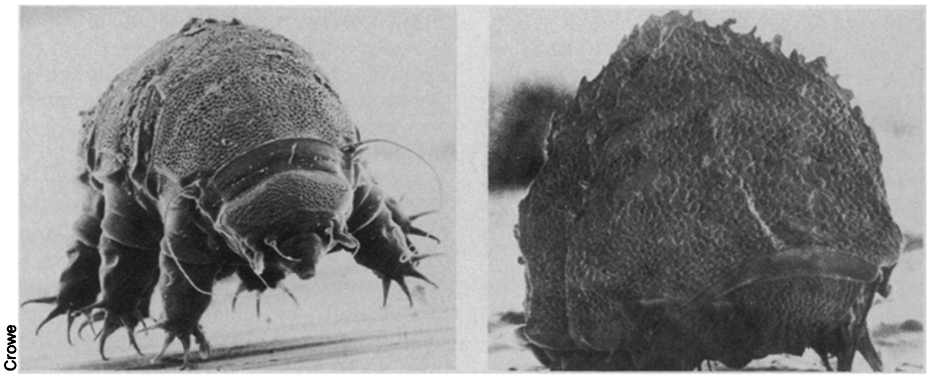
Death-Defying Dehydration

Sugars sweeten survival for dried-out animals, membranes and cells

By STEFI WEISBURD

Life without water seems an arid impossibility. After all, water is our main ingredient, making up 70 percent of the human body. Yet for some creatures, extreme dehydration is hardly the kiss of death; it's merely a way of putting active life temporarily on hold. From the desert resurrection plant to brine shrimp cysts to tiny, worm-like nematodes, there are scores of plants and animals that have learned to forgo nearly all of their body water, remaining suspended in a dry, death-like state for decades until they are revived with moisture.


Crowe

How dry I am: Under normal conditions this tardigrade, which is less than a millimeter long, contains 85 percent water by weight (left). But the animal, also known as a water bear, can survive even when water accounts for less than 2 percent of its body weight (right). This drought-induced dormancy also hardens the tardigrade against extreme heat and cold, strong vacuums, high doses of ionizing radiation and other damaging conditions of natural and laboratory environments.

"These organisms can be dry as dust," says biochemist John Crowe at the University of California at Davis. "You put them in water, and within a few minutes they're walking around. It's a downright mystical experience."

In the 25 years since Crowe revived his first desiccated microorganisms in college, he and his colleagues have unraveled much of the sophisticated biochemistry that enables these otherwise primitive animals and plants to survive periods of severe drought. He and other researchers are now borrowing and improving on nature's drought-resistant secrets to meet a variety of human needs. As a result of their work, scientists may someday be able to routinely "just add water" to reconstitute everything from freeze-dried orange juice to human sperm to blood substitutes used by combat medics in the treatment of wounded soldiers.

Crowe's work shows how "a clever guy who asks sensible and obvious questions about things he observes can sometimes find a trail into something interesting," notes John Baldeschwieler, a physical chemist at the California Institute of Technology in Pasadena and one of the scientists currently interested in applying Crowe's theories to engineer commercial products. "This is a super example of scientific inquiry at its best."

Crowe and his colleagues have been inspired by a zoo of organisms whose ability to enter a dry state of suspended animation and then to be revived by water is called anhydrobiosis. When moistened, many of these creatures rapidly swell and resume their full metabolic activities, often within minutes. In addition to the early life stages of several organisms—such as bacterial and fungal spores, baker's yeast, seeds of higher plants and some insect larvae — there are a number of bacteria and microscopic invertebrates that, throughout their lives, can survive dehydration. The tiny invertebrates include nematodes, which commonly inhabit moist soil, rotifers, which are primarily pond dwellers, and tardigrades, monstrous-looking creatures, which typically live in ponds and in the water films clinging to soil grains.

The ability to go dormant when the land goes dry holds an obvious evolutionary advantage for these creatures. How long they can stay in a dried state of suspended animation is not really known, however. After being kept dry for 120 years, some rotifers and tardigrades holing up in a piece of moss in London's British Museum were briefly revived when they were accidentally moistened. They staggered around a bit, then died. Had they been stored in an oxygen-free environment, says Crowe, their chances of survival might have increased, since oxygen interacts with membrane molecules, producing highly reactive molecules called free radicals, which destroy cell structure. "We've had some nematodes sealed up in tubes without oxygen since 1975 that still show a 80 to 90 percent survival rate," says Crowe. "But we really have no idea how long [dried specimens] can last."

Dehydrated dormancy also enables anhydrobiotic creatures to make a sort of Rip van Winkle leap in to the future, surviving well beyond their normal life spans. Scientists have estimated, for example, that without dormant periods a tardigrade would live for less than a year, while one that frequented the anhydrobiotic state might well reach the ripe old age of 60 years. Remarkably, dormancy also renders these organisms nearly impervious to ravages other than drought, including extreme heat and cold, strong vacuums and high doses of ionizing radiation.

But the real death-defying mystery has been how these creatures can survive at all in the absence of water. Water is thought to be essential to life because it maintains the three-dimensional structure of membranes, RNA, proteins and many other important biological molecules. One would expect that once water is removed, the structures would become distorted, cell contents would leak out, proteins would be denatured and the organism would die. Moreover, the amount of water these organisms lose during dehydration is phenomenal: In the case of a tardigrade, for example, water normally makes up 85 percent of its body weight, but the animal can live through periods when water accounts for less than 2 percent of its body weight.

The researchers in Crowe's lab came upon their first clue to the secrets behind anhydrobiotic tricks last decade when they noticed that the nematodes they were studying produced increasing amounts of a sugar called trehalose as the animals were slowly dried. To confirm that trehalose was the key, the group added it to a muscle membrane from the tail of a lobster, which normally would not survive severe drying.

"We found that [with trehalose] we could indeed preserve the functional and structural integrity of the membrane as it dried," says Lois Crowe, John Crowe's wife and co-worker. The researchers discovered that glucose and sucrose also protect against dehydration damage, although trehalose is more effective than these other sugars.

Since then, the Crowes and other researchers have tried to understand the molecular machinery of anhydrobiosis – how trehalose and other sugars interact with membranes and proteins to prevent dehydration damage. They've focused on liposomes, artificial membranes made of pure phospholipids. Phospholipids resemble two-tailed sperm, with their phosphate heads trailed by two hydrocarbon chains, and are among the many types of molecules normally found in biological membranes.

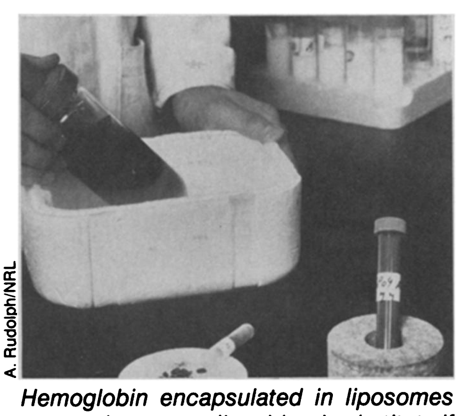
From a variety of studies, scientists have concluded that water molecules form a hydrogen-bonded network that separates the phospholipids' phosphate head groups from one another. When water is removed, the lipid heads and tails edge closer together, rearranging the membrane structure. One result may be that the membranes fuse together. Another possibility is that the entire membrane or some sections of it may change from a liquid state (called the liquid crystalline phase) into a solid (called the gel phase). In some cases, lipid reorganization can destroy the membrane-layer geometry by bunching the lipids into hexagonal arrays. Phase changes and fusion cause leakage of the carbohydrates, amino acids and proteins necessary to sustain a cell.

The Crowes and other scientists think that sugars such as trehalose save the day by acting as a kind of biological bookmark for the water. The hydroxyl (OH) groups on the sugars are thought to form hydrogen bonds with the phosphates, wedging themselves in between the head groups to maintain their spacing. This prevents fusion and keeps the membranes in a fluid phase even though they are dry.

Presumably, notes Lois Crowe, disaccharides such as trehalose (which is made of two glucose rings) and sucrose (which is made of one glucose and one fructose ring) are more effective protective agents than glucose and other monosaccharides because the former are larger molecules and so are better able to spread the lipids apart. And, adds John Crowe, it appears that the shape of the trehalose molecules makes them better protectors than sucrose.

The idea that the hydroxyl groups of the sugars directly bond to phospholipids is based on several lines of experimental evidence. It has been given further support by molecular modeling studies recently completed at the Naval Research Laboratory in Washington, D.C., by physical chemists Barbara Rudolph of Georgetown University in Washington and Indira Chandrasekhar of the Indian Institute of Science in Bangalore.

Starting with the atomic structures of glucose, sucrose, trehalose and one kind of phospholipid, these researchers performed a computer "docking" experiment. On the computer screen, they maneuvered the sugars and phosphate head groups together, searching for the most stable fit that maximizes the number of hydrogen bonds between the two kinds of molecules. The details unveiled by such molecular studies, says Rudolph, may help scientists to custom-design molecules that protect lipids against dehydration even better than do trehalose and the other sugars.



A. Rudolph/NRL

Hemoglobin encapsulated in liposomes may make an excellent blood substitute if it can be preserved well. Here a technician holds a sample about to be freeze-dried. A sample already freeze-dried with trehalose appears in the tube at lower left, and one that has been reconstituted with water is shown in the tube at lower right.

In addition to protecting liposomes, trehalose and other disaccharides have been found to protect proteins, especially enzymes, from dehydration, which can cause these molecules to unfold or fall apart. Again it appears that the hydroxyl groups of sugars form hydrogen bonds with proteins, but "we know far less about the molecular details of how these molecules interact," says John Crowe. "Proteins are a lot more complicated than phospholipids, and it will be a while before we fully understand what's going on here."

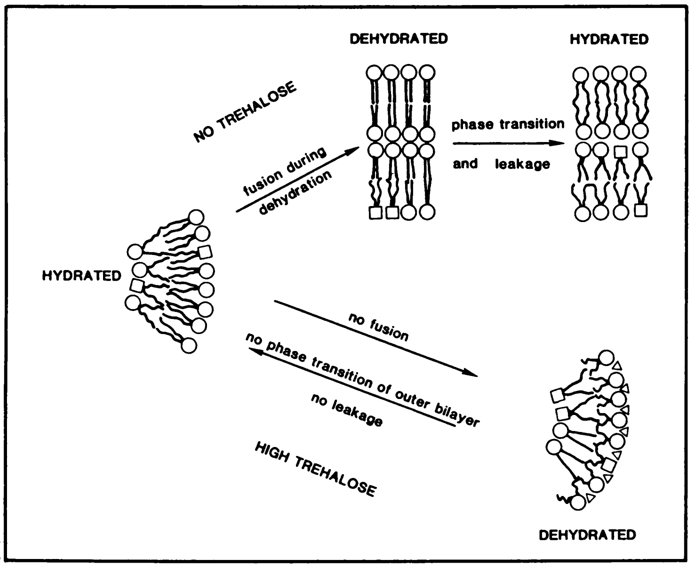
One added complexity is the Crowes' recent, accidental discovery that zinc (which was a contaminant in some of the trehalose samples used by the researchers) and some other ions added to a sugar enhance protein survival considerably, an effect that is not observed for the liposomes. For example, phosphofructokinase, an enzyme purified from rabbit skeletal muscle, is completely inactivated by one dehydration process. But the Crowes have shown that while a relatively high concentration of trehalose can result in a 50 percent recovery of the enzyme's activity, a much lower trehalose concentration added to some zinc ions restored more than 95 percent of the enzymatic activity. In general, they have found that the combination of zinc and sugars can produce protection when neither alone would do the job.

Now that scientists have uncovered some of the detailed biochemistry that makes the drought-resistant organisms tick, they are beginning to use that knowledge for human needs. The Crowes, for example, have applied for a patent on a trehalose-based process for preserving liposomes with freeze-drying. The resultant sugar-stabilized liposomes "can be reduced to a dry powder," says John Crowe, "and one only needs to add water and shake, and they're ready to use."

This process has caught the eye of Vestar, a Pasadena company that is developing a number of medical products using spherical liposomes to carry different agents into the body. Among these agents are imaging compounds for the *in vivo* diagnosis of tumors, antifungal agents and substances used in cancer chemotherapy. The ability to preserve such liposome-delivered products for extended periods is essential to their marketability, says Paul Schmidt, Vestar's senior director for research and development. "If the stuff is not stable, then doctors simply can't find ways to use it."

Schmidt thinks the Crowes' work "represents an innovative and imaginative approach to liposomal freeze-drying that will undoubtedly be useful." But while trehalose may be "a particularly efficacious additive in some cases, it may not be the best for all situations," he says, and Vestar is exploring a number of other protective additives as well.

Another medical area where the protective powers of trehalose and other sugars may find some use is the preservation of blood substitutes (SN: 9/26/87, p.200). The military in particular is keen on encapsulating hemoglobin – blood's oxygen-carrying protein – in liposomes that can then be freeze-dried and carried in a pack to treat combat casualties. One advantage of freeze-drying for the medics



Crowe

Liposomes are damaged when dried and then rehydrated, because the membranes fuse together and undergo a phase change. Both fusion and phase changes can cause membranes to leak. However, sugars (small triangles at lower right) lodge between the lipids in the liposomes, preventing both fusion and phase changes.

— who would have to carry the blood substitute into the field — is that the removal of water reduces weight and increases compressibility, according to Alan Rudolph, Barbara Rudolph's husband and a chemist at the Naval Research Laboratory.

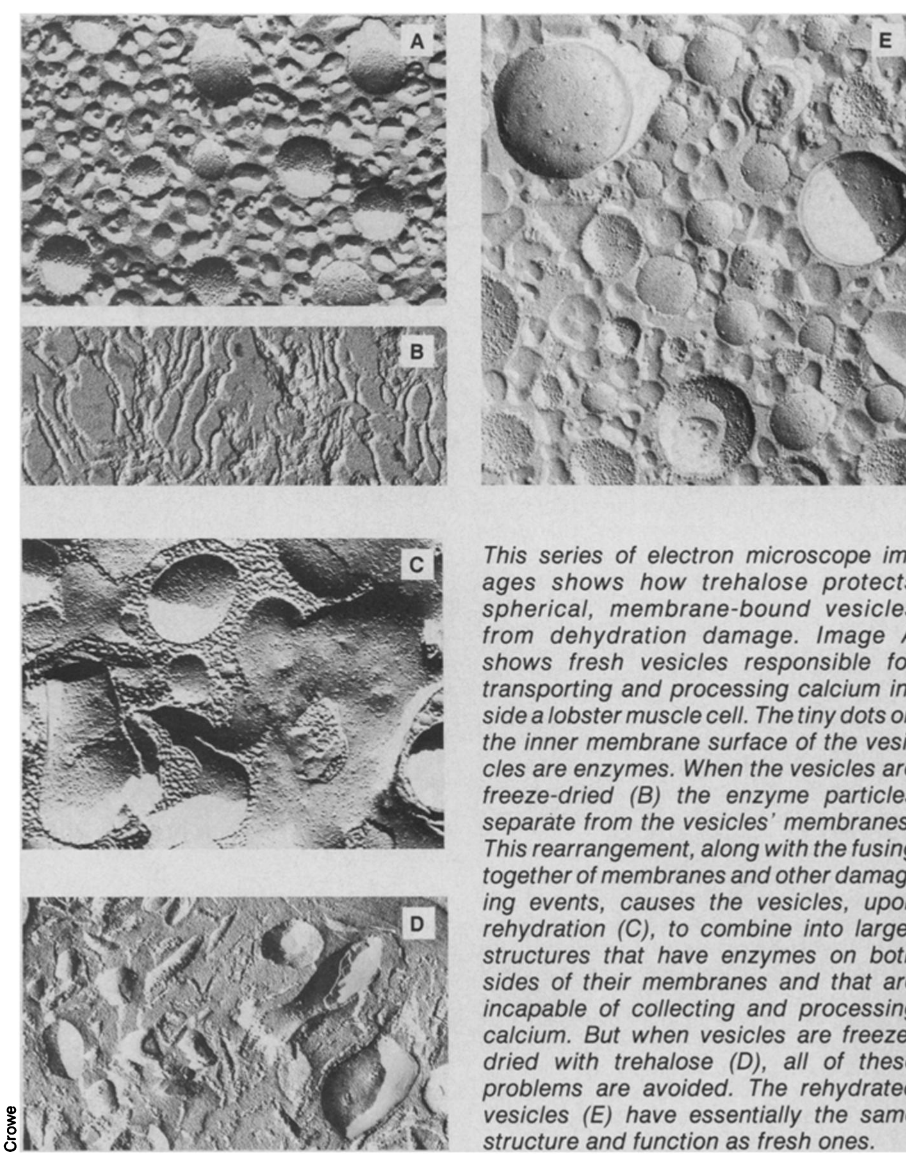
Alan Rudolph, who did his graduate work in the Crowes' laboratory, has been working on ways to use sugars to preserve both the liposome and the encapsulated hemoglobin molecules. In the samples his group has kept freeze-dried for several months, most of the liposomes have remained intact, keeping the hemoglobin inside. "Now we're trying to improve the condition of the hemoglobin following freeze-drying," he says. "We're losing 20 to 30 percent of the hemoglobin's [ability to carry oxygen] upon rehydration. We'd like to improve that and we're looking at ways to get more sugar inside the liposomes."

Before freeze-dried blood substitutes can be tried in humans, however, he says the toxicology of trehalose must be tested. "Nobody has really done those kinds of studies," he says, "and for that reason we're looking at sugars that may do as well but aren't necessarily as rare [as trehalose]." For example, sucrose's health effects are better known, and the common table sugar is also less expensive than trehalose.

One way of minimizing potential health effects and costs might be to reduce the amount of sugary additives required. At Caltech, Baldeschwieler and graduate student Raymond Goodrich have done just this. They have a filed a patent application for a method of directly and permanently attaching sugar molecules to cell surfaces by linking them through a chain of carbon and oxygen atoms to cholesterol molecules. Cholesterol is the primary ingredient in cell membranes and so is easily anchored into the membrane. In support of the Crowes' theory that sugars directly interact with phospholipids, Baldeschwieler and Goodrich say they have found that the carbon-oxygen "linker" must be long enough for the sugar molecules to cozy up to the phosphate head groups of the membrane.

The incorporation of sugars into membranes, the researchers believe, will afford better control of the freeze-drying properties of membranes and will eliminate the need for sugar additives. With this method, Baldeschwieler envisions someday being able to preserve many kinds of materials that have been difficult to freeze-dry, including orange juice and perhaps human tissues such as skin grafts. As for the use of sugar protectants in general, Crow adds to this list the preservation of human sperm and ova and other cells that researchers have had a hard time preserving through freezing. And on the very distant horizon, he says, lies the possibility of using sugar-like molecules to preserve entire organs for transplant.

Nearer at hand are many possible applications to aquaculture and agriculture. Understanding how sugars naturally protect against dehydration, says Lois Crowe, may help researchers to improve the survival of baker's yeast and brine shrimp and to dry encapsulated, pest-attacking nematodes that currently must be refrigerated to be kept alive (SN: 12/12/87, p.377).



Crowe

This series of electron microscope images shows how trehalose protects spherical, membrane-bound vesicles from dehydration damage. Image A shows fresh vesicles responsible for transporting and processing calcium inside a lobster muscle cell. The tiny dots on the inner membrane surface of the vesicles are enzymes. When the vesicles are freeze-dried (B) the enzyme particles separate from the vesicles' membranes. This rearrangement, along with the fusing together of membranes and other damaging events, causes the vesicles, upon rehydration (C), to combine into larger structures that have enzymes on both sides of their membranes and that are incapable of collecting and processing calcium. But when vesicles are freeze-dried with trehalose (D), all of these problems are avoided. The rehydrated vesicles (E) have essentially the same structure and function as fresh ones.

In a paper recently submitted to SCIENCE, the Crowes describe how sugars can prevent dry pollen grains from leaking their contents when they are rehydrated, a major cause of pollen and seed death. "We've been able to show that leakage is a membrane phenomenon involving a phase change [between gel and liquid crystalline states] in the membrane phospholipids," says John Crowe. Since the phase of the membrane is temperature-dependent, a dried grain in the gel phase can be converted to the liquid crystalline phase by heating. When the dried, heated grain is then placed in water, it stays in the liquid crystalline phase and survives. The sugars make this possible by reducing the temperature at which the pollen grains transform from the gel phase to liquid crystalline; with sugars, this transition temperature is about 30°C, but without sugars, the pollen grain must be heated to 60°C, a temperature that would destroy the grain.

Besides enhancing seed and pollen survival, the sugars' ability to tightly control the phases of many other kinds of molecular assemblies will give scientists a powerful tool for dictating other properties – such as sensitivity to light or whether or not a material polymerizes – of a wide range of biomaterials, Alan Rudolph believes. "The things we're now learning about the interactions between sugars and other molecules could in 20 years have an important impact on all these areas that involve the preservation of biological structures," he says.

While the Crowes and their contemporaries have made the most progress in extracting and using the dehydration secrets of anhydrobiotic creatures, they are not the first scientists to be fascinated with the phenomenon, which challenges the very notion of what it means to be alive. In 1702, the pioneering microscopist Anton van Leeuwenhoek first alerted biologists to the seemingly death-defying feats of tiny "animalcules" he had found in rooftop sediments. Writing to the Royal Society of London, he noted that the creatures became immobile and contracted into oval shapes upon drying, but that within half an hour of stirring the dried animals in a glass of water, he saw them swimming about.

For some years, many biologists believed that Leeuwenhoek's animals, and other animals that subsequently were shown to survive dehydration, actually died when they were dried and that they could be rescued from the clutches of death by water in a process that was then dubbed "anabiosis," or return to life. The existence of such animals became part of the arsenal of evidence used by some scientists in the 19th century to garner support for the doctrine of spontaneous generation, a now-defunct theory holding that life originates from lifeless matter.

Most modern scientists do not believe that dried animals revived by water have ever been dead. After all, some dehydrated animals, for a variety of reasons, may never spring back to life after moistening. "Does this mean they died while being dead?" asks John Crowe. In order to purge the anabiotic process of its spontaneous-generation taint, he says, scientists in this century renamed it cryptobiosis, meaning return to life, or anhydrobiosis, meaning life without water.

Still, he notes, scientists studying anhydrobiosis continue to grapple with the question of life or death. This is largely because one traditional measure of life has been metabolism – for example, do the anhydrobiotic animals consume oxygen and process other gases in their dried state? After numerous studies, says John Crowe, "the issue is still not fully resolved, although the weight of evidence says that [metabolism of dried animals] does indeed stop completely. So you get into a logical quandary if you define life in terms of metabolism."

What, then, is life? After probing around at the molecular level and seeing how sugars can act as structural understudies for water, John Crowe prefers instead to "define life as organized structure. As long as the structural integrity of the organism is intact, it's alive. When the structure is violated, it's dead." □

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Annual Review of Cell Biology, Vol. 3 – George E. Palade, Ed. Concentrates on topics central to cellular and molecular biology. Annual Reviews, 1987, 502 p., charts & graphs, $31.

Dinosaurs Past and Present, Vol. 2 – Sylvia J. Czerkas and Everett C. Olson, Eds. This lavishly illustrated volume concludes the investigations undertaken by a panel of scientists and artists at a symposium held at the Natural History Museum of Los Angeles County (see: SN: 10/4/86, p.216). The contributors here provide examples of how physical evidence can be interpreted, correctly or incorrectly, in the restoration of extinct animals. The final paper sheds additional light on the demise of the dinosaurs. U of Wash Pr, 1988, 149 p., color/b&w illus., $35.

Frogs & Toads of the World – Chris Mattison. A comprehensive overview of these fascinating and diverse creatures. They range from the largest known frog, the Goliath from West Africa, which can exceed 12 inches in length, to a frog found in Brazil that is 9.8 mm or 0.386 inch. Well illustrated, this book discusses the 21 families of frogs and toads, their life histories with their metamorphosis from tadpoles to frogs, their physiology, reproduction, food and feeding enemies and defense, distribution and movement. Facts on File, 1987, 191 p., color/b&w illus., $22.95.

Plants Plus – George Seddon and Andrew Bicknell. Explains in detail various forms of plant propagation for the indoor and outdoor gardener. Illustrates and describes annuals, herbaceous perennials, shrubs, bulbs, vegetables, herbs, soft fruit, bromeliads, cacti and succulents, ferns and orchids and gives the preferred method for propagation for each different plant. Rodale Pr, 1987, 160 p., color illus., paper, $14.95.

Portraits of Earth – Freeman Patterson. "Just as we study a face in order to know a person," says the author, "so we examine an earthscape ... in order to understand Earth itself." The magnificent photographs of breathtaking beauty express Patterson's deep love of the natural world. In the text he takes the readers behind the lens of the camera to show earth through his eyes and to help the readers sense its shapes, its lines and its textures. Sierra, 1987, 180 p., color illus., $35.

The Secret House: 24 Hours in the Strange and Unexpected World in Which We Spend Our Nights and Days – David Bodanis. An account of the astonishing physical and biological events that take place in a house during our sleeping and waking hours. Much of what is described is invisible to the naked eye or inaudible to the human ear. Reveals how processed foods are made, how polyester shirt fibers create an electrostatic field that guarantees dirty collars and cuffs, why potato chips snap, why pantyhose rip and a host of other interesting facts. Illustrated with electron micrographs, thermographs and computer-enhanced X-rays that give a unique view of the world around us. Originally published in hardback in 1986. S&S, 1988, 224 p., color/b&w illus., paper, $9.95.

The Tanagers: Natural History, Distribution, and Identification – Morton L. Isler and Phyllis R. Isler. With the exception of four species that breed in the United States, most tanagers dwell in the dense and often remote forests of Central and South America. In preparing this book, the authors observed tanagers in the field, compiled information from more than 1,000 references, studied museum specimens and gathered unpublished behavioral data from many contemporary ornithologists. A map accompanies each species account, and the 32 color plates illustrate 551 plumages and 23 flight patterns. The purpose of the book is to encourage field study and conservation of tanagers and their habitats. Smithsonian, 1987, 404 p., color/b&w illus., $70, paper, $49.95.