Copeia, 1993(3), pp. 644–650

Venom Delivery of Snakes as High-Pressure and Low-Pressure Systems

KENNETH V. KARDONG AND PABLO A. LAVIN-MURCIO

Two fundamentally different venom delivery systems are found among advanced snakes. One is a high-pressure system present in viperids and elapids wherein a pulse of venom is delivered quickly by a sudden pressure surge. The other is a low-pressure system found in some colubrids wherein release of oral secretions is more protracted. These differences help account for the great variation in medical signs and symptoms following bites of humans by colubrid snakes. Further, the high-pressure venom system of elapids and viperids represents an evolutionary innovation in snakes accompanied by a change from a mechanical to a chemical predatory strategy.

THE mechanism of delivery of toxic oral secretions by snakes has been studied mainly in the highly venomous families such as Elapidae (e.g., cobras, coral snakes) and Viperidae (vipers and pit vipers). But, within the Colubridae, the largest family of snakes, some species also possess toxic oral secretions. This has been known for some time (Alcock and Rogers, 1902; McAlister, 1963; Minton, 1979) but only recently has the medical significance become fully appreciated as clinical studies documented the extent of severe local tissue involvement around the bite (Minton, 1979; McKinstry, 1983) and even occasionally deaths of some stricken humans (FitzSimmons and Smith, 1958; Mittleman and Goris, 1974; Minton, 1990). This has prompted efforts to evaluate the venom systems in these species of colubrids. However, despite its potential for causing human envenomations, we show that the performance of the colubrid venom apparatus is fundamentally different from the venom apparatus of elapid and viperid snakes. To recognize these functional differences, we call the apparatus of colubrids a low-pressure venom system and that of elapids and viperids a high-pressure venom system. These differences in performance may affect our assessment of human health risks from colubrids and help clarify the major events involved in the evolution of venomous snakes.

These two general venom systems exhibit two distinct morphologies. Although elapids and viperids are taxonomically quite distinct (Cadle, 1982, 1987), the venom apparatus in both families is similar throughout these two groups (Haas, 1973). With the exception of some primitive elapids, the venom apparatus includes a hollow fang and an associated venom gland containing a large encapsulated reservoir; compressor glandulae (viperids) or adductor superficialis (elapids) jaw muscles insert directly on the capsule of the venom gland. By contrast, colubrids exhibit great morphological variation (Taub, 1966, 1967). In some species, there is no venom apparatus; in other species, a venom apparatus is apparent and is characterized by a long, posterior maxillary tooth, often grooved along its lateral surface. This long tooth is associated with Duvernoy's gland, an oral gland located in the temporal region that is homologous to the venom gland of other families (Kochva, 1965; Kochva and Gans, 1970). In a few species, striated jaw muscles insert into the Duvernoy's gland, but in most, muscles do not establish such a direct attachment (Kochva, 1978).

Two representative species were selected for study. One was the northern Pacific rattlesnake, *Crotalus viridis oreganus*, a viperid snake that uses its venom apparatus during feeding and in defense. Bites of humans can result in immediate severe tissue damage, long-term impairment of a stricken limb, and can be life threatening (Russell, 1980). The other species was the brown tree snake, *Boiga irregularis*, a colubrid snake with a Duvernoy's gland and grooved, enlarged maxillary teeth (Zalisko and Kardong, 1992). *Boiga irregularis* has been reported to deploy oral secretions to subdue prey (Greene, 1989), and bites of humans have occasionally caused alarming medical reactions, such as tissue ecchymosis, swelling, sloughing of tissue, and systemic respiratory distress (Fritts et al., 1990). In these two representative species, we compared the pressures developed during venom release and the morphological basis of each venom delivery system.

MATERIALS AND METHODS

The formalin-fixed heads of the snakes, five of each species, were examined under a dissecting microscope to trace the duct-to-fang association and to determine which, if any, jaw

muscles might affect duct association with the fang. Serial histological (H&E) cross-sections (8 μ) of two *B. irregularis* heads allowed careful elucidation of duct-to-fang association (Zalisko and Kardong, 1992).

In a lightly anesthetized (isoflurane) rattlesnake, a 20 cm length of narrow P.E. 50 polyethylene tubing was slipped over one fang tip, fixed with cyanoacrylate glue, and attached to a liquid Statham pressure transducer which was connected to a galvanometer-driven chart recorder. Valves allowed continuous back-filling of the entire catheter and hollow fang with distilled water. Care was taken not to stimulate premature release of venom. After the snakes recovered from anesthetization, both fangs were forced into a fully erect position over the lip of a cup (for technique, see Mebs, 1978), and the pressure was recorded from the catheterized fang in a total of five northern Pacific rattlesnakes, *C. v. oreganus*, 69.1–72.4 cm snout–vent length, collected in Whitman County, Washington.

The morphological examination of *B. irregularis* confirmed that the grooved, maxillary tooth was open. Consequently, unlike the closed rattlesnake solenoglyphic fang, no exit orifice could be fitted with a catheter in *B. irregularis*. Further, the very small diameter of the duct from Duvernoy's gland made direct catheterization of the duct impractical. Therefore, an alternative method of calculating secretory flow from Duvernoy's gland was used. Calibrated 50 μl micropipets held horizontal to the ground were used to collect Duvernoy's secretion following Vest (1981a) except that no aspiration was applied. To calculate the rate of flow, the time elapsed as secretion flowed was recorded from five brown tree snakes, *B. irregularis*, from Guam, 98.1–105.6 cm snout–vent length.

As argued elsewhere (Kardong, 1980, 1982a), calling the secretion of Duvernoy's gland a venom can invite confusion between the biological role of this secretion and its pharmacological properties. However, it is a tangential issue to the subject of pressures. Consequently, we follow herein the convention of calling the toxic Duvernoy's secretion a venom so we can more easily focus discussion on comparative pressures accompanying release of oral secretions by snakes rather than on the adaptive roles of the secretions themselves (1 bar = 10^5, Pascal = 14.5, psi = 0.987 atmosphere).

Results

Pressures.—Forces accompanying venom release were considerably different between the two species. In *Crotalus*, pressure recording experiments showed that this rattlesnake produced high pressure spikes up to 2.61 Bars ($\bar{x}$ = 1.26 Bars, SD ± 0.68 Bars, n = 11). Maximum peak pressure was reached within 0.22 sec (SD ± 0.18 sec), but postpeak decline lasted up to a minute before pressures again reached atmospheric (Fig. 1). However, in *Boiga*, the pressure developed during release of venom was low and prolonged, and flow was irregular. Direct venom flow might stop for up to a minute then resume. Once resumed, flow was continuous with no distinct pressure spike. The rate of venom collection was up to 0.176 μl/sec ($\bar{x}$ = 0.12, SD ± 0.09), a rate that could be sustained for up to a minute. However, this rate was not significantly different than the rate produced by capillary forces alone.

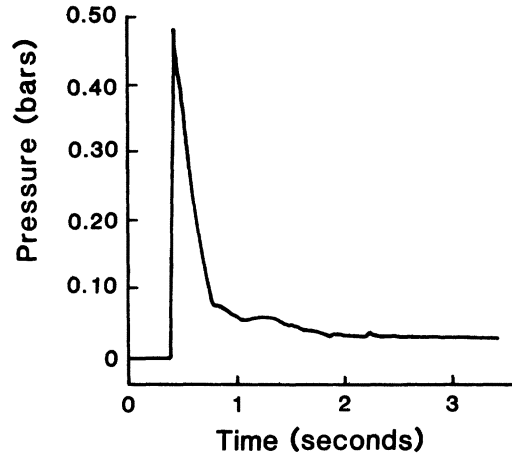

Fig. 1. Representative pressure spike recorded from the right fang of a rattlesnake, *Crotalus viridis*, during release of venom. Note the sudden rise in pressure (0.063 sec), reaching, on this occasion, 0.49 Bars but, thereafter, a protracted (several seconds) drop back to ambient pressure at the level of the transducer (0 Bars).

To calculate the capillary force, calibrated 50 μl micropipets were placed horizontally into a test tube with freshly collected Duvernoy's secretion, and the rate of filling was recorded. This rate, the result of capillarity in the micropipet, was compared to the rate of direct collection of Duvernoy's secretion from *Boiga*. There was no significant difference ($P < 0.64$) between the rate of Duvernoy's collection and the rate of micropipet filling directly. Placed vertically in distilled water, fluid rose in the micropipet to a height equivalent to a pressure of 0.001 Bars, taken here as the force of capillarity alone (at 20 C).

Morphology.—In *Crotalus*, the channel from the

venom gland to the exit orifice at the tip of the hollow fang was unbroken, except at the point where the venom duct connected with the entrance orifice at the base of the fang. At this duct/fang connection, the venom duct was held loosely within the fang sheath, a specialization of the oral epithelium in the vicinity of the fang (Fig. 2A). In turn, a broad tendon associated with the pterygoideus jaw muscle attached to the part of the fang sheath that held the venom duct. In the resting snake, the venom duct was not anatomically fused or functionally held to the fang. However, when the pterygoideus was grasped by forceps and pulled posteriorly, this tendon held the opening of the main venom duct tightly against the entrance orifice of the fang (Fig. 2B). Forward erection of the maxillary bone, upon which the fang rode, also helped to tighten the junction between venom duct and the base of the fang. This produced a tight, temporary seal between duct and fang. After expulsion of venom, when the fang was folded and returned to a rest position, this connection relaxed. Often after we allowed the rattlesnake to release its fangs from firm engagement with the edge of the cup, venom was seen leaking from this duct/fang junction as the tension about this junction relaxed.

In *Boiga*, the anatomical design of the venom delivery system was quite different from that of *Crotalus*. The pterygoideus muscle did not attach to the base of the fang, and therefore this muscle had no direct mechanical effect upon the duct (from Duvernoy's gland) or upon the teeth. Thus, no tight seal formed between the duct and the grooved maxillary teeth. Instead, the duct bringing secretion from Duvernoy's gland emptied into a sheath that formed a small pocket of oral epithelium around the grooved teeth but did not attach to them (Fig. 2C). During release of venom, increased local blood flow reddened and swelled the sheath as capillary beds in the sheath opened. This engorgement with blood caused the small pocket of oral epithelium to inflate into a cuff that encircled the grooved teeth and defined a receiving reservoir for arriving Duvernoy's secretion (Fig. 2D). When extracting Duvernoy's secretion, it was from this reservoir or directly from the duct that secretion was gathered. The grooved teeth are located on the posterior end of the maxillary bone and, thus, reside at the back of the mouth. Extreme opening of the mouth or working of the jaws over the prey may eventually bring them into engagement with the prey surface, but like most colubrids, *Boiga* exhibited no significant forward erection of the maxilla.

Discussion

Detailed examination of the morphology of the venom system, together with a recording of pressures accompanying venom release, clarified the basis for the differences in recorded pressures—high in rattlesnakes, low in brown tree snakes. In *Crotalus*, as in other viperids (Schaefer, 1976; Takács, 1986), the venom channel from venom gland to fang tip is anatomically unbroken, except where the duct connects with the base of the fang. However, during the strike, most jaw muscles contract (Kardong et al., 1986). When drawn taut during contraction of the pterygoideus, a tight, temporary seal at this connection is functionally established during full erection of the fang-bearing maxilla.

In *Boiga*, as in many other colubrids (Taub, 1967; Takács, 1986), the venom delivery system is quite different from viperids and elapids. The secretion channel in *Boiga* from Duvernoy's gland to tooth tip is both anatomically and functionally interrupted. The grooved maxillary tooth is, of course, open, and the duct does not even establish a temporary, tight seal with this tooth. Consequently, a significant pressure head, even if generated in the Duvernoy's gland, cannot be maintained all the way along this channel and into the prey.

We conclude that two distinct venom delivery systems are represented by the two snake species examined, *C. v. oreganus* and *B. irregularis*. During the strike of the rattlesnake, the direct action of jaw muscles upon the venom gland generates an initial pressure upon the enclosed venom reservoir; a tight seal, temporarily established between duct and fang, ensures that this pressure will be maintained throughout the conduction channel and serves to drive the venom into the prey. This results in venom delivery based upon a high pressure system. Although we did not study members of the Elapidae, the presence of direct muscle attachment to the venom gland together with a rapid strike and release behavior (Rosenberg, 1967; Kardong, 1982b) suggest the presence of a high-pressure system in elapids as well. There is variation in the specific mode of attachment of the elapid pterygoideus to the maxilla (McDowell, 1968; Young, 1987). Anatomical evidence suggests that the tight seal between venom duct and fang can also be temporarily established when the snake bites into the prey, as a result of pressing the fang sheath holding the duct firmly against the fang (Halstead et al., 1978). A tight temporary seal between duct and fang can, there-

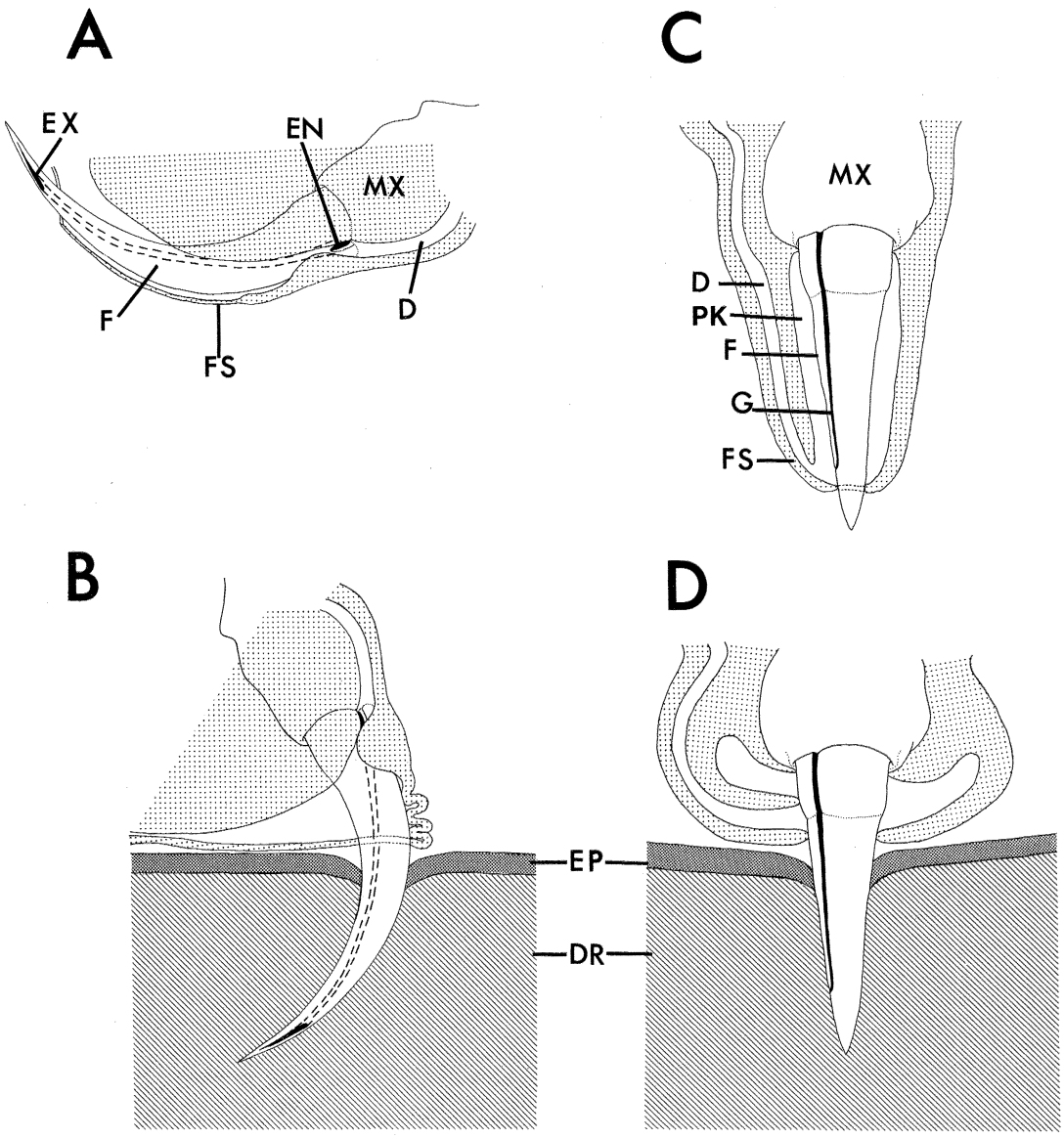

Fig. 2. A diagrammatic representation of fang penetration and accompanying venom release in two species of snakes. (A) Lateral view of rattlesnake, *Crotalus viridis,* fang in the folded rest position. The fang (F) is ankylosed to and rides with the maxillary bone (MX). The venom duct (D) empties into the entrance orifice (EN) at the base of the fang. During injection, venom travels down the hollow core of the fang (indicated by dashed lines) and departs at the tip via the exit orifice (EX). The fang sheath (FS) surrounds the resting fang. (B) Lateral view of rattlesnake fang in erect position. The venom duct is drawn into tight contact with the entrance orifice; the fang tip penetrates the epidermis, and, under high pressure, venom is forced into this tissue; the fang sheath does not enter but remains on the surface. (C) Anterior view of the right, posterior maxillary tooth of the brown tree snake, *Boiga irregularis.* This tooth (F) is ankylosed to the maxillary bone (MX). The duct (D) from Duvernoy's gland opens into the pocket of oral epithelium forming by the tooth sheath (FS); the lateral face of this enlarged maxillary tooth bears an open groove (G). (D) Anterior view of *Boiga* grooved maxillary tooth (F) during its penetration of the prey integument. No tight coupling of duct-to-fang occurs; instead the duct opens into the pocket (PK) of oral epithelium. The venom that enters the prey does so under low pressure introduced by the groove tooth.

fore, be established either by direct muscle action or by tension developed in the fang sheath during the bite.

By contrast, no such elevated pressures accompany venom release by *Boiga*. Adjacent striated jaw muscles may bulge when contracting, impinge on the walls of Duvernoy's gland, and so facilitate its emptying (Jansen and Foehring, 1983). But, because no muscles attach directly, no initial high pressure is generated as a result of mechanical forces. Further, no tight seal forms between Duvernoy's duct and grooved maxillary tooth. Consequently, the delivery of secretion is via a low-pressure venom apparatus.

These two types of venom systems may help account for the puzzling inconsistencies of venomous snake bites of humans. Among vipers and pit vipers, up to 50% of the bites of humans may be "dry bites" in which no venom enters the person (Parrish, 1959; Reid, 1970). It has been hypothesized that dry bites result from disruption of normal jaw mechanics during defense strikes (Kardong, 1986b). In venomous colubrids too, many bites of humans may be dry. In part, this may result from the position of the fangs at the back of the mouth, thus delaying their engagement. But our study also suggests that, even if engaged, the pressure behind the venom is low and may be sufficient only under unusual circumstances to deliver small amounts of venom into the victim (Vest, 1981b; Hayes and Hayes, 1985). For example, a high proportion of human victims showing signs of envenomation by *Boiga* are sleeping infants (Fritts et al., 1990). Because infants are less able to disengage a snake, the snake may have more time available to hold grooved teeth in place to deliver even small quantities under low pressure.

In a variety of morphological and behavioral features, colubrids are distinct from advanced venomous snakes (Kardong, 1979, 1982a). To this distinctiveness we can now add the presence, among some colubrids, of a low-pressure system delivering Duvernoy's secretion. If compared to the high-pressure venom delivery system of elapids and viperids, the low-pressure system of colubrids does not equal the rate and efficiency of delivery of oral secretion. Yet, if numbers are a measure of ecological success, then colubrids must be considered successful because the family includes more species than any other snake family. Alternatively, it might be more productive for evolutionary studies to evaluate the delivery of Duvernoy's secretion as a primary component in other roles rather than for strictly killing prey. For example, Duvernoy's secretion of colubrids might participate in a preingestion role where it prevents harsh chemicals released from prey from harming or fouling teeth (Gans, 1978) or, in postingestion, where introduced Duvernoy's secretion might enhance digestion (Kardong, 1982a; Rodriguez-Robles and Thomas, 1992). Redirecting our thinking about colubrids and their delivery systems can help clarify why they are so ecologically successful and evolutionarily derived (Cadle, 1987).

Differences in pressure of venom delivery also have implications for the evolution of clearly venomous elapid and viperid snakes. If elapids and viperids represent derived snakes (Rieppel, 1988) arising from nonvenomous ancestors, then one of the novel features to appear was the high-pressure venom system. Generally, elapids and viperids produce a more toxic venom than do other snakes (Mebs, 1978). But enhanced toxicity alone is not sufficient to account for the performance abilities of members of these families. To produce and use a high pressure, anatomical modifications are required. One of several such modifications was the development of an enclosed venom channel (hollow fang) within the tooth delivering venom; another was the establishment of a tight seal between the venom duct and the base of this channel within the fang. These morphological innovations are central to the control and deployment of the high pressure accompanying venom flow. Along with these morphological modifications, the high-pressure venom system allowed the accompanying evolution of a predatory style based upon quick delivery of a rapidly killing suite of oral secretions. The snake need not prolong contact (constriction, holding) with prey that is potentially injurious (Radcliffe et al., 1980; Kardong, 1986a). Switching from primarily mechanical (constriction, holding) to primarily chemical (venom) predation, the derived viperid and elapid snakes evolved a venom system and predatory behaviors that are marked departures from those found in other snake families.

Acknowledgments

We acknowledge M. McCoid and T. Fritts, who first drew our attention to the reddening around the active grooved tooth sheath of *Boiga*, and S. P. Mackessy, who first observed the occasional leaking of venom when rattlesnakes relaxed following venom collection. For critical comments on the manuscript, we thank W. Hayes, J. Herman, J. Larsen, J. Mallatt, and M. Rochelle. Supported in part by NSF grant BNS-8820091, United States Fish Wildlife grant, and CONACYT (Mexico).

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DEPARTMENT OF ZOOLOGY, WASHINGTON STATE UNIVERSITY, PULLMAN, WASHINGTON 99164-4236. Send reprint requests to KVK. Submitted 14 May 1992. Accepted 1 Sept. 1992. Section editor: G. R. Ultsch.

Copeia, 1993(3), pp. 650–660

Aclonal Reproduction by Polyploid Members of the Clonal Hybrid Species *Phoxinus eos-neogaeus* (Cyprinidae)

K. A. GODDARD AND R. J. SCHULTZ

Hybrids of the daces *Phoxinus eos* and *P. neogaeus* occur throughout the northern United States and southern Canada. Diploid hybrids and some diploid-triploid mosaic hybrids from East Inlet Pond, Coos County, New Hampshire, produce diploid all-identical ova of *P. eos-neogaeus* that develop into diploid young if sperm from either parental species serves only to stimulate development but triploid or diploid-triploid mosaic young if the sperm genome is actually incorporated. The hybrid population at East Inlet Pond appears to be perpetuated by this unique form of sperm-dependent, clonal reproduction.

Here we report two additional forms of reproduction based on laboratory spawnings of two mosaic and two triploid hybrid females collected from East Inlet Pond, New Hampshire. The two triploids produced diploid offspring that were indistinguishable from *P. eos* on the basis of several external morphological traits. In addition, the offspring possessed allozymes characteristic of *P. eos* at all six diagnostic loci tested, and they had histocompatibility antigens and an allele of Gpi-B absent from their mothers. Conversely, the coiling of their digestive tracts varied between the morphology characteristic of *P. neogaeus* and that of *P. eos*. Collectively, these data suggest that the triploid females produced haploid ova containing exclusively or mostly chromosomes of *P. eos* that were fertilized by sperm of *P. eos*.

The two mosaics produced offspring that carried the clonal genome of *P. eos-neogaeus*, indicating that they produced diploid all-identical ova of *P. eos-neogaeus* like the diploid and mosaic hybrids we have previously reported. However, they also produced some offspring that did not carry the clonal genome, apparently from nonidentical ova of unknown ploidy level.

Reproduction by the types of triploid and mosaic hybrids reported here may introduce greater variation into hybrid populations. These modes of reproduction may also provide a vehicle by which genes may be exchanged between the parental species, *P. eos* and *P. neogaeus*.

THE northern redbelly dace (*Phoxinus eos*) and the finescale dace (*Phoxinus neogaeus*) occur throughout the northern United States and southern Canada. Hybrids occur throughout the region where the two species overlap, from Maine and Nova Scotia to Montana and Alberta (e.g., Hubbs and Brown, 1929; Joswiak et al., 1985; Dawley et al., 1987). Individuals of