Toxicon, Vol. 31, No. 7, pp. 881–887, 1993.
Printed in Great Britain.

0041-0101/93 $6.00 + .00

DELIVERY OF DUVERNOY'S SECRETION INTO PREY BY THE BROWN TREE SNAKE, *BOIGA IRREGULARIS* (SERPENTES: COLUBRIDAE)

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(*Received* 23 *September* 1992; *accepted* 11 *January* 1993)

W. K. HAYES, P. LAVÍN-MURCIO and K. V. KARDONG. Delivery of Duvernoy's secretion into prey by the brown tree snake, *Boiga irregularis* (Serpentes: Colubridae). *Toxicon* **31**, 881–887, 1993.—Many colubrid snakes, like the more venomous elapid and viperid snakes, can produce and inject an oral secretion that is toxic and may present a human health risk. However, colubrid oral toxins are produced in a Duvernoy's gland and delivered not through a hollow fang, but instead by long, often grooved teeth under low pressure. The possible role of Duvernoy's secretion in functions other than rapid killing of prey make it important to know how and where this secretion is delivered during a feeding strike. We used ELISA analysis to determine the quantity and proportional distribution of Duvernoy's secretion delivered into the integument compared to the viscera during a feeding strike by the colubrid snake *Boiga irregularis*. We determined that only about 54% (1–5 mg) of the secretion actually reached the viscera and that the rest remained in the integument. The amount reaching the viscera is about three to eight times the i.p. LD_{50} for mice, but these snakes depend more on constriction than toxins to kill their prey. Consequently, delivery of Duvernoy's secretion by *B. irregularis* is hypothesized to be part of a digestive function and its toxic properties a by-product of this role.

INTRODUCTION

SOME colubrid snakes produce oral secretions that exhibit mild (VEST, 1981*a*,*b*) to even alarming toxicity (MCKINSTRY, 1983; SAKAI *et al.*, 1984), with reports of human deaths following bites by some of these colubrid species (MITTLEMAN and GORIS, 1974; MINTON, 1990). Yet colubrid snakes lack a venom gland. Instead many possess a Duvernoy's gland (TAUB, 1966, 1967), evolutionarily homologous to the venom glands of elapids and viperids (KOCHVA, 1965; KOCHVA and WOLLBERG 1970), but surprisingly distinct from a true venom gland. For example, Duvernoy's gland does not empty into a hollow fang. Instead, its duct opens into the oral cavity near posterior maxillary teeth that may or may not be grooved. Posterior maxillary teeth are often engaged during prey capture and swallowing (e.g. KARDONG, 1980, 1986). But an open-grooved or non-grooved tooth

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offers no protected, enclosed channel passing through the integument and into the prey. Consequently, the pliable skin of the prey can obstruct the tooth or its groove, making it less effective as an instrument for introduction of secretion from oral glands (KARDONG and YOUNG, 1991). Further, such teeth of colubrids cannot hold a pressure head generated by Duvernoy's gland in the same way that the hollow fang of elapids and viperids allows high-pressure delivery of a shot of venom (KARDONG and LAVÍN-MURCIO, 1993). Finally, Duvernoy's secretion may tranquilize prey or even lead to a protracted death. However, if surgically deprived of secretion from Duvernoy's gland during prey capture, some snakes nevertheless can and do kill prey by constriction as rapidly as when the secretion is available (ROCHELLE and KARDONG, 1993). For these reasons and for reasons related to the evolution of the venom apparatus, it has been argued that the function of the secretion from Duvernoy's gland should be evaluated separately from the venom of elapids and viperids (KARDONG, 1982*a*). To clarify the role of secretion from Duvernoy's gland in feeding and to assess its medical implications in cases of human envenoming, the question also arises as to just how much Duvernoy's secretion actually enters the prey. Our purpose then was to determine the quantity of Duvernoy's secretion delivered during a feeding strike, and to compare how much remained in the integument with how much actually reached the viscera.

We used the colubrid species *Boiga irregularis*, the brown tree snake. Because of its ecological and economic impact (FRITTS, 1988), the brown tree snake has received special attention, giving us information on its basic feeding ecology (SAVIDGE, 1986, 1987, 1988). Recent studies of the secretion of *Boiga* (Vest *et al.*, 1991; Weinstein *et al.*, 1991) and the morphology of its venom apparatus (Zalisko and Kardong, 1992; Kardong and Young, 1991) provide further details which relate to the quantity of venom released during feeding.

MATERIALS AND METHODS

Subjects

Nine adult brown tree snakes (122–184 cm in snout-vent length), collected on the island of Guam, were maintained in glass aquaria in a room with temperature (28°C ± 2°) and humidity (70%) controlled. The captive snakes were fed every 2 weeks on laboratory mice (*Mus musculus*) for 9 months prior to experiments.

Experiment 1

Each snake was permitted to strike and constrict an adult Swiss–Webster mouse (20.1–24.0 g) introduced into its home cage. Their feeding behavior was recorded until the mouse appeared to be dead (exhibited no reflexes). Then, the rodent was quickly retrieved, placed into a plastic bag, and frozen (−20°C); another mouse sacrificed earlier was offered to the snake for consumption.

The quantity of Duvernoy's secretion expended (dry mass) on each prey animal was measured to the nearest milligram by enzyme-linked immunosorbent assay (ELISA) of whole-mouse homogenates. After thawing, the mice were homogenized 3–4 min in a 14-speed Osterizer blender with 150 ml buffer (0.05% Tween-20 in phosphate-buffered saline (PBS); see HAYES *et al.*, 1992). Several 1.5 ml aliquots of homogenate were centrifuged 5 min in an IEC Clinical Centrifuge. The supernatant was frozen at −20°C until ELISAs were performed.

The ELISA methods are described elsewhere in detail (HAYES *et al.*, 1992). Briefly, venom extracted by micropipet (e.g. VEST, 1981*b*), but without aspiration from the *Boiga* specimens (4 months prior to study), was injected into two rabbits using the modified method of induced granuloma. This method (HILLAM *et al.*, 1974) takes advantage of a granuloma formed within a perforated, plastic golf ball surgically implanted under the skin of a rabbit. Fifteen days post-implementation, 200 μg of Duvernoy's secretion (antigen) in 1 ml PBS was injected directly into the lumen of the implanted ball; 2 weeks later a booster injection of another 200 μg was injected into the ball. Twenty-one days after the booster, up to 21 ml of clear, yellow fluid was directly withdrawn from the lumen of the ball using a two needle system. A sterile 22 gauge filter needle allowed air to pass into the lumen of the ball as fluid was being withdrawn through a second 22 gauge needle attached to a syringe. Polyclonal antibodies (IgG) were isolated from the hyperimmune fluid by protein A-sepharose affinity chromatography. A

portion of the immune IgG was conjugated to horseradish peroxidase (HPO; Sigma type VI, P 8375, RZ = 2.9) by the periodate method. After evaluation by ELISA, purification of the conjugate was deemed unnecessary.

Triplicate samples of homogenate from the nine struck mice were assayed on a single Dynatech Immulon I microtitre plate. The plate also contained triplicate samples of homogenate from nine control mice (20–24 g) injected with 0–4 mg venom in 0.5 mg increments. The venom, dissolved in 0.25–1.0 ml PBS, was delivered via tuberculin syringe (25 g needle) just below the skin of the right mid-dorsal region. These control mice, which were necessary for preparing a standard curve, were treated otherwise in an identical manner as the experimental mice.

To assay venom in the mouse homogenates, wells of the plate were coated overnight at 4°C with IgG in a 10 μg/ml PBS solution (99 μl/well). The wells were emptied (by slapping plate onto paper towels) and washed three times with Tween-20/PBS buffer (130 μl/well). Next (since a blocking step was found to be unnecessary; cf. HAYES *et al.*, 1992), the samples of mouse homogenate (1 : 1000 dilution in PBS) were added (99 μl/well) and incubated for 1 hr at room temperature. After the wells were emptied and washed as before, IgG-HPO conjugate was added (99 μl/well) and incubated another hour at room temperature. Finally, after wells were emptied and washed again, substrate solution [0.1 ml of 0.5% hydrogen peroxide added to 9.9 ml of 5-AS substrate (from Sigma) in 1 mg/ml 0.1 M sodium phosphate/EDTA buffer, pH 5.95] was added (99 μl/well). As the plate was agitated manually during the next hour, color development (absorbance) was read by an automatic microplate reader (Titertek Multiscan MCC/340) at 450 and 620 nm (dual wavelength mode).

To develop a standard curve, absorbance values for control homogenates were plotted against the concentration of venom and a regression equation was fitted using the program Quattro Pro (version 3.0). The regression equation was used to estimate the mass of venom expended (nearest mg) for each mouse based on the mean absorbance of the three homogenate samples from each carcass.

Experiment 2

Three months later, seven snakes (of the nine from experiment 1) were observed as before as each struck and constricted an adult male mouse (17–24 g) in its home cage. Immediately after death, the mouse was removed from the snake's mouth, stored within a plastic bag, and frozen at −20°C. Two months later, the mice were thawed, quickly and carefully skinned, and skin and viscera (skeleton, musculature, internal organs) placed in separate bags. As in experiment 1, each was homogenized with 150 ml wash buffer, centrifuged, and the supernatant frozen until ready for ELISA. Then, triplicate samples from three sets of homogenate (control, viscera, skin) were assayed on a single microtitre plate to determine concentrations of venom in both the viscera and the skin.

Validity of ELISA

The coefficients of determination (r^2) for our regression equations indicate the amount of variation in absorbance explained by the independent variable, quantity of venom present. The coefficients for the two experiments were, respectively, 0.81 and 0.84 (explaining more than 80% of the variance), which indicated that absorbance was a good indicator of the Duvernoy's secretion present in the samples.

Since the snakes deposited considerable quantities of saliva on the mice, we were concerned that our assay might have measured saliva in addition to venom. To confirm that the antibodies were not cross-reactive with *Boiga* saliva, we obtained saliva samples via micropipets as before from the oral epithelium of three of the live snakes. These samples were lyophilized, weighed, reconstituted in PBS, and assayed together with Duvernoy's secretion and venom from the midget-faded rattlesnake, *Crotalus viridis concolor* (as another control). In this ELISA, the wells were coated and washed as before. Duplicate samples of saliva and venom (1 mg/ml PBS) were then added and serially diluted in ten-fold dilutions (1 : 10) of PBS to 0.1 ng/ml. The wells were incubated 1.5 hr at room temperature and then washed. IgG-peroxidase conjugate was added next and incubated 2 hr at room temperature. After washing, substrate solution was added and color development was read as before.

Detectability, a useful indicator of cross-reactivity, is defined as the minimum concentration of venom (10") that can be detected by the ELISA (MINTON *et al.*, 1984; KAISER *et al.*, 1986; BOBER *et al.*, 1988). The level of detectability of *Boiga* Duvernoy's secretion was 0.001 μg/ml. Detectability for *Boiga* saliva was 1000 μg/ml, a difference of six orders of magnitude. *C. v. concolor* venom was not detectable. Thus, the antibodies appeared to be very specific for Duvernoy's secretion.

RESULTS AND DISCUSSION

The quantities of Duvernoy's secretion expended by brown tree snakes when striking mice and the survival times of prey are summarized in Table 1. The snakes expended similar total quantities of venom in the two experiments (means of 3.6 mg and 3.3 mg, respectively). These amounts of Duvernoy's secretion are less than but within an order of magnitude of those amounts of venom injected into mice by elapid and viperid snakes of

TABLE 1. DRY MASS OF DUVERNOY'S SECRETION (VENOM) EXPENDED BY THE BROWN TREE SNAKE (*Boiga irregularis*) DURING FEEDING AND TIME TO DEATH OF THEIR PREY, LIVE ADULT MICE (*Mus musculus*)

Dependent measures	Experiment 1 ($n = 9$) Mean	S.E.	Range	Experiment 2 ($n = 7$) Mean	S.E.	Range
Total venom expended (mg)	3.6	0.3	2–5	3.3	0.1	1.4–4.7
In viscera	—	—	—	1.8	0.2	1.0–2.7
In skin	—	—	—	1.5	0.2	0.5–2.2
Time to prey death (sec)	113	48	37–484	65	7.8	36–101

comparable size (HAYES *et al.*, 1992). However, the flow of Duvernoy's secretion in colubrid snakes is very slow compared to venom release by elapid and viperid snakes (CHISZAR *et al.*, 1992; HAYES, 1992; KARDONG and LAVÍN-MURCIO, 1993), and may have been continuous during the several minutes that the teeth engaged prey tissues.

Considering the minimum of 6.7 mg venom available in the Duvernoy's glands of adult *B. irregularis* (WEINSTEIN *et al.*, 1991; CHISZAR *et al.*, 1992; VEST *et al.*, 1991), the snakes of the first experiment expended approximately 54.5% of their available venom by the time their prey was dead. This value is equal to the highest percentage of available venom expended by any elapid or viperid snake for which comparable data are available (HAYES *et al.*, 1992); however, the venom is not released instantaneously as in other venomous snakes. An additional quantity of venom would likely have been secreted during the swallowing process had we permitted the snakes to do so. Unlike the venom glands of most elapids and viperids (KOCHVA, 1978), the Duvernoy's gland of *B. irregularis* lacks an extensive extracellular storage reservoir (ZALISKO and KARDONG, 1992). Therefore, this high percentage expended is a likely consequence of the low volume of ready venom within the lumen of the gland.

Results of experiment 2 indicate that approximately 54.5% of the total venom released was delivered into the viscera of prey, while 45.5% remained embedded in the skin (Table 1). The fact that total venom expenditure did not differ significantly between the two experiments gives us confidence that the separation of mouse skin from viscera in the second experiment did not confound our results. The value of 54.5% for envenoming efficiency is considerably below that of elapid and viperid snakes (MORRISON *et al.*, 1982, 1983; HAYES *et al.*, 1992). However, prior studies of envenoming considered only that venom on the surface of the integument which could be washed from it; these values for *Boiga* include venom embedded *in* the skin of prey, so comparisons must be treated cautiously.

The mean of 3.6 mg venom expended (experiment 1) when adjusted for mice averaging 21 g represents a dose of 171.4 mg/kg. The i.p. LD_{50} for *B. irregularis* is reported to vary between 10.5 and 34.1 mg/kg (VEST *et al.*, 1991; WEINSTEIN *et al.*, 1991, 1993). Thus, the amount of Duvernoy's secretion delivered during a feeding strike is well above the lethal dose (50%) necessary to kill mice. If only the portion of the secretion that entered the viscera (experiment 2) is considered ($1.8 \text{ mg} \pm 0.2$), this still gives an adjusted value of 90 ± 4 mg/kg, three to eight times the lethal dose (50%) depending upon which value is compared (34.1 or 10.5 mg/kg).

This success in getting a lethal dose of Duvernoy's secretion through the integument and into the viscera is particularly noteworthy for two reasons. First, delivery of Duvernoy's secretion during prey capture does not significantly contribute to rapid death of the prey. Where prey death was compared for *B. irregularis* with and without use of

Duvernoy's secretion, it was found that no difference occurred in the rate of prey death (ROCHELLE and KARDONG, 1993). The brown tree snake uses jaw pressure (ROCHELLE and KARDONG, 1993) and most prominently constriction (CHISZAR, 1991; ROCHELLE and KARDONG, 1993) to kill prey quickly. On occasions where prey escape or are removed before constriction brings death, they may become sluggish and even eventually die after many minutes or several hours (MCCOID, personal communication). This suggests that Duvernoy's secretion can tranquilize prey or even produce a protracted death. However, there is no published evidence that *Boiga* Duvernoy's secretion alone can equal the rapid prey death characteristic of the true venoms used during feeding by elapid and viperid snakes.

Second, the posterior maxillary teeth of *Boiga*, associated with the single duct from Duvernoy's gland (ZALISKO and KARDONG, 1992), bear an open groove. Thus, unlike the hollow teeth of elapids and viperids that form an enclosed venom channel within the fang, these grooved teeth of *Boiga* are an open channel and cannot sustain a pressure head to deliver a pulse of secretion deep into tissues (KARDONG and LAVÍN-MURCIO, 1993). Our results suggest that as a consequence, a significant portion (45.5%) of the delivered secretion remains lodged in the integument until the prey is dead.

Taken together, these results support the view that Duvernoy's secretion in most colubrids does not play a primary role in rapidly killing prey as do the venoms of elapids and viperids (KARDONG, 1982*b*). Further, the biological role of Duvernoy's secretions in most colubrids is most likely related to some other primary function, such as digestion (KARDONG, 1982*a*). The presence of proteolytic enzymes in Duvernoy's secretion from *Boiga* (WEINSTEIN *et al.*, 1992) supports this suggestion. The experimental evidence in other colubrid species of a digestion-promoting effect of Duvernoy's secretion (JANSEN, 1983; RODRÍGUEZ-ROBLES and THOMAS, 1992) further supports this hypothesis. Thus, toxicity of *Boiga* Duvernoy's secretion may be a simple by-product of this primary digestive role.

The digestive action of Duvernoy's secretion can act directly upon prey tissues to promote chemical digestion. We hypothesize that Duvernoy's secretion may additionally serve to open holes chemically in the tough integument of the swallowed prey to permit later entry of digestive enzymes released by the snake's digestive tract. Although we removed mice before swallowing began, the location of the grooved teeth results in their regular engagement with the integument of the prey as the jaws 'walk' over it, as in other colubrids (KARDONG, 1986). Consequently, during swallowing cycles, further quantities of Duvernoy's secretion from the gland could be introduced into the skin. Because the prey is dead when such swallowing begins, no active spread of the secretion by the circulatory system would occur, and for the most part the secretion would remain at the sites in the integument where the teeth initially deposited it.

If one biological role for Duvernoy's secretion is to open chemically the integument of the prey for entry of digestive tract enzymes, then this would help to account for why during prey capture such a large portion (45.5%) of the secretion remained embedded in the skin and why even further secretion is likely introduced during swallowing.

Acknowledgements—Very special thanks go to Dr NANCY MAGNUSON who generously contributed her time and laboratory resoures during the running of ELISA analyses. The School of Veterinary Medicine kindly loaned us use of an ELISA plate reader, J. FOLWELL helped with preparation of solutions, and M. ROCHELLE helped with husbandry of snakes. Supported in part by U.S. Fish & Wildlife Service and NSF (Animal Behavior, BNS-8820091), and by CONACYT (Mexico).

REFERENCES

BOBER, M. A., GLENN, J. L., STRAIGHT, R. C. and OWNBY, C. L. (1988) Detection of myotoxin α-like proteins in various snake venoms. *Toxicon* **26**, 665–693.

CHISZAR, D. (1991) The behavior of the brown tree snake: A study in applied comparative psychology. In: *Contemporary Issue in Comparative Psychology*, pp. 101–123 (DEWSBURY, D. A., Ed.). Sunderland: Sinauer Assoc.

CHISZAR, D., WEINSTEIN, S. A. and SMITH, H. M. (1992) Liquid and dry venom yields from brown tree snakes, *Boiga irregularis* (Merrem). In: *Contributions in Herpetology*, pp. 11–13 (STRIMPLE, P. D. and STRIMPLE, J. L., Eds). Cincinnati: Greater Cincinnati Herpetological Society.

FRITTS, T. H. (1988) The brown tree snake, *Boiga irregularis*, a threat to Pacific islands. *U.S. Fish Wildl Serv., Biol. Rep.* **88**(3), 36 pp.

HAYES, W. K. (1992) Factors associated with the mass of venom expended by prairie rattlesnakes (*Crotalus v. viridis*) feeding on mice. *Toxicon* **30**, 449–460.

HAYES, W. K., KAISER, I. I. and DUVALL, D. (1992) The mass of venom expended by prairie rattlesnakes when feeding on rodent prey. In: *Biology of Pitvipers*, pp. 383–388 (CAMPBELL, J. C. and BRODIE, E. D. JR, Eds). Tyler, TX: Selva.

HILLAM, R. P. L., TENGERDY, R. T. and BROWN, G. L. (1974) Local antibody production against the murine toxin of *Yersinia pestis* in a golf ball-induced granuloma. *Infect. Imm.* **10**, 458–463.

JANSEN, D. W. (1983) A possible function of the secretion of Duvernoy's gland. *Copeia* **1983**, 262–264.

KAISER, I. I., MIDDLEBROOK, J. L., CRUMRINE, M. H. and STEVENSON, W. W. (1986) Cross-reactivity and neutralization by rabbit antisera raised against crotoxin, its subunits and two related toxins. *Toxicon* **24**, 669–678.

KARDONG, K. V. (1980) Evolutionary patterns in advanced snakes. *Amer. Zool.* **20**, 269–282.

KARDONG, K. V. (1982*a*) The evolution of the venom apparatus in snakes from colubrids to viperids and elapids. *Mem. Inst. Butantan* **46**, 105–118.

KARDONG, K. V. (1982*b*) Comparative study of changes in prey capture behavior in the cottonmouth (*Agkistrodon piscivorus*) and Egyptian cobra (*Naja haje*). *Copeia* **1982**, 337–343.

KARDONG, K. V. (1986) Kinematics of swallowing in the yellow rat snake, *Elaphe obsoleta quadrivittata*: a reappraisal. *Jpn. J. Herp.* **11**, 96–109.

KARDONG, K. V. and LAVÍN-MURCIO, P. A. (1993) Venom delivery of snakes as high-pressure and low-pressure systems. *Copeia* **1993**, 644–650.

KARDONG, K. V. and YOUNG, B. A. (1991) Fangs and snakes: how do open grooves inject venom into enclosed spaces? *Amer. Zool.* **51A**.

KOCHVA, E. (1965) The development of the venom gland in the opisthoglyph snake *Telescopus fallax* with remarks on *Thamnophis sirtalis* (Colubridae, Reptilia). *Copeia* **1965**, 147–154.

KOCHVA, E. (1978) Oral glands of the Reptilia. In: *Biology of the Reptilia*, Vol. 8, pp. 43–161 (GANS, C. and GANS, K. A., Eds). New York: Academic Press.

KOCHVA, E. and WOLLBERG, M. (1970) The salivary glands of Aparallactinae (Colubridae) and the venom glands of *Elaps* (Elapidae) in relation to the taxonomic status of the genus. *J. Linn. Soc. (Zool.)* **49**, 217–224.

MCKINSTRY, D. M. (1983) Morphologic evidence of toxic saliva in colubrid snakes. A checklist of world genera. *Herp. Rev.* **14**, 12–15.

MINTON, S. A. (1990) Venomous bites by nonvenomous snakes: an annotated bibliography of colubrid envenomation. *J. wild. Med.* **1**, 119–127.

MINTON, S. A., WEINSTEIN, S. A. and WILDE III, C. E. (1984) An enzyme-linked immunoassay for detection of North American pitviper venoms. *Clin. Toxic.* **22**, 303–316.

MITTLEMAN, M. B. and GORIS, R. C. (1974) Envenomation from the bite of the Japanese colubrid snake *Rhabdophis tigrinus* (Boie). *Herpetologica* **30**, 113–119.

MORRISON, J. J., PEARN, J. H. and COULTER, A. R. (1982) The mass of venom injected by two Elapidae: the taipan (*Oxyuranus scutellatus*) and the Australian tiger snake (*Notechis scutatus*). *Toxicon* **20**, 739–745.

MORRISON, J. J., PEARN, J. H., CHARLES N. T. and COULTER, A. R. (1983) Further studies on the mass of venom injected by elapid snakes. *Toxicon* **21**, 279–284.

ROCHELLE, M. and KARDONG, K. V. (1993) Constriction vs. envenomation in prey capture by the brown tree snake *Boiga irregularis* (Squamata: Colubridae). *Herpetologica.* **49**, 297–300.

RODRÍGUEZ-ROBLES, J. A. and THOMAS, R. (1992) Venom function in the Puerto Rican racer, *Alsophis portoricensis* (Serpentes: Colubridae). *Copeia* **1992**, 62–68.

SAKAI, A., HONMA, M. and SAWAI, Y. (1984) Study on the toxicity of venoms extracted from Duvernoy's gland of certain Asian colubrid snakes. *Snake* **16**, 16–20.

SAVIDGE, J. A. (1986) The role of disease and predation in the decline of Guam's avifauna. Unpublished Ph.D. Thesis, University of Illinois, Champaign-Urbana.

SAVIDGE, J. A. (1987) Extinction of an island forest avifauna by an introduced snake. *Ecology* **68**, 660–668.

SAVIDGE, J. A. (1988) Food habits of *Boiga irregularis*, an introduced predator on Guam. *J. Herp.* **22**, 275–282.

TAUB, A. M. (1966) Ophidian cephalic glands. *J. Morph.* **118**, 529–542.

TAUB, A. M. (1967) Comparative histological studies on Duvernoy's gland of colubrid snakes. *Bull. Amer. Mus. Nat. Hist.* **138**, 1–50.

VEST, D. K. (1981*a*) Envenomation following the bite of a wandering garter snake (*Thamnophis elegans vagrans*). *Clin. Tox.* **18**, 573–579.

VEST, D. K. (1981*b*) The toxic Duvernoy's secretion of the wandering garter snake, *Thamnophis elegans vagrans*. *Toxicon* **19**, 831–839.

VEST, D. K., MACKESSY, S. P. and KARDONG, K. V. (1991) The unique Duvernoy's secretion of the brown tree snake (*Boiga irregularis*). *Toxicon* **29**, 532–535.

WEINSTEIN, S. A., CHISZAR D., BELL, R. C. and SMITH, L. A. (1991) Lethal potency and fractionation of Duvernoy's secretion from the brown tree snake *Boiga irregularis*. *Toxicon* **29**, 401–407.

WEINSTEIN, S. A., STILES, B. G., MCCOID, M. J., SMITH, L. A. and KARDONG, K. V. (1993) Variation and lethal potencies and acetylcholine receptor binding activity of Duvernoy's secretions from the brown tree snake, *Boiga irregularis*. *J. Nat. Tox.* In press.

ZALISKO, E. J. and KARDONG, K. V. (1992) Histology and histochemistry of the Duvernoy's gland of the brown tree snake, *Boiga irregularis*. *Copeia* **1992**, 791–799.