Snakebite Succor

Researchers foresee antivenin improvements

By RICK WEISS

In Baltic mythology, hell consists of an icy hall lined with snake heads, their jaws dripping a river of cold venom in which the damned must forever wade and swim.

In Egyptian mythology, the sun god Ra complained bitterly about getting bitten by a snake before he had even finished creating the Earth.

And everybody knows who takes the blame for that fateful offering of the Fruit of Knowledge. Snakes, it seems, have always mystified, and their bite has long been synonymous with misery and misfortune.

One might expect things to be different here in the 20th-century United States. After all, snakebite kits are available in camping stores everywhere. And emergency-room refrigerators sit stocked with vials of life-saving antivenins — specific antibodies that neutralize the poisonous proteins in venom — ready for injection into anyone unfortunate enough to need them.

But while few people in this country actually die from snakebites, physicians and scientists familiar with the situation say U.S. venomous snakebites remain a more serious health threat than many people realize. That's because the two commercially marketed antivenins (sometimes called antivenoms) for U.S. snakes cause painful and sometimes serious reactions in the vast majority of treated snakebite victims, researchers say. And until recently, no drug company was even trying to make a better product.

"I'm very sorry for the average snakebite victim in America," says Struan K. Sutherland, an antivenin expert at Commonwealth Serum Laboratories in Parkville, Australia. "We're of the opinion that management of snakebite in America is in complete chaos."

Sutherland doesn't stand alone in this opinion. Many snake-venom experts in the United States and England concur that while neither of the U.S. snake antivenins poses a life-threatening risk, both remain far from ideal — triggering a generalized and sometimes severe immunological reaction called serum sickness in about 75 percent of recipients. Unfortunately, they say, a combination of scientific challenges and the high costs of developing drugs for a limited market have conspired over the decades to prevent competition for those products, both made by Wyeth-Ayerst Laboratories in Philadelphia.

Now, two teams of scientists working separately have embarked upon serious efforts to change that. Using chicken eggs or sheep's blood rather than horse serum — the traditional source of antivenins — and high-tech purification techniques, each team hopes to enter and succeed in the small but potentially lucrative market for a better U.S. antivenin.

In the October BIO/TECHNOLOGY, Sean B. Carroll and his colleagues at the University of Wisconsin-Madison describe their efforts to produce rattlesnake antivenin in chicken eggs. They estimate their antivenin is 20 times purer than the equivalent horse-antibody product made by Wyeth.

Moreover, they note, chicken-based antibodies cannot trigger the highly inflammatory allergic cascade in humans that horse proteins can. "Antivenoms purified from chicken eggs may be pharmaceutically safer and more economical to produce than current horse antivenoms," they conclude. Working with a Madison-based drug company, Ophidian Pharmaceuticals Inc., they hope to begin FDA-approved human testing of their product sometime next year.

Meanwhile, equally enticing claims about a new sheep-derived product come from Findlay E. Russell, a herpetologist at the University of Arizona in Tucson, who is collaborating with other researchers to make a better North American snake antivenin. "Our product should be ten times more effective than the Wyeth material and should not create any [adverse] reaction," Russell says. In conjunction with a Nashville, Tenn.-based pharmaceutical concern, Therapeutic Antibodies Inc., the researchers hope to begin human testing in January.

Many experts deem these attempts at improvement long overdue. Says David Theakston, an expert in snake venoms at the Liverpool School of Tropical Medicine in England: "I've always told people that if they wanted to make money they should move to the United States and compete with Wyeth."

Russell

In the United States, the southern copperhead (Agkistrodon contortrix contortrix) *ranges as far north as southern Illinois and as far west as Texas. Copperheads can live more than 20 years and grow up to 1 meter long. Their bites, while representing a substantial fraction of venomous snakebites in the United States, are rarely fatal.*

The United States is no hotbed of venomous snakes, and even those most critical of U.S. antivenins quickly point out that from a global perspective, the U.S. situation is hardly catastrophic. Of the 115 species of snakes in this country, about 20 are dangerous, including 16 species of rattlesnakes, Russell says. Rattlers account for about 65 percent of the 8,000 venomous snakebites that occur here each year and for nearly all of the resulting nine to 15 deaths. A smaller fraction of bites comes from copperheads, fewer still come from cottonmouths and only three or four bites per year come from coral snakes.

Of course it's not the bite itself, but what's in it, that makes all the difference to someone who's bitten. Scientists have identified more than 100 proteins in rattlesnake venom, including potent tissue-degrading and neurotoxic compounds.

Russell

Left: The Arizona coral snake (Micruroides euryxanthus euryxanthus) *is one of several U.S. coral snake species. It packs a potent venom but rarely strikes at humans. Red stripes abutting yellow ones differentiate deadly coral snakes from benign species, giving rise to the adage: "Red on yellow, kill a fellow; red on black, okay Jack."*

Egen

Right: A nimble-fingered herpetologist milks venom from a rattlesnake.

Typically these poisons dissolve cell membranes and damage blood vessel walls, triggering fluid and electrolyte imbalances and coagulation abnormalities. Cardiac and renal complications often follow. Most snakebite fatalities occur 18 to 32 hours after envenomation, but death can occur within an hour or it may take several days.

"It can be a very unpleasant course. It can be hell," says Carroll. "Even if you are not dying you can be pretty sick and you can lose a foot or a hand."

For this reason, he and others note, rapid treatment with an appropriate antivenin can provide critical relief even when a bite is not life-threatening. Studies indicate that without antivenin treatment, hospital stays for venomous snake bites average twice as long (about 6.3 days) as those in which the victim received antivenin.

Although the specifics vary somewhat, the venoms of all snakes in the family Crotalidae—which includes rattlesnakes, copperheads and water moccasins—contain related poisons and all can be treated with a Wyeth antivenin. The venom of Eastern coral snakes differs enough to require its own antivenin, also produced by Wyeth. No antivenin exists for the Western coral snake, and no other company makes antivenins for any U.S. species.

Scientists and company officials at Wyeth repeatedly declined to discuss their antivenins with SCIENCE NEWS. But other scientists familiar with the procedure say antivenin production techniques have changed little in the past four decades. Scientists inoculate horses with small amounts of venoms "milked" from the fangs of poisonous snakes. Periodically, they collect a large sample of each horse's serum and harvest the antibodies that the animal has made to the snake-venom proteins. When injected into the blood of a person who has suffered a snakebite, those antibodies bind to circulating venom proteins and neutralize them—hopefully before the poisons do the bulk of their damage.

The tricky part of antivenin production lies in the purification, scientists agree. Using a variety of methods including protein precipitation, scientists try to separate from immunized animal blood a protein fraction rich in antibodies but lacking other, useless proteins.

Extraneous proteins bearing signature sequences that identify them as horse-derived have no human therapeutic value and can trigger serious reactions when injected into humans. Recognizing the proteins as foreign, the vast majority of people develop over a period of days or weeks some degree of the generalized immune frenzy called serum sickness. Common symptoms include fever, rashes, nausea and muscle weakness. In some cases, nerve inflammation and permanent muscle atrophy follow.

Moreover, a few people respond to horse-serum products by going into anaphylactic shock, a life-threatening immune overreaction characterized by immediate and intense flushing and itching, nausea and an inability to breathe.

Physicians test recipients' sensitivity to horse antivenins by applying a small amount under the skin before administering the entire intravenous dose. A strongly positive test leaves a physician choosing between the risks of anaphylactic shock and the risk of death or amputation that goes with some venomous snakebites. Making matters worse, the skin test itself can cause anaphylaxis. And about 3 percent of patients with clearly *negative* skin tests go on to develop severe immune reactions anyway when administered the full dose of antivenin, Russell says.

In part to minimize such reactions, Therapeutic Antibodies researchers make antivenin by injecting venoms into sheep rather than horses. "Sheep antibodies seem to cause fewer allergic reactions in man, and they are economical and easy animals to work with and are robust antibody producers," says A.J. Kazimi, the company's chief operating officer.

In addition, says Ned B. Egen, a University of Arizona herpetologist helping to develop the sheep antivenin, the team uses an enzyme called papain to partially digest the purified antibodies. Anti-

Other venomous varmints

Beyond the 20 species of poisonous snakes found in the United States, few venomous animals present serious threats to residents here. Nearly all spiders are venomous, but their fangs are generally too small and weak to break human skin. Two significant exceptions are the black widow spiders *Latrodectus mactans* and *L. variolus*, which cause about three U.S. deaths each year, and the brown recluse spider *Loxosceles reclusa*. Bites of U.S. tarantulas are considered medically benign.

Gary A. Polis

Carolina Biological Supply

Left: The deadly sculpturatus scorpion carries young on her back. Right: A female black widow guards cocoons.

All North American scorpions are relatively harmless except for the sculpturatus scorpion *Centruroides exilicauda*, found in Arizona, New Mexico and parts of California. Antivenins made from horse serum are available for this scorpion's sting and for bites of the black widow.

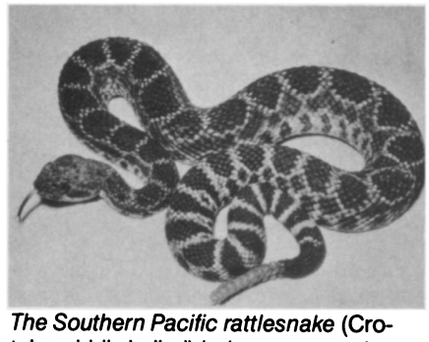
Compared with snake bites, bee stings cause three to four times more U.S. deaths each year. But these reactions are the result of individuals' immune sensitivity rather than any particular toxicity of the insect poisons themselves, so no therapeutic antivenins are available. —*R. Weiss*

bodies are shaped like the letter Y, he explains. The arms perform the work of venom neutralization, while the bottom leg, known as the Fc fragment, triggers the unwanted immune reactions in people. Papain not only dismembers the Fc fragment, but also divides the remaining V into two individual arms. The arms still do their job, but their individually small size allows them to spread more efficiently through the body.

In contrast, the Wisconsin team injects venoms into chickens, which produce antibodies that become concentrated in the yolks of those chickens' eggs. To purify the relevant antibodies, says Carroll, "you separate the yolks from the whites, just like you would in your kitchen."

After several steps of protein purification, the researchers pour the antibody-rich solution through a column lined with venom proteins. Only the venom-specific antibodies stick to this column, while extraneous proteins wash straight through. Later, by adding a special solvent, the researchers flush out and collect the retained, highly purified venom antibodies.

The process, called affinity purification, is state-of-the-art in antibody purification. Moreover, while egg proteins can trigger allergic reactions in some people, and the egg antivenins still retain their Fc fragments, the Wisconsin team anticipates no serious reactions to their product. That's because chicken Fc fragments can't trigger the so-called complement cascade in humans – the intense inflammatory reaction that underlies many of the more serious symptoms of serum sickness and anaphylaxis.



Russell

The Southern Pacific rattlesnake (Crotalus viridis helleri) *lurks among rocky crevices and tree roots in California.*

That's a real advantage of using chickens, concedes Egen, who is working on the competing system in sheep. "The method could have a lot going for it," he says. "But how the hell do you get enough antibody? This could take a lot of eggs."

In fact, says Bruce S. Thalley, who works with Carroll on the chicken-based antivenin, the Wisconsin team has already tripled their yield to about 3.3 milligrams of specific antibody per egg, and yields continue to improve. Still, with the average snakebite victim requiring 500 to 1,000 milligrams, that means the researchers need 12 to 25 dozen eggs to produce one therapeutic dose.

Not everybody agrees the Wisconsin approach will work. "People are more allergic to chickens than they are to horses," asserts John B. Sullivan Jr., a University of Arizona antivenin authority involved in the new sheep antivenin. "This whole thing with chickens may not hatch."

But the Wisconsin team defends their method. "Lots of those allergies are to things in egg whites," says Thalley. "We're pretty optimistic that so long as [our antivenin] is from egg yolk and it's highly purified, we won't have any problems."

Researchers in Australia and elsewhere say they hope *some* improved product gains U.S. market approval before long. Already, Sutherland says, researchers at his lab are looking beyond animal antibodies to an entirely new generation of antivenins made from genetically engineered proteins.

Of course, he adds – with a little pride coming through his tangy accent – it makes sense that Australians are hell-bent on developing extremely high-quality antivenins. "I don't mean to brag, but our antivenins have to be the finest," he says. "Australia is home to the 10 most dangerous snakes in the world." □

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tients, ophthalmic emissary veins do not operate to regulate brain temperature. We think (but this hypothesis has yet to be checked) that clonidine may "open" emissary veins, and that this mechanism may correct the lowering of the setpoint of temperature regulation during hot flashes. The brain-cooling mechanisms operate only in the state of hyperthermia. It is therefore not surprising that hot flushes induced by manipulation of the thermal state, as is the case in Freedmann's patients through the use of hot water pads, can be accessible to clonidine, whereas in other studies this was not the case.

A better knowledge of the physiological mechanisms of brain-temperature regulation will have important consequences not only in physiology and anthropology, but also in many fields of clinical medicine. In my opinion, a general review of selective brain cooling in a multidisciplinary journal is urgently needed.

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Saturn's spots

The statement "Astronomers observing the ringed planet have not glimpsed a comparable phenomenon on Saturn in 57 years" ("Spotting an ephemeral artifact on Saturn," SN: 10/13/90, p.228) is incorrect.

In 1960, J.H. Botham and A. Dollfus discovered a similar spot lasting five to six weeks. After it disappeared in May of that year, another large spot broke out in August at the same latitude, as confirmed by members of the British Astronomical Association. The 1933 spot you mention was discovered by British comedian W.T. Hay. Similar spots were seen in 1876 by Asaph Hall and in 1903 by E.E. Barnard. The only major difference in the spots of 1933, 1960 and 1990 was that the 1960 spots were in a latitude some 20° higher.

The periodicity of these events is well established – so much so that both SKY AND TELESCOPE and the Saturn section of the Association of Lunar and Planetary Observers were able to predict some time ago the appearance of a large spot in either 1989 or 1990. I saw the spot well displayed on Oct. 6 with 3-inch and 5½-inch refracting telescopes.

On the basis of the periodicity of these events, we can confidently state that a similar phenomenon will repeat itself around 2019 to 2021.

Rodger W. Gordon
Nazareth, Pa.

You are certainly correct that significant white spots on Saturn have occurred as recently as 1960. My statement that no comparable event has been seen since 1933 was based on an interview with astronomer Reta Beebe of New Mexico State University, who pointed out that the size of the current spot, even before its most recent expansion (documented by Hubble images), significantly exceeded that of the 1960 phenomenon.

It may well be true that the white spot was visible in October with a telescope smaller than 6 inches in diameter. But the astronomers I interviewed in October thought that amateur astronomers would likely need a 6-inch instrument to easily view the spot. – R. Cowen

Twilight musings

"Reflections on Refraction" (SN: 10/13/90, p.236) reminds me of a musing I often have when I see the sky at twilight: How much have atmospheric pollutants changed the visual impact of sunsets? I first had this thought while watching a particularly breathtaking sunset in air so clear that the boundary of sky and sea seemed like a razor slice.

If atmospheric pollution contributes significantly to the refractive process Bradley Schaefer is documenting, it seems this might have some bearing on the archeoastronomy questions discussed in your article.

Richard H. Tew
Encinitas, Calif.

Letter choice contested

Why would an editor devote precious space to a lecture on the gospel according to C.S. Lewis? I am referring to Peter H. Shaw's letter to the editor, titled "Altruism: A simpler explanation?" (SN: 9/15/90, p.163).

Shaw's objection to evolutionary explanations for altruism is obviously based on a fundamentalist interpretation of the Bible. In his letter, he quotes C.S. Lewis, author of fundamentalist Christian sword-and-sorcery novels and guru to creationist young-Earthers.

I am only an amateur paleontologist, but I have nothing to learn in the way of science from C.S. Lewis. None of us does.

Tom Cole
Chandler, Ariz.

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