SYNOPSIS. Even though water is required for the maintenance of biological integrity, numerous organisms are capable of surviving loss of virtually all their cellular water and existing in a state known as anhydrobiosis. Over the past three decades we and others have established that disaccharides such as trehalose and sucrose are almost certainly involved in stabilizing the dry cells. We discuss here some of the evidence behind the mechanism of this stabilization. Until the past few years this mechanism has been sufficiently appealing that a consensus has been developing that acquisition of these sugars in the cytoplasm may be both necessary and sufficient for anhydrobiosis. We show here that there are other routes to achieve the effects conferred by the sugars and that other adaptations are almost certainly required, at least in environmental conditions that are less than optimal. Under optimal storage conditions, the presence of the sugars alone may be sufficient to stabilize even mammalian cells in the dry state, findings that are already finding use in human clinical medicine.

Water is usually thought to be required for the continuation of life, but numerous organisms are nevertheless capable of surviving complete dehydration. The dry organisms, said to be in a state of anhydrobiosis, may persist in this unique state for years or even centuries, but resume active metabolism when they are rehydrated. Some such organisms are familiar, since they include seeds of agriculturally important plants. Others, including many species of soil-dwelling micrometazoan are less familiar in this regard, even though van Leeuwenhoek reported this phenomenon three centuries ago (see Keilin, 1959 for an interesting historical account).

We set out decades ago to elucidate the mechanisms by which anhydrobiotic organisms escape irreversible damage from dehydration. One might think that the adaptations that are involved would be so complex that a lifetime of investigation might not unravel them. Instead, it appeared for some time that only a few adaptations might be involved. Indeed, the fundamental mechanism appeared to depend only on the accumulation of disaccharides in the cells and tissues of the organism during drying. In general, anhydrobiotic animals, such as Artemia, nematodes, and yeast cells accumulate trehalose (reviewed in Crowe et al., 1998), whereas the dehydration resistant tissues of higher plants (i.e., seeds and pollen grains) accumulate sucrose (e.g., Koster and Leopold, 1988; Hoekstra et al., 1992). There are exceptions, however; some resurrection plants, such as Myrothamnus flabellifolia accumulate a combination of sucrose and trehalose (Bianchi et al., 1993; Drennan et al., 1993). These sugars are capable of preserving membranes, labile proteins, and even whole cells (including mammalian cells) in the absence of water (reviewed in Crowe et al., 1998), findings that have led to applications in biology and medicine.

Nevertheless, it is emerging that other adaptations may be involved as well. In this essay we provide a summary of what we know about stabilization of biomaterials by disaccharides in the dry state, ask whether these mechanisms are universal in living things, and present more recent evidence concerning additional adaptations, particularly in plants, in keeping with the theme of this symposium. We will concentrate on membranes in the following discussion, but much of what is said applies equally well to labile proteins.

DISACCHARIDES AND STABILIZATION OF DRY MEMBRANES

Two stress vectors appear to be involved in destabilizing membranes during drying: fusion and lipid phase transitions (for a more complete discussion of material in this section see Crowe et al., 1997, 1998).

Fusion

If membranes are dried without sugars, they can be seen with electron microscopy to undergo extensive fusion. On the other hand, if they are dried in the presence of sucrose or trehalose, fusion is completely inhibited. A remarkably small amount of the sugars is required to stop fusion—as little as 0.1 g trehalose/g lipid. But much more—as much as 1 g trehalose/g lipid—is required to stop leakage of water soluble content of the vesicles. Thus, it appears that at least one other stress vector is involved.

Lipid phase transitions

The polar headgroups of phospholipids are hydrated; about 10 water molecules are associated with a typical phosphatidylcholine (PC) headgroup. The physical state of these water molecules is not well understood, but their removal has profound consequences.
for the physical state of the bilayer. They spatially separate the polar headgroups, and when they are removed, the packing density of the headgroups increases. This increased packing, in turn, leads to increased opportunities for van der Waals interactions among the hydrocarbon chains. As a result, the temperature at which the chains melt to form the liquid crystalline phase \( T_m \) increases. For example, fully hydrated egg PC has a transition temperature of about \(-7^\circ \text{C}\). When this phospholipid is fully dehydrated \( T_m \) rises to about 70°C. Thus, egg PC is in gel phase at room temperature when it is dry, and will pass through the phase transition when it is rehydrated.

The significance of this phase transition during rehydration is that when phospholipids pass through such transitions the bilayer becomes transiently leaky. Thus, the leakage that normally accompanies this transition must be avoided if the contents of membrane vesicles and whole cells are to be retained. During drying this need not be a problem since \( T_m \) is not affected until all the bulk water has been removed. But during rehydration it is a serious problem; the membranes are placed in water and will undergo the phase transition in the presence of excess bulk water, thus allowing leakage. In addition, and perhaps even more importantly, phase separation of membrane components can occur in gel phase, an event that is often irreversible.

When phospholipids are dried in the presence of sucrose or trehalose \( T_m \) is depressed to a remarkable degree. In the case of egg PC mentioned above, \( T_m \) is driven down as low as \(-20^\circ \text{C}\)—at least 10°C lower than \( T_m \) for the fully hydrated lipid and about 90°C lower than \( T_m \) for the lipid dried without trehalose. Thus, such membranes are in liquid crystalline phase at room temperature, even though they are dry, and will not pass through a phase transition during rehydration. Furthermore, since the bilayer is maintained in a liquid crystalline phase even when it is dry, phase separations that often accompany prolonged storage in gel phase are obviated.

This mechanism has been shown to apply to intact cells (Crowe et al., 1989; Hoekstra et al., 1992; Leslie et al., 1994, 1995) as well as the liposomes (reviewed in Crowe and Crowe, 1993) with which it was first described. For instance, dry yeast cells are known to require rehydration at elevated temperatures, above about 40°C. Leslie et al. (1994, 1995) have shown that membrane lipids in the dry yeast cells have a phase transition between 30–38°C. If the cells are rehydrated at lower temperatures, they leak their contents and are killed during the rehydration. But if they are hydrated at 40°C or warmer, they do not leak. Trehalose in the cells, Leslie et al. (1995) established, depresses \( T_m \) from about 70°C to 30–38°C.

**Vitrification and Protection Against Damage from Dehydration**

Disaccharides often form glasses (vitrify) at low water contents. The carbohydrate glass is a solid-like, amorphous material that prevents many diffusion limited deterioration processes such as membrane fusion. Vitrification is a common feature of carbohydrates, but the temperature at which the glass devitrifies \( (T_g) \) depends on many factors. In general, \( T_g \) increases with increasing molecular weight and decreasing water content (Slade and Levine, 1991). For instance, at a given temperature, the polymer hydroxyethyl starch (HES) can be in the vitreous state \( (\text{i.e., below } T_g) \) at a much higher water content than can glucose. The presence of the carbohydrate glass is necessary during storage in the dry state, because storage above the \( T_g \) of a system leads to fusion, membrane leakage (Sun et al., 1996), and loss of cell viability (Sun and Leopold, 1994, 1997; Buitink et al., 1998).

**Polymers and Their Role in Stabilization**

High molecular weight polymers usually have elevated \( T_g \)'s, but they generally are not effective in preserving dry membranes. For example, hydroxyethyl starch (HES) of molecular weight ca. 500,000 has a \( T_g \) in excess of 150°C. Based on \( T_g \) alone, one might expect HES to be an excellent protective excipient. In reality, however, neither HES nor another carbohydrate polymer, dextran, can protect unilamellar vesicles from leaking their contents due to drying and rehydration (Crowe et al., 1994, 1997b). This results from the inability of the polymers to interact directly with the membrane headgroups and drive down the dry membrane \( T_m \) (Crowe et al., 1994, 1997b; Tsvetkova et al., 1998). Conversely, glucose, which can interact directly with the lipid headgroups, but has a low \( T_g \) value and thus devitrifies at relatively low temperatures, cannot prevent fusion caused during dehydration (Crowe et al., 1997b). But while neither glucose nor HES alone can protect vesicles during drying, a 1:1 combination of glucose and HES is able to both decrease the dry membrane \( T_m \) and form a stable glass, thus inhibiting fusion and leakage (Crowe et al., 1997b). Both the direct interaction and the glassy state are necessary, therefore, for full protection of membranes during drying and rehydration (Crowe et al., 1994, 1997b; Tsvetkova et al., 1998).

Based on the findings reported above, one might expect to find polymers, which are good glass formers, in combination with sugars in nature in anhydrobiotes. Potts (reviewed Potts, 1994) and his colleagues have for several years been working on the structure of an extracellular polysaccharide (EPS) produced by the cyanobacterium, Nostoc in preparation for dehydration. These cells also contain small amounts of a mixture of sucrose and trehalose, but probably not enough to stabilize the dry cells. When we measured \( T_m \) in the dry cells, it was found to be similar to that of the fully hydrated cells (Hill et al., 1997). In model systems, we found also that the EPS did not depress \( T_m \) in dry phospholipids. Thus, we inferred that the sucrose and trehalose were responsible for depressing \( T_m \) in the dry cells. Based on the discussion above, we expected the EPS to be a good glass former and thus to behave like HES. Instead, we could find no evidence of a glass.
transition up to the decomposition temperature for the EPS. However, we instead were able to detect a gel-sol transition in this material in the range of 50°C. Based on these findings, we are suggesting that the gel may play a similar role as vitrification, a possibility that we are currently investigating.

Similar findings have been made in a study on desiccation in the bacterium, *Rhizobium* (Oliver et al., unpublished data). These bacteria can be freeze dried in the presence of trehalose with nearly 100% recovery, even when the cells are stored under very adverse conditions such as high humidities and temperatures. By contrast, when the cells were freeze dried with sucrose, they initially showed high survival, but, unlike the samples with trehalose their viability rapidly deceased when they were exposed to high humidities and temperatures. (The mechanism by which trehalose imparts this remarkable stability will be described later.) One strain, however, showed high stability when it was freeze dried with sucrose and stored under the same unfavorable conditions. Subsequently, it emerged that this strain, like *Nostoc*, produces an extracellular polysaccharide. Like the EPS of *Nostoc*, the one from *Rhizobium* also shows a gel-sol transition, but no evidence of a glass transition. As in *Nostoc*, we propose that the gel may play a role in inhibiting proximity events such as membrane fusion.

It remains to be seen whether polymers that form glasses are indeed found in anhydrobiotes. The phenomenon that comes closest is that some anhydrobiotic plants are now known to have an elevated *T*<sub>m</sub> so low that the hydrated cells have a *T*<sub>m</sub> so low that it does not rise above physiological temperature during drying. Such cells survive drying with minimal amounts of disaccharides present. However, in order to achieve this low *T*<sub>m</sub> the membrane lipids are highly unsaturated and are particularly susceptible to free radical attack (McKersie et al., 1988, 1990) and perhaps to enzymatic deesterification (Oliver et al., 1995, 1997). Such species have only a brief lifetime in the dry state. More recently, Linders *et al.* (1997) showed that a species of *Lactobacillus* can be dried, even though it contains only small amounts of disaccharides. When phase transitions were measured in the intact cells, *T*<sub>m</sub> was seen to increase by only a small amount during drying. It emerged that the lipid composition of the membranes is probably responsible for this effect. The membranes have a high concentration of charged lipids, which presumably show electrostatic repulsion and thus a limited rise in *T*<sub>m</sub> during drying. These kinds of adaptations appear to be exceptional.

Hoekstra and his colleagues have described insertion of water soluble amphiphiles in membranes of pollen during dehydration (Buitink et al., 2001; Golovina et al., 1998; Hoekstra et al., 1999). The evidence appears strong that this occurs both *in vitro* and *in vivo*, but its significance is still uncertain. On the one hand, *T*<sub>m</sub> is reduced by adding these compounds to the bilayer, but on the other hand the amphiphiles themselves increase permeability of the bilayer. This interesting set of investigations on the role of amphiphiles in higher plant anhydrobiotes are on-going in Hoekstra’s group, but similar studies have yet to be attempted in other anhydrobiotic organisms.

**DOES Trehalose HAVE SPECIAL PROPERTIES?**

In our initial studies on stabilization of membranes by trehalose, we reported that trehalose was superior to the other sugars tested. Subsequently, we showed that trehalose is among the most effective sugars tested at stabilizing liposomes during drying (reviewed in Crowe and Crowe, 1993), although other sugars later proved to be equally effective, particularly at high concentrations. There has been considerable confusion on this point. At elevated concentrations, the differences between the sugars tend to disappear, leading to confusion about their relative effectiveness.

Nevertheless, numerous workers have reported that trehalose seems to have special abilities in preserving dry and frozen biological materials. Leslie *et al.* (1994) found that bacteria freeze-dried in the presence of trehalose showed remarkably high survival immediately after freeze drying. Furthermore, we found that the bacteria freeze-dried with trehalose retained a high viability even after long exposure to moist air. By contrast, when the bacteria were freeze-dried with sucrose they showed lower initial survival and when they were exposed to moist air, viability deceased rapidly.

Using liposomes as a model, we attempted to find a mechanism for the results obtained with bacteria. As with the bacteria, the liposomes exposed to 58% relative humidity rapidly leaked their contents when they were dried with sucrose, but not when they were dried with trehalose. Measurements on fusion of the liposomes showed that they had undergone extensive fusion in the moist air when dried with sucrose, but not with trehalose.

Examination of the state diagram for trehalose (Crowe et al., 1996), provides an explanation for this effect. *T*<sub>g</sub> for trehalose is much higher than that for
sucrose, in qualitative agreement with previous results of Green and Angell (1989). (Green and Angell reported a significantly lower $T_g$ than we found, almost certainly due to an under-estimate of the water contents of their samples.) As a result, one would expect that addition of small amounts of water to sucrose by adsorption in moist air would decrease $T_g$ to below the storage temperature, while at the same water content $T_g$ for trehalose would be above the storage temperature. This proved to be the case. Furthermore, Aldous et al. (1995) have suggested an additional interesting property of trehalose, which we were able to confirm. They suggested that, since the crystalline structure of trehalose is a dihydrate, some of the sugar might, during adsorption of water vapor, be converted to the crystalline dihydrate, thus sparing the remaining trehalose from contact with the water. This suggestion emerged as correct; with addition of small amounts of water the crystalline dihydrate immediately appeared, and $T_g$ for the remaining glassy sugar remained unexpectedly high.

We point out, however, that the elevated $T_g$ seen in trehalose is not anomalous, as has been claimed (Green and Angell, 1989). Indeed, trehalose lies at the end of a continuum of sugars that show increasing $T_g$, although the basis for this effect is not understood.

THE LIMITATIONS OF PROTECTION BY SUGARS

Although disaccharides are of great importance in the protection of anhydrobiotic tissues and organisms (for some interesting exceptions, see Hoekstra et al., 1992), there is more to the story. While the sugars can enable survival of the initial drying and rehydration events, they are not sufficient for survival during prolonged periods in the dry state. Seed storage provides an instructive example. Even with their full complement of carbohydrates, and even under optimal conditions of low temperature and moisture, seed viability decreases during storage following a negative sigmoidal curve (Roberts, 1979; Priestly, 1986).

This loss of viability is correlated with an accumulation of free fatty acids (FFAs) in the cell membranes (Priestly, 1986; Senaratna et al., 1987). The FFAs, which are present at significantly higher concentrations in the membranes of senescent seeds, cause an increase in the membrane order and $T_m$ (Senaratna et al., 1987; McKersie et al., 1976; McKersie and Thompson, 1977). These changes lead to increased membrane permeability and loss of cell solutes, leading to a decrease in seed viability (Priestly, 1986; Crowe et al., 1989; McKersie et al., 1989). Aging in pollen also correlates with an accumulation of FFAs in addition to lysophospholipid (LPL) (van Bilsen and Hoekstra, 1993). In model membrane studies, the LPL molecules were found to phase separate from phosphatidylcholine (PC), causing domain formation in the liposomal membranes and leakage of entrapped solutes (van Bilsen et al., 1994). Accumulation of FFAs, therefore, is a damaging event, and organisms adapted for prolonged survival in the dry state must be able to inhibit this process.

ENZYMATIC DEGRADATION IS POSSIBLE AT LOW WATER CONTENTS

Release of FFA into a membrane could be caused by either enzymatic or free radical-mediated deesterification of membrane lipids. Because of the low water contents in dehydrated cells, the free radical-mediated degradation appeared to be the more likely candidate. Further, McKersie and co-workers (1988) found that the changes that occur in aged seeds could be mimicked in vitro by free radical treatment of model membranes. More recently, however, enzymatic deestefification began to emerge as a possible contributor to membrane degradation over time. The two are not mutually exclusive, and both enzymatic and free radical-mediated mechanisms may occur in vivo.

Oliver et al. (1995, 1997) have studied the possibility that enzymatic hydrolysis of membrane lipids could proceed at low water contents. Interestingly, both phospholipases C and A$_2$ (PLC & PLA$_2$) were capable of enzymatic activity under conditions of low hydration—as little as 2 molecules/molecule lipid (Oliver et al., 1997). This enzymatic activity proceeded at a relative humidity of only 20% (Oliver et al., 1997). As ambient relative humidities in nature are quite likely to exceed 20%, this finding indicates that FFA accumulation in seeds, or other desiccation-tolerant tissues, along with its concomitant effect on enzymatic mechanisms. In freeze-dried liposomes allowed to incubate at elevated RH, the appearance of FFA (the reaction product of PLA$_2$) over time was correlated with a decrease in retention of trapped solutes (Oliver et al., 1995). These studies were done with liposomes dried with trehalose, so clearly the sugar cannot prevent the enzymatic activity. Thus, Oliver et al. have sought a mechanism by which anhydrobiotes might control this adventitious activity.

ARBUTIN INHIBITS PLA$_2$ ACTIVITY AT LOW WATER CONTENTS

Arbutin is a glycosylated hydroquinone found in extremely high concentrations (20% of the dry weight) in certain resurrection plants able to survive almost complete dehydration for extended periods of time (Suau et al., 1991; Bianchi et al., 1993). The physiological role and sub-cellular location of arbutin are not known, but it is thought to be necessary for survival in the dry state. Based on the structural similarity between arbutin and the PLA$_2$ inhibitor p-bromophenacyl bromide, Oliver et al. (1996) investigated the possibility that arbutin might be capable of PLA$_2$ inhibition and established that it is indeed. When liposomes were lyophilized in the presence of PLA$_2$ and varying amounts of arbutin, then allowed to rehydrate at 76% RH for 5 hr, full enzyme inhibition was seen at an arbutin to lipid ratio of 0.5 mole/mole (Oliver et al., 1996).

Since no direct interaction between PLA$_2$ and ar-
butin could be detected and because arbutin did not inhibit PLA₂ under hydrated conditions, it appears most likely that arbutin inhibits PLA₂ by steric exclusion of the enzyme from its substrate. Several lines of evidence indicate that arbutin interacts with the lipid bilayer, specifically by inserting its phenol moiety into the hydrophobic domain (Oliver et al., 1996, 1998; Hincha et al., 1999).

**The Effect of Arbutin Varies with Lipid Composition**

Experiments with air-dried vesicles disappointingly showed that arbutin increased leakage from PC vesicles during drying and rehydration (Oliver et al., 1998). This loss of membrane integrity was seen even in the presence of disaccharides, casting doubt on the hypothesis that arbutin could function as a protective compound. Subsequently, the same group of investigators found that the effect of arbutin on membrane stability depends entirely on membrane lipid composition (Hincha et al., 1999).

Because the intracellular location, and thus target membranes, of arbutin have not been determined, isolated chloroplast thylakoid membranes were chosen as a well-characterized possibility to study the effects of arbutin on membrane stability. The effect of arbutin on isolated spinach chloroplast thylakoids and on vesicles containing the thylakoid lipid monogalactosyl diacylglycerol (MGDG) was monitored after a freeze-thaw cycle. Although freezing and drying are very different stress vectors (Crowe et al., 1990), freezing does include dehydration stress due to the sequestration of free water as ice, and therefore can provide important information relevant to the effect of arbutin on membrane stability.

Interestingly, arbutin protected isolated thylakoid membranes from freeze-thaw damage (Hincha et al., 1999). Furthermore, in vesicles composed entirely of egg PC, arbutin caused freeze-induced damage in a dose-dependent manner, with only 20 mM arbutin causing complete leakage of entrapped solute. In contrast, when 20% MGDG was included in the membranes, arbutin instead protected the vesicles in a dose-dependent fashion; 5 mM arbutin was sufficient to protect the vesicles maximally (Hincha et al., 1999). Thus, the effect of arbutin could be changed from cryotoxic to cryoprotective depending on lipid composition.

Accumulation of FFAs is a damaging event, and organisms adapted for prolonged survival in the dry state must be able to inhibit this process. It seems likely that molecules like arbutin (including, perhaps, the amphiphiles studied extensively by Hoekstra et al. [reviewed in Hoekstra et al., 1999]) might play such a role in plants. Thus far, the mechanism by which animals and microorganisms escape such damage is totally unknown. Furthermore, phospholipase activity may represent only one of many such adventitious enzymatic reactions that proceed at low water activities. Under ideal storage conditions this might not be a problem, but since anhydrobiotes in nature rarely experience such ideal conditions, they must possess mechanisms for dealing with the results.

**Can We Apply These Lessons from Nature to Other Cells?**

The lessons learned from even the superficial studies on biochemical adaptation to anhydrobiosis have already led to applications of some significance in human welfare. For example, human blood platelets have a lifetime in the blood banks of five days, after which they are discarded. The platelets are stored at room temperature, and cannot even be chilled below room temperature without damaging them—an effect due to a membrane lipid phase transition just below 20°C (Tablin et al., 1996; Crowe et al., 1999; Tsvetkova et al., 1999). Wolkers et al. (2001) have recently discovered that trehalose can be introduced into the cytosol of human blood platelets, probably by a temperature mediated endocytotic pathway. They discovered that when the trehalose-loaded platelets are freeze-dried an astonishing proportion survive—about 90%—all of which appear to be functional platelets upon rehydration. These findings are already under clinical investigation in animal models, with a view towards clinical testing in humans within the next two years.

The prospects of protecting other cells with trehalose appears promising. A remarkably successful attempt at using trehalose to preserve mammalian cells during freezing is that of Beattie et al. (1998), who discovered that the insulin-producing cells from mammalian pancreas have a membrane lipid phase transition well above the freezing point. Since membranes are known to become transiently leaky during the phase transition, Beattie et al. used that leakiness to introduce trehalose into the cells, which were then successfully frozen and kept frozen for extended periods. The thawed cells were transplanted into rats, where they were found to remain viable for many months. These findings are being developed into a commercial product, which, it is proposed, will provide a stable, transplantable device for treatment of diabetes.

A recent paper by Eroglu et al. (2000) is logically similar to that of Beattie et al. (1998), but with a clever new twist. Instead of using a lipid phase transition to introduce the sugar inside the cell, Eroglu et al. engineered a pore-forming hemolytic protein, α-hemolysin, so that the pore could be switched on and off. By substituting a number of residues with histidines, the pore could be regulated; by adding μM quantities of Zn²⁺ the pore could be closed, and by removing the Zn²⁺, the pore could be re-opened. Since the pore protein spontaneously inserts into membranes, Eroglu found that they could simply incubate the cells in its presence, add trehalose in the absence of Zn²⁺, and introduce the sugar via the pore. Using this procedure, they were able to obtain very high rates of survival of two lines of mammalian cells in the frozen state. In another recent paper, Guo et al. (2000) engineered the genes for trehalose synthesis into mammalian cells and had the cells make their own sugar. This is a fairly old idea that seemed attractive
from the start; trehalose synthesis requires only two enzymes, and thus only two genes. (The substrates for trehalose phosphate synthase are normal metabolites in virtually all cells, so substrate availability would not seem to be a problem.) The genes were incorporated into an adenovirus vector, which was then introduced into a mammalian cell line. With multiple infections, the trehalose biosynthesis increased. The cells, which were then dried to a level where free water was no longer detectable, retained viability for five days. It seems reasonable to suspect that the level of viability might be improved by altering the storage conditions. More recently, De Castro and Tunnacclife (2000) have called these results into question, and reported they obtained no viable cells after a similar transfection. While it may be true that the transfected cells do not survive complete drying, it is also possible that differences in the methods for drying may account for this discrepancy. In addition, Panek and her colleagues have shown that a trehalose transporter is required in yeasts to transport trehalose out of the cell during drying (Cuber et al., 1997). Since stabilization requires that trehalose be on both sides of the membrane to stabilize it (reviewed in Crowe et al., 1998), this is a way of meeting that requirement. The gene for this transporter has now been cloned (Stambuk et al., 1998), so there is no obvious reason why it could not be incorporated into the cassette already in use for trehalose synthesis by Guo et al. (2000), a treatment that might well extend the period of viability.

**SUMMARY AND CONCLUSIONS**

We have learned a great deal about the fundamental stresses from dehydration that anhydrobiotic organisms survive. Nevertheless, our understanding of the adaptations that permit these organisms to survive these stresses is still remarkably superficial, particularly considering the fact that this phenomenon has been known for three centuries. The organisms that experience this fascinating state of life must per force possess related adaptations; indeed, the nearly universal presence of disaccharides is an example that cogently makes this point. However, as we pointed out in this essay it is clear that other routes exist to achieve the same end and that other adaptations are, without much question, required. Are the adaptations likely to be of such complexity we will never understand them? Perhaps, but we doubt that will be the case. Indeed, we already understand that under ideal storage conditions a single adaptation—accumulation of disaccharides—may be sufficient to maintain cellular stability in the dry state, findings that are already being applied in the world of human welfare.

**ACKNOWLEDGMENTS**

The work described in this essay was supported by numerous grants from NSF, NIH, ONR, and DARPA over the past 30 yr.

**REFERENCES**


Hill, D. R., T. W. Keenan, R. F. Helm, M. Potts, L. M. Crowe, and...


