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A Novel Method for the Release and Collection of Dermal, Glandular Secretions From the Skin of Frogs

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Introduction

The dorsal skin of frogs contains numerous glands broadly classified as mucous or granular; both gland types are under the control of sympathetic nerves, and discharge following a variety of stimuli, Sjöberg and Flock (1976). Lillywhite (1971) demonstrated the synchronous discharge of the glands, and their sympathetic control via inhibition by the β-blocker propranalol. Dockray and Hopkins (1975) stimulated the dermal sympathetic nerves of anesthetized African Clawed Frogs (*Xenopus laevis*), and demonstrated the release of the polypeptide caerulein from granular glands.

Mucous and granular glands are distributed over the entire dorsal surface but the granular glands may also be aggregated in specific areas to form conspicuous, elevated structures attributable to localized hypertrophy. In some cases vast quantities of secretions are arranged in a columnar fashion, for example, in the calf gland of *Limnodynastes dumerilii* (Crook and Tyler 1981). Tyler (1987) recognized eight forms of hypertrophied granular glands in Australian species classified according to their anatomical position.

A resurgence of interest in the nature of compounds in frog skin (particularly antibiotic polypeptides: Bevins and Zasloff, 1990) has focussed attention upon the method of extraction. In the 1960s granular secretions were obtained via a methanol extraction from sun-dried skins. In some species more than 1000 specimens were sacrificed to obtain sufficient quantities of the compounds for identification (Roseghini et al., 1976).

A method of obtaining dermal secretions was developed following the observation of Nakajima (1981) of discharge upon injection of noradrenaline into the dorsal, subcutaneous lymphatic sac. Gibson et al. (1986, 1991) recovered these secretions by injecting noradrenaline, immersing the frog in a 50-mM solution of ammonium acetate, and then scraping the precipitated secretion from the body.

Because of widespread concern at evidence that many frog species are in serious decline (Tyler, 1991), it is imperative that noninvasive techniques of extraction be developed. One such method is via surface electrical stimulation (SES).

Material and Methods

The SES technique was tested on diverse genera and species of Australian frogs: Family Hylidae, *Cyclorana australis*, *C. longipes*, *Litoria bicolor*, *L. latopalmata*, and *L. rubella*, which lack hypertrophied granular glands and *L. caerulea*, and *L. splendida*, which possess them. Family Leptodactylidae, *Crinia signifera*, *Limnodynastes ornatus*, and *L. tasmaniensis*, which lack hypertrophied glands, and *L. convexiusculus*, *L. dumerilii*, *Uperoleia inundata*, *U. littlejohni*, *U. micromeles*, and *U. rugosa*, which possess them.

The electrical stimulator used was a C. F. Palmer student model providing pulse durations of 2 or 4 msec, a pulse repetition rate of 50/sec and a maximum stimulus strength of 20 V. The instrument was developed for use with standard physiological preparations such as the sciatic/gastrocnemius nerve muscle preparation from the decerebated and spinalized frog. Battery-operated, acupuncture units are equally satisfactory.

To stimulate the release of granular gland secretion, we held the frog by the back legs, moistened the skin

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with distilled water, and rubbed a bipolar electrode of 21G platinum with a 3-mm gap gently in a circular manner upon the back of the animal. The strength of the stimulus varied with the size of the frog, and presumably the thickness and conductivity of the skin. The *Uperoleia* species with snout to vent lengths of as little as 20 mm required a pulse duration of 2 msec and no more than 3 V. In contrast, *Litoria caerulea* with a body length of 100 mm could require a 4-msec pulse duration and 20 V.

The stimulation parameters were varied from subthreshold to achieve secretion release with minimal discomfort to the subject.

Results

The speed of onset of gland discharge was highly variable, with a delay of 5–15 sec before anything was visible. Because the skin is loose and with contact with the underlying musculature only via septa of transparent connective tissue, it was evident that the expression of secretions was mediated only via intrinsic cutaneous muscles. As soon as the secretions were released, a second operator washed them into a clean bowl beneath the animal, using a stream of distilled and deionized water. On average, 50–75 mL water was required. The entire procedure was completed in 30–40 sec, after which secretion flow was reduced markedly and none remained on the surface. The animal was unharmed, and it was found that it could be "milked" again following replenishment of the glands after 2–4 weeks. No attempt was made to stimulate at shorter intervals.

In the case of the large *L. caerulea* and *L. splendida* as much as 50 mgm of polypeptides could be recovered at a single milking. Comparison of the composition of the peptides obtained by electrical stimulation of *L. caerulea* was similar to that in a homogenate provided by Dr. M. Zasloff.

Discussion

The technique described here permits the extraction of frog skin secretions without necessitating the sacrifice of the donors. We acknowledge that it is difficult to assess the level of distress experienced by the frogs, because they tend to struggle when restrained in any manner, but we note that the struggling did not increase conspicuously during the electrical stimulation. We have found that the suitability of battery-operated acupuncture stimulators permits the use of the technique in the field, so eliminating the need for transportation and maintenance of captive individuals.

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References

Bevins DL, Zasloff M (1990) Peptides from frog skin. *Ann Rev Biochem* 59:395–414.

Crook GA, Tyler MJ (1981) Structure and function of the tibial gland of the Australian frog *Limnodynastes dumerili* Peters. *Trans Royal Soc South Australia* 105:49–52.

Dockray GF, Hopkins CR (1975) Caerulein secretion by dermal glands in *Xenopus laevis*. *J Cell Biol* 64:724–733.

Gibson BW, Poulter L, Williams DH, Maggio JE (1986) Novel peptide fragments originating from PGL and the caerulein and xenopsin precursors from *Xenopus laevis*. *J Biol Chem* 261(12): 5341–5347.

Gibson BW, Tang D, Mandrell R, Kelly M, Spindel ER (1991) Bombinin-like peptides with antimicrobial activity from skin secretions of the Asian toad, *Bombina orientalis*. *J Biol Chem* 266(34): 23103–23111.

Lillywhite HB (1971) Thermal modulation of cutaneous mucous discharge as a determinant of evaporative water loss in the frog, *Rana catesbeiana*. *Z Vergl Physiol* 73:84–104.

Nakajima T (1981) Active peptides in amphibian skin. *Trends Pharmacol Sci* 2:202–205.

Roseghini M, Erspamer V, Endean R (1976) Indole-, imidazole- and phenyl-Alkylamines of the skin of one hundred amphibian species from Australia and Papua New Guinea. *Comp Biochem Physiol* 540:31–43.

Sjöberg E, Flock A (1976) Innervation of skin glands in the frog. *Cell Tissue Res* 172:81–91.

Tyler MJ (1987) Frog and cane toad skin secretions. In *Toxic Plants and Animals. A Guide for Australia*. Eds., J Covacevich, P Davie, and J Pearn. Brisbane: Queensland Museum, pp 329–339.

Tyler MJ (1991) Declining amphibian populations—a global phenomenon? An Australian perspective. *Alytes* 9(2):34–50.