TOXINOLOGICAL RESEARCH IN WESTERN EUROPE REPORT OF A VISIT IN 1986

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Toxinology is the science of natural venoms originating from different living organisms. Toxins occur in plants, microorganisms (e.g. bacterial toxins), wasps, bees and ants (Hymenoptera), scorpions and spiders (Arachnoidea), and many marine organisms (sea anemones, jellyfish, scorpionfish, etc.). They are also found in some amphibians (salamanders, toads) and reptiles (Helodermatidae, snakes). In this last context, toxinology is closely associated with herpetology, since most of the venoms used today in various kinds of toxinological research originate from snakes. Furthermore, thousands of deaths every year are the result of venomous snake bites and these also fall into the field of clinical and experimental toxinology. The world's toxinologists are associated under the aegis of the International Society on Toxinology (IST), which issues a journal, *Toxicon.*

In October of 1986, the author had the opportunity to make a study visit to some institutes and universities in Western Europe where research on snake venoms and/or venomous snakes is being carried out. The visit was partially supported by the College of Pharmacy, Medical School of Szeged, Hungary. During the period of a month, a total of 29 institutes were visited in eight countries. In the following account some of those which could be of interest to BHS members are briefly introduced.

Italy: My visit started in Florence, at the Museum of Zoology, where B. Lanza, the President of Societas Europaea Herpetologica (SEH), introduced me to the Museum's collection. His studies involve the amphibians and reptiles of East Africa, especially Somalia, from whence he has described some new species (Lanza, 1978). At the same institute G. Delfino is carrying out investigations on the cutaneous venom glands of *Bombina variegata* and on the venom apparatus of *Polistes gallicus* (Hymenoptera, Vespidae) at the electron microscopic level.

At the Department of General Physiology, University of Torino, I met L. Cedrini who is studying the various effects of Gaboon viper *(Bitis gabonica)* venom on heart and muscle preparations. The evidence that *B. gabonica* venom is able to change the features of the transmembrane action potentials and causes the arrest of isolated preparations from guinea-pig hearts suggests a direct cardiotoxicity of this snake venom (Alloatti and cedrini, 1981).

France: The first station I visited in France was Latoxan (Laboratorie des Toxines Animales et Animaux Venimeux) in Rosans, situated in the French Alps 120 km north of Marseille. This laboratory probably has the largest collection of venomous creatures in Europe. Most of the animals have been captured by the Latoxan's own experts on their home territories.

The geographical place of origin is an important factor in the further use of venom in toxinological studies, because venoms from snakes of the same species collected from different areas may have different pharmacological properties. I received a very friendly and professional reception from Y. Doljansky, A.M. Saint-Michael and Y. Mittaine. The snake representatives of the *Elapidae, Viperidae* and *Crotalidae* are milked regularly by electrical stimulation through the application of electricity to the maxillary region, thereby stimulating contractions of the buccal muscles surrounding the venom gland and delivery of the venom. Besides venom production the laboratory puts a great deal of effort into breeding their animals. Apart from snakes, Latoxan also provides scorpion (e.g. *Androctonus* spp., *Buthotus* spp.) and amphibian (e.g. *Bufo bufo, B. calamita, Salamandra salamandra)* venoms.

From Rosans I moved on to Beauvoir-sur-Niort (150 km north of Bordeaux) and visited G. Naulleau at the centre d'Etudes Biologiques des Animaux Sauvages. Naulleau's research concentrates on the ethology and ecology of the genus *Vipera.* In order to investigate these aspects, he has at his institute numerous different outdoor cages (some of them are directly

connected to indoor terraria) equipped with special instruments registering various environmental factors affecting the vipers' behaviour and physiology. Another of his studies concentrates on the effects of temperature on digestion in *Vipera*. Naulleau (1983) has shown that among the five European vipers he studies, only *V. berus* can digest prey completely at 10°C. The other species can digest completely at 15°C, except *V. seoanei,* which perhaps needs a higher temperature. At every temperature level, *V. berus* digests faster than other species, except at 30°C, where temperature disturbs both the time and quality of the digestion. With the exception of *V. berus* the digestion period in every species shortens when temperature rises.

At the Pasteur Institute in Parish I met C. Bon, whose present research is mainly on the biochemistry of crotoxin. This is the major toxic protein of the South American rattlesnake, *Crotalus durissus terrificus*. Poisoning from the bite of North American rattlesnakes can lead to the onset of shock and, in anesthetised animals, many of these venoms produce circulatory failure associated with vasodilation and hypovolemia. In contrast, poisoning by the venom of *C. d. terrificus* involves neurotoxic symptoms and respiratory paralysis (Gopalakrishnakone *et al.*, 1980). The lethal effect of crotoxin has generally been attributed to a presynaptic blockage of the neuromuscular transmission. At this level, it causes a reduction of acetylcholine release by the nerve terminals simmilar to that observed with elapid neurotoxins (ß -bungarotoxin, notexin, taipoxin from the venoms of *Bungarus multicinctus, Notechis scutatus* and *Oxyuranus scutellatus* respectively).

The protein, crotoxin, has played its part as a milestone in the history of snake venom research. In 1938 Karl H. Slotta and Heinz Fraenkel-Conrat at the Instituto Butantan in São Paulo (Brazil) crystallized crotoxin and determined its molecular structure. This discovery provided the impetus for world-wide research on animal venoms.

Bon's studies in Paris also include other neurotoxins. Bon described the ceruleotoxin which blocks the neuromuscular transmission from the venom of *Bungarus fasciatus* postsynaptically (Bon and Changeux, 1975; Bon and Saliou, 1982). This toxin is very potent and is responsible for at least 35% of the total toxicity of the venom. When intravenously injected into mice, ceruleotoxin produces a flaccid paralysis of the skeletal muscles and death occurs by respiratory failure (Bon, 1976).

Spain: In Barcelona I met D. Gonzales, a clinical toxinologist from the Universidad Autonoma de Barcelona, Facultad de Medicina. His research includes work on the epidemiological and clinical aspects of various venomous animals of Spain. According to Gonzales (1982) the viperid species responsible for snake bites during 1965-1980 in Spain were *Vipera latasti* (54.8%), *V. aspis* (35.1%) and *V. seoanei* (10,1%). It is noteworthy that the opisthoglyphous *Malpolon monspessulanus* can sometimes also cause severe poisoning, however most of the bites by this colubrid species result only in local symptoms, like oedema and paresthesia (Gonzales, 1979).

Switzerland: From Barcelona, an overnight train took me to Geneva where I consulted with P. Sizaret at the Biological Standardization Department of the World Health Organization (WHO). In 1979, WHO held a Coordination Meeting on Venoms and Antivenoms in Zürich which was devoted to collecting data on the clinical effects of snake bites and scorpion stings and experience in their treatment. The snakes proved to be most important in causing major health problems are *Naja naja, Notechis scutatus, Echis carinatus, Vipera russelli, Crotalus adamanteus, Bothrops atrox asper* and *Trimeresurus flavoviridis* (WHO, 1981).

I next visited the Swiss Tropical Institute in Basle where A. Moser is carrying out telemetric studies on the populations of *Vipera berus* in the eastern Swiss Alps by tracking transmitter-tagged individuals. This method could also be of importance to research on snake bite epidemiology (Moser and Freyvogel, 1986).

The other organization in Basle of interest in this context is Pentapharm Ltd., which manufactures pharmaceuticals and diagnostics from snake venoms. At this firm, K. Stocker is carrying out research on thrombin-like snake venom proteinases (enzymes that coagulate fibrinogen). Several preparations of fibrinogen-coagulant snake venom enzymes have today found a current application. They can be used as enzymatic tools in fibrinogen and platelet research, as diagnostic reagents for the characterization of impaired fibrin formation in patients, as haemostatic drugs or as agents for therapeutic defibrinogenation (Stocker *et al.*, 1982). Stocker's present studies

involves the ancrod (Arwin(R)) from the venom of *Agkistrodon rhodostoma* and batroxobin (Defibrase(R)) from *Bothrops atrox moojeni (B. moojeni).* Both are currently being used as defibrinogenating drugs in man for the treatment of vascular occlusive diseases. Also in Pentapharm Ltd., J. Meier is studying the venom apparatus of snakes, namely the fangs of *Dispholidus typus* and *Thelotornis kirtlandi.* Despite both snake species being opisthglyphous, their bites can sometimes result in death, and so they also have some medical importance. One of the founders of modern herpetology, Professor R. Mertens, died in 1975 from a rare bite by *T. Kirtlandi.* Lung oedema and renal failure were the actual cause of his death on the 18th day after the bite (Kornalik *et al.,* 1978). Meier is also active in the field of snake venom pharmacology.

United Kingdom: My visit in England started at the Reptile and Amphibian Section of the Department of Zoology, British Museum (Natural History). C. J. McCarthy guided me throughout the Museum's collection and showed me various preserved specimens of venomous snakes which I was interested in. We also had a short discussion on the phylogeny of venomous snakes based on their venom apparatus, and I was also able to examine microscopically some fangs and other maximllary teeth of *Bungarus.* McCarthy is presently investigating the taxonomy and phylogeny of proteroglyph snakes.

I visited three toxinologists in London. Firstly I met B. Banks at the Physiology department, University College London. Her research includes the pharmacology of Hymenoptera venoms. At the Department of Physiology, Queen Elizabeth College (University of London), I met B. J. Hawgood, a physiologist investigating the action of snake venom. Her work, however, is concentrating on crotoxin. As mentioned above, this venom component has a presynaptic site of action. And so, based on Gopalakrishnakone and Hawgood (1984), the morphological changes in the diaphragm motor nerve terminals induced by crotoxin complex, which include a reduction in synaptic vesicle population, the appearance of omega shaped indentations in the axolemma and swelling of mitochondria, are associated with clinical signs of developing muscular paralysis during systemic intoxication. No postsynaptic or myofibrillar changes were observed at the stage when respiration ceased. Another part of Hawgood's studies includes work on Mojave toxin isolated from the venom of *Crotalus s. scutulatus* which has some biochemical similarities to crotoxin. Mojave toxin appears to have multiple sites of action and in addition to neurotoxic and myonecrotic activity, signs of cardiovascular involvement were observed by gopalakrishnakone *et al.* (1980). Mojave toxin and crotoxin also have antigenic similarities as shown by the ability of antiserum produced against cortoxin to provide protection against Mojave intoxication in mice (Gopalakrishnakone *et al.,* 1980). At the same department, N.A. Marsh is carrying out studies on the cardiovascular effects of *Bitis gabonica* venom. The results in collaboration with co-workers (Adams *et al.,* 1981) indicate the presence of two different components in *B. gabonica* venom affecting the cardiovascular system: a vasodilatory component responsible for the fall in peripherial resistance and hence arterial blood presure and a cardiotoxic component responsible for the progresive reduction in stroke volume.

During my stay in London, I was provided with very pleasant accommodation by T. Langton, Staff Herpetologist with the Fauna and Flora Preservation Society, who is active in the conservation of amphibians and reptiles. I also had the opportunity to meet up with M.R.K. Lambert, Chairman of the BHS.

I next travelled to Liverpool and visited the Liverpool School of Tropical Medicine (LSTM) where D. Theakston and D. Iddon introduced me to their work. Theakston's research expands into many fields of snake venom toxinology, including epidemiology, immunology, etc. According to Theakston and Reid (1982) there is a yearly total of almost 10,000 deaths due to snake bite in the Nigerian savanna and about 23,000 deaths per year in West Africa as a whole. It is interesting that contrary to the widespread view of the lay public and even some medical personnel, antivenom if used correctly can reverse systemic poisoning even when given hours or even days after the bite. It is therefore wise to wait for the appearance of signs of systemic poisoning before administrating antivenom, rather than using it routinely (Reid and Theakston, 1983). WHO has designated the LSTM as a Collaborating Centre for the Control of Antivenoms, and this centre now holds a collection of reference venoms from several important snake species.

The Netherlands: From England I returned by sea to the Continent. In Leiden, Netherlands,

I met P. Dullemeijer at the Zoological Laboratory, University of Leiden. His world-renowned research includes work on the morphology, biomechanics and evolutationary biology of animals, and in this context he has outstanding results on the functional morphology of snake venom apparatus and in the biomechanics of the feeding mechanisms of snakes.

F.R. Germany: I started my visit in West Germany at the Behringweke A.G. in Marburg which is a great antivenom producer for the treatment of European, North and Central African, as well as Near and Middle Eastern, venomous snake bites.

In Waibstadt (near Heidelberg) I was able to meet J. Fehres at the Antitoxin Dr. Helmbold GmbH, where, under the direction of W. Helmbold, the production of antivenom in goats (antivenoms for humans are generally raised in horses) is being carried out for the management of Southeast Asian snake bites.

Next, in Hannover, I visited to G. Habermehl at the Department of Chemistry, Hannover School of Veterinary Medicine. He is President of the European Section of IST and is now active in plant toxin research. The most famous case of plant poisoning is without any doubt that of the sentence against the Greek philosopher Socrates who had to drink an extract of *Conium maculatum* with wine. From he and his student Platon come the first exact descriptions of a fatal poisoning including all of the symptoms by the alkaloid coniin (Habermehl, 1986).

Unfortunately, on account of a business trip that he was making at the time, I could not meet D. Mebs (University of Frankfurt) the Secretary-Treasurer of IST. His research work is also of great importance to Western European toxinology. Apart from his extensive pharmacological studies on snake venoms, including a chapter on reptilian venoms in the *Biology of the Reptilia* series, Mebs recently created and recommended a system for the classification of various snake venom toxins, which now exceed 300. The proposed nomenclature of venom components includes, beside the genus name of the snake, the principal biological function and a number when more than one toxin exists followed by the complete scientific name, i.e. Naja neurotoxin 1 *(Naja naja)* (Mebs, 1986).

Austria: On my return journey to Budapest, I visited F. Tiedemann at the Museum of Natural History, Vienna, with whom I discussed the status of the endangered *Vipera ursinii rakosiensis.* Regrettably, this subspecies seems to be extinct in Austria, and the situation for probably the last populations of this viper in Hungary is also not encouraging.

ACKNOWLEDGEMENTS

I would like to express my gratitude to everyone that I visited for the kind way in which they received me, some of whose names are not mentioned above. Dr. M.R.K. Lambert's helpful suggestions on the manuscript and his linguistic corrections are gratefully acknowledged. My special thanks are due to Miss Edina Dorothy Hidveghy (Veres Palne Gimnazium, Budapest), Mr. Lajos Komar and Mr. György Czeher for their invaluable assistance in the arrangements of this visit.

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Ed. Note. Through the kind aegis of the British Council in Budapest, Zoltán Takács became a member of the BHS in 1986. He has completed his first year at the Medical School of Szeged, in Szeged, Hungary, specialising in pharmacology.