coast of Victoria and New South Wales, Australia (Repenning *et al.* 1971). Marion Island is closer to South Africa (1 972 km, great circle route to Cape Agulhas) than to Tasmania (7 642 km, great circle route to western Tasmania) and it is likely that the specimen recorded here originated in South Africa, its movement possibly aided by the southward flowing Agulhas Return Current whose southern limit is in the region of 40°S (Heydorn, Bang, Pearce, Flemming, Carter, Schleyer, Berry, Hughes, Bass, Wallace, van der Elst, Crawford & Shelton 1978). The direct route from Tasmania to Marion Island is against the prevailing currents (West Wind Drift and Return Agulhas Current — Heydorn *et al.* 1978), reducing the likelihood of this seal originating in Tasmania.

Within their normal distribution, marked *A. pusillus* individuals have been recorded to cover distances of 1 300 km within a few months (Rand 1959) following the coastline, and Payne (1979) recorded *A. tropicalis* vagrants covering distances of up to 3 000 km. Shaughnessy & Ross (1980) recorded a total of 23 *A. tropicalis* individuals arriving in South Africa and although the origin of these seals is at present unknown, the possibility does exist that they could have come from Marion Island which is the reverse of the present case. This record of *A. pusillus* on Marion Island is the most southerly record for this species.

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The histology of the venom-secreting apparatus of the puff-adder, *Bitis arietans*

A.R. Lake, J. Hattingh*, R.E. King and T.R. Trevor-Jones

Departments of General Anatomy and General Physiology, Dental School, University of the Witwatersrand, Johannesburg 2001
*To whom correspondence should be addressed

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Recently, Rosenberg (1967) described the histology, histochemistry and emptying mechanisms of the venom glands of more than 20 different elapid species including those of some sea snakes. He showed that these snakes all possess an accessory venom gland in addition to the main gland. The accessory gland was composed of uniform mucous epithelium which was usually P.A.S. positive and surrounded the primary venom duct. The main venom gland consisted of many tubules which usually contained large amounts of secretory product. The lining of the tubules was usually a flat epithelium, but little cellular detail could be observed. Kochva, Shayer-Wollberg & Sobol (1967) and Kochva & Gans (1970) investigated the histology of some 20 species of viperid snakes and found that the venom glands were all of a similar shape and glandular structure except in the mole viper where the accessory gland was absent. De Lucca, Huddad, Kochva, Rothschild & Valeri (1974) demonstrated a relationship between the secretory activity, the morphology of the epithelial cells lining the venom glands and the secretory cycle. The cells varied from low cuboidal to almost squamous epithelium in unmilked snakes, to tall columnar secretory epithelium in milked snakes with the variation between the two cell types depending on the amount of stored venom.

Very little information is available concerning the histology of the venom secreting apparatus of the puff-adder, *Bitis arietans*. King & Hattingh (1979) investigated the main venom gland of this snake in the resting and stimulated state. In the resting state the tubules comprising the venom gland were lined with columnar secretory cells. After repeated milkings the histological appearance of the gland changed and the lining epithelim of the individual tubules became taller, more slender and the tubules themselves more

foliaceous. A pronounced increase in the pigment was observed in the connective tissue septa. The aim of the present investigation was to study the histology of the entire venom secreting apparatus of the puff-adder as a first step in understanding the role the accessory venom gland plays in venom production.

Five mature puff-adders were obtained from a local snake dealer. The animals were all clinically healthy and the material for study was removed immediately after sacrifice (unmilked animals). The venom apparatus was fixed and processed as previously described (King & Hattingh, 1979). The sections were stained with haematoxylin and eosin, and Masson's trichrome for general morphology, and the following histochemical methods were used: Periodic acid-Schiff (P.A.S.) for carbohydrates, alcian blue pH 2,6 for acid mucopolysaccharides and a combination of alcian blue and P.A.S.

The puff-adder venom secreting apparatus may be divided into (i) a posterior main gland; (ii) a tapering intermediate neck region which contains the primary duct; (iii) an anteriorly situated accessory venom gland, and (iv) a terminal or secondary venom duct (Figure 1). This latter duct opens anterolaterally inside the fang sheath adjacent to the superior aperture in the fang and the duct is not continous with, or attached to, the fang. The venom apparatus is bilateral and extends supralabially from the *crotaphyte* muscle (*compressor glandulae*, Rosenberg 1967). Approximately one third of the main venom gland is embedded in this muscle. The main gland and neck region is pyriform in shape, oval in cross-section and compressed lateromedially. The anterior part of the main gland tapers, becoming slender ventral to the orbit, where the primary duct commences. Immediately anterior to the orbit is a bulbous expansion, the accessory gland, which surrounds the anterior part of the primary duct. The secondary, or terminal duct, is approximately 3 to 5 mm in length, curves ventromedially and enters the fang sheath anterolaterally.

Figure 1 Diagrammatic representation of the venom secretory apparatus. A = crotophyte muscle; B = main venom gland; C = neck region; D = primary duct; E = accessory gland; F = secondary duct; G = fang sheath; H = fang with central canal.

The venom apparatus is enclosed in a tough connective tissue capsule. The muscle ligaments and connective tissues associated with the venom apparatus insert into the outer surface of this capsule. The capsule forms connective tissue septa for support of the simple tubular gland elements. These septa contain a considerable amount of yellow-black pigment, an extremely rich capillary network and an extensive nerve supply. The septa in the neck region of the gland contain isolated smooth muscle slips. The framework of the main gland and neck area of the venom apparatus consists of multiple, radially arranged tubules which are neither simple nor complex, but of an intermediate type, supported by connective tissue septa. The tubules are orientated in a postero-anterior direction converging towards the centre of the gland where they open into a narrow lumen, which forms the primary duct.

The tubules of the main gland and neck region are lined with an epithelium which varies from low cuboidal to low columnar and which are foliaceous at the free margin. The lumen of the tubular glands is filled with a secretory product which is acidophilic and contains darker staining rods and cocci chains (indicating the presence of bacteria). The venom or secretory product adjacent to the secretory

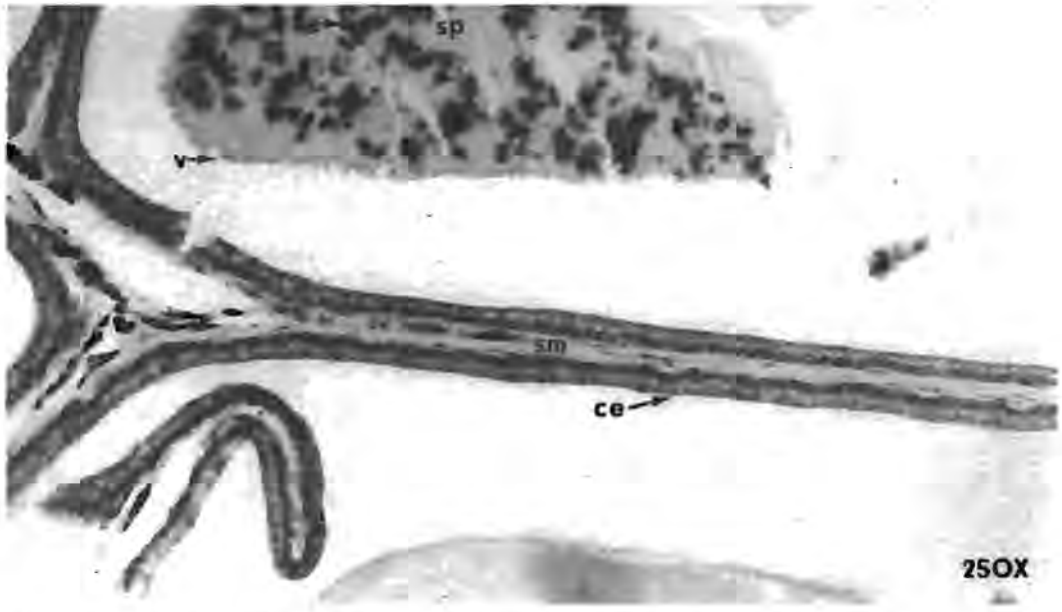

Figure 2 Low columnar epithelium of glands in neck region (CE). Stored product (SP) containing bacteria (B) and vacuoles (V). Smooth muscle slips (SM) are also present. Masson's Trichrome.

epithelium often contains vacuoles (Figure 2). This, however, may be an artifact owing to shrinkage of the fixed material.

The cytoplasm of the columnar secretory epithelium contains basophilic granules and clear secretory vacuoles which are large in some cells. The nuclei vary from spherical to elliptical and the cytoplasms appears to be slightly acidophilic, but this is masked by the large number of contained basophilic granules. P.A.S. sections of the neck and main gland show that the secretory vacuoles inside the cells appear to have a red central granular content with clear surrounding material. The remainder of the cytoplasm of the epithelial cells contains yellow granules. The secretory product in the tubular glands is P.A.S. positive which is markedly positive peripherally, indicating the area of most recent secretion. Alcian blue shows that the cytoplasm of the secretory epithelium contains dark staining blue granules with blue to dark purple secretion vacuoles and that some secretory vacuoles also contain dark purple granules. Two types of cells are present: (i) cells with blue staining nuclei, dark staining blue granules and vacuoles as mentioned above (these constituted the greater proportion), and (ii) a few cells with a pale blue foamy cytoplasm and translucent secretory vacuoles.

The height of the epithelial cells of the tubules may be correlated with their secretory activity. The tubules lined with the taller more foliaceous cells showed far less stored product than the tubules lined with low cuboidal cells with an ill-defined foliaceous border. A fine capillary network is associated with the base of some epithelial cells in the main gland and neck region. Masson's trichrome shows that the horizontal cells reminiscent of myo-epithelial cells are in fact blood cells which fill the lumen of the fine capillaries related to the base of the epithelial cells of the tubular gland elements.

The duct epithelium is cuboidal secretory epithelium with the same characteristics as that lining the main gland. The accessory gland (Figures 3 & 4) is divided into a posterior region and an anterior region and surrounds the anterior part of the primary duct. The posterior part of the accessory gland shows a large central duct around which are arranged diverticula lined with low columnar epithelium. P.A.S. shows that the secretory activity varies in the cells — some contain red granules and others fewer yellow-pinkish granules. The secretory product is P.A.S. positive. Alcian blue sections are positive and indicate the presence of mucopolysaccharides. The lining epithelial cells show two types of vacuoles, light blue and light purple, and the remaining cytoplasm contains purple granules. The anterior part of the accessory gland has a tall columnar epithelium with goblet-type cells. The material in some of the goblet cells appears basophilic (granular) and in others clear. The goblet cell secretion is P.A.S. positive, being a dark purple. Alcian blue shows that the epithelial cells contain blue granules and that the goblet cells are either clear or contain a light purple product. The combined P.A.S.-alcian blue reaction indicates that the lumenal margins of the epithelial cells in both the anterior and posterior regions of the accessory gland are lined by a mucopolysaccharide and that the secretory product produced is P.A.S. positive (Figure 4).

The terminal or secondary duct is lined with a tall columnar secretory epithelium with elliptical nuclei, supported by underlying connective tissue. P.A.S. shows a positive secretion product being released at the distal margin of the cells. The cytoplasm of the cells contains dark red granules. Alcian blue shows cells containing blue and purple granules in the cytoplasm. The free margin has a blue secretion in the process of being released. The combined P.A.S.-alcian blue reaction indicates a blue mucopolysaccharide lining on the free margin of the cells and a P.A.S. positive secretion

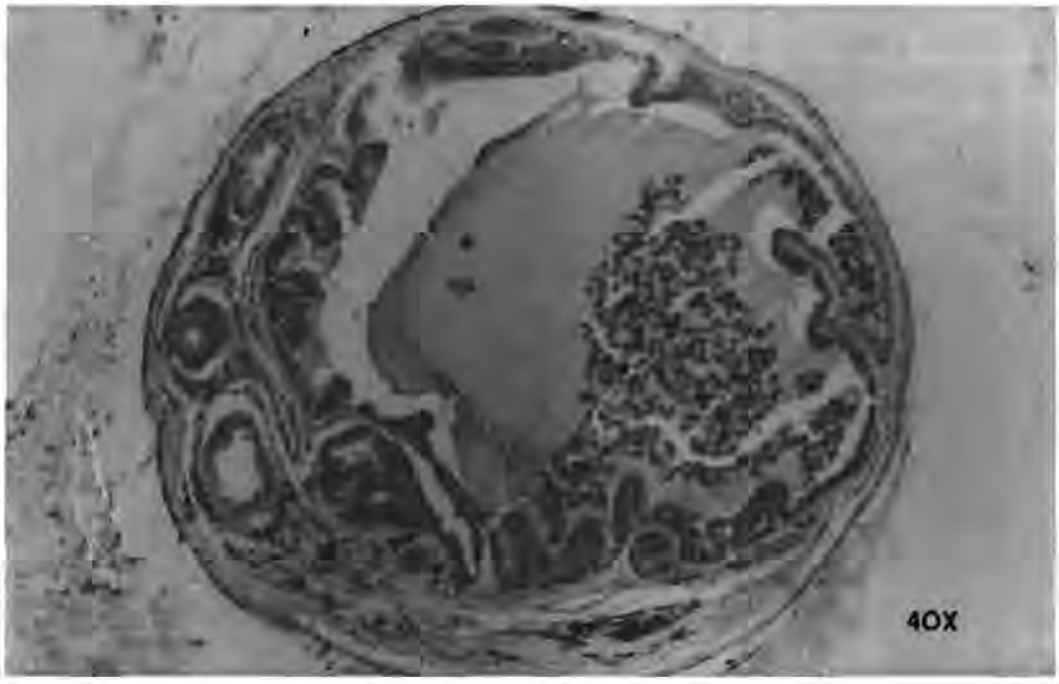

Figure 3 Posterior region of accessory gland with diverticula lined with cuboidal epithelium surrounding a central duct filled with secretory product (SP). H and E.

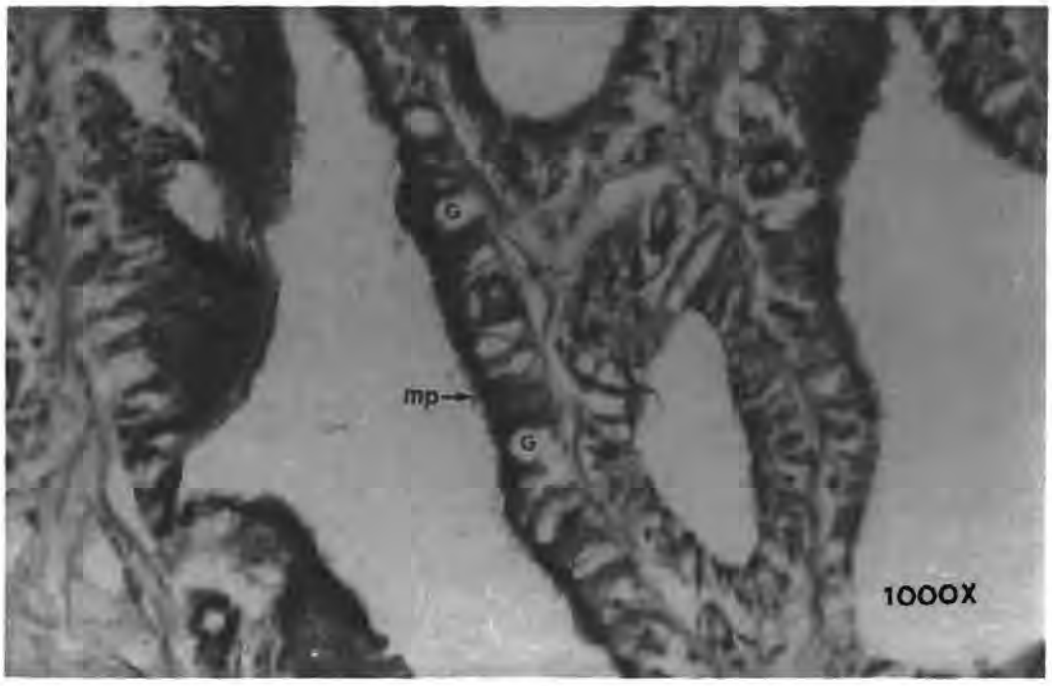

Figure 4 Anterior portion of accessory gland showing mucopolysaccharide secretion (mp) and goblet-type cells (G). P.A.S.-alcian blue.

adjacent to the mucopolysaccharide lining.

The fang sheath has an epithelium which varies from stratified cuboidal towards the free margin of the sheath to a simple squamous epithelium towards the base. The connective tissue underlying the epithelium consists of collagen and elastic elements with an extensive capillary network. P.A.S. is negative, but a few cells in the stratified cuboidal layer show cytoplasm with P.A.S. positive granules. Alcian blue shows that cells of the stratified cuboidal layer and squamous epithelial layer contain deep blue to purple granules as well as large secretory granules. The free margin of the cells appears to have a blue staining mucopolysaccharide lining (Figure 5).

There is an extensive nerve plexus in the connective tissue septa of the main gland and neck region. The accessory gland is well supplied with a separate nerve supply indicating, possibly, that this gland is controlled independently of the main gland.

The morphology of the venom apparatus bears no resemblance to mammalian salivary glands as the gland parenchyma consists of partially divided, essentially simple tubular glands lined with low cuboidal to tall columnar secretory epithelial cells. The accessory gland produces a mucopolysaccharide substance which appears to form a protective coating on the epithelium lining the accessory glands. This coating also lines the tall columnar cells of the terminal

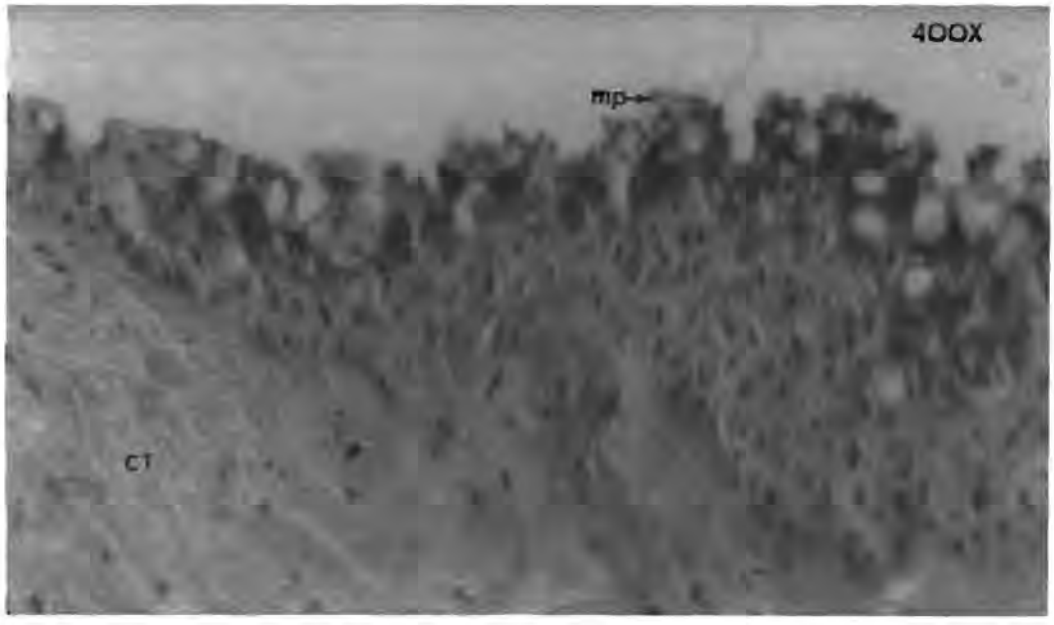

Figure 5 Fang sheath cuboidal epithelium (CE) with mucopolysaccharide lining on the free margin (mp) and an extensive capillary bed in the underlying connective tissue (CT). P.A.S.-alcian blue.

duct but is absent from the epithelium of the main venom gland. The large number of capillaries associated with the underlying supporting tissue of the fang sheath epithelium, suggests that there is some mechanism for the reabsorption of fluid, thereby concentrating the venom before it is ejected by the fang. The fang sheath probably functions as a cuff surrounding the base of the fang thus allowing for a closed pressure system for the ejection of the venom by means of the fang. This pressure is developed by the *crotaphyte* muscle which is continuous with the venom apparatus connective tissue sheath. The connective tissue sheath in the neck region and the supporting septa have smooth muscle slips which could act in conjunction with the *crotaphyte* muscle. These findings agree with those of Gans & Kochva (1965), Rosenberg (1967) and De Lucca *et al.* (1974).

Kochva & Gans (1970) showed a uniformity in glandular structure in the venom apparatus of more than 20 viperid species, except for the mole vipers which do not have a differentiated accessory gland. The venom glands have four distinct regions, the main gland which occupies the posterior two-thirds of the gland, the primary duct, the accessory gland and the terminal duct that leads to the fang sheath. These findings are similar to those found in *Bitis arietans* except that the accessory gland is separated from the main gland by a distance of approximately one centimetre. Similarly, the accessory glands show two distinct regions, the anterior part lined with a typical mucous epithelium containing goblet cells, and the posterior part lined with a flat to cuboidal epithelium, the shape of which appears to correlate with the secretory activity (Gennaro, Callahan & Loring 1963; Gans & Kochva 1965; Odor 1965). Rosenberg (1967) showed that elapid snakes also have an accessory gland. These accessory glands, however, did not have an anterior and posterior region since the morphology of the gland showed no variation.

The function of the accessory glands remains to be fully investigated. Gennaro *et al.* (1963) have shown that an extract of the accessory gland enhances the toxicity of the main venom gland, but subsequent mixing experiments of the main venom gland product and the accessory gland secretion failed to show any inhibition or activation of enzymes in the venom of viperid snakes (BDolah 1979). In addition, the rods and cocci chains seen by us (King & Hattingh 1979) in the venom by aseptically culturing this product indicate that the venom produced by the main gland is not cytotoxic to all cell types and that it may subsequently be activated by additional secretion which is added to the venom or by some other mechanism not yet understood. The mechanisms involved in the production of venom in these snakes are clearly not yet fully understood.

Acknowledgements

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Range extensions of blennioid fishes on the southern African west coast

M.J. Penrith* and Mary-Louise Penrith

State Museum, Windhoek, South West Africa/Namibia

Present address: National Zoological Gardens of S.A., P.O. Box 754, Pretoria 0001, Republic of South Africa

*To shom correspondence should be addressed

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The intertidal zone of the south and west coasts of southern Africa is notable for the complete dominance of blennioid fishes. Clinids of the subfamily Clininae are the most numerous fishes in the south and south-west, and blennies of the subfamily Blenniinae dominate in the northern portion of the west coast.

The distribution and ranges of clinids have been recorded by Penrith (1969, 1970) and those of blennies by Penrith & Penrith (1972). Subsequently Winterbottom (1976) recorded a number of clinid range extensions from the Cape Peninsula eastwards, but no additional information on blennioid fish distribution on the coast west of the Cape Peninsula has been published.

During December 1981 a number of localities were sampled in the company of Dr P.J. Miller (Bristol University) during an unsuccessful search for gobioid fishes. Some unexpected new records of clinid fishes were obtained, and in view of these results it was decided to rework some of the localities to the north of Toscanini (Figure 1).

Localities where collections were made, and where blennioid fishes were collected outside their recorded range were: