Histology of the venom gland of the puff-adder (*Bitis arietans*)

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The histology of the venom gland of the puff-adder (*Bitis arietans*) has been investigated in the resting and stimulated state. No accessory venom gland was found to be associated with the main venom gland or duct in the same position as has been reported for other snakes. In the resting state the parenchyma of the venom gland was found to consist of tubules lined by a single layer of tall columnar secretory cells. After being stimulated to secrete by repeated milkings, the histological appearance of the gland changed and the epithelium was found to be more foliaceous and the component cells to be taller and more slender. A pronounced increase of pigment was also observed in the connective tissue septae. Micro-organisms were observed in the secretion pools of resting glands and by culturing the venom it could be shown that these were *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Klebsiella ozaenae*. The presence of these organisms would suggest that the venom is not cytotoxic to all cells at this level of production.
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Die histologie van die gifklier van die pofadder (*Bitis arietans*) is ondersoek in die rustende sowel as die gestimuleerde staat. Geen bykomstige gifklier, geassosieer met die hoof gifklier soos beskryf vir ander slange, kon gevind word nie. In die rustende toestand bestaan die parenkiem van die gifklier uit tubules uitgevoer met 'n enkele laag silindriese sekretoriese selle. Na stimulasie deur herhaalde melkings, verander die histologiese voorkoms van die klier en die epiteel word meer blaaragtig en die selle dunner silindries. 'n Merkwaardige vermeerdering in pigment kan waargeneem word in die bindweefsel septae. Mikro-organismes kom voor in die sekretoriese produk van rustende kliere en deur die gif te kweek is gevind dat *Staphylococcus aureus*, *Staphylococcus epidermidis* en *Klebsiella ozaenae* teenwoordig was. Die voorkoms van hierdie organismes dui aan dat die gif nie sitotoksies is vir alle selle op hierdie vlak van sekresie nie.
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In recent years there has been an upsurge of interest in the venom glands of snakes. Much of this emphasis has centred around the glandular activity (Robertson & Delpierre 1969; Russel & Eventov 1964; Marsh & Gladstone 1974) and the suggestion by Gans and Kochva (1970) that some snakes do not possess a single venom gland, but that a complex of glandular portions on each side of the head exists. The same authors in 1965 had described an accessory gland that straddled the connection from the main gland to the secondary duct leading to the fang. It was further postulated that the venom produced by either the main or accessory gland is non-toxic and that this substance only becomes toxic after mixing of the two glandular secretions. It is also notable that most observations up to the present are related to viperid snakes and that little effort has been made to correlate structure, function and changes that take place after repeated stimulation of the glands. In the present communication light microscopical appearances of the puff-adder venom gland are reported before and after stimulation of the gland by milking.

Methods

Six mature puff-adders (*Bitis arietans*, Viperidae), clinically healthy at the time of collection, were used in the study. They were obtained from a local snake dealer who caught them in the Rustenberg region of Transvaal. The snakes were acclimatized to laboratory conditions for three to four weeks prior to being investigated. Two individuals were anaesthetized with ether, their glands aseptically dissected free after ligation of the venom ducts as near as possible to the fangs and the venom contained in the glands allowed to drop into sterile specimen bottles for later use. Two further individuals were milked once by allowing them to bite into a plastic membrane covering a beaker, and their glands then dissected out immediately after this procedure. The remaining two snakes were milked daily (as above) approximately at the same time for five consecutive days and then sacrificed, after which the glands were also removed. The glands were either fixed in 10% formol saline as a whole or were bisected; one half of which was fixed in 10% formol saline and the other half immediately deep-frozen in an ultrafreeze at −70 °C. Following fixation the specimens were

dehydrated in ascending grades of ethyl alcohol, cleared in chloroform and embedded in paraffin wax, after which sections were cut at a thickness of 6 μm. Selected sections were stained with haematoxylin and eosin and Masson's Trichrome. Frozen sections were cut in a Cryostat and stained with PAS and alcian blue at pH 2,6.

The venom collected aseptically from the glands (see above) and placed into sterile specimen bottles was plated onto blood agar plates immediately after it was obtained. Aerobic cultures were incubated at 37 °C for 24 – 48 h. Anaerobic cultures were incubated in Baird and Tatlock jars with an atmosphere of 20,7% carbon dioxide. Results were analyzed after an incubation period of one week and all colonies were stained with Gram's stain.

Results

One venom gland was found to be located on each side of the head in all cases. They were 'kidney shaped' with a slight expansion posteriorly and varied between 1 – 1,5 cm in length and 0,5 – 0,75 cm in breadth. The expansion was capped by what was once known as the *crotaphite* muscle, but now is referred to as the *compressor glandulae* (Rosenberg 1967) (Fig. 1). The anterior end tapered markedly from the body of the gland and then formed a narrow portion which was the beginning of the main venom duct. The narrow portion and the duct were located ventral to the orbit.

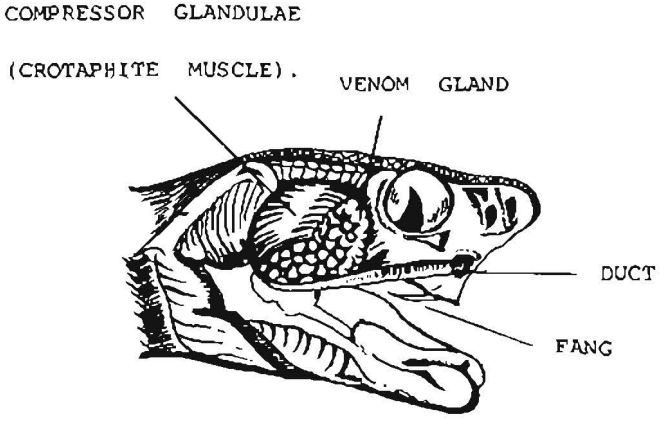

Fig. 1 Diagram of superficial dissection of the head, showing relationship of venom gland and compressor muscle.

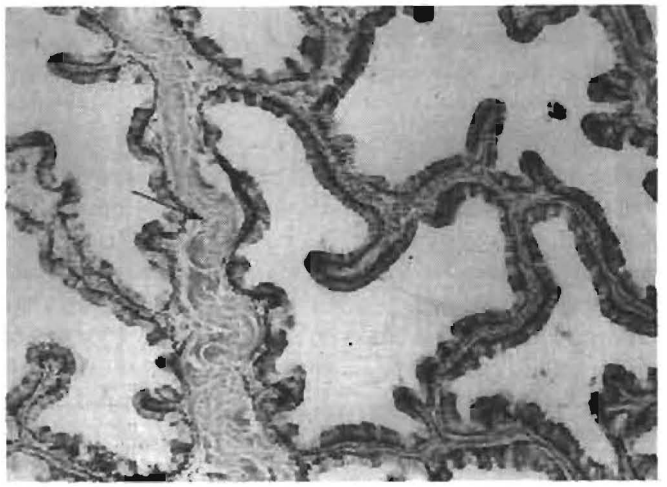

Fig. 2 Connective tissue septa (arrowed) clearly demarcating two lobes. H/E . . . X 100.

The gland was found to be invested by a thin connective tissue capsule. Septae from the capsule projected into the gland and divided it into 5–6 clearly demarcated lobes (Fig. 2). In addition to providing support for the secretory epithelium, these septae carried nerves and vessels and contained pigment (Fig. 3). No myo-epithelial cells could be

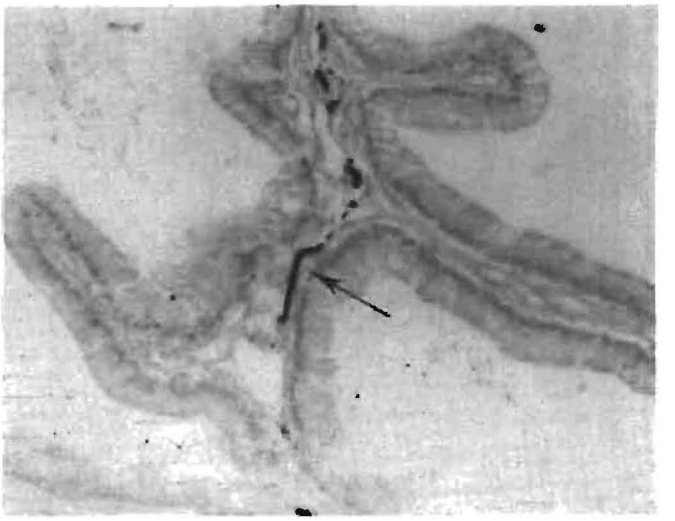

Fig 3 Negative alcian blue reaction shows prominent pigmentation in the connective tissue. Alcian blue . . . X 400.

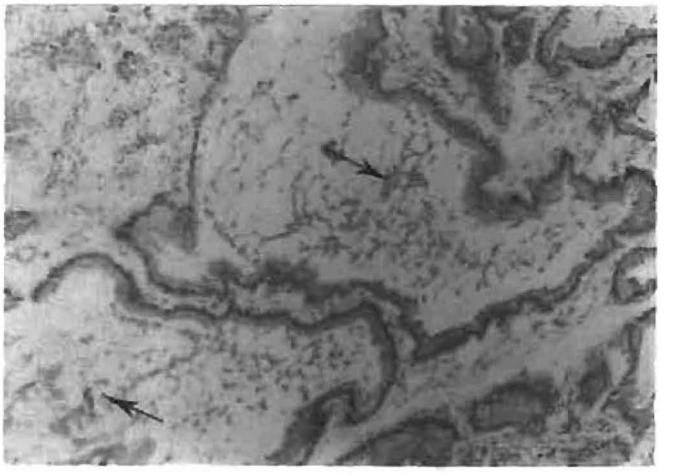

Fig. 4 Coagulated secretion in the irregular secretory tubules of the venom gland. H/E . . . X 100.

demonstrated. The parenchyma of the gland consisted of many irregular secretory tubules lined by a single layer of tall columnar secretory cells. Secretion product was found within most of the secretory tubules in the resting gland (Fig. 4). Staining with PAS and alcian blue at a pH of 2,6 gave negative reactions for the cells and the secretion product (Figs. 3 & 4). Within the secretion pools there were scattered micro-organisms (Fig. 5). After one milking the gland appeared clinically similar to the resting gland. Structurally there was little difference except for the absence of secretion product in the tubules. However, there were numerous secretion droplets gathered at the tips of the columnar cells (Fig. 6).

After five daily milkings the glands underwent a drastic change. They became reduced in size by at least one third and were hard and compact. Histologically the lumens were

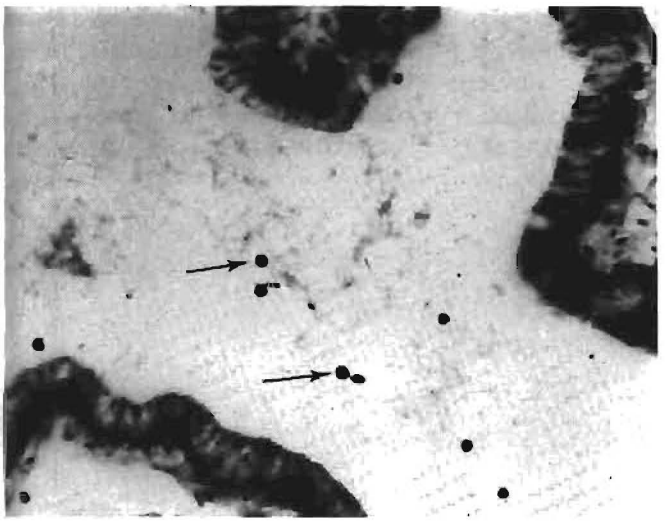

Fig. 5 Scattered micro-organisms in depleted secretory tubules. H/E . . . × 400.

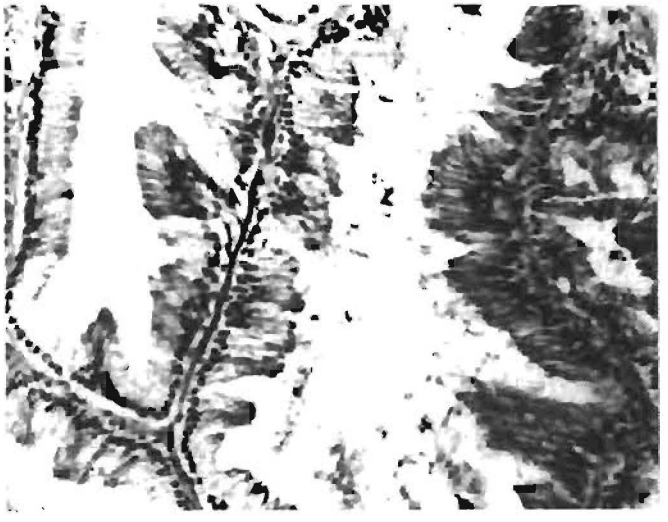

Fig. 6 Resting gland, showing secretion droplets at tips of columnar cells. H/E . . . × 400.

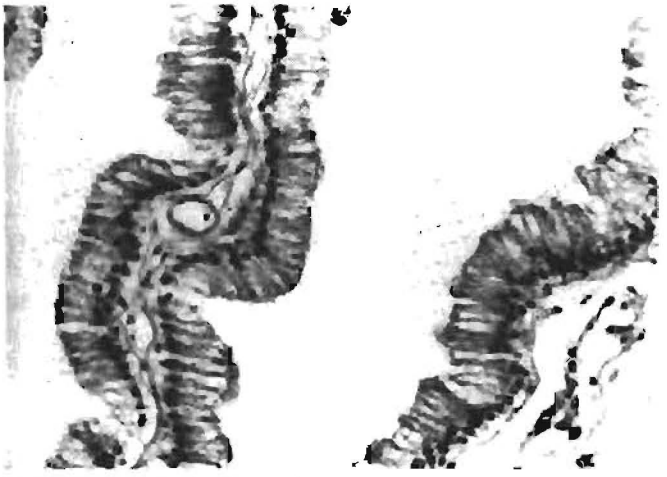

Fig 7 Depleted gland with empty lumens and tall slender columnar cells lining secretory tubules. H/E . . . × 400.

more spacious, but empty (Fig. 7). The infoldings of epithelium were more foliaceous and component cells taller and more slender. A pronounced increase of pigment was apparent, and a similar absence of reaction for PAS and alcian blue was demonstrated (Fig. 3).

Discussion

BDolah (1979) describes the morphology of venom glands in *Elapidae*, the genus *Atractaspis* and *Viperidae* and claims characteristics for each group. However, he emphasizes results obtained on viperid snakes and it is clear that the most conspicuous feature of the venom gland in most vipers is the absence of differentiated accessory glands. Twenty other species of 'genuine' vipers display a uniformity of venom gland structure and it appears that the gland has four distinct regions: the main gland, the primary duct, accessory glands and secondary ducts. The accessory glands are further divided into two parts. Unlike these and other reports (Rosenberg 1967; Gans & Kochva 1970) on the viperids, an accessory venom gland could not be seen in the puff-adder in the same position in the present study. Previous work by the authors mentioned showed that the accessory gland envelops the main venom duct in the region where the main venom gland gives rise to the venom duct. Although the main venom gland and venom duct was carefully dissected free as a unit here, this accessory gland could not be demonstrated and no difference in the histology of any part of the main venom gland was observed. If an accessory venom gland does exist in the puff-adder, it is definitely not in the same position as reported in the other species of snakes.

The structural components of the venom gland consisted of tall columnar secretory cells that have no histological resemblance to a mammalian parotid salivary gland whatever. Gans and Kochva (1970) refute any implication that these glands represent a reptilian parotid since they evolve independently. However, they do claim a joint origin for the venom glands, fangs and Duvernoy's gland as being adjuncts to the dental lamina of the maxilla. The micro-organisms identified after culture of the venom were the same as previously observed, namely, *Staphylococcus aureus, Staphylococcus epidermidis* and *Klebsiella ozaenae* (Hattingh, Williams & Beissner 1979). The significance of these micro-organisms in venom is unknown at this stage, but their presence does suggest that the secretion of the venom gland is not cytotoxic to all cells at this level of production. This may therefore indicate that an accessory secretion is necessary to make the venom cytotoxic, which would agree with the suggestion made by Gans and Kochva (1970).

The pigment found in the septae of resting and stimulated glands is thought to be lipofuscin and the quantity seems to be increased after the stress of frequent milking. The significance of this observation is not clear and it is also not known whether this pigment is reabsorbed after a suitable period of rest.

Although the tubules of the glands appear empty after milking, the presence of numerous secretory droplets suggest that the glands cannot be completely depleted. This agrees well with field and laboratory observations that venom can be expressed from the venom glands even after repeated milkings. The fact that the histological appearance of the glands change after repeated milking correlates well with published observations on the puff-adder indicating that frequent milking of the snakes has no effect on inter-individual venom variations, but does largely abolish intraindividual venom variation with a more constant electrophoretic pattern as a result (Willemse *et al.* 1979). In the

mentioned study it was also shown that the mean venom protein concentration decreases by approximately 63% after 4 – 5 days of successive milking. The fact that the resting secretion of the venom gland contains a very high protein content is reflected in the fact that negative staining responses for PAS and alcian blue were observed in the present study. From these results it can therefore be postulated that, at this level of secretion, the main venom components are protein, and a further study is expected to yield strongly positive staining reactions for these proteins in the main venom gland. It would be of interest to compare the staining properties of the venom in the glands of the snake species containing accessory glands with the present observations. Such a study will provide useful information concerning the composition of the secretions at the level of the main venom gland.

Acknowledgement

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