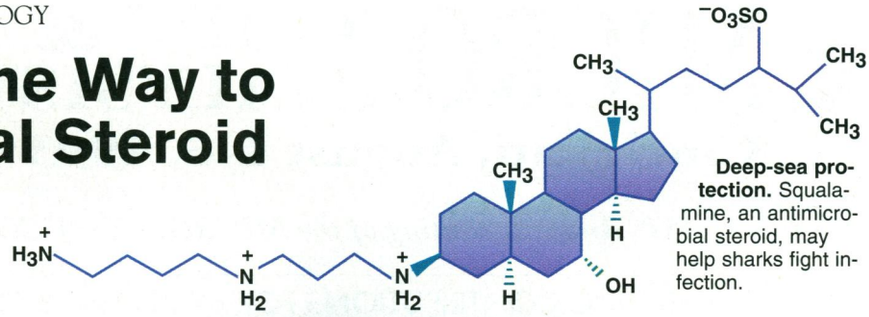
MICROBIOLOGY

Déjà Vu Guides the Way to New Antimicrobial Steroid



Deep-sea protection. Squalamine, an antimicrobial steroid, may help sharks fight infection.

After giving a guest lecture at the Mount Desert Island Biological Laboratory in Maine in July 1989, molecular biologist Michael Zasloff found himself watching dogfish sharks swim circles in a holding pen. As Zasloff listened to a marine biologist describe how pregnant sharks flush their fallopian tubes with seawater to get rid of fetal waste, he wondered whether the sharks had any way to sterilize the microbe-filled water that soaked their fetuses. As he watched, déjà vu came over him: Only a couple of years earlier Zasloff had pondered why African clawed frogs with fresh surgical wounds rarely became infected in murky holding tanks—and he'd quickly discovered the magainins, a family of peptides present in frog skin that kill bacteria, protozoa, and fungi. Could sharks be producing a similar antimicrobial compound?

The answer is now in, and it's yes. Zasloff and graduate student Karen Moore of the University of Pennsylvania report in the 15 February issue of the *Proceedings of the National Academy of Sciences* the isolation of a compound found in sharks that kills a broad range of microorganisms. This compound turns out to have a surprising structure: It's a steroid. Although a handful of antimicrobial steroids have been isolated from plants, it's the first time one has been identified in an animal. And its discovery may shed some light on a persistent puzzle concerning how the shark defends itself against microbes.

That's intriguing enough. But what really has medical scientists buzzing is the fact that the agent, which the researchers have dubbed squalamine because it was isolated from the dogfish shark, *Squalus acanthias*, is good at killing *Candida*. *Candida* is a fungus that causes stubborn infections in people whose immune systems are suppressed—including AIDS and cancer patients. "Systemic fungal infections are the bane of the existence of oncologists, and we don't have good treatments for such infections," says Franklin Epstein, a nephrologist at Harvard Medical School. The main problem is that the antifungal drugs now on the market tend to be toxic. Squalamine's structure suggests that it won't be, says Epstein, but its toxicity and effectiveness have yet to be tested in mammals.

After Zasloff's Proustian moment at Mount Desert, he and Moore wasted little time in trying to isolate a shark antimicrobial. They turned to the dogfish shark, a widely studied species, and within months isolated a compound from the animal's stomach, as well as from various other organs, that proved very effective in antimicrobial assays. In culture, squalamine is about as potent as ampicillin, a widely used antibiotic, at killing bacteria such as *Escherichia coli* and strains of *Staphylococcus* and *Streptococcus*.

BARBARA KENT/MOUNT DESERT ISLAND BIOLOGICAL LABORATORY

Based on his previous experience with the magainins, Zasloff expected the new anti-microbial to be a peptide, but chemical analysis quickly proved otherwise. Using mass spectroscopy and nuclear magnetic resonance, Zasloff and Moore showed that the molecule has two chemical constituents that are no strangers to the animal kingdom: a steroid that resembles a molecule in the cholesterol-synthesizing pathway, and the other a compound called spermidine, an amine that interacts with nucleic acids and may play a role in stabilizing membranes.

Aside from its potential as a human therapeutic agent, squalamine has shark biologists excited: They see the steroid as a possible "missing link" in the shark's defenses against infections. Those defenses have long perplexed some biologists, who contend that the animal's immune system is not up to the job of protecting it against infections. Sharks produce an extremely limited repertoire of antibodies compared to those of higher vertebrates, many researchers argue. "You can almost imagine the shark as an immunocompromised creature," Zasloff says. The upshot of this is that scientists have long been searching for other means the animals might use to ward off infections.

The discovery of squalamine now "puts the shark's immune system in a different perspective," says immunologist Louis Du Pasquier of the Basel Institute for Immunology in Switzerland. Squalamine, says Du Pasquier, might serve the shark as a frontline defense against pathogens, killing them directly before cells in the immune system even see them. He speculates that the steroid defenses may come into play when sharks are swimming in cold water and their body temperatures drop, making their immune systems even less effective. Carl Luer, a molecular biologist at Mote Marine Laboratory in Sarasota, agrees that squalamine may provide a defense in addition to antibodies, although its exact role is unclear.

Still, other scientists take issue with the idea that squalamine necessarily plays a key role in shark disease defenses. "The shark's immune system is more potent than people think—their natural levels of antibodies against virtually everything are very good," contends John Marchalonis, a molecular immunologist at the University of Arizona. He says that squalamine may, perhaps, play a regulatory role in the shark if it interacts with immune cells. The reason, he says, is that squalamine could act like corticoid steroids, which are known to kill T cells in response to stress. Zasloff acknowledges that squalamine could act this way, but, he argues, it may also be able to kill microbes directly, perhaps by perforating their membranes, the way magainins do.

For the time being Zasloff is going to leave it to immunologists to figure out squalamine's biological role. Zasloff, who's now president of the research arm of Magainin Pharmaceuticals Inc., based in Plymouth Meeting, Pennsylvania, says his main concern is developing the steroid into a drug. One problem his lab will tackle, he says, is figuring out how squalamine kills microbes. He also wants to fully characterize the steroid's antibiotic activity as well as look for related compounds that may also have antimicrobial activity. Squalamine or its kin may even be present in humans, he says. Noting that it's made of two ubiquitous compounds, Zasloff concludes, "The chances of it not being found elsewhere are pretty slim."

–Richard Stone