ANTHONY T. TU

VENOMS

CHEMISTRY AND MOLECULAR BIOLOGY

VENOMS: Chemistry and Molecular Biology

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A WILEY-INTERSCIENCE PUBLICATION
JOHN WILEY & SONS, New York • London • Sydney • Toronto

Library of Congress Cataloging in Publication Data:

Tu, Anthony T 1930–
Venoms: chemistry and molecular biology.

Includes index.
1. Snake venom – Physiological effect. 2. Snake venom. 3. Venom. 4. Hydrolases. I. Title.
QP941.S6T8 591.1'9'245 76–30751
ISBN 0-471-89229-7

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

VENOMS:
Chemistry and Molecular Biology

To the Memory of

My Mother, Songsui Lin Tu (1901–1968)

and

My Ph.D. Research Adviser, Professor Hubert S. Loring (1908–1974)
Stanford University

PREFACE

There are numerous books, review articles, and multiauthored monographs on the subject of venoms, which are excellent sources for specialists' research and have their archival values. However, because so many different people have contributed portions of the information, it is difficult to obtain a comprehensive, unified view of venoms. For scientific laymen who want a general idea about "What is venom?" there is a need for a book written by a single author.

When asked by the publisher about the possibility of writing such a book, I knew that it would be a monumental task to assemble all references, to digest the contents and analyze the data, to reconstruct these materials, and to present them in a logical and unified form.

In this book, I have tried to be comprehensive so that specialists can use it as a source of information. At the same time, I have also tried to be selective so as to present general and systematic views to scientists in general, from the vast store of information on venoms. To balance these two basically opposing aims has been difficult.

Overall, materials presented here are expansions of my lecture course, "Chemistry and Pharmacology of Animal Toxins," given at Colorado State University, and my many review articles, lectures at different colleges and universities, and special lectures at scientific meetings. I sincerely hope that this book will stimulate more interest in venoms and increase the understanding of them.

I was fortunate to have Dr. Charlotte L. Ownby, Assistant Professor of Physiology, Oklahoma State University, participate on Chapter 27 on the chemical neutralization of snake venoms. Thanks are also extended to the following persons, who helped in the many phases of the writing of this book: Dr. A. Bieber, Dr. G. Happ, Dr. N. Iritani, Dr. B. Joyce, Dr. D. Leuker, Dr. C. Ownby, Dr. D. Will, Ms. A. Kano, Ms. L. Rimsay, Mr. J. Fox, Mr. J. Pardee, Mr. M. Stringer, and Mr. J. Yadlowski.

I am grateful also to many colleagues – Drs. P. J. Delori, W. B. Elliott, D. M. Fambrough, N. Frontali, E. Heilbronn, J. Ishay, D. Munjal, T. Okonogi, R. Perez-Polo, Y. Setoguchi, J. W. Simpson, and R. Yarom – who supplied valuable photographs; without these, the book could not have been published. I thank Teresa Szidon and Dee Kohut for the final typing of the manuscript.

Finally, my thanks are extended for encouragement by Kazuko Yamomoto Tu, my wife, and Dr. Tsungming Tu, my father, who has done many years of snake venom research.

My deepest regret is that my mother, Songsui Lin Tu, and my Ph.D. research adviser, Professor Hubert S. Loring of Stanford University, cannot see this book.

Anthony T. Tu

Fort Collins, Colorado
January 1977

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CONTENTS

I SNAKE VENOMS: General Background and Composition

Human beings have traditionally, and justifiably, been fearful of and puzzled by the violent action of venoms from such small creatures as venomous snakes. The amount of venom injected by these snakes is usually very small, but in some cases the results are fatal. Symptoms arising in the victim result from the combined effects of complex protein components present in the venom.

Venom is not composed of a single substance common to all poisonous snakes, although almost all venoms consist of approximately 90% protein. The proportions of the different substances in venom and their specific characteristics vary among the species. However, usually the closer the phylogenetic relationship of the snakes, the more similar are the venom properties and composition.

Any given snake venom usually contains more than one toxic principle, and these tend to act in combination in an actual poisoning. The overall toxicity is due to enzyme as well as to nonenzymatic proteins. However, the main lethal action, especially in Elapidae and Hydrophiidae snakes, can be attributed to neurotoxins that are not enzymes. This does not mean that enzymes are unrelated to the toxic actions of venoms. Many venom enzymes actively participate in blood coagulation, anticoagulation, hemorrhage, hemolysis, autopharmacological action, and lysis of cell and mitochondrial membranes.

Of the nearly 2000 different types of snakes that exist, about 300 are known to be venomous. The venomous snakes are classified according to morphological characteristics and comprise five families: Crotalidae (crotalids, pit vipers), Viperidae (viperids, vipers), Elapidae (elapids), Hydrophiidae (sea snakes), and Colubridae (colubrids).

Crotalidae comprise six genera: *Crotalus, Sistrurus, Agkistrodon, Bothrops, Lachesis*, and *Trimeresurus. Crotalus* and *Sistrurus* are the rattlesnakes and can be found only in North, Central, and South America. *Bothrops* occurs only in Central and South America. *Agkistrodon* includes the copperheads and moccasins. This is the only genus of snake that can be found in both the New and the Old World. *Lachesis* has only one species and is distributed from Central to South America. *Trimeresurus* is the Asiatic pit viper, which lives only in Asia; there are 31 species in this genus.

Viperidae are known commonly as viperids or vipers and can be found in Africa, Europe, and Asia. They are not found in Australia or on the American continent. Viperidae comprise the genera *Vipera, Atractaspis, Bitis, Causus, Cerastes, Echis, Adenorhinos, Atheris, Eristicophis, Pseudoceratstes*, and *Azemiops*. Africa and the Middle East are particularly rich in varieties of Viperidae. In Asia, there is only one genus of *Vipera.*

Elapidae include well-known cobras, mambas, and kraits. All of the poisonous snakes in Australia and New Guinea belong to the family Elapidae. There are only two genera of Elapidae in North America and Central America. In North America, Elapidae are coral snakes, which belong to the genera *Micruroides* and *Micrurus*. In South America, there is *Leptomicrurus* in addition to the other two genera mentioned. These are the only Elapidae that migrated to the New World from Asia through the Bering land bridge many millions of years ago. Australia and New Guinea, on the other hand, are rich in genera belonging to this family: *Acanthophis, Brachyaspis, Demansia, Denisonia, Elapognathus, Glyphodon, Hoplocephalus, Micropechis, Notechis, Oxyuranus, Parademansia, parapistocalamus, Pseudapistocalamus, Pseudechis, Rhinoplocephalus, Toxicolamus*, and *Vermicella*. Other genera of Elapidae are *Elapsoidea, Naja, Walterinnesia, Aspidelaps, Boulengerina, Dendroaspis, Elaps, Elapsoidea, Hemachatus, Paranaja, Pseudohaje, Bungarus, Calliophis, Maticora, Ophiophagus, Apistocalamus, Aspidomorphus, Brachyurophis, Ormodon, Rhynchoelaps, Tropidechis*, and *Ultrocalamus, Ophiophagus*, the king cobra by common name, is the largest poisonous snake in the world, reaching more than 10 ft in length. *Bungarus* (the krait) is an Asiatic poisonous snake.

Hydrophiidae are sea snakes and live in tropical and subtropical sea waters bordering the Indian and Pacific oceans. They are not found in the Atlantic Ocean or the Mediterranean Sea. Only one genus, *Pelamis*, occurs in the coastal waters of Central and South America.

Classification of sea snakes is not complete and is still in a state of confusion. In this book, the classification by Smith, *Monograph of the Sea-Snakes* (British Museum, 1926), is followed. The family Hydrophiidae has two subfamilies, Laticaudinae and Hydrophiinae. Laticaudinae include such genera as *Laticauda, Aipysurus*, and *Emydocephalus*. Hydrophiinae include *Hydrelaps, Kerilia, Thalassophina, Enhydrina, Hydrophis, Acalyptophis, Thalassophis, Kolpophis, Lapemis, Astrotia, Pelamis*, and *Microcephalophis*. There are many species within the genus *Hydrophis*, and species identification is very difficult.

Colubridae constitute by far the largest family of snakes and consist of 250 genera and over 1000 species. But not all of them are poisonous. Poisonous Colubridae include the genera *Dispholidus* and *Thelotornis*, both of which are found in Africa. They are rear-fanged snakes and, because of the awkward position of the fangs, seldom envenomate victims by natural bite.

1 Composition of Snake Venoms: Nonprotein Components

About 90 to 95% of dry snake venom consists of proteins. Protein fractions are biologically more important than nonprotein ones as most of the biological activities reside in protein fractions. Protein fractions contain major as well as minor toxins, nontoxic proteins, and enzymes. Most enzymes are hydrolytic in nature with the notable exception of L-amino acid oxidase, which causes oxidative deamination of amino acids. Because of the hydrolytic nature of venom enzymes, it is thought that they facilitate tissue damage on the prey to help eventual digestion (exodigestion). In some cases, venom enzymes are considered to play an important role in the self-defense of snakes. It is hard to define the exact role of enzymes in snake venoms, but the fact is that venoms do contain a number of enzymes.

In this chapter, only the nonprotein portions of snake venoms will be discussed. Subsequent chapters will be devoted to the various proteins.

For convenience, the nonprotein components will be divided into inorganic and organic constituents. Organic constituents are further classified into free amino acids and small peptides, nucleotides and related compounds, carbohydrates, lipids, and biogenic amines.

Table 1 Metal Contents (Micrograms Metal per Gram Venom) of Snake Venoms before and after Dialysis, Analyzed by Atomic Absorption

Venom (Origin)	Hr*	Ca	Zn	Mg	Na	K	Cu	Mn	Fe	Other metals†
Elapidae										
Naja naja (India)	0	1000	1600	840	60200	150	0	200	0	0
	48	105	360	650	24800	100		521		
N. naja atra (Formosa)	0	1000	380	650	43600	300	0	13	0	0
	48	138	170	317	25250	109		3		
Bungarus fasciatus (Thailand)	0	1620	196	810	26500	391	0	0	0	0
	48	137	139	500	24700	110				
Viperdae										
Bitis arietans (South Africa)	0	2306	1000	700	41500	500	0	500	0	0
	48	1200	846	274	500	439		52		
B. gabonica (South Africa)	0	2900	690	636	36400	220	0	0	0	0
	48	1080	680	277	750	220				
Vipera russelli siamensis (Thailand)	0	1987	1800	976	34100	760	0	0	0	0
	48	1306	809	306	654	310				
Crotalidae										
Agkistrodon acutus (Formosa)	0	3000	1200	450	36977	1070	175	0	0	0
	48	2668	522	409	12780	965	42			
A. contortrix laticinetus (U.S.A.)	0	2438	964	493	18600	1463	10	49	36	0

Crotalus atrox	0	4196	1394	701	57300	410	0	0	0	0
(U.S.A.)	48	3780	1093	344	24600	320				
C. adamanteus	0	1610	773	107	42300	750	0	0	0	0
(U.S.A.)	48	1604	452	97	8400	750				
C. basciliscus	0	1989	1400	376	16800	670	0	0	0	0
(Mexico)	48	1990	990	310	10200	638				
C. durissus	0	3003	1203	1470	36700	13500	0	0	0	0
(Central America)	48	2968	700	775	12800	3970				
C. durissus terrificus	0	2390	1856	342	45700	1660	0	0	0	0
(South America)	48	2280	1380	204	1780	1440				
C. durissus totonacus	0	1633	840	117	28800	590	0	0	0	0
(Mexico)	48	1590	680	100	1500	550				
C. horridus horridus	0	4930	980	973	53000	420	0	0	0	0
(U.S.A.)	48	3629	800	406	21900	400				
C. horridus atricaudatus	0	150	680	129	49900	350	0	0	0	0
(U.S.A.)	48	97	657	91	10010	240				
C. viridis viridis	0	4560	1847	240	26400	710	0	0	0	0
(U.S.A.)	48	2730	1050	209	1200	600				
Sistrurus milarius barbouri	0	4000	2010	446	39500	2540	200	0	0	0
(U.S.A.)	48	2750	1525	297	1550	2159	90			

* Length of time that crude venoms were dialyzed against distilled water before analysis.

† Mo, Bi, Se, Pt, Pd, Ag and Au.