**:967–971.
145. **Goodsell, D. S.** 1991. Inside a living cell. Trends Biochem. Sci. **16**: 203–206.
146. **Goosen, M. F. A.** 1992. Fundamentals of animal cell encapsulation and immobilization. CRC Press, Inc., Boca Raton, Fla.
147. **Gottesman, S.** 1987. Regulation by proteolysis, p. 1308–1312. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
148. **Gouesbet, G., H. Abaibou, L. F. Wu, M.-A. Mandrand-Berhelot, and C. Blanco.** 1993. Osmotic repression of anaerobic metabolic systems in *Escherichia coli*. J. Bacteriol. **175**:214–221.
149. **Gould, G.** 1986. Water and the survival of bacterial spores, p. 143–156. *In* A. C. Leopold (ed.), Membranes, metabolism and dry organisms, 1st ed. Cornell University Press, Ithaca.
150. **Graham, P. H.** 1992. Stress tolerance in *Rhizobium* and *Bradyrhizobium*, and nodulation under adverse soil conditions. Can. J. Microbiol. **38**:475–484.
151. **Green, J. L., and C. A. Angell.** 1988. Phase relations and vitrification in saccharide-water solutions and the trehalose anomoly. J. Phys. Chem. **93**:2880–2882.
152. **Greenberg, J. T., P. Monach, J. H. Chou, P. D. Josephy, and B. Demple.** 1990. Positive control of a global antioxidant defense regulon activated by superoxide-generating agents in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **87**:6181–6185.
153. **Grilli Caiola, M., A. Canini, F. Galiazzo, and G. Rotilio.** 1991. Superoxide dismutase in vegetative cells, heterocysts and akinetes of *Anabaena cylindrica*. Lemm. FEMS Microbiol. Lett. **80**: 161–166.
154. **Gunther, T., and H. W. Peter.** 1979. Polyamines and osmoregulation in *Escherichia coli*. FEMS Microbiol. Lett. **5**:29–31.
155. **Gupta, A., A. P. Morby, J. S. Turner, B. A. Whitton, and N. J.**

Robinson. 1993. Deletion within the metallothionein locus of cadmium-tolerant *Synechococcus* PCC 6301 involving a highly iterated palindrome (HIP1). Mol. Microbiol. **7:**189–195.
156. **Gutierrez, C., M. Ardourel, E. Bremer, A. Middendorf, W. Boos, and U. Ehmann.** 1989. Analysis of DNA sequence of the osmoregulated *treA* gene encoding the periplasmic trehalase of *Escherichia coli* K12. Mol. Gen. Genet. **217:**347–354.
157. **Hagemann, M., D. Golldack, J. Biggins, and N. Erdmann.** 1993. Salt-dependent protein phosphorylation in the cyanobacterium *Synechocystis* PCC 6803. FEMS Microbiol. Lett. **113:**205–210.
158. **Halliwell, B.** 1987. Oxidants and human disease. FASEB J. **1:**358–364.
159. **Harris, R. F.** 1981. Effect of water potential on microbial growth and activity, p. 23–96. *In* J. F. Parr, W. R. Gardner, and L. F. Elliot (ed.), Water potential relations in soil microbiology. Soil Science Society of America, Madison, Wis.
160. **Harris, R. F., W. R. Gardner, A. A. Adebayo, and L. E. Somm.** 1970. Agar dish isopiestic equilibration method for controlling the water potential of solsid substrates. Appl. Microbiol. **19:**536–537.
161. **Haselkorn, R., and W. J. Buikema.** 1992. Nitrogen fixation in cyanobacteria, p. 166–190. *In* G. Stacey, R. H. Burris, and H. J. Evans (ed.), Biological nitrogen fixation. Chapman & Hall, New York.
162. **Hassan, H. M., and H.-C. H. Sun.** 1992. Regulatory roles of Fnr, Fur, and Arc in expression of manganese containing superoxide dismutase in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **89:**3217–3221.
163. **Hemmingsen, S. M., and L. P. Cloney.** 1993. Molecular chaperones, particularly chaperonins, as compatible solutes. Cryobiology **30:**230–231.
164. **Hengge-Aronis, R., W. Klein, R. Lange, M. Rimmele, and W. Boos.** 1991. Trehalose synthesis genes are controlled by the putative sigma factor encoded by *rpoS* and are involved in stationary-phase thermotolerance in *Escherichia coli*. J. Bacteriol. **173:**7918–7924.
165. **Hengge-Aronis, R., R. Lange, N. Henneberg, and D. Fischer.** 1993. Osmotic regulation of *rpoS*-dependent genes in *Escherichia coli*. J. Bacteriol. **175:**259–265.
166. **Herdman, M., M. Janvier, R. Rippka, and R. Y. Stanier.** 1979. Genome size of cyanobacteria. J. Gen. Microbiol. **111:**73–85.
167. **Hershkovitz, N., A. Oren, and Y. Cohen.** 1991. Accumulation of trehalose and sucrose in cyanobacteria exposed to matric water stress. Appl. Environ. Microbiol. **57:**645–648.
168. **Hill, D. R., S. L. Hladun, S. Scherer, and M. Potts.** 1994. Water stress proteins of *Nostoc commune* (Cyanobacteria) are secreted with UV-A/B-absorbing pigments and associate with 1,4-β-D-xylanxylanohydrolase activity. J. Biol. Chem. **269:**7726–7734.
169. **Hill, D. R., A. Peat, and M. Potts.** Biochemistry and structure of the glycan secreted by desiccation-tolerant *Nostoc commune* (cyanobacteria). Protoplasma, in press.
170. **Hill, D. R., and M. Potts.** Unpublished data.
171. **Hochachka, P. W., and M. Guppy.** 1987. Metabolic arrest and the control of biological time. Harvard University Press, Cambridge, Mass.
172. **Hoekstra, F. A.** 1993. Membrane protection in desiccation-tolerant organs of higher plants. Cryobiology **30:**228–229.
173. **Hoekstra, F. A., J. H. Crowe, and L. M. Crowe.** 1991. Effect of sucrose on phase behavior of membranes intact pollen of *Typha latifolia* L., as measured with fourier transform infrared spectroscopy. Plant Physiol. **97:**1073–1079.
174. **Hoekstra, F. A., J. H. Crowe, and L. M. Crowe.** 1992. Germination and ion leakage are linked with phase transitions of membrane lipids during imbibition of *Typha latifolia* pollen. Physiol. Plant. **84:**29–34.
175. **Hoekstra, F. A., J. H. Crowe, L. M. Crowe, T. van Roekel, and E. Vermeer.** 1992. Do phospholipids and sucrose determine membrane phase transitions in dehydrating pollen species. Plant Cell Environ. **15:**601–606.
176. **Hoekstra, F. A., L. M. Crowe, and J. H. Crowe.** 1989. Differential desiccation sensitivity of corn and *Pennisetum* pollen linked to their sucrose contents. Plant Cell Environ. **12:**83–92.
177. **Hoelzle, I., and J. G. Streeter.** 1990. Increased accumulation of trehalose in rhizobia cultured under 1% oxygen. Appl. Environ. Microbiol. **56:**3213–3215.
178. **Houssin, C., N. Eynard, E. Schechter, and A. Ghazi.** 1991. Effect of osmotic pressure on membrane energy-linked functions in *Escherichia coli*. Biochim. Biophys. Acta **1056:**76–84.
179. **Imlay, J. A., and S. Linn.** 1988. DNA damage and oxygen radical toxicity. Science **240:**1302–1309.
180. **Imshenetsky, A. A., N. F. Pisarenko, L. A. Kuziurina, and V. M. Yakshina.** 1977. Physiology of xerophytic micro-organisms growing under martian conditions, p. 47–52. *In* R. Holmquist and A. C. Strickland (ed.), Life sciences and space research. 1st ed. Pergamon Press, Oxford.
181. **Ingraham, J.** 1987. Effect of temperature, pH, water activity, and pressure on growth, p. 1543–1554. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
182. **Irvin, R. T.** 1990. Hydrophobicity of proteins and bacterial fimbriae, p. 137–177. *In* R. J. Doyle and M. Rosenberg (ed.), Microbial cell surface hydrophobicity. American Society for Microbiology, Washington, D.C.
183. **Israeli, E., E. Giberman, and A. Kohn.** 1974. Membrane malfunctions in freeze-dried Escherichia coli. Cryobiology **11:**473–477.
184. **Israeli, E., A. Kohn, and J. Gitelman.** 1975. The molecular nature of damage by oxygen to freeze-dried *Escherichia coli*. Cryobiology **12:**15–25.
185. **Israeli, E., B. T. Shaffer, J. A. Hoyt, B. Lighthart, and L. M. Ganio.** 1993. Survival differences among freeze-dried genetically engineered and wild-type bacteria. Appl. Environ. Microbiol. **59:**594–598.
186. **Ito, T.** 1992. The effects of vacuum-UV radiation (20–190nm) on microorganisms and DNA. Adv. Space Res. **12:**249–253.
187. **Jaenicke, R.** 1987. Folding and association of proteins. Prog. Biophys. Mol. Biol. **49:**117–237.
188. **Jaenicke, R.** 1991. Protein stability and protein folding. Ciba Found. Symp. **161:**206–221.
189. **Jaenicke, R.** 1991. Protein stability and molecular adaptation to extreme conditions. Eur. J. Biochem. **202:**715–728.
190. **Jaenicke, R.** 1991. Protein folding: local structures, domains, subunits, and assemblies. Biochemistry **30:**3147–3161.
191. **Jaenicke, R.** 1993. Molecular adaptation of proteins to extremes of physical conditions. Cryobiology **30:**211–212.
192. **Jäger, K., and M. Potts.** 1988. In vitro translation of mRNA from *Nostoc commune* (cyanobacteria). Arch. Microbiol. **149:**225–231.
193. **Jäger, K., and M. Potts.** 1988. Distinct fractions of genomic DNA from cyanobacterium *Nostoc commune* that differ in the degree of methylation. Gene **74:**197–201.
194. **Janick, J., S. L. Kitton, and Y.-H. Kim.** 1989. Production of synthetic seed by desiccation and encapsulation. In Vitro Cell. Dev. Biol. **25:**1167–1172.
195. **Jenkins, D. E., S. A. Chaisson, and A. Matin.** 1990. Starvation-induced cross protection against osmotic challenge in *Escherichia coli*. J. Bacteriol. **172:**2779–2781.
196. **Jernigan, R. L., A. Sarai, K.-L. Ting, and R. Nussinov.** 1986. Hydrophobic interactions in the major groove can influence DNA local structure. J. Biomol. Struct. Dyn. **4:**41–48.
197. **Johnston, C. G., and J. R. Vestal.** 1991. Photosynthetic carbon incorporation and turnover in Antarctic cryptoendolithic microbial communities: are they the slowest-growing communities on Earth? Appl. Environ. Microbiol. **57:**2308–2311.
198. **Johnston, D. S., and F. Castelli.** 1989. The influence of sugars on the properties of freeze-dried lysozyme and haemoglobin. Thermochim. Acta **144:**195–208.
199. **Jung, J. U., C. Gutierrez, and M. R. Villarejo.** 1989. Sequence of an osmotically inducible lipoprotein gene. J. Bacteriol. **171:**511–520.
200. **Keenleyside, W. J., D. Bronner, K. Jann, B. Jann, and C. Whitfield.** 1993. Coexpression of colanic acid and serotype-specific capsular polysaccharides in *Escherichia coli* strains with group II K antigens. J. Bacteriol. **175:**6725–6730.
201. **Kennedy, E. P.** 1987. Membrane-derived oligosaccharides, p. 672–679. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B.

Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
202. **Kieft, T. L., E. Soroker, and M. K. Firestone.** 1987. Microbial biomass response to a rapid increase in water potential when dry soil is wetted. Soil Biol. Biochem. **19:**119–126.
203. **Kierstan, M. P. J., and M. P. Coughlan.** 1985. Immobilisation of cells and enzymes by gel entrapment, p. 39–48. *In* J. Woodward (ed.), Immobilised cells and enzymes: a practical approach, 1st ed. IRL Press, Oxford.
204. **Kilbertus, G., J. Proth, and B. Vervier.** 1979. Effets de la dessiccation sur les bacteries gram-negatives d'un sol. Soil Biol. Biochem. **11:**109–114.
205. **Kim, Y.-H., and J. Janick.** 1989. ABA and polyox-encapsulation or high humidity increases survival of desiccated somatic embryos of celery. Hortscience **24:**674–676.
206. **Kitto, S. L., and J. Janick.** 1985. Hardening treatments increase survival of synthetically-coated asexual embryos of carrot. J. Am. Soc. Hort. Sci. **110:**283–286.
207. **Koch, A. L., and M. F. S. Pinette.** 1987. Nephelometric determinations of turgor pressure in growing gram-negative bacteria. J. Bacteriol. **169:**3654–3663.
208. **Kogut, M., and N. J. Russell.** 1987. Life at the limits: considerations on how bacteria can grow at extremes of temperature and pressure, or with high concentrations of ions and solutes. Sci. Prog. **71:**381–399.
209. **Koike, J., T. Oshima, K. A. Koike, H. Taguchi, R. Tanaka, K. Nishimura, and M. Miyaji.** 1992. Survival rates of some terrestial microorganisms under simulated space conditions. Adv. Space Res. **12:**271–274.
210. **Kondo, T., C. A. Strayer, R. D. Kulkarni, W. Taylor, M. Ishiura, S. S. Golden, and C. H. Johnson.** 1993. Circadian rhythms in prokaryotes:luciferase as a reporter of circadian gene expression in cyanobacteria. Proc. Natl. Acad. Sci. USA **90:**5672–5676.
211. **Kornblatt, J. A., and G. H. B. Hoa.** 1990. A nontraditional role for water in the cytochrome *c* oxidase reaction. Biochemistry **29:** 9370–9376.
212. **Kosanke, J. W., R. M. Osburn, G. I. Shuppe, and R. S. Smith.** 1992. Slow rehydration improves the recovery of dried bacterial populations. Can. J. Microbiol. **38:**520–525.
213. **Koshi, G., K. Rajeshwari, and L. Philipose.** 1977. Preservation of streptococci and other bacteria by sand desiccation and filter paper techniques. Indian J. Med. Res. **65:**500–502.
214. **Kuntz, I. D., and A. Zipp.** 1977. Water in biological systems. N. Engl. J. Med. **297:**262–266.
215. **Kushner, S. R.** 1987. DNA repair, p. 1044–1053. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
216. **Lafleur, M. V. M., and J. Retel.** 1993. Contrasting effects of SH-compounds on oxidative DNA damage: repair and increase of damage. Mutat. Res. **295:**1–10.
217. **Laudenbach, D. E., and A. Grossman.** 1991. Characterization and mutagenesis of sulfur-regulated genes in a cyanobacterium: evidence for function in sulfate transport. J. Bacteriol. **173:**2739–2750.
218. **Le Campion-Alsumard, T.** 1978. Les Cyanophycees endolithes marines. Systematique, ultrastructure, ecologie et biodestruction. Oceanol. Acta **2:**143–156.
219. **Lechner, J., and F. Wieland.** 1989. Structure and biosynthesis of prokaryotic glycoproteins. Annu. Rev. Biochem. **58:**173–194.
220. **Lee, C. W. B., S. K. Das Gupta, J. Mattai, G. G. Shipley, O. H. Abdel-Mageed, A. Makriyannis, and R. G. Griffin.** 1989. Characterization of the L_λ phase in trehalose-stabilized dry membranes by solid-state NMR and X-ray diffraction. Biochemistry **28:**5000–5009.
221. **Lee, C. W. B., J. S. Waugh, and R. G. Griffin.** 1986. Solid-state NMR study of trehalose/1,2-dipalmitoyl-sn-phosphatidylcholine interactions. Biochemistry **25:**3737–3742.
222. **Leistner, L., and W. Rödel.** 1976. Inhibition of micro-organisms in food by water activity, p. 219–237. *In* F. A. Skinner and W. B. Hugo (ed.), Inhibition and inactivation of vegetative microbes, 1st ed. Academic Press Ltd., London.
223. **Lemassu, A., M.-A. Laneelle, and M. Daffe.** 1991. Revised structure of a trehalose-containing immunoreactive glycolipid of *Mycobacterium tuberculosis*. FEMS Microbiol. Lett. **78:**171–176.
224. **Leslie, S. B., S. A. Teter, L. M. Crowe, and J. H. Crowe.** 1994. Trehalose lowers membrane phase transitions in dry yeast cells. Biochim. Biophys. Acta **1192:**7–13.
225. **Li, C., and J. Adler.** 1993. *Escherichia coli* shows two types of behavioral responses to osmotic upshift. J. Bacteriol. **175:**2564–2567.
226. **Li, J., and D. K. Ross.** 1993. Evidence for two kinds of hydrogen bond in ice. Nature (London) **365:**327–329.
227. **Lindahl, T., and B. Nyberg.** 1972. Rate of depurination of native deoxyribonucleic acid. Biochemistry **11:**3610–3618.
228. **Lindberg, C., and G. Horneck.** 1992. Thymine photoproduct formation and inactivation of intact spores of *Bacillus subtilis* irradiated with short wavelength UV (200–300nm) at atmospheric pressure and in vacuo. Adv. Space Res. **12:**275–279.
229. **Lindsay, J. A., and W. G. Murrell.** 1983. A comparison of UV induced DNA photoproducts from isolated and non-isolated developing bacterial forespores. Biochem. Biophys. Res. Commun. **113:**618–625.
230. **Liochev, S. I., and I. Fridovich.** 1992. Effects of overproduction of superoxide dismutases in *Escherichia coli* on inhibition of growth and on induction of glucose-6-phosphate dehydrogenase by paraquat. Arch. Biochem. Biophys. **294:**138–143.
231. **Lohman, T. M.** 1986. Kinetics of protein-nucleic acid interactions: use of salt effects to probe mechanisms of interaction. Crit. Rev. Biochem. **19:**191–245.
232. **Lounnas, V., B. Montgomery Pettitt, and G. N. Phillips, Jr.** 1994. A global model of the protein-solvent interface. Biophys. J. **66:** 601–614.
233. **Mackenzie, A. P.** 1977. Non-equilibrium freezing behavior of aqueous systems. Philos. Trans. R. Soc. London Ser. B **278:**167–189.
234. **Marshall, B., P. Flynn, D. Kamely, and S. B. Levy.** 1988. Survival of *Escherichia coli* with and without ColE1::Tn*5* after aerosol dispersal in a laboratory and a farm environment. Appl. Environ. Microbiol. **54:**1776–1783.
235. **Mathews, C. K.** 1993. The cell-bag of enzymes or network of channels. J. Bacteriol. **175:**6377–6381.
236. **Mazur, P.** 1980. Limits to life at low temperatures and at reduced water contents and water activities. Origins Life **10:**137–159.
237. **McCartney, B., and M. Potts.** Unpublished data.
238. **Meury, J., and M. Kohiyama.** 1992. Potassium ions and changes in bacterial DNA supercoiling under osmotic stress. FEMS Microbiol. Lett. **99:**159–164.
239. **Michel, D., A. Furini, F. Salamini, and D. Bartels.** 1994. Structure and regulation of an ABA- and desiccation-responsive gene from the resurrection plant *Craterostigma plantagineum*. Plant Mol. Biol. **24:**549–560.
240. **Miller, K. J., E. P. Kennedy, and V. N. Reinhold.** 1986. Osmotic adaptation by Gram-negative bacteria: possible role for periplasmic oligosaccharides. Science **231:**48–51.
241. **Mitscherlich, E., and E. H. Marth.** 1984. Microbial survival in the environment: bacteria and rickettsiae important in human and animal health, 1st ed. Springer-Verlag KG, Berlin.
242. **Mittler, R., and E. Tel-Or.** 1991. Oxidative stress responses and shock proteins in the unicellular cyanobacterium *Synechococcus* R2 (PCC-7942). Arch. Microbiol. **155:**125–130.
243. **Miyake, C., F. Michihata, and K. Asada.** 1991. Scavenging of hydrogen peroxide in prokaryotic and eukaryotic algae: acquisition of ascorbate peroxidase during the evolution of cyanobacteria. Plant Cell Physiol. **32:**33–43.
244. **Moore, B. G., and R. G. Tischer.** 1965. Biosynthesis of extracellular polysaccharides by the blue-green alga *Anabaena flos-aquae*. Can. J. Bot. **11:**877–885.
245. **Morikawa, M., H. Daido, T. Takao, S. Murata, Y. Shimonishi, and T. Imanaka.** 1993. A new lipopeptide biosurfactant produced by *Arthrobacter* sp. strain MIS38. J. Bacteriol. **175:**6459–6466.
246. **Motta, T. B., and A. M. Sechi.** 1992. Phospholipid polar heads affect the generation of oxygen active species by Fe^{2+} autooxidation. Biochem. Int. **26:**987–994.

247. **Mugnier, J., and G. Jung.** 1985. Survival of bacteria and fungi in relation to water activity and the solvent properties of water in biopolymer gels. Appl. Environ. Microbiol. **50:**108–114.
248. **Nakagawa, M., Y. Takamura, and O. Yagi.** 1987. Isolation and characterization of the slime from a cyanobacterium, *Microcystis aeruginosa* K-3A. Agric. Biol. Chem. **51:**329–337.
249. **Nei, T.** 1969. Freezing and drying of microorganisms. University of Tokyo Press, Tokyo.
250. **Neidleman, S. L.** 1989. Enzymes under stress. ASM News **55:**67–70.
251. **Newell, J.** 1987. Putting nature into reverse. Biobulletin **5:**1–2.
252. **Nichols, J. M., and D. G. Adams.** 1983. Akinetes, p. 387–412. *In* N. G. Carr and B. A. Whitton (ed.), The biology of cyanobacteria, 1st ed. Blackwell Scientific Publications Ltd., Oxford.
253. **Nicholson, P., R. W. Osborn, and C. J. Howe.** 1987. Induction of protein synthesis in response to ultraviolet light, nalidixic acid and heat shock in the cyanobacterium *Phormidium laminosum*. FEBS Lett. **221:**110–114.
254. **Nicholson, P., J. P. A. Varley, and C. J. Howe.** 1991. A comparison of stress responses in the cyanobacterium *Phormidium laminosum*. FEMS Microbiol. Lett. **78:**109–114.
255. **Niedhardt, F. C.** 1987. Chemical composition of *Escherichia coli*, p. 3–6. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
256. **Nienow, J. A., and E. I. Friedmann.** 1993. Terrestial lithophytic (rock) communities, p. 343–412. *In* E. I. Friedmann and A. B. Thistle (ed.), Antarctic microbiology. John Wiley & Sons, Inc., New York.
257. **Nikaido, H. M., and M. Vaara.** 1987. Outer membrane, p. 7–22. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
258. **Nilsson, B., and S. Anderson.** 1991. Proper and improper folding of proteins in the cellular environment. Annu. Rev. Microbiol. **45:**607–635.
259. **Nobel, P. S.** 1983. Biophysical plant physiology and ecology, 3rd ed. W. H. Freeman & Co., San Francisco.
260. **Noone, M. J.** 1991. Preserving the appearance of asphalt shingle roofs. Prof. Roofing **R:**14–16.
261. **Norris, J. R., R. C. W. Berkeley, N. A. Logan, and A. G. O'Donnell.** 1981. The genera *Bacillus* and *Sporolactobacillus*, p. 1712–1742. *In* M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes, vol. 1, 1st ed. Springer-Verlag KG, Heidelberg, Germany.
262. **Nunoshiba, T., E. Hidalgo, Z. Li, and B. Demple.** 1993. Negative autoregulation by the *Escherichia coli* SoxS protein: a dampening mechanism for the *soxRS* redox stress response. J. Bacteriol. **175:**7492–7494.
263. **O'Connor, L. T., and D. C. Savage.** 1993. Characterization of an activity from the strict anaerobe *Roseburia cecicola* that degrades DNA when exposed to air. J. Bacteriol. **175:**4681–4687.
264. **Oksanen, C. A., and G. Zografi.** 1993. Molecular mobility in mixtures of absorbed water and solid poly(vinylpyrrolidone). Pharm. Res. **10:**791–799.
265. **Olie, J. J., and M. Potts.** 1986. Purification and biochemical analysis of the cytoplasmic membrane from the desiccation-tolerant cyanobacterium *Nostoc commune* UTEX 584. Appl. Environ. Microbiol. **52:**706–710.
266. **Oliver, M.** 1992. Miracle moss. Science **257:**322.
267. **Oliver, M. J., and J. D. Bewley.** 1984. Plant desiccation and protein synthesis. VI. Changes in protein synthesis elicited by desiccation of the moss *Tortula ruralis* are effected at the translational level. Plant Physiol. **74:**923–927.
268. **Orcutt, D. M., B. C. Parker, and W. R. Lusby.** 1986. Lipids in blue-green algal mats (modern stromatolites) from Antarctic oasis lakes. J. Phycol. **22:**523–530.
269. **Osa-afiana, L. O., and M. Alexander.** 1982. Differences among cowpea rhiobia in tolerance to high temperature and desiccation in soil. Appl. Environ. Microbiol. **43:**435–439.
270. **Otting, G., E. Liepinsh, and K. Wüthrich.** 1991. Protein hydration in aqueous solution. Science **254:**974–980.
271. **Palmer, R. J., and E. I. Friedmann.** 1990. Water relations and photosynthesis in the cryptoendolithic microbial habitat of hot and cold deserts. Microb. Ecol. **19:**111–118.
272. **Palmer, R. J., Jr., J. A. Nienow, and E. I. Friedmann.** 1987. Control of matric water potential by temperature differential. J. Microbiol. Methods **6:**323–326.
273. **Papageorgiou, G. C., and T. Lagoyanni.** 1986. Immobilization of photosynthetically active cyanobacteria in glutaraldehyde-crosslinked albumin matrix. Appl. Microbiol. Biotechnol. **23:**417–423.
274. **Papendick, R. I., and G. S. Campbell.** 1981. Theory and measurement of water potential, p. 1–22. *In* J. F. Parr, W. R. Gardner, and L. F. Elliot (ed.), Water potential relations in soil microbiology. Soil Science Society of America, Madison, Wis.
275. **Parker, J. G.** 1993. On the inactivation of bacteria by singlet oxygen—another view. Biochim. Biophys. Acta **1157:**209–210.
276. **Parr, J. F., W. R. Gardner, and L. F. Elliot.** 1981. Water potential relations in soil microbiology. Soil Science Society of America, Madison, Wis.
277. **Patrice, M., D. Ochin, and R. Tailliez.** 1985. Rates of drying and survival of *Rhizobium meliloti* strains during storage at different relative humidities. Appl. Environ. Microbiol. **50:**207–211.
278. **Peat, A., and M. Potts.** 1987. The ultrastructure of immobilised desiccated cells of the cyanobacterium *Nostoc commune* UTEX 584. FEMS Microbiol. Lett. **43:**223–227.
279. **Peat, A., N. Powell, and M. Potts.** 1988. Ultrastructural analysis of the rehydration of desiccated *Nostoc commune* HUN (Cyanobacteria) with particular reference to the immunolabelling of NifH. Protoplasma **146:**72–80.
280. **Pedersen, J. C.** 1992. Survival of *Enterobacter cloacae*: field validation of a soil/plant microcosm. Microb. Releases **1:**87–93.
281. **Pedersen, J. C., and C. S. Jacobsen.** 1993. Fate of *Enterobacter cloacae* JP120 and *Alcaligenes eutrophus* AEO106(pR0101) in soil during water stress: effects on culturability and viability. Appl. Environ. Microbiol. **59:**1560–1564.
282. **Pedersen, J. C., and T. D. Leser.** 1992. Survival of *Enterobacter cloacae* on leaves and in soil detected by immunofluorescence microscopy in comparison with selective plating. Microb. Releases **1:**95–102.
283. **Pentecost, A.** 1985. Relationships between light, temperature and photosynthesis in a temperate *Microcoleus* (cyanobacterium) mat. Microbios **43:**141–148.
284. **Perona, J. J., C. S. Craik, and R. J. Fletterick.** 1993. Locating the catalytic water molecule in serine proteases. Science **261:**620–621.
285. **Phillips, T. A., V. Vaughn, P. L. Bloch, and F. C. Neidhardt.** 1987. Gene-protein index of *Escherichia coli* K-12, edition 2, p. 919–966. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
286. **Pinette, M. F. S., and A. L. Koch.** 1987. Variability of the turgor pressure of individual cells of the gram-negative heterotroph *Ancylobacter aquaticus*. J. Bacteriol. **169:**4737–4742.
287. **Platt, M. W., K. J. Miller, W. S. Lane, and E. P. Kennedy.** 1990. Isolation and characterization of the constitutive acyl carrier protein from *Rhizobium meliloti*. J. Bacteriol. **172:**5440–5444.
288. **Pollock, M. R., and M. H. Richmond.** 1962. Low cyst(e)ine content of bacterial extracellular proteins: its possible physiological significance. Nature (London) **194:**446–449.
289. **Potthast, K.** 1978. Influence of water activity on enzymic activity in biological systems, p. 323–342. *In* J. H. Crowe and J. S. Clegg (ed.), Dry biological systems, 1st ed. Academic Press, Inc., New York.
290. **Potts, M.** 1977. Ph.D. thesis. Durham University, Durham, England.
291. **Potts, M.** Unpublished data.
292. **Potts, M.** 1979. Nitrogen fixation (acetylene reduction) associated with communities of heterocystous and non-heterocystous blue-green algae in mangrove forests of Sinai. Oecologia (Berlin) **39:**359–373.
293. **Potts, M.** 1980. Blue-green algae (Cyanophyta) in marine coastal

environments of the Sinai Peninsula: distribution, zonation, stratification and taxonomic diversity. Phycologia 19:60–73.
294. **Potts, M.** 1985. Protein synthesis and proteolysis in immobilized cells of the cyanobacterium *Nostoc commune* UTEX 584 exposed to matric water stress. J. Bacteriol. **164**:1025–1031.
295. **Potts, M.** 1986. The protein index of *Nostoc commune* UTEX 584 (cyanobacteria): changes induced in immobilized cells by water stress. Arch. Microbiol. **146**:87–95.
296. **Potts, M.** 1993. Stabilities of macromolecules during water stress in cyanobacteria. Cryobiology **30**:232–233.
297. **Potts, M., S. V. Angeloni, R. E. Ebel, and D. Bassam.** 1992. Myoglobin in a cyanobacterium. Science **256**:1690–1692.
298. **Potts, M., and M. A. Bowman.** 1985. Sensitivity of *Nostoc commune* UTEX 584 (cyanobacteria) to water stress. Arch. Microbiol. **141**:51–56.
299. **Potts, M., and M. A. Bowman.** Unpublished data.
300. **Potts, M., M. A. Bowman, and N. S. Morrison.** 1984. Control of matric water potential (Ψ_m) in immobilised cultures of cyanobacteria. FEMS Microbiol. Lett. **24**:193–196.
301. **Potts, M., and E. I. Friedmann.** 1981. Effects of water stress on cryptoendolithic cyanobacteria from hot desert rocks. Arch. Microbiol. **130**:267–271.
302. **Potts, M., and N. S. Morrison.** 1986. Shifts in the intracellular ATP pools of immobilised *Nostoc* cells (Cyanobacteria) induced by water stress. Plant Soil **90**:211–221.
303. **Potts, M., R. Ocampo-Friedmann, M. A. Bowman, and B. Tüzün.** 1983. *Chroococcus* S24 and *Chroococcus* N41 (cyanobacteria): morphological, biochemical and genetic characterization and effects of water stress on ultrastructure. Arch. Microbiol. **135**:81–90.
304. **Potts, M., J. J. Olie, J. S. Nickels, J. Parsons, and D. C. White.** 1987. Variation in phospholipid ester-linked fatty acids and carotenoids of desiccated *Nostoc commune* (cyanobacteria) from different geographic locations. Appl. Environ. Microbiol. **53**:4–9.
305. **Potts, M., H. Sun, K. Mockaitis, P. J. Kennelly, D. Reed, and N. K. Tonks.** 1993. A protein-tyrosine/serine phosphatase encoded by the genome of the cyanobacterium *Nostoc commune* UTEX 584. J. Biol. Chem. **268**:7632–7635.
306. **Potts, M., and B. A. Whitton.** 1977. Nitrogen fixation by blue-green algal communities in the intertidal zone of the lagoon of Aldabra Atoll. Oecologia **27**:275–283.
307. **Potts, M., and B. A. Whitton.** 1979. pH and Eh on Aldabra Atoll. 2. Intertidal photosynthetic microbial communities showing zonation. Hydrobiologia **67**:99–105.
308. **Potts, M., and B. A. Whitton.** 1980. Vegetation of the intertidal zone of the lagoon of Aldabra, with particular reference to the photosynthetic prokaryotic communities. Proc. R. Soc. London Ser. B **208**:13–55.
309. **Prestrelski, S. J.** 1993. The structure of proteins in lyophilized formulations using fourier transform infrared spectroscopy. Cryobiology **30**:222–223.
310. **Priestley, D. A.** 1986. Seed aging. Implications for seed storage and persistence in the soil. Comstock Publishing Associates, Cornell University Press, Ithaca, N.Y.
311. **Pritzer, M., J. Weckesser, and U. J. Jürgens.** 1989. Sheath and outer membrane components from the cyanobacterium *Fischerella* sp. PCC 7414. Arch. Microbiol. **153**:7–11.
312. **Proteau, P. J., W. H. Gerwick, F. Garcia-Pichel, and R. Castenholz.** 1993. The structure of scytonemin, an ultraviolet sunscreen pigment from the sheaths of cyanobacteria. Experientia **49**:825–829.
313. **Purcell, L. C., and T. R. Sinclair.** 1993. Soybean (*Glycine max*) nodule physical traits associated with permeability responses to oxygen. Plant Physiol. **103**:149–156.
314. **Quiocho, F. A., D. K. Wilson, and N. K. Vyas.** 1989. Substrate specificity and affinity of a protein modulated by bound water molecules. Nature (London) **340**:404–407.
315. **Rand, R. P.** 1992. Raising water to new heights. Science **256**:618.
316. **Rand, R. P.** 1993. Don't forget the solvent—measuring water's contribution to molecular assembly and conformational change. Cryobiology **30**:216–217.
317. **Rattray, E. A. S., J. I. Prosser, L. A. Glover, and K. Killham.** 1992. Matric potential in relation to survival and activity of a genetically modified microbial inoculum in soil. Soil Biol. Biochem. **24**:421–425.
318. **Rau, D. C., B. Lee, and V. A. Parsegian.** 1984. Measurement of the repulsive force between polyelectrolyte molecules in ionic solution: hydration forces between parallel DNA double helices. Proc. Natl. Acad. Sci. USA **81**:2621–2625.
319. **Redenbaugh, K.** 1992. Synseeds: applications of synthetic seeds to crop improvement. CRC Press, Inc., Boca Raton, Fla.
320. **Reeck, G. R., and L. Fisher.** 1973. A statistical analysis of the amino acid compositions of proteins. Int. J. Pept. Res. **5**:109–117.
321. **Reichenbach, H., and M. Dworkin.** 1981. Introduction to gliding bacteria, p. 315–379. *In* M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes, vol. 1, 1st ed., Springer-Verlag KG, Heidelberg, Germany.
322. **Reslow, M., P. Adlercreutz, and B. Mattiason.** 1988. The influence of water on protease-catalyzed peptide synthesis in acetonitrile/water mixtures. Eur. J. Biochem. **177**:313–318.
323. **Richards, W. G., P. M. King, and C. A. Reynolds.** 1989. Solvation effects. Protein Eng. **2**:319–327.
324. **Ritter, D., and J. H. Yopp.** 1993. Plasma membrane lipid composition of the halophilic cyanobacterium *Aphanothece halophytica*. Arch. Microbiol. **159**:435–439.
325. **Roberson, E. B., and M. K. Firestone.** 1992. Relationship between desiccation and exopolysaccharide production in a soil *Pseudomonas* sp. Appl. Environ. Microbiol. **58**:1284–1291.
326. **Rodgers, G. A.** 1977. Nitrogenase activity in *Nostoc muscorum*: recovery from desiccation. Plant Soil **46**:671–674.
327. **Rodriguez-Valera, F.** 1992. Biotechnological potential of halobacteria. Biochem. Soc. Symp. **58**:135–147.
328. **Romeo, T., J. Moore, and J. Smith.** 1991. A simple method for cloning genes involved in glucan biosynthesis: isolation of structural and regulatory genes for glycogen synthesis in *Escherichia coli*. Gene **108**:23–29.
329. **Roser, B.** 1991. Trehalose drying: a novel replacement for freeze-drying. BioPharm **8**:47–53.
330. **Roszak, D. B., and R. R. Colwell.** 1987. Survival strategies of bacteria in the natural environment. Microbiol. Rev. **51**:365–379.
331. **Roth, I. L.** 1977. Physical structures of surface carbohydrates, p. 5–26. *In* I. Sutherland (ed.), Surface carbohydrates of the prokaryotic cell. Academic Press Ltd., London.
332. **Rudolph, A. S.** 1988. The freeze-dried preservation of liposome encapsulated hemoglobin: a potential blood substitute. Cryobiology **25**:277–284.
333. **Rudolph, A. S., R. O. Cliff, and B. J. Spargo.** 1993. The use of compatible solutes in the long-term preservation of lipid microstructures. Cryobiology **30**:236–237.
334. **Rudolph, B. R., I. Chadresekhar, B. P. Gaber, and M. Nagumo.** 1990. Molecular modelling of saccharide-lipid interactions. Chem. Phys. Lipids **53**:243–261.
335. **Rumley, M. K., H. Therisod, A. C. Weissborn, and E. P. Kennedy.** 1992. Mechanisms of regulation of the biosynthesis of membrane-derived oligosaccharides in *Escherichia coli*. J. Biol. Chem. **267**:11806–11810.
336. **Russell, N. J.** 1990. Cold adaptation of microorganisms. Philos. Trans. R. Soc. London Ser. B **326**:595–611.
337. **Russell, N. J., and N. Fukunaga.** 1990. A comparison of thermal adaptation of membrane lipids in psychrophilic and thermophilic bacteria. FEMS Microbiol. Rev. **75**:171–182.
338. **Sadasivan, L., and C. A. Neyra.** 1985. Flocculation in *Azospirillum brasilense* and *Azospirillum lipoferum*: exopolysaccharides and cyst formation. J. Bacteriol. **163**:716–723.
339. **Sakane, T., I. Fukuda, T. Itoh, and A. Yokota.** 1992. Long-term preservation of halophilic archaebacteria and thermoacidophilic archaebacteria by liquid drying. J. Microbiol. Methods **16**:281–287.
340. **Santell, L., R. L. Rubin, and E. G. Levin.** 1993. Enhanced phosphorylation and dephosphorylation of a histone-like protein in response to hyperosmotic and hypoosmotic conditions. J. Biol. Chem. **268**:21443–21447.
341. **Sar, N.** 1987. Direction of spreading (DOS): a simple method for measuring the hydrophobicity of bacterial lawns. J. Microbiol. Methods **6**:211–219.
342. **Sartor, G., E. Mayer, and G. P. Johari.** 1994. Calometric studies

of the kinetic unfreezing of molecular motions in hydrated lysozyme, hemoglobin, and myoglobin. Biophys. J. **66**:249–258.
343. **Scherer, S.** 1991. Mikroorganismen in extremen Lebensräumen: cyanobakterien in Wüstengebieten. Biol. Unserer Zeit **4**:220–222.
344. **Scherer, S., T. W. Chen, and P. Böger.** 1988. A new UV-A/B protecting pigment in the terrestial cyanobacterium *Nostoc commune*. Plant Physiol. **88**:1055–1057.
345. **Scherer, S., and M. Potts.** 1989. Novel water stress protein from a desiccation-tolerant cyanobacterium: purification and partial characterization. J. Biol. Chem. **264**:12546–12553.
346. **Scherer, S., and Z.-P. Zhong.** 1991. Desiccation independence of terrestial *Nostoc commune* ecotypes (Cyanobacteria). Microb. Ecol. **22**:271–283.
347. **Schmid, E. N.** 1981. Fate of the *Escherichia coli* capsule during embedding procedures for electron microscopical studies. Microsc. Acta **84**:353–360.
348. **Schneider, J.** 1976. Biological and inorganic factors in the destruction of limestone coasts. Contrib. Sedimentol. **6**:1–112.
349. **Schneider, S., and U. J. Jürgens.** 1991. Cell wall and sheath constituents of the cyanobacterium *Gloeobacter violaceus*. Arch. Microbiol. **156**:312–318.
350. **Seckler, R., and R. Jaenicke.** 1992. Protein folding and protein refolding. FASEB J. **6**:2545–2552.
351. **Setlow, P.** 1992. I will survive: protecting and repairing spore DNA. J. Bacteriol. **174**:2737–2741.
352. **Setlow, B., and P. Setlow.** 1977. Most of the coenzyme A in dormant spores of *Bacillus megaterium* is in disulfide linkage to protein. Biochem. Biophys. Res. Commun. **75**:45–50.
353. **Shehata, F. M., and B. A. Whitton.** 1982. Zinc tolerance in strains of the blue green alga *Anacystis nidulans*. Br. Phycol. J. **17**:5–12.
354. **Siegele, D. A., and R. Kolter.** 1992. Life after log. J. Bacteriol. **174**:345–348.
355. **Simon, P.** 1980. DNA content of heterocysts and spores of the filamentous cyanobacterium *Anabaena variabilis*. FEMS Microbiol. Lett. **8**:241–245.
356. **Skriver, K., and J. Mundy.** 1990. Gene expression in response to abscisic acid and osmotic stress. Plant Cell **2**:503–512.
357. **Slade, L., and H. Levine.** 1991. Beyond water activity: recent advances based on an alternative approach to the assessment of food quality and safety. Crit. Rev. Food Sci. Nutr. **30**:115–360.
358. **Sleesman, J. P., and C. Leben.** 1976. Bacterial desiccation: effects of temperature, relative humidity, and culture age on survival. Phytopathology **66**:1334–1338.
359. **Smith, M. D., C. I. Masters, and B. E. B. Moseley.** 1991. Molecular biology of radiation-resistant bacteria, p. 258–280. *In* R. A. Herbert and R. J. Sharp (ed.), Molecular biology and biotechnology of extremophiles. Blackie & Son, Ltd., Glasgow, Scotland.
360. **Sneath, P. H. A.** 1962. Longevity of micro-organisms. Nature (London) **195**:643–646.
361. **Sohal, R. S., S. Agarwal, A. Dubey, and W. C. Orr.** 1993. Protein oxidative damage is associated with life expectancy of houseflies. Proc. Natl. Acad. Sci. USA **90**:7255–7259.
362. **Somero, G. N.** 1993. Conserving macromolecular stability and function: complementary roles of adaptive changes in proteins and the "micromolecules" of their milieu. Cryobiology **30**:203–204.
363. **Southam, G., and T. J. Beveridge.** 1992. Characterization of novel, phenol-soluble polypeptides which confer rigidity to the sheath of *Methanospirillum hungatei* GP1. J. Bacteriol. **174**:935–946.
364. **Southam, G., and T. J. Beveridge.** 1992. Detection of growth sites in and promoter pools for the sheath of *Methanospirillum hungatei* GP1 by use of constituent organosulfur and immunogold labeling. J. Bacteriol. **174**:6460–6470.
365. **Spaink, H. P., D. M. Sheeley, A. A. N. van Brussel, F. Glushka, W. S. York, T. Tak, O. Geiger, E. P. Kennedy, V. N. Reinhold, and B. J. J. Lugtenberg.** 1991. A novel highly unsaturated fatty acid moiety of lipo-oligosaccharide signals determines host specificity of *Rhizobium*. Nature (London) **354**:125–128.
366. **Spargo, B. J., L. M. Crowe, T. Ioneda, B. L. Beaman, and J. H. Crowe.** 1991. Cord factor (α,α-trehalose 6,6′-dimycolate) inhibits fusion between phospholipid vesicles. Proc. Natl. Acad. Sci. USA **88**:737–740.
367. **Stadtman, E. R., and C. N. Oliver.** 1991. Metal-catalyzed oxidation of proteins. J. Biol. Chem. **266**:2005–2008.
368. **Stal, L. J., and R. H. Reed.** 1987. Low-molecular mass carbohydrate accumulation in cyanobacteria from a marine microbial mat in response to salt. FEMS Microbiol. Lett. **45**:305–312.
369. **Stanier, R. Y., and C. B. van Niel.** 1962. The concept of a bacterium. Arch. Mikrobiol. **42**:17–35.
370. **Starr, M. P., and J. M. Schmidt.** 1981. Prokaryote diversity, p. 3–42. *In* M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes, vol. 1, 1st ed. Springer-Verlag KG, Heidelberg, Germany.
371. **Steiner, B. M., G. H. W. Wong, P. Sutrave, and S. Graves.** 1984. Oxygen toxicity in *Treponema pallidum*: deoxyribonucleic acid single-stranded breakage induced by low doses of hydrogen peroxide. Can. J. Microbiol. **30**:1467–1476.
372. **Storz, G., M. F. Christman, H. Sies, and B. N. Ames.** 1987. Spontaneous mutagenesis and oxidative damage to DNA in *Salmonella typhimurium*. Proc. Natl. Acad. Sci. USA **84**:8917–8921.
373. **Stout, V., A. Torres-Cabassa, M. R. Maurizi, D. Gutnick, and S. Gottesman.** 1991. RcsA, an unstable positive regulator of capsular polysaccharide synthesis. J. Bacteriol. **173**:1738–1747.
374. **Streeter, J. G.** 1985. Accumulation of α,α-trehalose by *Rhizobium* bacteria and bacteroids. J. Bacteriol. **164**:78–84.
375. **Stulp, B. K., and M. Potts.** 1987. Stability of nucleic acids in immobilized and desiccated *Nostoc commune* UTEX 584 (cyanobacteria). FEMS Microbiol. Lett. **41**:241–245.
376. **Styrvold, O. B., and A. R. Strøm.** 1991. Synthesis, accumulation, and excretion of trehalose in osmotically stressed *Escherichia coli* K-12 strains: influence of amber suppressors and function of the periplasmic trehalase. J. Bacteriol. **173**:1187–1192.
377. **Sutherland, J. M., M. Herdman, and W. D. P. Stewart.** 1979. Akinetes of the cyanobacterium *Nostoc* PCC 7524: macromolecular composition, structure and control of differentiation. J. Gen. Microbiol. **115**:273–287.
378. **Tanaka, K., Y. Takayanagi, N. Fujita, A. Ishihama, and H. Takahashi.** 1993. Heterogeneity of the principal σ factor in *Escherichia coli*: the *rpoS* gene product, σ^{38}, is a second principal σ factor of RNA polymerase in stationary-phase *Escherichia coli*. Proc. Natl. Acad. Sci. USA **90**:3511–3515.
379. **Taranto, P. A., T. W. Keenan, and M. Potts.** 1993. Rehydration induces rapid onset of lipid biosynthesis in desiccated *Nostoc commune* (cyanobacteria). Biochim. Biophys. Acta **1168**:228–237.
380. **Tartaglia, L. A., C. J. Gimeno, G. Storz, and B. N. Ames.** 1992. Multidegenerate DNA recognition by the OxyR transcriptional regulator. J. Biol. Chem. **267**:2038–2045.
381. **Tease, B. E., and R. W. Walker.** 1987. Comparative composition of the sheath of the cyanobacterium *Gloeothece* ATCC 27152 cultured with and without combined nitrogen. J. Gen. Microbiol. **133**:3331–3339.
382. **Therisod, H., A. C. Weissborn, and E. P. Kennedy.** 1986. An essential function for acyl carrier protein in the biosynthesis of membrane-derived oligosaccharides of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **83**:7236–7240.
383. **Timasheff, S. N.** 1992. A physiological basis for the selection of osmolytes by nature, p. 70–84. *In* G. N. Somero, C. B. Osmond, and C. L. Bolis (ed.), Water and life, 1st ed. Springer-Verlag KG, Berlin.
384. **Timasheff, S. N.** 1993. Stabilization of proteins by cosolvents in aqueous media. Cryobiology **30**:218–219.
385. **Tomaselli, L., and L. Giovanetti.** 1993. Survival of diazotrophic cyanobacteria in soil. World J. Microbiol. Biotechnol. **9**:113–116.
386. **Tripathi, S. N., and E. R. S. Talpasayi.** 1980. Sulphydryls and survival of subaerial blue-green algae. Curr. Sci. **49**:31–32.
387. **Tsaneva, I. R., and B. Weiss.** 1990. *soxR*, a locus governing a superoxide response regulon in *Escherichia coli* K-12. J. Bacteriol. **172**:4197–4205.
388. **Tsvetkov, T. D., L. I. Tsonev, N. M. Tsvetkova, R. D. Koynova, and B. G. Tenchov.** 1989. Effect of trehalose on the phase properties of hydrated and lyophilized dipalmitoylphosphatidylcholine multilayers. 1989. *Cryobiology* **26**:162–169.
389. **Tudor, J., and F. Conti.** 1977. Characterization of bdellocysts of *Bdellovibrio* sp. J. Bacteriol. **131**:314–322.

390. **Van Loosdrecht, M. C. M., J. Lyklema, W. Norde, and A. J. B. Zehnder.** 1990. Influence of interfaces on microbial activity. Microbiol. Rev. **54:**75–87.
391. **Vergnaud, J. M.** 1991. Liquid transport processes in polymeric materials: modeling and industrial applications, 1st ed. Prentice-Hall, Inc., Englewood Cliffs, N.J.
392. **Vertucci, C. W., and A. C. Leopold.** 1986. Physiological activities associated with hydration level in seeds, p. 35–49. *In* A. C. Leopold (ed.), Membranes, metabolism and dry organisms. Comstock Publishing Associates, Cornell University Press, Ithaca, N.Y.
393. **Vishniac, H. S.** 1993. The microbiology of Antarctic soils, p. 297–341. *In* E. I. Friedmann (ed.), Antarctic microbiology. John Wiley & Sons, Inc., New York.
394. **von Hippel, P. H., and O. G. Berg.** 1989. Facilitated target location in biological systems. J. Biol. Chem. **264:**675–678.
395. **Wada, A., Y. Yamazaki, N. Fujita, and A. Ishihama.** 1990. Structure and probable genetic location of a "ribosome modulation factor" associated with 100S ribosomes in stationary-phase *Escherichia coli* cells. Proc. Natl. Acad. Sci. USA **87:**2657–2661.
396. **Waks, M.** 1986. Proteins and peptides in water-restricted environments. Proteins Struct. Funct. Genet. **1:**4–15.
397. **Walsby, A. E.** 1969. The permeability of blue-green algal gas-vacuoles' membranes to gas. Proc. R. Soc. London Ser. B **173:** 233–255.
398. **Walsby, A. E.** 1982. Cell-water and cell-solute relations, p. 237–262. *In* N. G. Carr and B. A. Whitton (ed.), The biology of cyanobacteria. Blackwell Scientific Publications Ltd., Oxford.
399. **Wanner, B. L.** 1987. Phosphate regulation of gene expression in *Escherichia coli*, p. 1326–1333. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
400. **Ward, D. M., R. Weller, J. Shiea, R. W. Castenholz, and Y. Cohen.** 1989. Hot spring microbial mats: anoxygenic and oxygenic mats of possible evolutionary significance, p. 3–15. *In* Y. Cohen and E. Rosenberg (ed.), Microbial mats: physiological ecology of benthic microbial communities. American Society for Microbiology, Washington, D.C.
401. **Webb, S. J.** 1960. Factors affecting the viability of air-borne bacteria. II. The effect of chemical additives on the behavior of air-borne cells. Can. J. Microbiol. **6:**71–87.
402. **Webb, S. J.** 1960. Factors affecting the viability of air-borne bacteria. III. The role of bonded water and protein structure in the death of air-borne cells. Can. J. Microbiol. **6:**89–105.
403. **Webb, S. J.** 1965. Bound water in biological integrity. Charles C Thomas, Springfield, Ill.
404. **Webb, S. J.** 1966. The influence of oxygen and inositol on the survival of semidried microorganisms. Can. J. Microbiol. **7:**733–742.
405. **Webb, S. J.** 1967. Mutation of bacterial cells by controlled desiccation. Nature (London) **213:**1137–1139.
406. **Webb, S. J.** 1968. Effect of dehydration on bacterial recombination. Nature (London) **217:**1231–1234.
407. **Webb, S. J.** 1969. The effects of oxygen on the possible repair of dehydration damage by *Escherichia coli*. J. Gen. Microbiol. **58:** 317–326.
408. **Webb, S. J., M. D. Dumasia, and J. S. Bhorjee.** 1965. Bound water, inositol, and the biosynthesis of temperate and virulent bacteriophages by air-dried *Escherichia coli*. Can. J. Microbiol. **11:**141–150.
409. **Webb, S. J., and J. L. Walker.** 1968. The influence of cell water content on the inactivation of RNA by partial desiccation and ultraviolet light. Can. J. Microbiol. **14:**565–572.
410. **Weckesser, J., K. Hofmann, U. J. Jürgens, B. A. Whitton, and B. Raffelsberger.** 1988. Isolation and chemical analysis of the sheaths of the filamentous cyanobacteria *Calothrix parietina* and *C. scopulorum*. J. Gen. Microbiol. **134:**629–634.
411. **Weetall, H. H., B. Robertson, D. Cullin, J. Brown, and M. Walch.** 1993. *Bacteriorhodopsin* immobilized in sol-gel glass. Biochim. Biophys. Acta **1142:**211–213.
412. **Weissenborn, D. L., N. Wittekindt, and T. J. Larson.** 1992. Structure and regulation of the *glpFK* operon encoding glycerol diffusion facilitator and glycerol kinase of *Escherichia coli* K-12. J. Biol. Chem. **267:**6122–6131.
413. **Welch, T. J., A. Farewell, F. C. Neidhardt, and D. H. Bartlett.** 1993. Stress response of *Escherichia coli* to elevated hydrostatic pressure. J. Bacteriol. **175:**7170–7177.
414. **Whitley, P., and G. von Heijne.** 1993. The DsbA-DsbB system affects the formation of disulfide bonds in periplasmic but not in intramembraneous protein domains. FEBS Lett. **332:**49–51.
415. **Whitton, B. A.** 1987. Survival and dormancy of blue-green algae, p. 109–167. *In* Y. Henis (ed.), Survival and dormancy of microorganisms, 1st ed. John Wiley & Sons, Inc., New York.
416. **Whitton, B. A.** 1992. Diversity, ecology, and taxonomy of the cyanobacteria, p. 1–51. *In* N. H. Mann and N. G. Carr (ed.), Photosynthetic prokaryotes. Plenum Press, New York.
417. **Whitton, B. A., A. Donaldson, and M. Potts.** 1979. Nitrogen fixation by *Nostoc* colonies in terrestial environments of Aldabra Atoll, Indian Ocean. Phycologia **18:**278–287.
418. **Whitton, B. A., M. Potts, J. W. Simon, and S. L. J. Grainger.** 1990. Phosphatase activity of the blue-green alga (cyanobacterium) *Nostoc commune* UTEX 584. Phycologia **29:**139–145.
419. **Whyte, W., and L. Niven.** 1986. Airborne bacteria sampling: the effect of dehydration and sampling time. J. Parenter. Sci. Technol. **40:**182–188.
420. **Wiggins, P.** 1990. Role of water in some biological processes. Microbiol. Rev. **54:**432–449.
421. **Wildhaber, I., U. Santarius, and W. Baumeister.** 1987. Three-dimensional structure of the surface protein of *Desulfurococcus mobilis*. J. Bacteriol. **169:**5563–5568.
422. **Wilson, J. M., and D. M. Griffin.** 1975. Water potential and the respiration of microorganisms in the soil. Soil Biol. Biochem. **7:** 199–204.
423. **Winston, P. W., and D. H. Bates.** 1960. Saturated solutions for the control of humidity in biological research. Ecology **41:**232–237.
424. **Wolfe, J., M. F. Dowgert, B. Maier, and P. L. Steponkus.** 1986. Hydration, dehydration, and the stresses and strains in membranes, p. 286–305. *In* A. C. Leopold (ed.), Membranes, metabolism and dry organisms, 1st ed. Cornell University Press, Ithaca, N.Y.
425. **Wolfenden, R. V., P. M. Cullis, and C. C. F. Southgate.** 1979. Water, protein folding, and the genetic code. Science **206:**575–577.
426. **Wolk, C. P.** 1983. Heterocysts, p. 359–386. *In* N. G. Carr and B. A. Whitton (ed.), The biology of cyanobacteria, 1st ed. Blackwell Scientific Publications, Ltd., Oxford.
427. **Woodward, J.** 1985. Immobilised cells and enzymes: a practical approach. IRL Press, Washington, D.C.
428. **Wunderlich, M., R. Jaenicke, and R. Glockshuber.** 1993. The redox properties of protein disulfide isomerase (DsbA) of *Escherichia coli* result from a tense conformation of its oxidized form. J. Mol. Biol. **233:**559–566.
429. **Wynn-Williams, D., C. Ellis-Evans, and R. Leakey.** 1992. Microbial ecology in Antarctica. SGM Q. **Nov:**99–104.
430. **Xie, W.-Q., and M. Potts.** Unpublished data.
431. **Xie, W.-Q., B. A. Whitton, J. W. Simon, K. Jäger, D. Reed, and M. Potts.** 1989. *Nostoc commune* UTEX 584 gene expressing indole phosphate hydrolase activity in *Escherichia coli*. J. Bacteriol. **171:** 708–713.
432. **Yager, T. D., and P. H. von Hippel.** 1987. Transcript elongation and termination in *Escherichia coli*, p. 1241–1275. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
433. **Zimmerberg, J., and V. A. Parsegian.** 1986. Polymer inaccessible volume changes during opening and closing of a voltage-dependent ionic channel. Nature (London) **323:**36–39.