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PRELIMINARY CHARACTERIZATION OF
THE DEFENSIVE SECRETION OF *DIPLO-*
DACTYLUS (REPTILIA: GEKKONIDAE).—

The subgenus *Strophurus* of the Australian genus *Diplodactylus* possesses a defensive gland that forms most of the core of the tail (Rosenberg and Russell, 1980; Russell and Rosenberg, 1981a). The defensive nature of the secretion has been verified by laboratory observations (Bustard, 1980; Richardson and Hinchliffe, 1983) but its chemical nature is known only from a few histochemical reactions.

The material is very sticky and this may be its most effective property (Eisner, 1970:189 discusses sticky secretions). No toxic effects have been observed on chicks (Rosenberg and Russell, 1980), the lizards themselves (Russell and Rosenberg, 1981b) or rats (Richardson and Hinchliffe, 1983). The secretion of some species is black while that of others is pale yellow. The defensive secretion has "a very distinct musty odor" (Rosenberg and Russell, 1980) or "an

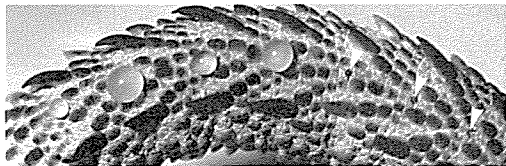


Fig. 1. *D. ciliaris*. Dorsal view of tail just after stimulation. Note three small drops of secretion (arrows) in addition to large spheres. $\times 4$.

unpleasant odor resembling that of crushed legume seeds" (Richardson and Hinchliffe, 1983).

Initial histochemical tests yielded negative results for glycoprotein and lipid (Rosenberg and Russell, 1980). These results have been corroborated by those of Richardson and Hinchliffe (1983) who reported negative results on tests for neutral lipid, carbohydrate, proteins containing tyrosine and acid mucopolysaccharides. The occasional presence of minute, PAS positive particles within intracellular vacuoles, a weak PAS reaction in the lamellar region of the cell, and the proteinaceous nature of the secretion have been noted (Richardson and Hinchliffe, 1983).

In this communication we describe a method for obtaining the secretion and we present results of a preliminary electrophoretic analysis.

Materials and methods.—Lizards were cooled to 10 C for two hours and packed in crushed ice 20 min prior to collection of secretion. The tail was cleared of ice and its skin gently prodded with a stimulating electrode. Secretions were collected on the outer surface of micropipettes that were sealed in vials and kept at -20 C. Material was collected from *D. ciliaris*, *D. rankini* and *D. strophurus*.

One dimensional polyacrylamide gel electrophoresis was carried out in the presence of sodium dodecyl sulphate (SDS) (Laemmli, 1970). The separating gel consisted of a 7–15% polyacrylamide linear gradient while 4% polyacrylamide was used in the stacking gel. Electrophoretic separation was performed using an LKB 2001 vertical slab gel unit. The molecular weights of polypeptides were estimated by co-electrophoresing standard proteins available in a molecular weight calibration kit (Bio-Rad) that consists of phosphorylase b-92,500; bovine serum albumin-66,200; ovalbumin-45,000; carbonic anhydrase-31,000; trypsin inhibitor-21,500 and lysozyme-14,400.

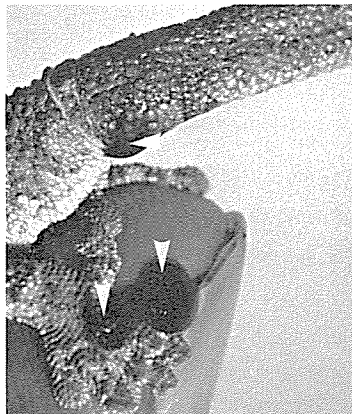


Fig. 2. *D. strophurus*. Lateral view of regenerated tail. The black secretion (arrows) has been ejected from the zone of weakness between the tail and the stump of the original tail. Specimen fired its secretion before it could be chilled. $\times 3.2$.

Frozen secretions were dissolved in 0.0625 M Tris-HCl buffer, pH 6.8, containing 2% SDS and 5% β -mercaptoethanol by boiling for 10 min. The samples were electrophoresed at 20 mA per gel for 6–8 hours, gels fixed in 30% methanol-10% acetic acid, stained for protein with Coomassie brilliant blue R-250 (Sigma), and destained in 30% methanol-10% acetic acid. After initial destaining was complete, slab gels were stained for glycoproteins using a modified periodic acid-Schiff technique (Zacharius et al., 1969), as adapted by Dewald et al. (1974).

Results.—The collection of secretion from cooled lizards proved to be effective and did not harm the specimens. Very often the act of prodding alone (current switched off) caused localized, reflexive contraction of caudal muscles and the secretion oozed out through the epidermal rupture zone (Fig. 1). Lizards that were not chilled delivered their secretion in an explosive manner that defied controlled collection.

Secretion was collected from the caudal gland of non-regenerated (i.e., original) tails of *D. ciliaris* and *D. rankini*. A dramatic expulsion of black secretion from the gland in a regenerated tail of *D. strophurus* indicated the probable retention of defensive capabilities even after regeneration of an autotomized tail (Fig. 2).

Electrophoretic profiles of defensive secretion from *D. ciliaris*, *D. rankini* and *D. strophurus* are presented in Fig. 3. Approximately 20 bands

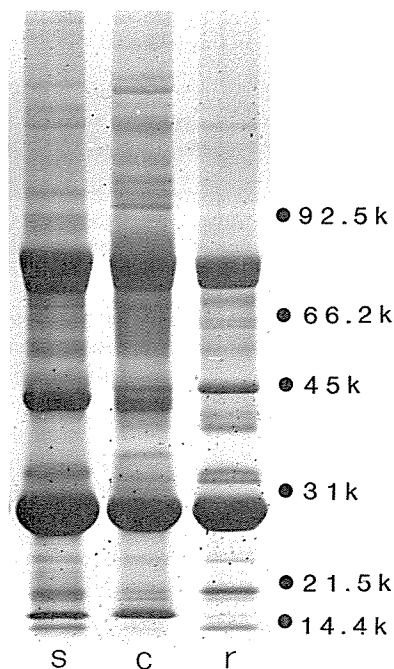


Fig. 3. SDS-gel electrophoresis of caudal gland secretions run at 20 mA. s = *D. strophurus*, c = *D. ciliaris*, r = *D. rankini*. Dots indicate positions of low molecular weight markers in thousands of daltons.

were resolved, depending on the volume of the sample applied. Concentrations of protein were not determined as only minute quantities were available for analysis. One of the polypeptides (MW ~ 80,000) shown in Fig. 3 from *D. strophurus* gave a positive reaction for glycoprotein while all the other bands were stained by Coomassie blue.

Three major bands were observed in similar positions for all three species: band I (MW ~ 30,000), band II (MW ~ 45,000) and band III (MW ~ 80,000). The minor bands are described in relation to the above a) All three species showed five minor bands in similar positions with molecular weights less than I, b) Four minor bands between I and II were stained. *D. ciliaris* and *D. rankini* show a double band just above I, *D. strophurus* has a wide band above I, and *D. ciliaris* shows one sharp band midway between I and II. On the other hand, *D. rankini* has a double band below II. *D. strophurus* and *D. ciliaris* both have dense bands just below II. c) All three species show similar patterns of minor bands between II and III. d) The greatest difference between the three species is evident

in the region above III. Relatively high molecular weight polypeptides in the secretions from *D. strophurus* and *D. ciliaris* are stained while those from *D. rankini* are not apparent. There are about five bands that are apparently unique to *D. ciliaris* and two that are unique to *D. strophurus* in the region above III.

Discussion.—Chilling of entire specimens and stimulating the tail enabled us to easily collect secretion from the caudal gland. The secretion oozed onto the surface, formed spherical drops (Fig. 1) and was recovered for testing. The method was repeatable and did not cause mechanical injury to the specimen.

The proteinaceous nature of the secretion has been shown and at least one glycoprotein component demonstrated for *D. strophurus*. The electrophoretic patterns of the secretions from the three species are quite similar. All of the major bands and most of the minor bands occupy similar positions along the length of the gel (Fig. 3). The similarity is not too surprising as all three species are members of the *Strophurus* species group (Kluge, 1967; Storr, 1979). The current study demonstrates the presence of caudal glands in *D. rankini* and thereby corroborates Storr's (1979) placement of this species in the *Strophurus* species group.

Electrophoretic patterns of tail secretions produced by *D. elderi* and *D. michaelsoni*, the only known members of the two remaining species groups within the subgenus *Strophurus* (Russell and Rosenberg, 1981a) are still to be determined. Also of interest may be the secretion of the newly described *D. wilsoni*, stated by Storr (1983) to be a link between the *D. strophurus* and *D. michaelsoni* species groups. For this subgenus, electrophoretic profiles of the proteins in caudal secretions may be a useful taxonomic adjunct at the species and population level.

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