

BIOLOGY OF THE REPTILIA

Edited by

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BIOLOGY
OF THE
REPTILIA

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Preface

The venomous nature of snakes is one of the best known of popular attributes of these animals and the source of much folklore and mystery. Recently these subjects have received renewed attention and not just from physicians interested in treatment and from those interested in snakes as an end in themselves. Rather, biochemists found the venoms an important tool, and pharmacologists have used them to analyze the actions of various tissue systems.

The resulting information explosion, with symposia seemingly published biannually, raises the question about what, if anything, should be covered in the framework of the present series. Rather than collating a series of individual research reports, contributions of broadly based reviews, summarizing the major subfacets of this field were solicited. We are happy to have had the assistance of Dr Kochva in reviewing the literature and recent research on the morphology, histology and ultrastructure of venom glands; Willard B. Elliott in summarizing and organizing the enormous biochemical and immunological literature; and Dietrich Mebs for a review of the pharmacology of reptilian venoms. Also, an article by Dr Mahmoud Latifi was solicited to deal with the issue of antiserum production, not from the experimental viewpoint but as practiced in one major government serum laboratory. On the other hand, we are not including a specific discussion of the treatment of snake bite as this still involves some major disagreements, and treatment should always reflect previous experience with the bite of the particular race or species of snake. Some of the pertinent literature has been cited by W. B. Elliott. Some of the evidence produced in these chapters tends to support the hypothesis that the venom gland systems are closely associated with the digestive processes of snakes. Consequently, it seemed appropriate to include a paper by Dr Rafał Skoczylas discussing reptilian digestion processes, as this represents an obvious complement and counterpoint to the other reports. Finally, I add an introductory account summarizing some evolutionary consideration of reptilian venoms and the literature dealing with them, as well as appendices listing the species that might be of concern to man.

The volume again attempts to organize the data in terms of the current names of the animals with which we are dealing. We are grateful to Drs G. Zug and Heinz Wermuth for their critical reading of the manuscripts and proofs, as well as assistance with transliteration of names. Their decision

obviously assumes that the species were correctly identified in the first place. Drs Paul Boquet, Paul A. Christensen, Brian D. Clark, Horace W. Davenport, Herbert C. Dessauer, Willard B. Elliott, Joseph Gennaro, Elazar Kochva, Dietrich Mebs, Sherman Minton, Ch. Moroz, Akira Ohsaka, Thomas S. Parsons, Noel Rose, Herbert I. Rosenberg, Findlay Russell, Aaron M. Taub, Peter Tolson and A. DeVries read and commented on manuscripts or parts thereof. Linda Hilton, Erna Renner, and Kristine Vaitkus retyped some of the manuscripts. I am particularly pleased to record my appreciation to my wife for her discerning assistance with the editorial work. The Department of Zoology and later the Division of Biological Sciences of The University of Michigan assisted with the considerable bills for postage.

January, 1978

Carl Gans

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Reptilian Venoms: Some Evolutionary Considerations

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I. Introduction

Approximately one-third of all reptiles produce some buccal toxins, i.e. substances that have a deleterious effect on those organisms that normally encounter the reptile as predator or as prey. Thus modified salivary glands have been found in all families of higher snakes, as well as in lanthanotids, varanids and helodermatids among the lizards. The widespread appearance of variably toxic venoms in those snakes and lizards popularly (and correctly) considered to be harmless to man also indicates that the system probably did not evolve as a deterrent to large predators.

The present volume documents how far we have come toward an understanding of the biochemistry, immunology and pharmacology of reptilian venoms. Chromatography and electrophoresis have helped to separate venoms into their components. Histochemistry, radioautography and electron microscopy are beginning to unravel the nature of the secretion process. Biochemistry is teaching us the structural nature and affinities of the individual venom components, and pharmacology is now beginning to correlate each gross effect with the actions of a specific component. Immunology permits an assessment of the relative stability of the surface of the various proteins and polypeptides, and immunological relatedness as determined by complement fixation is a sensitive test permitting assessment of particular peptides. The dates of the papers referred to in subsequent sections also indicate that the preponderant fraction of the information was obtained during the last two decades, and that we appear to have been inundated by the results of an explosion of data. The International Society on Toxinology, publishers of *Toxicon*, also appear to sponsor almost annual conferences, each of which results in one or more volumes of proceedings, and the nature and effect of venoms attract ever wider interest (cf. Miller, 1977). This sudden

and immense fund of information may make it useful to pause a moment and take stock.

The present brief article attempts to do this by expressing some comments on the presumptive origin and evolution of the venom system, stressing the meaning and limitations of the data that have been accumulated thus far and suggesting some pathways for ongoing studies.

II. Evolution of the System

The anterior parts of the buccal membranes (i.e. the stomodeum) of vertebrates derive embryonically from ectoderm. Their capacity for glandular proliferation is thus similar to that of the skin but presumably not limited to this, as the pharyngeal region begins to incorporate endodermal components as well.

Fishes have a relatively limited number of oral glands, but many teleosts produce venomous integumentary secretions (Cameron and Endean, 1973). The buccal membranes tend to produce some mucous secretions, possibly equivalent to those of the external skin. These secretions would appear to function primarily in the conditioning and protection of the oral surface. Gill ventilation and inertial reduction of food items presumably impose similar demands on these surfaces as on the outside of the fish. Food objects do not appear to be especially lubricated or chemically processed, and even the esophagus (Krause, 1923; Andrew, 1959) shows but few glandular regions.

Similar patterns are seen in aquatic amphibians. One sees the first serous glands, in the classical sense, in frogs (Oeder, 1905; *Rana*, *Bufo*, *Xenopus*, Francis, 1961). The secretion of the salientian intermaxillary gland contains both amylase and protease (Francis, 1961). It has been suggested that those secretions produced here represent part of a functional complex. The glands discharge their secretion into ciliated grooves edged with taste papillae; in many species these grooves curve around patches of vomerine teeth. It is suggested that the system permits the animal to taste objects *after* these have entered the mouth. Detection of prey by frogs would be primarily visual (motion detection) with only occasional or no olfactory components (Ingle, 1969). Such a description, of course, represents something of an oversimplification; learning (Brower and Brower, 1962; Heatwole and Heatwole, 1968) and cues, picked up by the tongue (Dean, 1975), may also be involved. It is suggested that more subtle decisions on palatability are apparently determined by an assay of materials that are dissolved from portions of the prey, previously abraded by the vomerine teeth.

The extent to which equivalent behaviour occurs or equivalent secretions

are produced in other terrestrial amphibians (indeed to which the mechanism applies to all frogs) is unclear, although the enzymes also occur in *Triturus* and *Salamandra* (Francis, 1961). It is also uncertain whether fossil terrestrial amphibians had such enzyme-secreting systems. The need for a "taste-after-ingesting" system presumably relates to shifts from olfaction via the vomero-nasal organ to a more advanced stage of olfactory bulb (Parsons, 1970). Such shifts might possibly eliminate the need for taste functions residing along the palate. The amphibian condition presumably may not tell much about the situation in the first large species of terrestrial vertebrates.

Terrestrial reptiles show a number of different patterns. In turtles the buccal surface is keratinized and covered by stratified squamous cells. Deeper areas show some mucous glands and organized glandular tissue. The buccal system of crocodilians shows only mucous-secreting glands (Saint Girons, 1976). In all of these systems the function is primarily one of lubrication.

The mucous glands of snakes and lizards have become enlarged and organized; thus snakes have strips of glandular tissue lying deep both to the supralabial and infralabial scales and emptying along the labial side of the tooth row, via a sequential series of evenly spaced secretory pores. The buccal cavity of squamates, particularly that of snakes and of those lizards having a Brille, also receives secretory products that have served to moisten and bathe the organ of Jacobson. This fluid includes all secretions of the Harderian gland, which may or may not have previously passed through the sub-Brillar space. All of these liquids, whether labial or nasal and possibly everted by passive pressure, are clearly available for the conditioning of buccal tissues; their function in lubricating prey is particularly clearly seen when snakes regurgitate partially swallowed mice or other food objects. The contents of the labial glands then mark the furthest extent of the labials on the surface of the prey.

This lubricating function is not restricted to the labial and Harderian glands, but there are also cells and glandular groupings lining the lingual sheath of numerous lizards and all snakes. The potential for functional modification of oral glands is documented by the apparent modification of these lingual glands into a salt-secreting system (Dunson, 1976) in members of two unrelated families of marine snakes.

The true venom glands of elapid, hydrophiid and viperid snakes lie on the sides of the head (and neck) and send their ducts along the labial side of the maxilla (see Kochva, this volume) to enter a complex pocket that surrounds the bases of modified maxillary teeth so that the venom may course down a deep groove or hollow tube (Edmund, 1969; Schaefer, 1976). A variety of colubrid snakes, as well as some lizards, show similarly modified Duvernoy's glands that discharge serous secretion to the bases of more or less modified

fangs (Taub, 1967). The structured venom glands of the genus *Heloderma* empty along the modified dentary teeth as do the glands of dipsadine snakes (Taub, 1966).

It has been clear, since the classic study of Alcock and Rogers (1902), that the Duvernoy's glands of colubrid snakes allow these animals to incapacitate their prey, and that their secretion thus facilitates feeding. It is fairly clear that their primary function is not killing but the immobilization of prey. Any secretion, discharged upon the skin of the prey just lateral to the position of the teeth, would lie on the site next to be bitten in advancing or inertial ingestion movements. Diverse foreign proteins, particularly enzymes and complex organic molecules, would probably cause local tissue reaction and, with this, increase the distraction caused by a bite. At higher doses and more specific chemical structure they might also have had central effects, but these are unnecessary. Many animals respond to predator attack by immobility. This has advantages in reducing predator interest or in permitting sudden escape from a general attack; it may also have provided the advantage for specialized predators to develop an attack pattern and substances that will tend to induce tonic immobility.

What advantage does a snake derive from inducing this tonic immobility? The serpentine ingestion mechanism derives a premium from distending a movably articulated series of slender struts around a bulky prey object (Gans, 1961). Fracture is an obvious risk; however, a 3% increase in the diameter of the prey, that can be handled, means at least a 6% increase in the total volume of prey ingested. A 5% diametric increase means a 11% increase in volume, and a 10% increase means a 21% increase in volume. Each such step carries with it a concomitant reduction in the number of "search-recognize-capture-ingest" operations needed to fulfill nutritional needs. Devices that decrease the risk of fracture or damage to the cephalic system permit further stretching, though they will remain limited by the need to facilitate the initial bite. Consequently, anything that a snake does to keep the prey from struggling while being captured and ingested lets the head become more slender, lets the complexity of linkages increase, and permits the use of soft and flexible, rather than of rigid, tissues to stretch around the prey and, with this, increases the effectiveness of the system. It may well be that the enlarged teeth (= fangs) on the posterior ends of the maxillae were adaptive in maintaining a firm grasp on struggling prey. Perhaps selection acted to associate the developing venom glands with these preexisting large teeth, rather than enlarging the teeth in the vicinity of evolving venom glands (K. Kardong, in litt.). The need for preingestion incapacitation probably provided a selective advantage, not only to the development of incapacitating substances but also for the constricting mechanisms utilized in diverse families of snakes. In this sense it is probably significant that the combination

of constriction and venom glands occurs almost exclusively in the colubrids. The best constrictors lack venom and the truly poisonous snakes are indifferent constrictors at best. The development of complex poisoning mechanisms, also adaptive as predator deterrent and involving highly toxic secretions rapidly and controllably injected by motile fangs, was probably secondary to a mechanism that served mainly to make prey easy to swallow.

There has been a great deal written on whether venoms indeed serve as predator deterrents, and the issue perhaps deserves brief mention here. It has often been argued that the truly dangerous snakes could not have evolved their venoms as predator deterrents, because (a) the predator will be killed if bitten by such a snake and hence cannot learn to associate the appearance of the particular snake with the noxious reaction, and (b) even when the predator is killed the snake is likely also to be harmed or even killed in "repelling" the attack. There have also been a whole series of statements noting the improbability that certain small burrowing or "nocturnal" snakes would ever encounter the predators to which their aposematic (= nastiness-advertising) signals are presumably addressed. Various complex mechanisms, such as mimicry cycles in which the predators are "trained" mainly by rear-fanged or less deadly snakes and the truly poisonous forms participate as so-called Emsleyan mimics (cf. Emsley, 1966), have been adduced to compensate for these difficulties. Actually there is a significant body of evidence indicating that truly poisonous snakes are avoided or treated with caution by a spectrum of small predators including fishes (Kropach, 1975), birds (Smith, 1975) and mammals (Gans, 1965). In a minority of cases, this may involve the kind of simple one-to-one learning often assumed. In many more, it apparently involves empathic or observational learning (Gans, 1965; Jouventin *et al.*, 1977) as well as innate avoidance of poisonous snakes (Smith, 1975). But while the case is only now being proven, the occurrence of the phenomena should not be surprising. Venoms presumably arose early in the evolution of snakes, and there long must have been a high selective advantage for those predators on snakes that reduced the frequency of encounters with such potentially dangerous components of their environment. Still, we need detailed studies of the distribution and predator interaction of all similarly colored forms in a particular, local situation before we are likely to resolve such problems (Savage and Vial, 1974; Huheey, 1976).

Another point that must be stressed in considering the evolution of envenomating systems is that all presently surviving snakes are specialized creatures and that the conditions they now exhibit are unlikely to have remained "intermediate", and presumably (whether in venom production or any other system) less than "optimally" effective without cause. The "intermediacy" observed is likely to reflect only a single aspect of a coadapted