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Department of Biology, Concord College, Athens, West Virginia 24712.

DEFENSIVE BEHAVIOR AND SKIN GLANDS OF THE NORTHWESTERN SALAMANDER, *AMBYSTOMA GRACILE*

EDMUND D. BRODIE, JR. AND LINDA S. GIBSON

ABSTRACT: Concentrations of granular glands are present as parotoid glands and a tail ridge in *Ambystoma gracile*. Each granular gland has a collar of smooth muscle around its neck and a smooth muscle sheath around the entire gland. The secretion of these glands is viscous, insoluble, toxic, and irritates the lining of the mouth and eyes. Specialized defensive postures are effective in orienting glandular regions toward predators. Head butting and tail lashing bring the glandular secretions into contact with the predator.

DEFENSIVE behavior as related to noxious skin secretions in amphibians seems to be of two basic types. The first, posturing to display warning coloration, is exhibited by forms with evenly distributed skin glands. Fire-bellied toads, *Bombina*, and newts exhibit this type of behavior in the form of an "unken" reflex. The second type of defensive behavior involves posturing in a way to orient heavy concentrations of glands toward danger, usually a predator. This behavior, not necessarily related to aposematic coloration, is common in the genus *Bufo* and has been noted for the salamander, *Ensatina eschscholtzi* (Hubbard, 1903).

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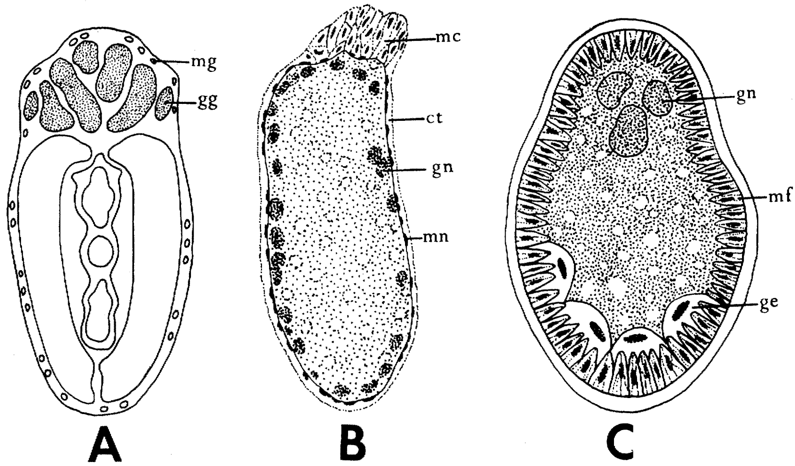

FIG. 1.—Granular glands of *Ambystoma gracile*: A. Cross section through tail, 4 ×; B. Longitudinal section of granular gland, 80 ×; C. Cross section through muscle collar, 180 ×. Granular glands (gg), mucous glands (mg), muscle collar (mc), connective tissue (ct), gland nucleus (gn), muscle nucleus (mn), muscle fiber (mf), gland epithelium (ge).

Ambystoma gracile is a large dark brown salamander with conspicuous concentrations of glands in the parotoid region and on the dorsum of the tail. Specialized behavior, orienting the concentrations of glands toward danger, would allow these areas and their secretions to be more effective. We describe these concentrations of glands, the action of their secretion, and related defensive behavior.

HISTOLOGY OF GLANDS

The tail ridge (Fig. 1A) and the parotoid regions of *A. gracile* skin contain heavy concentrations of granular glands. There are smaller and less dense concentrations on the costal folds, below the parotoid patches, and along the ventral surface of the tail tip. Distribution of granular gland concentrations is illustrated in Figs. 2 and 3.

Granular glands are oval but may be deformed by neighboring glands. The tail ridge of a representative animal, 75 mm in snout–vent length, had granular glands 195–2260 μ long (avg 1350 μ) and 90–730 μ in width (avg 430 μ); smaller granular glands were located along the lateral edges of the tail ridge. Larger salamanders have a deeper ridge and probably larger individual glands. Occasionally there are smaller granular glands above the large glands. There are usually 7–8 granular glands across the width of the tail ridge at any point (Fig. 1A) and about 30 per millimeter of tail length.

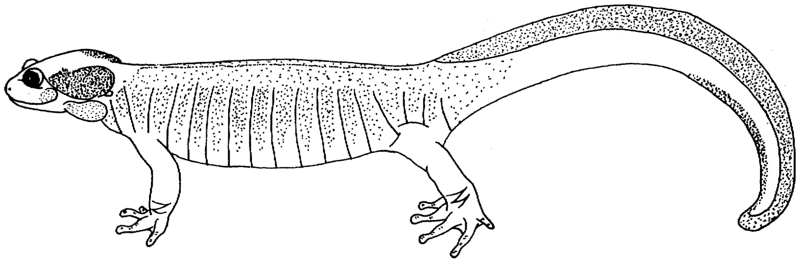

FIG. 2.—Defensive posture of *Ambystoma gracile* when endangered from the rear. Granular gland concentrations are stippled.

Mucous glands are located between the necks of the granular glands and around the entire tail (Fig. 1A); they are about 90 by 120 μ.

The gland cells of granular glands comprise a single layer which is surrounded by smooth muscle cells and a connective tissue sheath (Fig. 1B). In secreting cells the walls are broken down in typical holocrine secretion, obliterating the lumen. Large gland nuclei are found only around the outer edges of the gland. A large "collar" of muscle, two fibers thick and three fibers long, surrounds the neck of the granular glands (Figs. 1B, 1C). These fibers extend longitudinally and may retain secretion in the gland, i.e., relaxation of these muscles would allow passage of the secretion. Muscle nuclei around the body of the gland indicate a single layer of muscle cells surrounding the gland. Both constrictor and dilator muscles are found around the ducts of the granular glands.

Muscle cells could be distinguished from connective tissue cells by spiraled nuclei (when contracted) and spindle-shaped cytoplasm. Cross sections of the glands occasionally show spiraled nuclei in a longitudinal aspect indicating the presence of muscle fibers running horizontally around the gland.

NATURE AND TOXICITY OF SECRETIONS

The secretion from the glandular regions of *gracile* is milky, viscous, extremely adhesive, and hardens within seconds after being secreted. The secretion is insoluble in water, ethyl alcohol, and acetone.

The tail ridge of *gracile* was macerated in mammalian Ringer's solution, but little if any of the secretion went into solution; most adhered to the mortar and pestle.

When eight Townsend's voles, *Microtus townsendi*, and six white rats were injected intraperitoneally with the above mixture, the results were variable; 6 voles and 4 rats died in 15 min to 5 hr. The variability in results is probably due to the insolubility of the secretion since it has been shown that water soluble secretions from other salamanders give uniform results (Brodie, 1968a, 1968b). Injections of the skin preparation caused hypersensitivity to sound and touch, instability, loss of righting, and convulsions prior to death. Rodents injected with similar volumes of Ringer's solution showed none of these symptoms.

Four *gracile* were unaffected by injections of their own skin secretions, but it is possible, as with *Taricha granulosa*, that they would be susceptible to large doses (Brodie, 1968a). Reciprocal injections indicated that neither *A. gracile* nor *T. granulosa* is resistant to the skin secretions of the other; two individuals of each species died less than 10 min after injection with the other's skin secretion.

Since *Ambystoma macrodactylum* occasionally exudes small quantities of glandular secretions from the dorsal surface of the tail, similar experiments were performed using macerated tail skin of this species. Four *Microtus townsendi* injected with *macrodactylum* skin reacted like those injected with *gracile* skin. Neither *gracile* nor *macrodactylum* was affected by injections of the other's skin.

A white rat, fed a mixture of *gracile* skin and Ringer's, was affected by hypersensitivity and instability, but recovered within 3 hr.

Distastefulness of the glandular secretion may be more important than toxicity in defense against predators. *A. gracile* were fed to cats, rats, a great-horned owl, and garter snakes. Cats would not eat or bite *gracile* voluntarily and were apparently repulsed by odor. When cats were forced to bite the tail, they pawed at their mouths.

When a rat attacked and bit an adult *gracile* on the parotoid gland, the salamander twisted and rubbed secretion on the rat's head. In less than 5 sec, the rat released the salamander. In 30 sec the rat was effectively blinded since its eyes were swollen shut; its mouth and nose were encrusted with the hardened secretion, and breathing was labored. One eye was partially open after 2.5 hr. After 5 hr both eyes were partially open but were inflamed and draining heavily; breathing remained labored. After 17 hr the eyes appeared normal and the small amount of encrusted secretion still remaining around the mouth seemed not to disturb the rat.

A drop of glandular secretion applied to the senior author's tongue resulted in drying, loss of sense of touch, and a burning sensation which lasted for 15 min in the area of application.

A great-horned owl (*Bubo virginianus*) voluntarily ate an adult *gracile* and four *macrodactylum* without ill effect.

Responses of garter snakes, *Thamnophis sirtalis fitchi*, toward *gracile* were variable. One snake explored the parotoid region with its tongue and moved away. This was repeated four times after which the salamander was replaced by a frog; the frog was quickly examined and eaten. Another snake attacked and swallowed an entire *gracile* tail (which was severed from the salamander) without ill effects or evidence of distaste.

Defensive Behavior

The following is a composite description of the behavior of more than 15 adults from the vicinity of Corvallis, Benton Co., Oregon. Defensive postures were elicited by pinching their toes or prodding them gently with a pencil.

When collected, *gracile* often remain rigid without attempting to escape. When stimulated on the hindquarters or tail, *gracile* elevates the body on all four legs and arches the tail (Fig. 2). In this posture the salamander appears larger and is able to lash its tail from side to side with great speed and accuracy. If stimulation continues, the salamander leans toward the source and secretes from the tail ridge. They are so adept at tail lashing that it is difficult to touch the leg or body without being struck by the tail. Some secretion is usually left on the object contacted. The head is usually held forward (Fig. 2) but is sometimes flexed (Fig. 3). Some salamanders do not arch the tail but whip it from side to side along the ground in response to stimulation; one did not use the tail at all but turned its head rapidly toward all stimuli.

When stimulated on the forequarters or head, *gracile* flexes and tilts the head, butting with the parotoid gland (Fig. 3). The eyes are tightly closed and the body is tilted so the concentrations of glands on the costal folds face the irritation. When further stimulated the parotoid glands and smaller glandular areas on the head secrete. The tail is seldom arched and there is usually no secretion from the tail ridge.

When a *gracile* is grasped, it twists and rubs its glandular regions on the attacker, exuding large quantities of secretions. In some cases the salamander secretes only from those regions in contact with the attacker. Some *gracile* require only the presence of a foreign object to elicit defensive behavior; others posture only after much mechanical stimulation. After several months in captivity some animals lose the response.

Larval *gracile* have poorly developed glandular patches which secrete if the animal is severely stimulated. The defensive postures and movements described above do not appear until the glandular regions become obvious about a month after metamorphosis. When tail slapping and head butting first appear, they are not always directed at the stimulus, as they invariably are in adults. This suggests that adult defensive behavior is at least partially learned.

Discussion and Conclusions

The granular glands of *A. gracile* are surrounded by a coat of smooth muscle like those of *A. tigrinum* and *A. annulatum* (Mason,

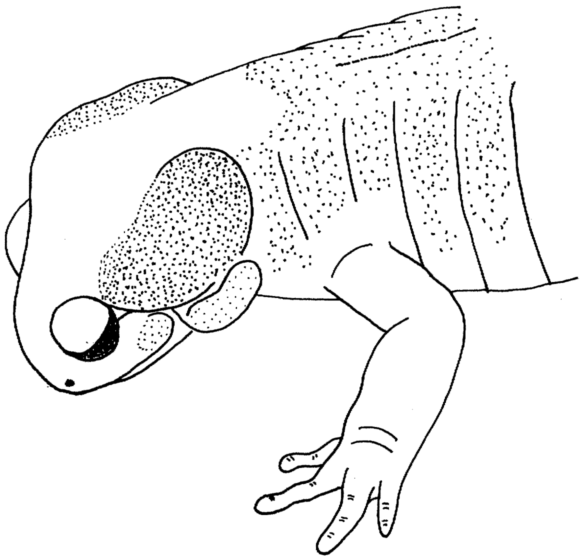

FIG. 3.—Defensive posture of *Ambystoma gracile* when endangered from the front. Granular gland concentrations are stippled.

Hall, and Roofe, 1965), but also have a collar of muscle around the neck of the gland. As in *Ensatina eschscholtzi* (Esterly, 1904), both constrictor and dilator muscles are present around the ducts on the granular glands.

A. gracile relies upon secretions from glandular areas and related defensive behavior to ward off attacks by predators. The adhesive and insoluble properties of the secretion are probably as important in the salamander's defense as distastefulness and toxicity. An attacking predator with this substance in its eyes is blinded since the eyes swell closed. The insolubility may, however, reduce the amount of toxin taken into the bloodstream of a predator after ingesting the salamander.

Skin secretions are of defensive value in at least two other *Ambystoma*. Potential predators are repulsed by taste from *A. maculatum* (Barach, 1951) and by odor from *A. macrodactylum croceum* (Anderson, 1963), but specialized behavior was not noted in these species. Defensive behavior similar to that described for *A. gracile* has been reported for *A. jeffersonianum* and *A. tigrinum melanostictum*. *A. jeffersonianum* raises its hindquarters, holds its tail vertically, and waves the tail from side to side while keeping its head and forebody near the ground (Rand, 1954). During this display the tail exudes a "sticky, white substance." Apparently *A. jeffersonianum* does not release gland contents from different portions of the body independently. Carpenter (1955) suggests that similar tail-waving behavior of *A. t. melanostictum* is aposematic, displaying the black and yellow pattern. Mason et al. (1965) report the presence of granular glands on the tail of this subspecies, and it is possible that this behavior may also serve to draw attention to the tail, which is "expendable" and may contain a noxious substance. The vertical tail-waving display of *A. jeffersonianum* and *A. tigrinum* is passive in nature and distinct from the head butting and tail lashing of *A. gracile*. Noxious skin secretions and defensive behavior are apparently common among ambystomatid salamanders but are most highly developed in *A. gracile*. *A. rosaceum* has weakly developed concentrations of glands on the tail dorsum and in the parotoid region (Anderson, 1961), but no defensive behavior has been noted.

The type of defensive behavior in salamanders seems to correspond with the distribution of concentrations of glands. Granular glands of *Ensatina eschscholtzi* are concentrated along the dorsal surface of the tail (Esterly, 1904) and its defensive behavior (Hubbard, 1903) is similar to that of *A. gracile* when danger is from the rear.

Salamandra salamandra possess well-developed parotoid glands (Phisalix-Picot, 1900) and exhibit a defensive posture similar to *A. gracile* when danger is from the front. *S. salamandra* also butts with the head and parotoid region when danger is from the rear. The head butting of *A. gracile* and *S. salamandra* is similar to the defensive behavior of many species of *Bufo* (Hinsch, 1926; Noble, 1931). This reaction by *Bufo* is thought to aid the toad by increasing its apparent size but is no doubt also useful in placing the parotoid glands and their toxic secretions near the predator (Hanson and Vial, 1956).

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Department of Zoology, Oregon State University, Corvallis, Oregon 97331 (PRESENT ADDRESS OF BRODIE: *Zoology Section, Clemson University, Clemson, South Carolina* 29631).

THE EFFECT OF TEMPERATURE ON THE METABOLISM OF THE PRAIRIE RINGNECK SNAKE, *DIADOPHIS PUNCTATUS ARNYI* KENNICOTT

ARTHUR L. BUIKEMA, JR. AND KENNETH B. ARMITAGE

ABSTRACT: Metabolism was measured in relation to body weight, temperature, and temperature acclimation. Weight regression coefficients (b) determined for nonacclimated snakes at 5 C intervals between 10 and 35 C averaged 1.03, for acclimated snakes, 0.68. The value of b tended to vary with temperature in a consistent pattern. The acutely determined R-T curve of snakes held at 22 C was double sigmoid. Acclimation occurred in snakes held above 20 C, but not in snakes held at 20 C or lower. Acclimation apparently involved the translation of the R-T curve of warm acclimated animals. Q_{10} was higher at higher temperatures for warm acclimated than for cold-acclimated snakes.

TEMPERATURE conformers, such as reptiles, which inhabit temperature climates are subjected to a wide range of environmental temperate. Undeniably, the evolution of behavioral thermoregulation has been paramount in determining the distributional limits and survival of such conformers. However, some reptiles also undergo physiological adjustments when exposed to various temperatures (Bartholomew and Tucker, 1963, 1964; Bartholomew et al., 1965; Dawson and Bartholomew, 1956; Gelineo and Gelineo, 1955; Hutchison et al., 1966; Steward, 1965; and Wilhoft and Anderson, 1960).

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