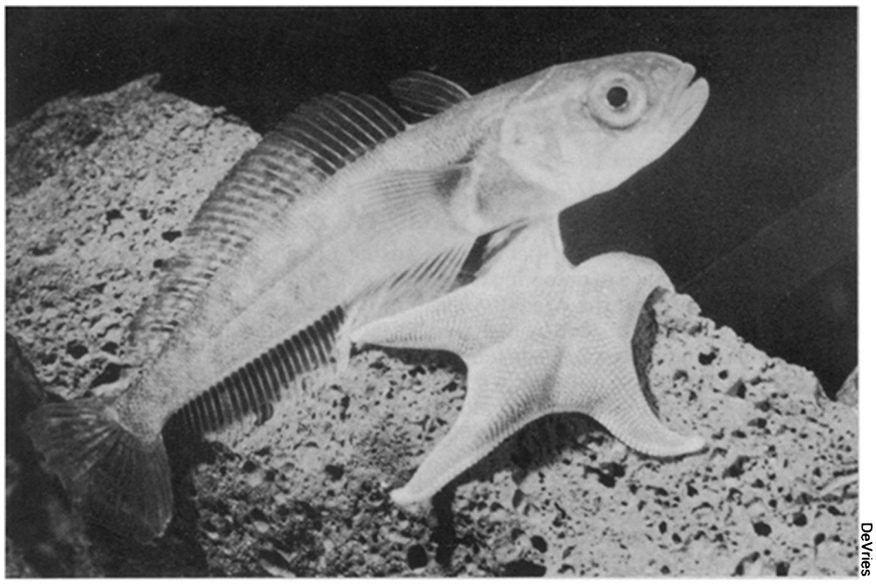


DeVries

This perchlike fish, Trematomus borchgrevinki, *produces an antifreeze in its blood to keep from freezing in ice-cold Antarctic water.*

A Biological Antifreeze

Antifreeze proteins found in the blood of polar fish alter the way ice crystals grow

By IVARS PETERSON

The chilly waters of polar oceans harbor many fish that seem to be immune to freezing. These Arctic and Antarctic species are cold-blooded — their body temperature matches that of their environment. Yet they swim through water laden with ice crystals or lurk in icy underwater caverns. In this ice-cold water, their body fluids and tissue should freeze, but don't.

To keep from freezing, it turns out that polar fish produce and store in their blood a set of large protein molecules that have powerful "antifreeze" properties. The addition of these antifreeze proteins lowers the blood's freezing point to a temperature below the freezing point of seawater.

These antifreeze proteins, however, don't behave like commonly used antifreeze additives, such as ethylene glycol, sodium chloride and other salts. They have the unique property of lowering the blood's freezing point while having little effect on the frozen blood's melting point.

As a result, fish blood carrying these special proteins has a freezing point that is lower than its melting point — an effect not seen with other antifreeze compounds. Protein-carrying fish blood, then, could freeze at, say, -2° C, and once frozen, may melt at -1° C, a somewhat higher temperature. In contrast, salts typically depress the freezing and melting points equally.

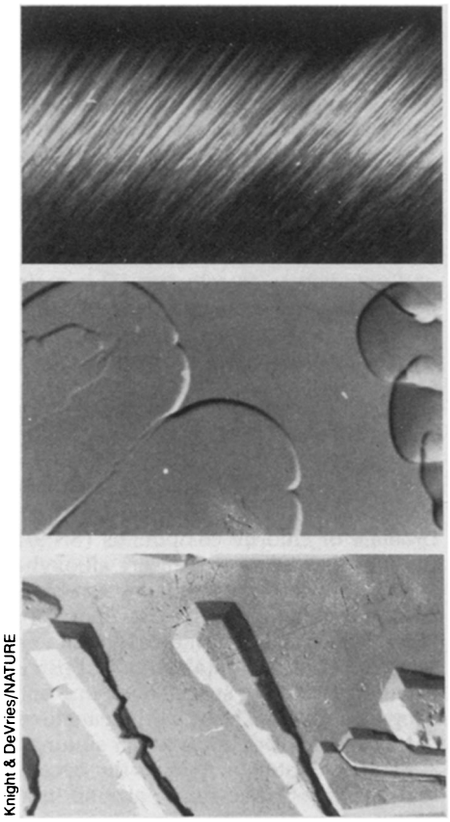
Although scientists have known about these unusual antifreezes for more than a decade (SN: 4/21/73, p.257; 8/23/75, p.124), they're still not sure how the proteins work. "The problem was well defined in the 1970s," says Charles A. Knight of the National Center for Atmospheric Research in Boulder, Colo. "It's the kind of thing that one would think ought to have been solved by now, but it hasn't been. The question of how the stuff really works is wide open."

Recently, researchers interested in crystal growth in general have started to take a closer look at the action of antifreeze proteins. "If one could learn a bit more about it," says physicist John Hallett of the Desert Research Institute in Reno, Nev., "it might provide some insight into the way ice crystals grow anywhere" — from the ionosphere to ice cream.

Several new results, along with some research reported a few months ago at an American Chemical Society meeting in Anaheim, Calif., provide important hints about how antifreeze proteins may prevent crystals from growing. Some of these studies show how ice crystal shape is altered when protein-bearing liquids freeze. Other recent experiments focus on the way ice crystals scatter light when an antifreeze protein is present.

When water is cooled to its freezing point, ice begins to form around any nucleus — a dust speck or microscopic ice crystal — suspended in the liquid. The addition of simple salts like sodium chloride or substances like glucose lowers the liquid's freezing point. These particles apparently get in the way of the jostling water molecules. The more particles there are, the less likely it is that water molecules will aggregate to form an ice crystal. As the salt concentration increases, the freezing point decreases further.

As a result, seawater has a lower freezing point than pure water. Fish, however,



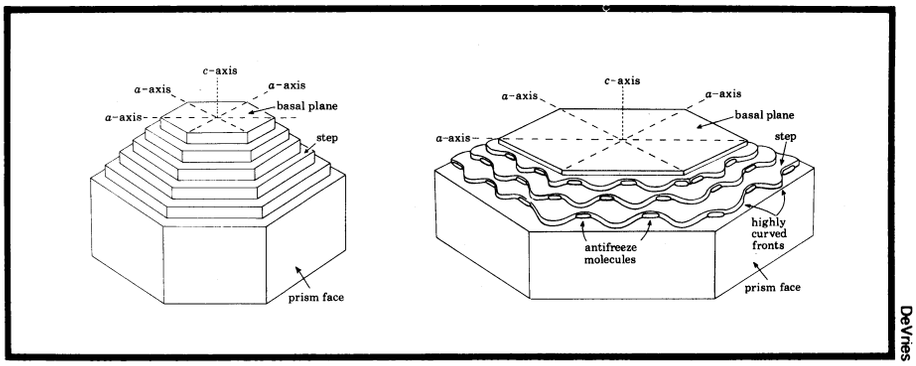
Knight & DeVries/NATURE

Antifreeze glycopeptides affect the way ice crystals grow in a film of supercooled liquid between glass plates. When a 1-percent glycopeptide solution freezes, needlelike ice crystals form (top). In contrast, pure water freezes into the pattern shown in the middle photograph. Unusual crystal faces appear when the glycopeptide concentration is very low (bottom).

can't stand such a high salt concentration in their blood. Although their blood contains some salts, they need an additional additive to keep from freezing in polar waters. That's where antifreeze proteins come in.

These proteins, first isolated from a perchlike Antarctic fish by physiologist Arthur L. DeVries, now at the University of Illinois at Urbana-Champaign, are made up of amino acids and sugars linked to form long, bristly chains. Molecules contain 12 to 150 amino acids, strung together in a repetitive pattern, with sugar components that branch out from the central chain. The sugar portions carry numerous hydroxyl (OH) groups that tend to interact with water molecules. These glycopeptides, as they are called, can be found in blood and other fluids inside almost all fish that inhabit polar waters.

Unlike salts, however, the glycopeptides substantially lower only the freezing point and have little effect on the resulting solid's melting point. Moreover, the freezing-point depression isn't directly proportional to the amount of glycopeptide dissolved in the blood. Instead, its effectiveness peaks when antifreeze levels reach about 4 percent by weight. This suggests that an antifreeze glycopeptide does not affect ice crystal growth in the same way salts do.



DeVries

Ice crystal growth occurs when water molecules join the crystal at growth steps on the basal plane (left). Adsorption of antifreeze molecules at steps on the basal plane appears to block growth in the region where the antifreeze covers the step (right). One result is the growth of fine, needlelike crystals in the direction of the c axis.

The adsorption of antifreeze glycoproteins on an ice surface, says biochemist Robert E. Feeney of the University of California at Davis, could inhibit ice growth by producing a barrier between the ice surface and water molecules. "Crystal growth can occur only if the solution temperature is lower than some critical value at which the barrier can be overcome," he says.

DeVries has a similar view. Glycopeptides, he says, may inhibit the formation of large, biologically disruptive ice crystals by coating certain faces and edges on minute ice crystals that happen to get into a fish's body. As these molecules drape themselves over a crystal surface, ice is forced to grow only in the small spaces between them.

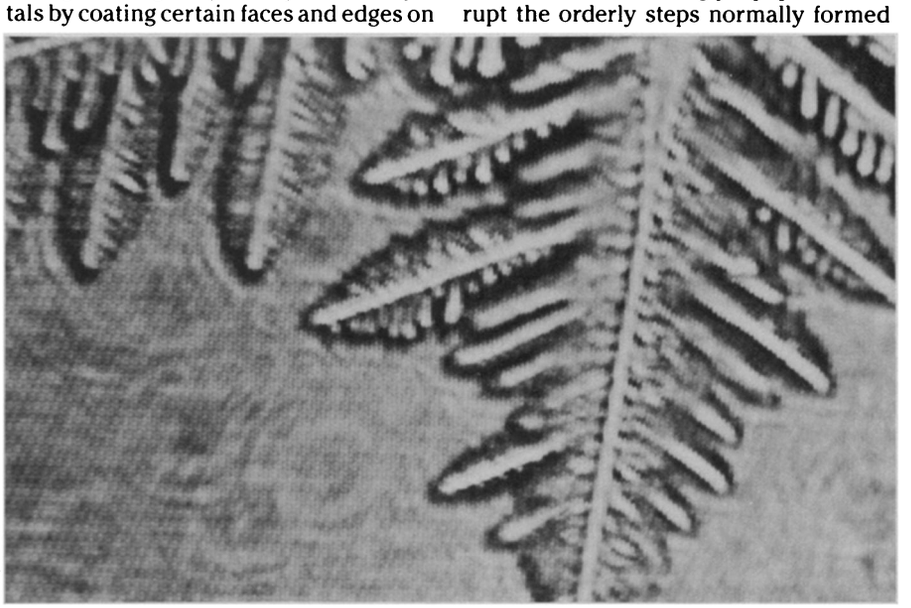
The researchers differ, however, over how firmly the antifreeze proteins bond to ice surfaces. Feeney and his colleagues see a dynamic process involving a competition between the rates at which water and protein molecules either arrive at or leave an ice crystal surface. DeVries and his co-workers assume that protein molecules, once settled into place, tend to stay in those locations.

These hypotheses are difficult to confirm. "There's no good method for looking at what goes on at the ice-water interface," says DeVries. That means doing experiments that provide clues indirectly.

DeVries and his colleagues, for example, have looked at the way ice crystals in an antifreeze glycopeptide solution scatter light. They observed that the ice surfaces appear to get rougher as the temperature of a protein-containing solution is lowered. This is consistent, says DeVries, with the idea that the glycopeptides disrupt the orderly steps normally formed by layers of water molecules settling into place to create a stack of hexagonal plates.

"We think that the binding [of the antifreeze] to the ice divides the growth steps into many small steps that become highly curved," says DeVries. Water molecules that find themselves at the edges of these steps would have fewer neighbors with which to bond, a condition that essentially stops crystal growth, unless the temperature of the surrounding liquid falls.

"What we'd like to know is what the configuration of that surface really is," says DeVries. "That's a hard thing to do because you can't look at the surface of ice with an electron microscope."


K. Harrison

Ice crystals, when free to grow in pure water, normally create dendritic patterns.

No one yet knows exactly how antifreeze molecules bind to an ice crystal. One possibility is that some of the glycopeptide hydroxyl groups line up with the oxygen atoms found at the "corners" of hexagons in rings formed by the arrangement of water molecules in ice crystals. Alternatively, the carbonyl (CO) groups sitting in a peptide chain may also bind to a crystal at various sites.

Another question concerns the shape of these antifreeze glycopeptides in solution. Recent measurements made with nuclear magnetic resonance indicate that a typical protein is neither an extended rigid rod nor a randomly twisted coil. Instead, the molecule may be like a flexible rod, coiled in a fairly regular manner so that all the sugar groups branch out from one side.

The discovery that glycopeptides change the way ice freezes provides some useful clues. "When ice does grow, it begins to grow abnormally," says Feeney, who has been working with Hallett and several colleagues and students to document the effects of antifreeze proteins on ice crystal structure.

Normally, ice crystals formed from pure water look a lot like snowflakes. In a glycopeptide solution, such ice crystals lose their hexagonal form. Instead, long needles grow out from the crystals, even

when the antifreeze amounts used are so small that the freezing point isn't lowered significantly.

"This tells us that [the glycopeptide] is interfering with the surface of the ice at the molecular scale," says Hallett. Moreover, the crystal shape change means that the antifreeze proteins affect some crystal faces more than others. Hence, crystal growth occurs only in certain directions because the glycoproteins prefer to land in certain, well-defined spots on an ice crystal.

When an antifreeze solution is supercooled below -5° C before crystallization is allowed to occur, ice crystals again take on their usual hexagonal habit. In this case, says Hallett, the ice crystal layers form so quickly that the bulky glycopeptide molecules fail to respond soon enough. "You can argue that the water-molecule process is beating the antifreeze process," he says. "Once growth starts at the lower temperature, there just isn't time for new antifreeze glycoprotein molecules to diffuse in and block all the sites."

Knight and DeVries have looked at the same effect when ice crystals are grown from solution on the surface of glass plates. They observed that under certain conditions, antifreeze-contaminated crystals show gem-like faceted faces, which don't normally show up in ice crystals formed in pure water.

Knight and other researchers have also noticed that the presence of minute amounts of antifreeze glycopeptides helps prevent recrystallization — a result that has caught the attention of ice cream manufacturers. Recrystallization occurs when the temperature of a frozen mass increases but still remains below the material's melting point. Under these conditions, large ice crystals grow at the expense of small ones. That changes a product like ice cream from a smooth, creamy delight into a prickly, unpalatable concoction.

"It happens quite quickly in ice at temperatures not too far below freezing," says Knight. Nevertheless, "just the tiniest amount of this stuff stops that with spectacular efficiency."

The problem is that there isn't much antifreeze glycopeptide around. Researchers are looking for other materials that have similar properties. Some insects, for instance, may produce such an antifreeze compound.

Finding simpler molecules that perform in the same way as fish blood glycopeptides would make it easier to study and understand these materials, says Knight. "If you understand how they work, then you could say this sort of molecule is going to do it and that one isn't. It's that fundamental knowledge which is lacking." □