LESSONS FROM HISTORY

Challenging paradigms is risky business: reflections on C. C. Snyder's "A Definitive Study of Snakebite" —a commentary

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Recent improvements in research technologies for protein purification have expanded our understanding of the molecular and cellular toxins that comprise snake venoms. Many findings have suggested novel strategies for drug development and inspired renewed respect for the finesse with which snakes—and snake venoms—have evolved. Despite advances in basic science venom research, a universally accepted animal model for comparing different therapies for treating snake venom poisoning has not been established. Many animal models remain problematic in both design and execution. Results from experiments using mammalian surrogates pose challenges for the clinician interested in extrapolating results from the animal laboratory to the emergency department. For example, many studies performed between the 1950s and the late 1970s made use of age-, sex-, and mass-matched canines, an *in vivo* model that often requires a large sample size due to heterogeneity between animals. In contrast, experiments using pigs benefit from mass and physiologic responses that are comparable to those of humans. Unfortunately, the high costs of husbandry and test animals force many studies to operate with marginal sample sizes and an abbreviated period of experimental monitoring. Rabbit studies offer the advantage of lower cost, while serving as an established model for studies on complement and TH1 cytokine derangement, 2 intriguing methods with which snake venom appears to induce hemodynamic instability in the envenomated animal. Unfortunately, rabbits possess unacceptable intrinsic resistance to the venoms of many New World pit vipers, making comparisons with human patients difficult.

The controversy surrounding the accuracy of envenomation research is not limited to animal experimentation. For example, many bench-top studies make use of lyophilized venom obtained from large wholesalers, who in turn obtain venom from a wide range of sources. Despite assurances from suppliers of freeze-dried venoms, such

preparations are often contaminated with foreign proteases, venom degradation products, snake gland epithelial cells, and cytokine-inducing lipopolysaccharide. Indeed, our *in vivo* studies have provided growing evidence that natural venoms, obtained directly from captive-bred Southeast Asian elapids, are more potent and produce more complex chromatography profiles than lyophilized preparations obtained from the same colony animals. The point is important in that many animal studies performed decades previously made use of unaltered venom, which was obtained directly from the snake.¹

More remarkable than our appreciation of the complexities of envenomation research is the observation that studies performed decades earlier were often accurate, prophetic, and accomplished without the benefit of modern laboratory techniques. One fascinating yet controversial study that epitomizes the successes of many early investigators, was published by C. C. Snyder et al in April 1968.¹ To understand both the value and the subsequent criticism of this study, one must first appreciate how recommendations for treating snakebite were established at that time.

Prior to the late 1960s the management of pit viper envenomation was determined more by the clinical impressions of local authorities than by scientific investigation. Many reports were published in journals that omitted peer review or strict adherence to principles of clinical research. On occasion, articles were published that recommended therapies now regarded as damaging or without clinical merit.^{2–4} The certainty of the author's clinical opinion and the circulation of the article were principal determinants in the acceptance of a specific treatment. For example, between 1962 and 1968 more than 68% of archive patients referred to the University of Alabama Medical Center were treated using dangerous techniques, such as cold water immersion/cryotherapy, cruciate incisions, scarification of fang marks, excision of venom "pools," or tourniquets.⁵ These thera-

pies reflected the recommendations of local physicians who were regarded as authorities and of lecturers presenting at a recent surgical conference. It is worthy to note that once published, potentially damaging therapies would endure in clinical practice despite subsequent evidence suggesting the treatment was either damaging or without clinical merit. We should also recall that this phenomenon persists in the recent literature, as evidenced by the publication of recent embarrassing clinical reports on treatment.⁶

At the time of Snyder's publication, the initial characterization of US pit viper venoms was being completed by Russell and Puffer.⁷ Despite exemplary work characterizing principal venom peptides, the research techniques of the time were not sufficiently sensitive to demonstrate many subtle, yet clinically important differences in the venom of different species of pit vipers. We now have clear evidence that the composition of snake venoms varies among snake hybrids, as well as with differences in range, environment, and age of the animal.^{8,9} Similarly, few physicians treating snakebite had bridged the knowledge gap between clinical medicine and medical herpetology. Such lapses in scientific understanding set the stage for the incorrect identification of offending species and the publication of miraculous cures, which could only be explained by nonvenomous snakebites or dry bites from venomous species.

The 1968 study by Snyder and colleagues was one of the first reports to identify the dangers of using clinical opinion, rather than scientific study, as the basis for recommending different treatments. Another distinction that set Snyder's team apart was their familiarity with the behavior of US pit vipers, a talent demonstrated by their observation that the success of many alleged treatments was likely the result of dry bites from venomous species. While this observation was originally suggested by others,¹⁰ it was this group's work that demonstrated how venomous snakebite *sans venom* could promulgate misleading case reports.

In comparison with other publications of the time, Snyder's team also demonstrated a clear understanding of the myriad variables at work when different snakes, with distinct venoms, variably envenom a variety of human phenotypes, who in turn undergo a wide range of treatments. On page 330 of the original article the author writes:

Mindful of these variables, we outlined an investigation a decade ago to study the effects of snake venoms, to evaluate conventional treatments as well as other methods of therapy and to ascertain which treatments contribute to the best results.

The conclusions reported in "A Definitive Treatment of Snakebite" were based on the results of a canine

model and observations obtained from a small series of envenomed patients. In the animal studies, the efficacy of many contested therapies was assessed using artificial injection of lethal doses of ¹³¹I-radiolabeled, crude venom obtained from *Crotalus adamanteus* (eastern diamondback rattlesnake) and *Agkistrodon* (*Ancistrodon*) *piscivorus* (eastern cottonmouth). The authors stated that additional studies were performed in which venomous snakes delivered the envenomation injury; however, these results were not commented on. The results focus on a series of experiments in which 139 dogs were artificially envenomed and then divided into treatment cohorts that included rest, exercise, cryotherapy, incision (cruciate and linear), venous tourniquet, bulb suction, immunotherapy using Wyeth's Antivenin (*Crotalidae*) Polyvalent (ACP), and the use of surgical excision of tissue surrounding the fang marks.

Despite the number of study objectives, the authors achieved remarkable success in evaluating many forms of treatment. Many conclusions about treatment would be validated by experiments performed years later. Perhaps the greatest role that the study played in furthering our clinical understanding of snake envenomation was in vivo confirmation that many treatment techniques were damaging. In particular, the study's dog model provided an explanation of how cycled release of venous tourniquets might explain the rapid deterioration observed when the severely envenomed patient has a tourniquet removed prior to the initiation of protective measures, such as hemodynamic support and infusion of antivenom. This study was also the first death knell of cruciate incisions. In the discussion, the authors explained how crosshatched incisions compromise blood flow to the flaps, thus exacerbating necrosis and increasing the risk of infection. A third contribution was in vivo evidence contradicting the value of cryotherapy, a disastrous form of treatment that is still practiced in several rural regions of the Americas.

Many of the therapies examined by Snyder's team were to outperform studies conducted years later. In particular, this study made use of incision and bulb suction devices, which achieved retrieval rates of ¹³¹I-labeled venom in excess of 53% at 10 minutes, an amount far greater than that reported by studies that used the more powerful Sawyer Extractor suction device (Safety Harbor, Fla).¹¹ It is not clear if the high retrieval of venom, manifest as increased gamma counts, reflects variability in iodination of venom, the use of fresh rather than lyophilized venom, or the use of venom from species (such as *C. adamanteus*) that produce considerable bleeding from fang marks (or incisions).

Of 41 human cases of envenomation reported in the article, 23 patients were treated entirely by the investi-

gators. Eleven of these patients were bitten by *C adamanteus*—an important point given the severity of bites from this species. Another 8 patients were envenomed by *A piscivorus*, and 4 patients were envenomed by pygmy rattlesnakes (*Sistrurus*). All patients were treated with venous tourniquets (excluding the 1 facial bite), intra-arterial infusion of ACP antivenom, and the use of elliptical excision of the envenomation site. What is unusual about the species of pit vipers implicated in these snakebites is the absence of bites from *C horridus atricaudatus* (canebrake rattlesnake), a common and dangerous pit viper found throughout many areas of Florida. Also omitted is an explanation as to how the identity of the offending reptile was confirmed (eg, serum antigen assay, obtaining the snake).

The impact of “A Definitive Study of Snakebite” was compromised by the inclusion of a series of animal experiments and clinical cases that were treated with excision of tissue surrounding the fang marks. As discussed by the authors, elliptical excisions of skin, subcutaneous fat, and muscle in the area surrounding the fang marks were performed in an attempt to remove venom before it had traveled from the bite site. In the canine studies, ¹³¹I-labeled venom was injected into the hind-quarters of an unknown number of dogs, and elliptical full-thickness excisions were performed between 10 and 120 minutes postinjection. When analyzed by Geiger scintillography, excised tissue was found to contain 79% of the total venom injected into the animal, suggesting this technique as an effective method of treatment. The report also included a small number of patients envenomed by pit vipers and treated with excision therapy with alleged good results.

In the decades following this article, many investigators directed their criticism solely at the comments concerning excision therapy. In the process, critics may have failed to recognize the report’s contribution to our understanding of snakebite. A second point is that the high gamma counts recorded in excised tissue suggest that venom may have been removed by the surgeon’s scalpel. Perhaps Snyder and colleague’s consideration of a surgical approach to the treatment of snakebite is easier to comprehend when we recall that in the 1960s, Wyeth’s new equine ACP was still undergoing refinement, and there was considerable debate about the dosage required to treat bites from US pit vipers. The high cost of Wyeth ACP resulted in conservation of the drug, which often lead to underdosing of many moderate and severe envenomations. Physicians were also concerned about acute and delayed hypersensitivity reactions resulting from the use of this equine immunotherapy; the perception that ACP was a dangerous drug was widespread. With these concerns in mind, it is easier to com-

prehend why clinicians felt pressured to search for alternative therapies. In 1996, our research team had the opportunity to relive the experiences of doctors who lacked confidence in antivenom when we discovered that lyophilized “antivenom” imported into Laos for use in a clinical trial was, in fact, a counterfeit mixture of baking powder and unknown reagents. When notified of the problem, the response of the Lao surgeons was to recommend that excision therapy be used until quality antivenom became available. For physicians practicing in resource-constrained environments, including modern hospitals unprepared for the sometimes radical quantities of antivenom required to treat severe cases, consideration of all evidenced-based therapies for treating snakebite needs to be undertaken. Such patients benefit from both the inclusion of all forms of effective treatment and the exclusion of damaging or useless therapies. With this in mind, Dr Snyder’s “A Definitive Study of Snakebite” serves as a reminder that our current literature might also contain a blend of valid observations and treatment recommendations that will be disproved by the researchers of tomorrow.

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