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Natural Toxins

Proceedings of the 6th International Symposium on Animal, Plant and Microbial Toxins, Uppsala, August 1979

Editors

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Preface

Poisons produced by living organisms have fascinated man for many centuries, and various of them have been exploited for good and devious ends from ancient times down to the present. Unlike most of the man-made environmental poisons that pervade the industrialized world today, most of the natural poisons produced by living things are unstable in nature, and nearly all cases of accidental poisoning by such toxins involve direct contact with the organisms that produce them. In most of the industrialized nations of the world, natural toxins are not serious problems because, owing to climatic conditions and urbanization, poisonous insects and animals are rare, and in the case of microorganisms, because contacts have been minimized by better hygiene and controls on the production and distribution of foodstuffs and water or because the unpleasant consequences of such contacts have been largely eliminated by immunization. However, the tropical climates of most developing countries favor proliferation of all forms of life. Serious and even fatal diseases in man and livestock caused by toxins of various microorganisms are often endemic, and more than 100 000 human deaths occur each year as a consequence of bites or stings by poisonous snakes, insects and spiders. Diarrhea diseases caused by various microorganisms are the major cause of infant mortality in the world. Furthermore, the mycotoxins produced by certain molds render a very large fraction of the cereal and peanut production of many developing countries unfit for consumption by man or beast. The latter toxins are especially dangerous owing to the insidious nature of their effects.

For two reasons, natural toxins have received steadily increasing attention during the last two decades: firstly, because we realize that better methods for their detection and the diagnosis, treatment and prevention of the diseases that they cause are among the prerequisites for social and economic progress in many of the developing nations of the world. The second reason is the realisation that the often awesome potency of the most powerful natural toxins is due to very specific interference with vital molecular processes involved in the maintenance of cell integrity and in the communication among different cells. Natural toxins are thus emerging in their own right as extremely valuable tools for the study of some of the most fundamental mechanisms of life.

The concensus among the participants was that the 6th International Symposium on Animal, Plant and Microbial Toxins held in Uppsala in August 1979 under the co-sponsorship of the International Society on Toxinology and the University of Uppsala was a great success both scientifically and socially. The scientific success relects in no small part the high relevance of the subject matter and the competence of the chairmen who put together the 16 different sessions. The meeting was attended by 319 registered participants, and including non-registered locals attendance exceeded 400 on most days. The next international meeting of the International Society on Toxinology will be held in Brisbane, Australia, during July 1982.

Of the 235 abstracts submitted for the meeting, 209 arrived in time for publication in the special issue of TOXICON (volume 17, supplement 1, 1979). 207 papers were actually presented at the meeting: 78 orally and 129 in poster form. Although nearly all of the papers presented were worthy of publication, it did not seem feasible to publish the entire proceedings, which might have run to well over 2000 pages. Working within a page limit of 800 - 1000 pages, we therefore decided to invite submission of manuscripts of only the invited oral papers, which were mainly of review character, and the special workshop presentations, which were tightly organized and also contained considerable amounts of review material. We felt that the remaining free communications, which mainly represented new, original research on a broad range of topics would inevitably be published elsewhere anyway. In any case, the publication of the abstracts meant that all participants had the opportunity

to record their participation in print.

Of the 124 manuscripts thus requested for these proceedings, 83 were received and appear here under the authorship of 228 authors. Although we had correctly estimated the time required for the editorial work at two full months, most of the manuscripts arrived late in October 1979, toward the end of the period that we had reserved for the job, and owing to teaching and other committments we were unable to return in earnest to the task until mid-Spring of this year. A delay of somewhat more than one month in the submission of the manuscripts has thus delayed completion of the book by about half a year. We hope that you readers will find the volume worth waiting for.

Uppsala, June 1, 1980

David Eaker Torkel Wadström

VENOM GLANDS, VENOM SYNTHESIS, VENOM SECRETION AND EVOLUTION

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ABSTRACT

This paper reviews the embryonic development, structure and function of the compound oral glands of non-venomous and venomous snakes in comparison with other exocrine glands, mainly the pancreas. It discusses the phylogenesis of the snake venom glands and proposes a hypothesis for the co-evolution of the two-component, phospholipase-containing toxin and the anti-toxic factor(s) found in the blood serum of snakes.

KEYWORDS

Venom; snake; evolution; phospholipase A; toxin; embryonic development; exocrine glands; anti-toxin; enzyme inhibitor; Viperidae.

INTRODUCTION

In his paper on the evolution of enterosecretory proteins, Adelson (1971) states that "genetic changes in the time and location of expression of the functionally different, related genes led to the evolution of functionally specialized regions of the gut" and that "the ability to secrete related proteins remained constant among the gut-derived glands." He sees as a special case "the ability of several specialized non-entodermal tissues to secrete proteins related to entodermal proteins", which could evolve by "a change in the pattern of gene-activation..... allowing expression of a formerly repressed entodermal gene in a non-entodermal tissue". Extensive evidence in support of these suggestions has since been accumulated (Dayhoff and co-workers, 1975), and hypotheses on the evolution of toxins from certain pancreatic enzymes have been proposed (Eaker, 1975; Heinrikson, Krueger and Keim, 1977; Ivanov and Ivanov, 1979; Strydom, 1977). Given these hypotheses one might expect to find similarities also in the morphology of the glands secreting these compounds, i.e. the pancreas and the compound oral glands of snakes; these similarities should then be more evident in the more primitive glands of the non-venomous species.

EMBRYOLOGY AND MORPHOLOGY

Snakes have developed a variety of exocrine glands in the mouth. The two major types are found in the supra-labial region and are represented by Duvernoy's glands in the colubrid snakes and by the venom glands in the Viperidae and Elapidae *sensu lato* (Kochva, 1978b).

We shall start this comparison with the embryonic development of Duvernoy's glands and the venom glands of Viperidae. In all species thus far examined the gland develops from a common, ectodermal primordium, together with the dental lamina of the maxilla (Fig. 1a). At later stages, the primordium of Duvernoy's gland branches in a symmetrical pattern, while in the Viperidae branching is restricted to the posterior region of the gland (Figs. 1b-d). From what meager evidence is available, the development of the venom glands of the Elapidae and the Atractaspidinae seem to resemble the pattern of Duvernoy's glands rather than the viperid venom glands.

In general terms, the embryonic development already provides some clues suggesting that the venom glands of the Viperidae are the more specialized and should be expected to differ from other glands also in the adult. The similarities with other exocrine glands such as the pancreas, should be looked for among Duvernoy's glands of the non-venomous snakes. The great variability of these glands should facilitate the search and make it possible to find glands with a general pattern not dissimilar to that of the pancreas. An example of such a gland is given in Fig. 2. The venomous snakes, and mainly the vipers, show a different morphology

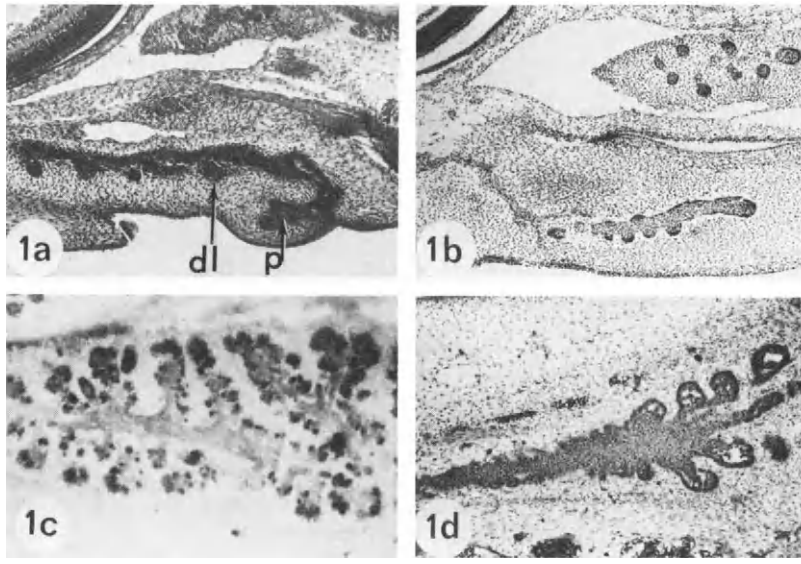

Fig. 1. Embryonic development of oral glands. a) Early stage of dental lamina with gland primordium in *Natrix tesselata*; approx. 80x. b) First branching of gland primordium in *Spalerosophis cliffordi*; approx. 50x. c) Later branching of gland in *Spalerosophis*; approx. 40x. d) Branching of venom gland primordium in *Vipera palaestinae*; approx. 60x. dl - dental lamina; p - primordium of Duvernoy's gland.

that can be clearly seen even at lower magnifications of the light microscope (Figs. 3a-c). At the ultrastructural level the differences are more evident: In all glands examined, including the Elapidae, the cells are filled with secretory granules; only the Viperidae and Crotalidae show a very small number of granules, compensated for by the wide lumina that store large amounts of venom (Figs. 4a-c). *Vipera palaestinae* and the other Viperidae and Crotalidae thus show a gland that is well adapted for having a large amount of venom in store to use effectively even in several consecutive strikes, in connection with a simple and efficient way of replenishing the dose(s) injected (Kochva, 1978a).

Looking at the amounts of venom found in the gland lumina and the small number of secretory granules in the cells, the question was asked whether these secretory granules are the only avenue of venom secretion or whether there was an alternative, direct route of secretion, e.g. from the cisternae of the rough endoplasmic reticulum into the lumen. The latter pathway was suggested for other exocrine glands (cf. Isenman and Rothman, 1979).

In order to answer this question, some histochemical and immunohistochemical techniques were applied, at the level of the light and electron microscopes (Figs. 5a-d). The results show that all secretory cells contain the venom components examined, some of which could be identified in the same secretory granules. Admittedly, not all our evidence is direct and overlapping, but it appears nevertheless that the venom is secreted through these granules, despite their scarcity.

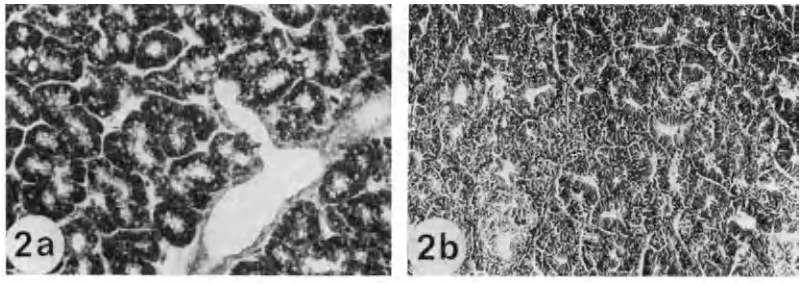

Fig. 2. Comparison of pancreas and Duvernoy's gland. a) Pancreas of *Vipera palaestinae*; approx. 200x. b) Duvernoy's gland of *Aparallactus modestus*; approx. 200x.

PHYSIOLOGY

Quantitative evidence on the synthesis and secretion of venom is now available from work done on the Cascavel, *Crotalus durissus terrificus* at Riberão Preto by Marchi, Haddad and De Lucca (1978), on the Tsefa, *Vipera palaestinae*, by Oron and Bdolah (1978b) in Tel Aviv and on the sea snake *Laticauda semifasciata* by Takeda, Yoshida and Tamiya (1974). Radioautographic and morphometric calculations (Fig. 6) support the assumption that the intracellular transport of venom proteins in both *Crotalus* and *Vipera* follows the conventional pattern of exocrine glands, as documented by Palade (1975) for the mammalian pancreas. In *Crotalus* there is a variable number of intracisternal granules (Brasileiro, 1976) that follows a different labelling pattern.

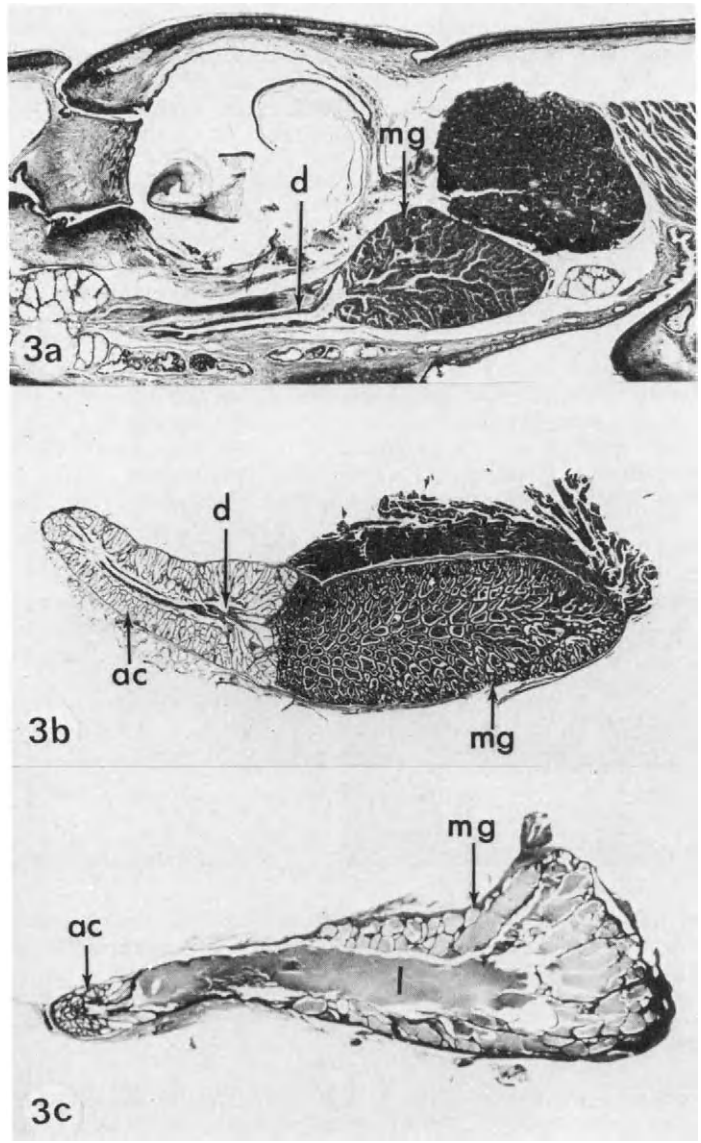

Fig. 3. Adult morphology of oral glands. a) Duvernoy's gland of *Chilorinophis*; approx. 40x. b) Venom gland of *Walterinnesia aegyptia*; approx. 12x. c) Venom gland of *Vipera palaestinae*; approx. 20x. ac - accessory gland; d - duct; l - lumen; mg - main gland.

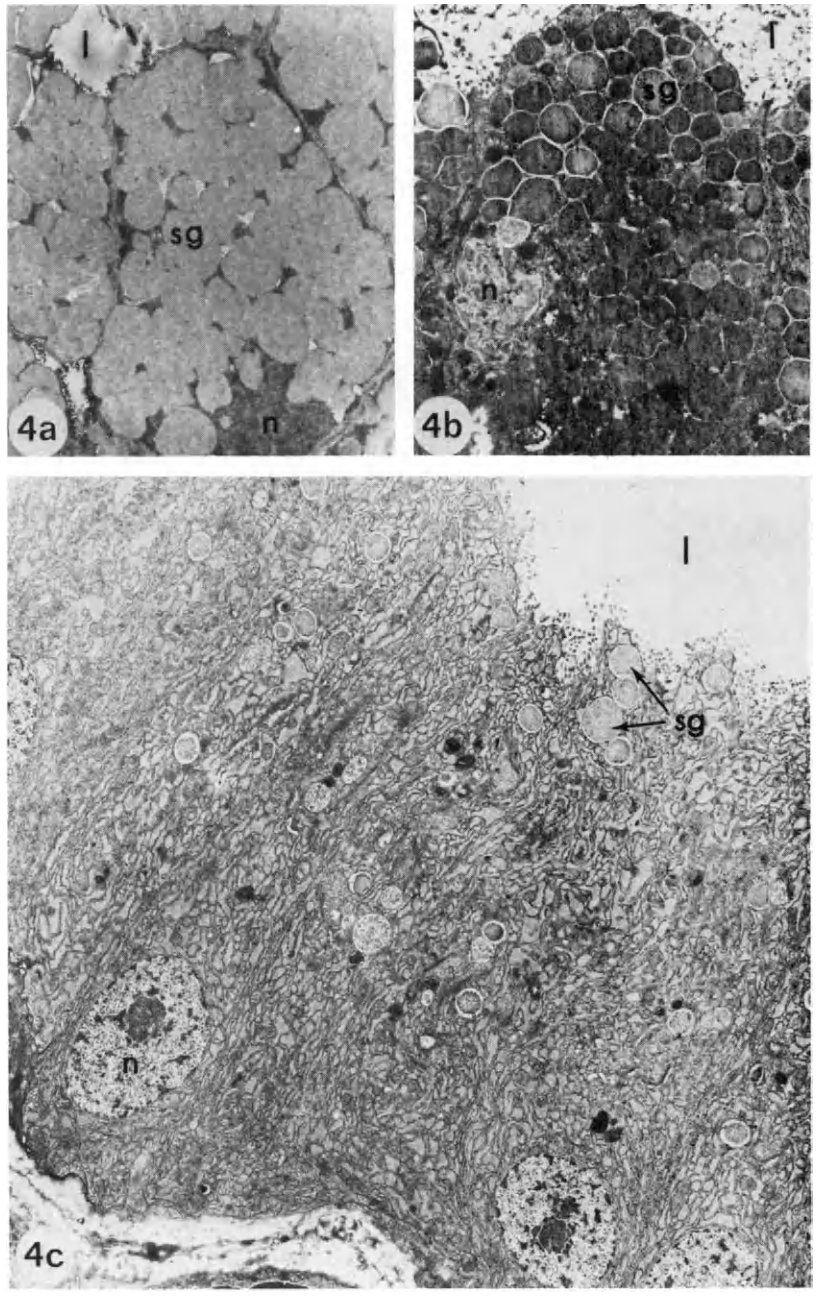

Fig. 4. Electron micrographs of oral glands. a) Duvernoy's gland of *Dispholidus typus*; approx. 4000x. b) Venom gland of *Naja melanoleuca*; approx. 5000x. c) Venom gland of *Vipera palaestinae*; approx. 3000x. l - lumen; n - nucleus. sg - secretory granules.

The major differences between the pancreas and the viperid venom gland, thus, lie in the storage of secretion in the glands. While in the pancreas and salivary glands the secretion is stored intracellularly in the so-called zymogen granules, in the viperid venom gland secretion is mainly accumulated in the extended lumina of the gland. After manual extraction of the venom (milking) there is an increased rate of secretion which is a result of a high rate of venom synthesis and of intracellular transport. As the gland lumina become filled with venom, both processes slow down considerably (Oron and Bdolah 1978b). The secretion cycle in the Elapidae venom gland is virtually unknown.

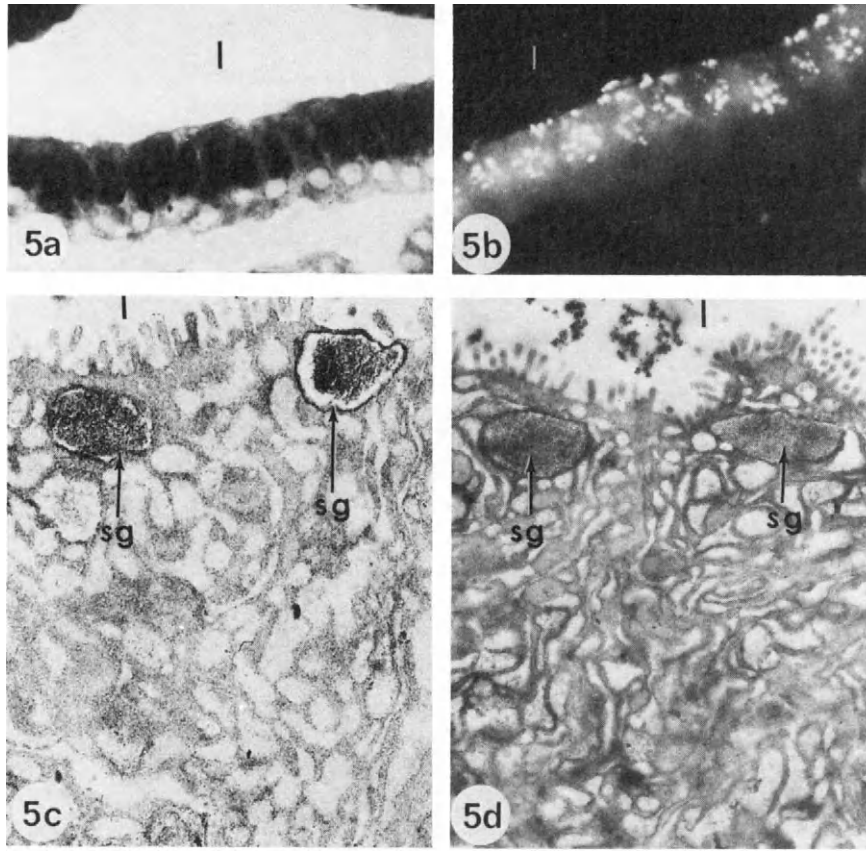

Fig. 5. Localization of venom components in *Vipera palaestinae*. a) Histochemical staining for L-amino acid oxidase; approx. 900x. b) Immuno-fluorescence staining for the two-component toxin; approx. 800x. c) Immuno-peroxidase staining for phosphodiesterase; approx. 14000x. d) Immuno-peroxidase staining for L-amino acid oxidase; approx. 8000x. l - lumen; sg - secretory granule.

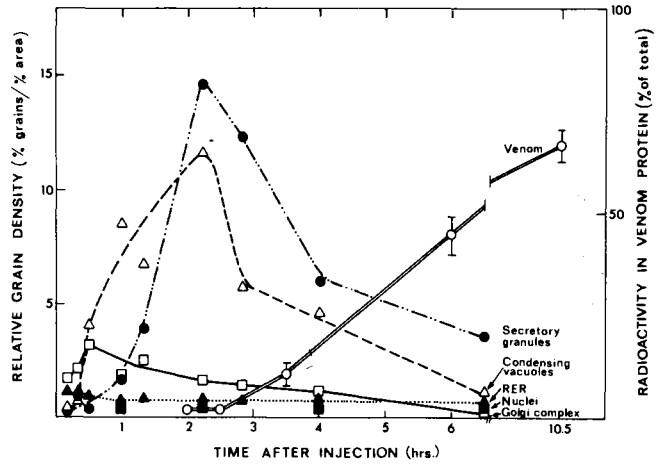

Fig. 6. Labelling kinetics of cell organelles and of secreted venom in the active venom gland of *Vipera palaestinae*. The snakes were labelled by a chronic cannulation of the left systemic arch (Oron and Bdolah 1978a) and sacrificed at the indicated intervals. Relative grain densities were calculated from radioautographs of the glands and the radioactivity in the secretcd venom was monitored at various intervals after milking (Oron and Bdolah 1978b).

EVOLUTION

From what has been said so far, it appears that the compound oral glands of snakes have preserved some basic characteristics of other exocrine glands, such as the pancreas, glands that have appeared early in vertebrate evolution. These characteristics are more evident in the non-venomous, colubrid snakes, but are also found in the Elapidae. In the latter group, the venom apparatus in general is in a less advanced stage of development as compared to the Viperidae, which show a high specialization of both the venom glands and skull components related to the venom injecting mechanism (Kochva, 1978b). It is therefore interesting to note that some of the elapid venom components - cardiotoxins, short and long neurotoxins - are considered to be the last step in toxin evolution, while the phospholipase-containing toxins appear to be their predecessors (Strydom, 1977).

In the two-component toxin system of *Vipera palaestinae*, both components, the basic, non-enzymic protein and the acidic phospholipase A are required for lethality. The first point we would like to stress is that the phospholipase can be replaced effectively by heterologous enzymes taken from venoms of elapid and viperid snakes, but not by the enzyme from pig pancreas (Table 1; Simon, Bdolah and Kochva, 1980). The second point deals with the anti-toxic factor found in the blood serum of *Vipera* and of other snakes, a factor that inhibits phospholipase activity and, at the same time, abolishes the toxicity of the venom (Table 2). The anti-toxic factor(s) that is found in the blood serum of venomous and non-venomous snakes and in some mammals neutralizes a variety of venoms except elapid venoms (Table 3, Ovadia and Kochva, 1977).

These data in the context of the current ideas on the evolution of venom (Eaker, 1975; Heinrikson, Krueger, and Keim, 1977; Strydom, 1977) suggest the following hypothesis: The ancestors of snakes had a pancreas that secreted a phospholipase

and had also a phospholipase-inhibitor in their blood. When compound glands appeared in the lower jaw of some lizards and in the upper jaw of snakes, they carried with them, among other things, a phospholipase. This enzyme has been found so far in the lizard *Heloderma* (Shier and co-workers, 1979) and in the colubrid snakes *Malpolon* (unpublished) and *Leptodeira* (Mebs, 1968), but not in *Thelothornis* (Kornalik, Taborska and Mebs, 1978). The presence of the phospholipase inhibitor made it possible for the enzyme to become toxic by preventing damage to the snake in case the toxin should find its way into the blood stream. We thus present here a scheme of molecular co-evolution of two proteins, enzyme and inhibitor, toxin and anti-toxin, that had obvious functions in the ancestors, without having to involve "preadaptation" or "neutral" mutations as a way of explanation.

TABLE 1 Substitution of Phospholipase (PLA) of the *Vipera palaestinae* Two-Component Toxin by Heterologous Enzymes (Simon, Bdolah and Kochva, 1980)

Source of PLA		Mixture injected PLA (µg)	A (µg)	Toxicity (surviving mice)
V. palaestinae	pI 4.5	10	10	10/18
Pseudocerastes fieldi	pI 7.2	20	10	3/18
	pI 5.5	20	10	2/18
Walterinnesia aegyptia	pI 7.8	50	20	1/6
	pI 4.5	25	20	1/6
Sus scrofa, pancreas		100	10	6/6

A - basic, non-enzymic component of *Vipera*

TABLE 2 Inhibition of Phospholipase A (PLA) Activity by *Vipera palaestinae* Antitoxic Factor (Simon, Bdolah and Kochva, 1980)

Incubation mixture	Lecithin splitting (%)	Lethality (surviving mice)
Exp. I		
Toxin	92	0/10
Toxin + antitoxic factor 1:100	39	10/10
Toxin + bovine albumin 1:100	96	0/10
Toxin + rabbit serum 1:100	88	0/10
Exp. II		
Isolated PLA	90	-
Isolated PLA + antitoxic factor	20	-
Isolated PLA + antihemorrhagic factor	60	-

TABLE 3 Neutralization of *Viperidae* and *Elapidae* venoms by Sera of Different Animals (Ovadia and Kochva, 1977)

Origin of sera	Origin of venom: Viperidae			Elapidae		
	Vipera palaestinae	*Echis colorata*	*Pseudocerastes fieldi*	*Aspis cerastes*	*Walterinnesia aegyptia*	*Naja nigricollis*
	No. surviving mice / No. injected mice					
Snakes						
Viperidae						
Vipera palaestinae	9/10	8/10	7/10	10/10	0/10	0/10
Pseudocerastes fieldi	10/10	9/10	10/10	10/10	0/10	1/10
Vipera ammodytes	10/10	8/10	10/10	10/10	0/10	0/10
Elapidae						
Walterinnesia aegyptia	10/10	9/10	7/10	10/10	0/10	10/10
Naja nigricollis	10/10	0/10	10/10	5/10	0/10	10/10
Colubridae						
Malpolon monspessulanus	9/10	8/10	2/10	10/10	0/10	0/10
Natrix tessellata	9/10	3/10	8/10	4/10	0/10	0/10
Mammals						
Herpestes ichneumon	0/10	7/10	1/10	10/10	0/10	0/10
Mesocricetus auratus	8/10	4/10	1/10	1/10	0/10	0/10

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PHARMACOLOGY OF VENOMS

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ABSTRACT

The action of a venom on an organism is dependent upon a number of variables: the route of administration, absorption, distribution, passage across a succession of membranes, accumulation at receptor site(s), metabolism, and excretion. In addition, in determining the action of a venom, such factors as autopharmacological changes, the action of venom metabolites, and the importance of different animal and tissue kinds must be considered. These various influences on the pharmacological activities of animal venoms are discussed.

KEYWORDS

Route of administration; absorption; distribution; passage across membranes; accumulation at receptor site(s); metabolism, excretion; autopharmacology; lethality; immunity.

INTRODUCTION

It would be nice to think that Kipling had the International Society on Toxinology in mind when he wrote:

"It is unjust that when we have done
All that a serpent should,
You gather our poisons, one by one,
And break them down to your good."

Man's interest in breaking down venoms is several fold. Firstly, he is interested in what these diversified and intriguing substances are composed of. Secondly, he is interested in how these substances exert their deleterious effects, as well as their beneficial ones. Thirdly, he is interested in why these poisons have evolved as they did. Finally, he is interested in the potential of venoms as drugs for the treatment of disease states, and as tools in biology for the study of cellular and subcellular function, as well as for general physiopharmacologic processes.

The pharmacologist, in formulating his investigations, should exercise the consciousness of a fundamental biologist by seeking to understand how and why venoms came into being, and how the evolution of their function and chemistry relates to the evolution of the anatomical development of the venom apparatus. In his investigative work he must be concerned with the biological activities of the isolated and characterized fractions of the venom. He must know the techniques for screening the individual venom components for their specific pharmacologic properties and, hopefully, he should be wise enough to screen for the wide spectrum of activities

that has been endowed in the venoms.

He must be aware of the potential for any autopharmacologic responses precipitated by the release of normal tissue components, and he must also be cognate of the possibility that venom fraction metabolites might be formed within the envenomated organism and might, in themselves, produce deleterious reactions. Most importantly, he must be conscious of the fact that synergistic and, possibly, antagonistic reactions might occur as a result of interaction between individual venom components. It seems wise to keep in mind that there is no piece of experimental evidence which demonstrates that the total pharmacologic effect of a whole venom is equal to the sum of the properties of the individual fractions or functions nor, from a philosophical posture, does such a conclusion seem plausible.

It is one of the unfortunate facts in the study of the chemistry and pharmacology of venoms that the structure and design are most easily investigated by taking the venom apart. This has two shortcomings; it means that a destructive process must be substituted for a constructive, progressive and integrative one; and, secondly, the essential quality of the whole venom may be destroyed before one has made a suitable acquaintance with it. Often times the process of examination becomes so exacting that the end is lost sight of in our preoccupation with the means, so much so that in some cases the means becomes substituted for the end.

A few of us may still recall the decided attempt in 1954 to press for a classification of snake venoms based solely on their enzyme content. Today, the pendulum appears to have swung to the opposite pole. The basis for classifying snake venoms should be founded on data derived from all biological considerations, including those presented by systemics, taxonomy, biology, anatomy, physiology, pharmacology, biochemistry, immunology and clinical medicine with, perhaps, a dash of philosophical adventure thrown in. We should be exceedingly conscious of the possibility that our experiments, however well performed, may not be a true reflection of the manner in which nature herself went about constructing the properties of venoms.

MECHANISMS

In general, it can be said that the disposition of a venom, that is, what is done with it (not what it does) is associated with the chemical nature of its component parts. The action of the function of a venom, on the other hand, is dependent upon the pharmacologic properties of the individual and collective components, as well as their metabolites, and on any autopharmacologic changes the combined properties produce. Although much emphasis has been placed on receptor sites, it must be remembered that some components, such as enzymes, may attack any macromolecule in any tissue within their enzymatic specificity: combination with a receptor site is not necessary.

The fate of a venom or venom component in an organism is dependent upon a number of variables, including its route of administration, absorption, distribution, passage across a succession of membranes,

accumulation at receptor site(s), metabolism, and excretion. All of these factors play some role in determining the effect of a venom or venom component within the organism. During the past two decades it has become increasingly clear that there are very significant variations in the roles of these factors in different species of animals. Differences which, in some cases, are more important than the differences usually attributed solely to the weight of the experimental animal.

The toxinologist, as the pharmacologist, is confronted with the question of whether or not a particular difference between animals of various kinds is due to variations in the effectiveness of the toxin at a receptor site or to its passage across membranes, absorption, distribution, accumulation, metabolism, or to its excretion. Of equal importance is the consideration of the possible conversion of toxins to less or, perhaps, more pharmacologically active metabolites.

With respect to the route of administration of a venom and its bioavailability, this not only affects the latent period and duration of an effect but also the toxicity. The physico-chemical property of the sample, its pH, the vehicle, the particle size, and the concentration are important considerations in the distribution and absorption within the organism. The route of administration has different values in different animals. As seen in Table 1, the LD_{50} of a venom is usually less when the toxin is injected intravenously than when given subcutaneously, intramuscularly, or intraperitoneally. However, with some venoms there may be exceptions to this in mice, while in cats and dogs the intravenous route almost always appears to be the far more life-threatening.

TABLE 1 Variations in LD_{50} by Different Routes of Injection

VENOM	INTRAVENOUS	INTRAPERITONEAL	SUBCUTANEOUS
Crotalus viridis helleri	1.29	1.60	3.65
Crotalus adamanteus	1.68	1.90	13.73
Crotalus atrox	4.18	3.71	17.75
Crotalus scutulatus	0.21	0.23	0.31
Agkistrodon piscivorus	4.17	5.10	25.10
Agkistrodon contortrix	10.92	10.50	26.10
Sistrurus miliarius	2.91	6.89	25.10

All determinations in 20 g female mice of the same group, done within a one-hour period and observed for 48 hours.

As seen in Table 1, closely related venoms may display marked differences related to the route of administration. For instance, the

subcutaneous LD_{50} for Crotalus viridis helleri venom is approximately three times the intravenous LD_{50}, while with Sistrurus venom the difference is almost nine fold. Surprisingly, the intraperitoneal LD_{50} for Agkistrodon contortrix venom is less than the intravenous LD_{50}. The route of administration and bioavailability is thus an important factor in determining its pharmacologic activity.

Before a venom can be absorbed it must be in a form suitable for passage through several membranes prior to reaching the circulation or some specific site. The membranes through which it may pass act as semipermeable barriers to its penetration. They may selectively permit the passage of certain venom fractions, while blocking the permeation of others.

When a venom is injected into a vein its primary target will be the blood-vascular system, although, obviously, every tissue may be affected in one way or another. When the same venom is injected into subcutaneous tissues, absorption through lymph channels, as well as through the capillary bed and other membranes may affect the toxin's activity, as well as its target tissue or tissues. When the toxin is injected into the peritoneum, again, different membranes become involved.

Some venoms and venom fractions have been injected intraventricularly or directly into the cerebrum. Such studies have elicited considerable academic interest, as well they might. Some reports attempt to show properties of the blood-brain barrier as it relates to the diffusion of venoms. However, the delicacy of this barrier and the factors which can influence it by the very nature of an experimental technique may invalidate the experimenter's results. Such factors as mechanical force, volume of injectable, concentration, pH, and vehicle can greatly affect results, and are too frequently minimized. In addition, the amounts of venom or venom fraction that have been applied to the central nervous system in some experiments are far in excess of physiological parameters or the amount of toxin that could reach the center following parenteral injection or injection by a snake.

Another difficulty relating to bioavailability and absorption is sometimes reflected in disagreements relating to whether or not a venom fraction exerts its effect on a neuromuscular junction, a heart fiber, a vascular strip or some other site. It is not unusual to hear this point debated. The difficulty lies in the fact that it is not possible, in studying the effect of a specific dose of snake venom or venom fraction on various in vitro preparations, to come to unequivocal conclusions on an in vivo effect. In the in vivo preparation the concentration of the toxin reaching the various sites will be different; the amount of toxin needed to elicit specific responses will be different; the ability of the site receptor to absorb, metabolize and excrete the toxin will be different; and the ability of the whole organism, by its own physiopharmacology, to adjust to the action of the poison can be very different. If one compares the doses of venom used in some nerve-muscle or coagulation preparations with those that are administered in in vivo studies, or compares the data with changes seen in humans following envenomation, it is obvious that the factors which may affect the biological activity of a venom may be altered

considerably by the physiopharmacology of the intact animal.

Nowhere is this more evident than in studies on venoms and neuromuscular transmission. The investigator who uses a dose of "neurotoxin" isolated from three adult cobras to elicit a particular *in vitro* neuromuscular phenomenon (and one not seen in the *in vivo* animal preparation or in humans), and who seeks to project his data to conclusions on humans (which frequently occurs), does little but confuse our understanding of the development of venom poisoning. Although the experimenter's interest may not directly relate to this concern, he has the moral responsibility to oversee the use of his data and its application. Finally, it is a matter of grave concern that few toxinologists attempt to pursue the several possible sites of mechanisms of action of a single venom fraction.It hardly seems valid to accept the dogma that venom fractions are organ specific ("neurotoxins, cardiotoxins or hemotoxins"), based on single studies of isolated tissue preparations, but the hypothesis is so deeply rooted in traditional toxinology that it persists, in spite of strong evidence to the contrary.

In considering the passage of toxins across membranes, this can be accomplished by one or several mechanisms: passive diffusion, facilitated diffusion, active transport, or pinocytosis. Studies to date would seem to indicate that passive diffusion and perhaps facilitated diffusion are the principal mechanisms by which venom components reach their target sites. In passive diffusion the drug is transported across the membrane by the concentration gradient of the solute. The rate of diffusion is proportional to the gradient and is dependent upon lipid solubility, degree of ionization, molecular size, and the area of the absorptive surface. The rate of entry into the vascular bed is usually determined for lipid soluble venom fractions by their water-oil partition coefficients, and for lipid-insoluble fractions by their molecular size.

The various membranes of the body are remarkably similar in their chemical structure and spatial arrangement, regardless of their location. This is sometimes overlooked, when such terms as "cytotoxin," "myotoxin," " nephotoxin," "necrotoxin," and the like are used. Primarily, membranes are composed of proteins, phospholipids, and cholesterol. It is thought that the membrane protein is primarily involved in the transport process of venom fractions, although recent work appears to indicate that the phospholipids may play a role. The lipids confer both hydrophilic and hydrophobic properties thereby giving stability to the membrane and providing the characteristics of permeability.

In cases of facilitated diffusion it has been suggested that a "carrier component" combines reversibly with the venom molecule at the membrane's outer surface and that the carrier-substrate complex diffuses more rapidly across the membrane, releasing the molecule (or toxin) at the membrane's inner surface. For certain drugs it is known that this process of facilitated diffusion is highly selective, accepting only those substances which have a relatively specific molecular configuration. There is some evidence to believe that some fractions of venoms are transported by facilitated diffusion.

A third way in which a drug may be transported across a membrane