thought to increase with the learning capacity of the predator and his ability to communicate this information. If cases of mimicry could be demonstrated in this context, these would present a strong indication for the protective and selective value of poisons.

There is a further group of toxic animals for which the value of toxicity is not yet understood. They are classified as 'ichthyocrinotoxic'[10] e.g. boxfish (*Ostracion spec.*), which show dermal glands at the bases of the tail and fins, and in connection with the buccal orifice. The presence of dermal glands, producing toxins, without means of application, is similar to the situation in amphibians. It is questionable if a special term, like 'ichthyocrinotoxic', is necessary and justifiable. Even if it is, the term would seem somewhat misleading, in that it appears to place the fish concerned into the context of 'ichthyosarco'- 'ichthyohaemo-' and 'ichthyootoxic', i.e. poisonous fish. The production of toxic substances in special glands, the contents of which are excreted into the outside world, however, clearly demonstrates the venomous (as opposed to poisonous) character of the animals concerned.

As to the functions of the venom apparatus it has been suggested[10] that it is used for self-defense as well as for the control of population dynamics. The venom might serve as a repellent to other fish and even to its own species. Its application may be seen in the context of the solitary mode of life of the boxfish. Further to this, the idea has been put forward that the venom might serve for the control of ectoparasites[10]. If these assumptions should prove correct, new dimensions would be added to the field of toxinology.

Conclusions

Some of the papers to follow in the present series of communications on toxinology will show that in the field of chemistry, physiology, pharmacology and immunology, as well as molecular biology, animal venoms provide us with some particularly useful models. Obviously, this is one of the main reasons for the growing interest shown by numerous scientists in animal toxins.

With reference to medicine, more research is needed in the field just mentioned with the aim of improving medical care. In addition, however, it is postulated that research on the behaviour of venomous animals towards man, and research into the quantities of venom actually applied to man, be intensified. Also, on the basis of results in this context, people most exposed could be provided with more and better information about prevention.

Work of this sort requests the collaboration of biologists, who observe toxic animals in their natural habitat and who investigate in particular when, and under what prerogatives, the animals make use of toxins in their natural surroundings. Thus we end up with what has been said in the introduction to these notes: toxic animals are to be studied as entities and toxicity has to be looked at from all aspects essential for life, possibly including parasite and population control.

Should the very last point prove valid, fascinating links could be established between toxinology and ecology and in turn migt become important for nature conservation. Thus, toxinology is but a budding field, the limits of which, can yet only be assumed.

Chemistry of Some Potent Animal Toxins

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Introduction

My theme is to give a short review of the present knowledge of animal poisons, especially neurotoxins. The subject is a vast one, as there are thousands of poisonous animals with many different types of toxins. I have therefore chosen to limit myself to discussing only the most active toxins, which are almost exclusively neurotoxins. This selection is perhaps more justifiable on psychological rather than on scientific grounds, since the primeval interest in poisonous animals and their toxins inherently involves the attitude: the more poisonous – the more interesting. It is suitable to begin with a description of snake venom neurotoxins, since no animal has played such a role in religion and superstition as the snake, and the snake is still today the most feared animal[1]. There are, however, also other reasons to give snake venoms priority in this article: we know more about snake venom neurotoxins than about any other group of animal poisons.

[1] R. Morris and D. Morris, *Men and Snakes* (Hutchinson and Co. Ltd, London 1965).

Snake venom neurotoxins

a) *Curarimimetic neurotoxins.* Snakes of the families *Elapidae* (cobras, mambas, kraits, etc.) and *Hydrophidae* (sea-snakes) have toxins which impair the neuromuscular transmission by blocking the nicotinic acetylcholine receptors at the myoneural synapses, roughly mimicking the action of curare. The venom neurotoxins have a much higher affinity for the acetylcholine receptor than curare and are consequently more toxic. The intravenous LD_{50} doses for the neurotoxins are in the range 50–100 μg/kg mouse and 200 μg/kg for D-tubocurarine chloride. The difference in potency is even more evident from a comparison of the lethal doses on a molar basis (Table II). In experimental animals, the toxins cause death by respiratory paralysis, with violent spasms during the final stages of asphyxia.

The curarimimetic neurotoxins are low molecular weight basic proteins tightly cross-linked by disulfide bridges and they fall into two distinct size groups. The smaller toxins (mol. wts. ca. 7,000) consist of 60 to 62 amino acid residues in a peptide chain cross-linked by 4 disulfides, and the larger ones (mol. wts. ca. 8,000) contain 71 to 74 amino acids and 5 disulfides.

The high degree of disulfide cross-linking probably accounts for the unusual stability of these protein neurotoxins, which can survive heating in saline for 30 min at 100 °C[2] or exposure to 8 M urea for 24 h[3] without any significant loss of toxicity. They are, however, rapidly inactivated by strong alkali probably as a result of de-sulfurization and/or disulfide interchange.

Sea-snakes have an extremely high content of neurotoxins, which can comprise as much as 85% of the protein content of the venom[4]. Terrestrial snakes have a lower neurotoxin content, but as the venom output per individual snake is considerably higher, and it is easier to maintain colonies of these snakes, their venoms are generally more available. Every venom contains more than one neurotoxin. The toxin *siamensis* 3 or the *siamensis* toxin, the main neurotoxin of *Naja naja siamensis* (*kaouthia*) (Thailand cobra) is perhaps the easiest available individual neurotoxin. It comprises between 20 and 30% of the total weight of the dry venom, which is commercially available at a cost of 50–75 US dollars per gram. The siamensis toxin is also comparatively easy to isolate in pure form from the crude venom[5,6].

The two types differ from each other with respect to:

1. *Affinity* for the acetylcholine receptor. A neuromuscular block caused by a small neurotoxin can be reversed by washing the neuromuscular preparation with neostigmine[7–9], whereas the block is much stronger, or essentially irreversible, with the larger neurotoxins[9–12].

2. *Immunogenicity.* Antiserum against a toxin does not neutralize toxins of the other size group but gives probably good protection against any other toxin of its own type[13,14].

3. *Vulnerability.* The smaller neurotoxins appear to be much more sensitive to chemical modification than the larger ones. Ozonization of a 4-disulfide neurotoxin resulted in 92% loss of activity, whereas ozonization of a 5-disulfide toxin yielded a derivative with 50% residual activity[15]. Lyophilization under unsuitable conditions (e.g. poor vacuum, low pH, or high concentrations of ammonium acetate) may cause extensive (more than 50%) polymerization of the small toxins[16], whereas for a large toxin the usual figure is only 5%[5]. From the position of the extra disulfide in a large neurotoxin, it is intuitively understood why the 5-disulfide neurotoxins are more resistant. A small neurotoxin has a long stretch of 16 amino acid residues between the 3rd half-cystine and the 4th one (alignment No. 29–45, Figure 1). This is also the longest stretch

Table I. Toxicity of batrachotoxin and related compounds

Position of substituents in pyrrole moiety	LD_{50} (μg/kg mouse, s.c.)
Batrachotoxin 2,4-dimethyl-3-carboxylate	2
Homobatrachotoxin (in skin secretion)	
2-ethyl-4-methyl-3-carboxylate	3
2,5-dimethyl-3-carboxylate	2.5
4,5-dimethyl-3-carboxylate	280
2,4,5-trimethyl-3-carboxylate	1
2,4-dimethyl 5 ethyl-3-carboxylate	8
2,4-dimethyl-5-acetyl-3-carboxylate	250
N,2,4,5-tetramethyl-3-carboxylate	>1000
Batrachotoxinin A (without pyrrole moiety)	1000

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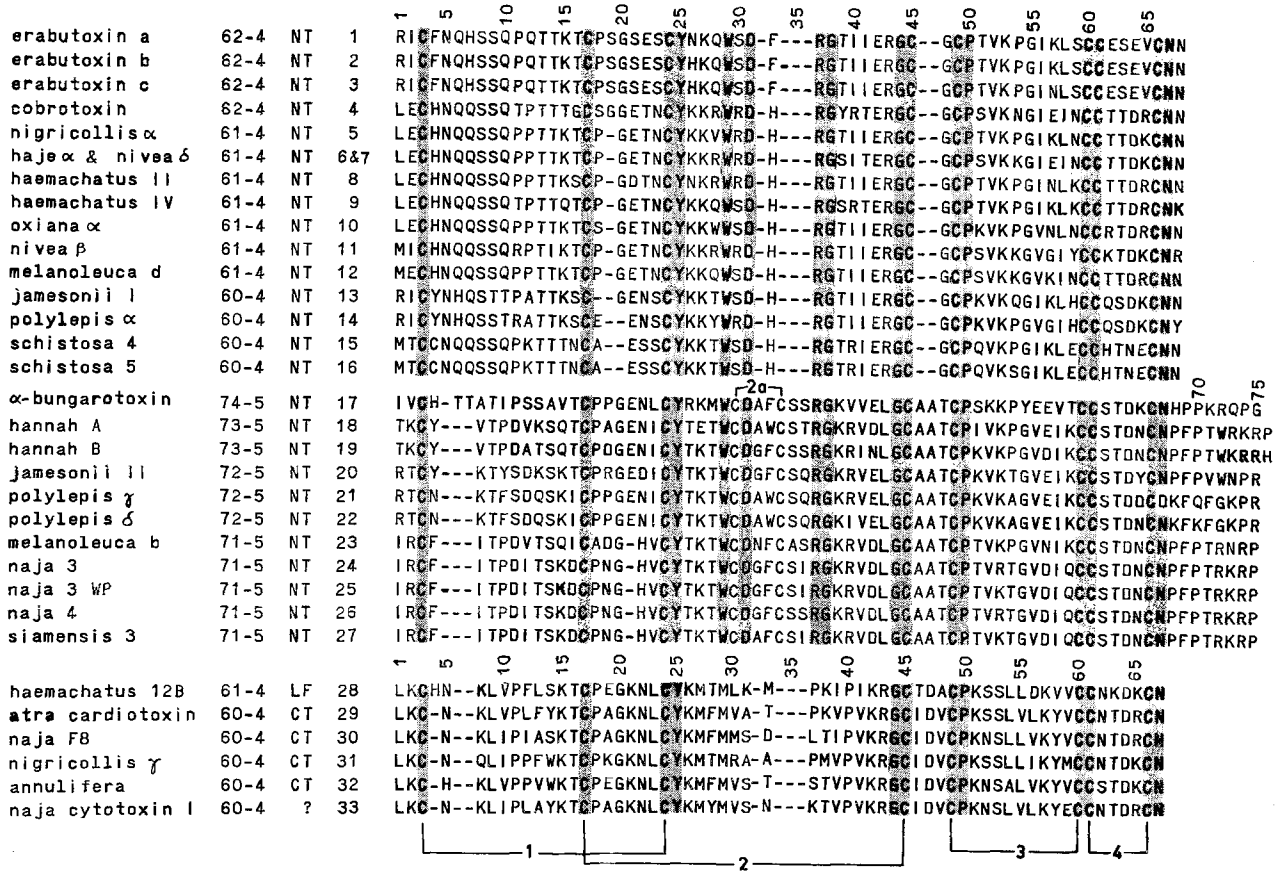

Fig. 1. Alignment of sequences of curarimimetic snake venom neurotoxins and phylogenetically related proteins. The IUPAC one-letter code for the amino acids is used: A, Ala; B, Asx; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; Y, Tyr; V, Val; W, Trp; Z, Glx. The notations 60–4 etc. in the first column denote the structural type; number of amino acid residues and disulfides. The abbreviations in the second column are: NT, neurotoxin; CT, cardiotoxin; and LF, lytic factor. The shaded areas mark the invariant amino acids. For toxin 21 compare the footnote. The disulfides are: 1. Cys 3–Cys 24; 2. Cys 17–Cys 45; 3. Cys 49–Cys 60; and 4. Cys 61–Cys 66. The extra disulfide 2a in the large neurotoxins connects Cys 31 to Cys 34. In the toxins *schistosa* 4 and 5 disulfide 1 connects instead Cys 4 to Cys 24. The different sequences are compiled from the following sources: 1 and 2[21,22], 3[4], 4[23,24], 5[25], 6 and 7[26,27] 8 and 9[28], 10[29], 11[30], 12[31], 13[32], 14[33], 15 and 16[34], 17[35], 18 and 19[36], 20[37], 21[33], 22[18], 23[31], 24–27[37], 28[38], 29[30], 30 and 31[40], 32[18] and 33[42].

between 2 disulfide bridges in the molecule. In a large neurotoxin, however, this long stretch has been given an additional stabilization by the extra disulfide between the residues 30 and 35 (2a in Figure 1).

Elapid venoms contain also other low molecular weight basic proteins which are homologous to (Figure 1) and phylogenetically closely related to the neurotoxins[17,18]. They have a comparatively low toxicity, about 750 μg/kg intravenously in mice. They cause hemolysis (lytic factors), fibrillation of heart muscle (cardiotoxins), depolarize muscle membranes, inhibit the Na^+– and K^+– activated ATPase[19] (the sodium pump?).

An alignment of sequences to a maximal homology as done in Figure 1 shows that most amino acids have changes frequently, whereas some have remained unaltered during evolution. There are certainly important reasons for the conservation of these amino acids. Invariant amino acids may be constituents of the active site, they may be essential for maintaining the active conformation, or serve as nucleii for the folding of the peptide chain after the synthesis. The reasons may also be found on the DNA-level, such as a constant base sequence being required for the conformation of the gene and this sequence will then code for an invariant amino acid[20]. Attempts to map out the active site by chemical modifications have been made, but have not yet provided any direct evidence for the participation of particular amino acids in the toxin-receptor interaction.

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Intact disulfides are essential for maintaining the active conformation, since activity is lost upon reduction of the disulfides and is regained upon reoxidation[3]. The invariant Tyr 25 is not accessible for chemical modification unless the molecule is unfolded with urea or guanidine hydrochloride[42,43]. Tyr 25 is apparently located in the interior of the molecule and is therefore unlikely to participate in the interaction with the receptor. Iodination of Tyr 25 in *cobrotoxin* does not cause inactivation[43], a result which confirms the assumption that the invariant tyrosine is not part of the active site.

The invariant Gly 38, Gly 49, and Pro 50 are hardly functionally essential, but they may have a structurally important role. A glycine in the sequence Cys-Gly-Cys (Nos. 45, 48 and 49, Figure 1) invariant in the small neurotoxins may illustrate the importance of glycine in a case where sterical restrictions exist. The 2 adjacent disulfide bridges apparently require that the single amino acid between them lacks a side-chain.

Trp 29 is invariant in the neurotoxins but is never found in the non-neurotoxic proteins in that position. Trp 29 is, however, not functionally essential, as it can be modified by a variety of means (treatment with 2-hydroxy-5-nitrobenzyl bromide[44,15], ozonization[15,41], formylation[45]) with retention of 50% of the toxicity.

The neurotoxins and acetylcholine appear to have the same target, the acetylcholine receptor. The active site of the neurotoxins should therefore have reactive groups resembling the quaternary ammonium group and the carbonyl group of acetylcholine. It has been shown that free amino groups are not essential for the activity (a free N-terminal group is invariant). Acetylation of any one of the 6 amino groups in the siamensis toxin reduces the toxicity by only one-third[46]. Histidine can also be excluded as an essential cationic group, it is not invariant and it is even absent in some toxins[47,33]. In fact, the only invariant cationic group other than the N-terminal amino group is the guanidino group of Arg 37 which is therefore the most likely candidate for the postulated essential cationic group which should be functionally analogous to the quaternary ammonium group of acetylcholine.

The glycine residue that always follows the invariant Arg 37 probably plays an important role. Due to the absence of a side-chain, it provides no sterical restriction on the preceding guanidino group and such a conformational freedom might be required of a group that participates in the binding of the toxin to its target.

The possible essential carbonyl group might be a carbonyl in the peptide backbone, but a side-chain carbonyl should be more accessible for the interaction with the receptor. There are 2 invariant side-chain carbonyls, in Asp 31 and Asn 67. Polylepis γ (No. 21) has aspartic acid at 67. This might be incorrect. Polylepis γ elutes on the cation exchanger Amberlite CG-50 at pH 8 (no charge on histidine) after polylepis α[33]. The toxins might have the same charge, but it is hardly possible for toxin γ to have the charge + 5 (from sequence data) and elute after toxin α with a charge of + 6. A rather frequent error in sequence determinations is that the number of amides is too low. It seems therefore that toxin γ should have at least one amide more than reported. Considering also that all the other 32 sequences shown in Figure 1 have asparagine at 67, it is then very probably that this also should apply to polylepis γ. Asp 31 has its side-chain carbonyl substituted with a negatively charged group, whereas in Asn 67 the carbonyl is part of an amide as in carbamylcholine. The side-chain of Asp 31 can also be modified with glycine methyl ester and the toxin (*cobrotoxin*) retains 75% of its original toxicity[48]. If there exists an essential carbonyl group, the most probable one is therefore in the side-chain of Asn 67.

The strength of the binding between the toxin and the receptor is probably not accountable only to the interaction involving a guanidino group and a carbonyl moiety. The binding should then be largely electrostatic, but the receptor-toxin complex is not dissociated by high salt concentrations[49]. These two groups serve perhaps as recognition sites for the initial attachment of the toxin to its target and the final strong binding is

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brought about by allosteric protein-protein interactions involving large areas of both the toxin and the receptor.

The acetylcholine receptor has been isolated from the electric organs of *Torpedo* and *Electrophorus* by affinity chromatography on cholinergic substances covalently bound to agarose gels[49–51]. The receptor is a protein capable of binding 56 μg of the mono-acetyl (^{3}H) derivative of the siamensis toxin (mol. wt. 7861) per mg of protein. The active receptor should then have an equivalent weight of 140,000[49], and it has been shown to contain subunits[50,52,53]. A remarkable feature of the toxin-receptor interaction is that the toxin does not combine with the receptor at temperatures below 11 °C[12,49].

Other snake venom neurotoxins

The curarimimetic neurotoxins are not the only neurotoxins found in snake venoms. There are also toxins which have a presynaptic effect, impairing the release of acetylcholine. Such neurotoxins appear to be rare, and so far only a few of them have been isolated and investigated. I will shortly discuss them all.

Crotoxin from the venom of *Crotalus durissus terrificus* (South American rattlesnake) is one of the first neurotoxins isolated from snake venom[54]. The molecular weight was reported as 30,000[55], the isoelectric point as pH 4.7[56], and the LD_{50} (i.v.) as 50 μg/kg mouse[57] (the lowest value found in the literature). The toxin is a complex between a non-toxic acidic protein termed *crotapotin*[58] and a basic phospholipase of a rather high toxicity (LD_{50} ca. 500 μg/kg mouse, mol. wt. ca. 13,000)[57,58]. The two constituents of crotoxin can be separated by ion-exchange chromatography. Recombination restores the activity. Crotoxin appears to interfere with neuromuscular transmission at both pre- and postjunctional sites: the presynaptic effect predominates in amphibian muscles and the postsynaptic one in mammalian muscles[59,60].

β-bungarotoxin[61,62] from *Bungarus multicinctus* (banded krait) is a basic protein and has a molecular weight of 28,500 and an LD_{50} (i.p.) of about 25 μg/kg mouse. Information about possible subunits or phospholipase activity are not available. The toxin acts exclusively on the presynaptic side by inhibiting the release of acetylcholine[63].

Notexin from the Australian tiger snake *Notechis scutatus scutatus* is a basic protein of 119 amino acids (mol. wt. 13,574) in a single peptide chain cross-linked by 7 disulfides. The LD_{100} (i.v.) is 25 μg/kg mouse[64]. The toxin blocks the release of acetylcholine[65], but it has also a myotoxic effect[66]. Injection of toxin into the limb of rat causes within 1 day an increase of over 30% in the wet weight of the injected muscle, primarily due to oedema, and by 3 days a decrease to about 60% of the normal weight. The primary effect appears to be an inhibition of the transmitter release which eventually results in muscle dystrophy.

The most purified notexin preparations show a weak phospholipase A activity. No traces of impurities have been detected in the determination of the N-terminal sequence by the Edman degradation[67], but the method hardly allows detection of contaminants lower than 5–10%. The enzyme activity could therefore be due to such a low degree of contamination. The notexin seems, however, to be related to a basic phospholipase A, recently isolated from the venom of *Naja nigricollis*, consisting of a peptide chain of 117 amino acids and 7 disulfides[67]. The LD_{100} (i.v.) is about 500 μg/kg mouse, which is the same as for the phospholipase constituent of crotoxin. The

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	1							5								10								15
Notexin	Asn	Leu	Val	Gln	Phe	Ser	Tyr	Leu	Ile	Gln	Cys	Ala	Asn	His	Gln	Lys	Pro	Pro						
Naja nigricollis phospholipase	Asn	Leu	Tyr	Gln	Phe	Lys	Asn	Met	Ile	Tyr	Cys	Val	Phe	Pro	Ser									

Fig. 2. Similarities in the N-terminal sequences between notexin from the tiger snake *Notechis scutatus scutatus* and a basic phospholipase A from *Naja nigricollis*.

acidic phospholipases of elapid venoms usually are non-toxic. Injection of the phospholipase into muscle causes the same kind of effects as with notexin, but the increase in the wet weight is only about 10% and the decrease correspondingly smaller[66]. A comparison of the partial sequences of the notexin and the phospholipase indicates also a phylogenetic relationship, 6 amino acids are invariant among the first 15[67].

Taipoxin[68] from the Australian taipan *Oxyuranus scutellatus* is the most potent snake venom toxin known. The LD_{50} (i.v.) is only 2 μg/kg mouse. The toxin is a weakly acidic protein (Ip about 5) and dissociates into 2 subunits at pH 3, which can then be separated by gel filtration on Sephadex G-75. The complex has an *apparent* molecular weight of about 42,000, the subunit α of about 30,000 and β of 12,000. Recombination does not restore the activity. The molecular weights for taipoxin and subunit α are probably too high as they are glycoproteins and therefore tend to elute earlier than the globular proteins of corresponding molecular weights which were used for the calibration of the gel filtration column used to estimate the molecular weights. Both subunits are toxic, α has an LD_{100} (i.v.) of 200 μg/kg and β 20 μg/kg. A weak phospholipase activity is observed in the taipoxin and in both of its constituents, but this might be due to contamination. The taipoxin decreases the acetylcholine release from nerve terminals especially that evoked by nerve impulses[69].

Another presynaptic mode of action is displayed by the *black widow spider venom*, which destroys the nerve terminals causing 'avalanches' of transmitter release until the store of acetylcholine is depleted[70–72]. No chemical data for spider toxins are presently available.

Apart from the above toxins, only the botulinus toxins are known to inhibit the transmitter release. The venom neurotoxins are likely to be useful tools for investigating the mechanism for the release of acetylcholine. It is interesting to note that the presynaptic snake venom neurotoxins are much more potent than the curarimimetic toxins (Table II). One possible explanation might be that there are few release sites for acetylcholine as compared to the number of receptors.

Scorpion toxins

Scorpions may constitute a much more serious health problem than snakes. In Mexico during the periods 1940–1949 and 1957–1958 more than 20,000 people were killed by scorpions, about 2,000 by snakes, and 274 by spiders[73].

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Table II. Comparative toxicities

	Lethal dose (μg/kg mouse)[a]	Molecular weight	Lethal dose (mol/kg mouse)
Botulinus toxin type A	2.6×10^{-5}[b]	150,000	1.7×10^{-16}
Botulinus toxin type B	1.0×10^{-5}[b]	167,000	0.6×10^{-16}
Tetanus toxin	2.8×10^{-5}[b]	140,000	2.0×10^{-16}
Abrin	2.8	65,000	4.3×10^{-11}
Palytoxin	0.15	3,300	4.5×10^{-11}
Taipoxin	2	42,000	4.8×10^{-11}
β-bungarotoxin	25	28,500	88×10^{-11}
Notexin	25	13,574	180×10^{-11}
Crotoxin	50	30,000	170×10^{-11}
Naja naja siamensis neurotoxin	75	7,819	9.6×10^{-9}
d-tubocurarine chloride	200	696	290×10^{-9}
Toxin I of *Androctonus australis Hector*	17	6,822	2.5×10^{-9}
Toxin II of *Androctonus australis Hector*	9	7,249	1.2×10^{-9}
Batrachotoxin	2[c]	538	3.7×10^{-9}
2,4,5-trimethyl homologue of batrachotoxin	1[c]	552	1.8×10^{-9}
Tetrodotoxin	8	319	25×10^{-9}
Saxitoxin	8	281	28×10^{-9}
Potassium cyanide	10,000	65	1.5×10^{-4}

[a] Expressed as LD_{50} (i.v.), except when stated otherwise. [b] Minimum lethal dose. [c] LD_{50} (s.c.). *Botulinus toxins* A[108] and B[109] (neurotoxins, inhibitors of acetylcholine release) from the bacteria *Clostridium botulinum*. *Tetanus toxin*[110] (neurotoxin acting on the spinal cord suppressing inhibitor impulses) from *Clostridium tetanii*. *Abrin*[111] (inhibitor of protein synthesis) from *Semen jequiriti*, the seeds of *Abrus precatorius*.

Death from a scorpion sting is most likely due to small basic protein toxins in the same size range as the curarimimetic snake venom neurotoxins[74–76], but they are generally much more toxic. The intravenous LD_{50} doses lie between 9 and 90 μg/kg mouse[77], as determined in presence of albumin. The toxins are not stable at low concentrations, but addition of proteins has a stabilizing effect[74]. The toxins cause a depolarization[78,79] of nerve and muscle membranes. This seems to be due to an increased sodium permeability[80–83], which may depend on an initial liberation of calcium[83,84]. The toxins have no effect on the acetylcholine receptors. The effects of scorpion toxins are antagonized by reducing the external concentration of sodium, by increasing the concentration of calcium, or by tetrodotoxin[83,85]. The scorpion toxins resemble batrachotoxin which also causes depolarization and is antagonized by the same means.

A remarkable feature of the amino acid composition is the high content of aromatic amino acids (5–14 per molecule), especially tyrosine (3–8). The amino acid sequences of 2 toxins from *Androctonous australis* Hector (North African scorpion) are known[86], and they differ only by a valine/isoleucine replacement (Figure 3). More than 20 residues of the N-terminal sequence have been determined for 7 other toxins, and a great sequential homology is apparent[77].

The scorpion toxins are basic proteins, but whether that also implies the presence of an essential cationic group is even more speculative than with the curarimimetic snake venom neurotoxins. Arg 2 (Figure 3) is, however, not invariant, but is replaced by lysine in some of the partially sequenced toxins. No data from chemical modifications are available and nothing is known about the nature of the target, and there is therefore no rational basis for any further speculation regarding the active site of the toxins.

The occurrence of the first half-cystine at position 12 in these toxins leaves a long 'tail' of 11 amino acid residues which is not stabilized by a disulfide. The remainder of the molecule, about 50 residues, is then crosslinked by 4 disulfides and is probably very rigid. It is interesting to note that a bulky tryptophan residue (Trp 46) can be accommodated between 2 half-cystine residues that are presumably involved in 2 different disulfide bridges. In the small curarimimetic neurotoxins, however, the disulfide bridges involving half-cystines 45 and 49 (alignment numbering, Figure 1) seems to require that the residue between them (Gly 48) lacks a side-chain.

Tetrodotoxin, saxitoxin, and batrachotoxin

Tetrodotoxin and saxitoxin block the transient inward flow of sodium through nerve and muscle membranes, and thus inhibit the action potential which normally follows stimulation. Batrachotoxin causes depolarization by increasing the sodium influx, and its action is antagonized by tetrodotoxin. None of the toxins act on the acetylcholine receptors, as the sensitivity of the postsynaptic membrane to acetylcholine is not changed in the presence of the toxins. Several review articles have been published on the pharmacology of tetrodotoxin, saxitoxin[87–93], and batrachotoxin[94,95].

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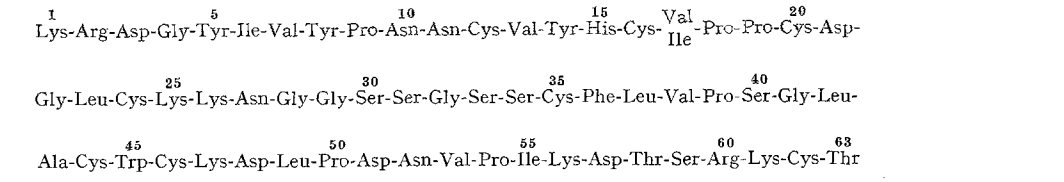

Fig. 3. The amino acid sequences of toxin I (Val 17) and toxin I′ (Ile 17) from the North African scorpion *Androctonus australis Hector*.

Tetrodotoxin occurs in fishes of the order *Tetraodontiformes* (ref. [106] Vol. 2, p. 680) (puffer fish, globefish, porcupine fish, etc.) most species of which are distributed in the Pacific and the Indian Ocean, and in newts of the family *Salamandridae*, such as the California newt *Taricha torosa*[96]. The liver, ovaries, viscera, and in some cases the muscles of fishes and the eggs and tissues of newts are toxic. The LD_{50} (i.v.) is 8 µg/kg mouse.

Saxitoxin (Figure 4)[97] has the same potency as tetrodotoxin and it is responsible for *paralytic shellfish poisoning*. It is found in clams, such as the Alaskan butter clam *Saxidomas giganteus*, only when the mussels have been feeding on the dinoflagellate *Gonyaulax catenella*[98].

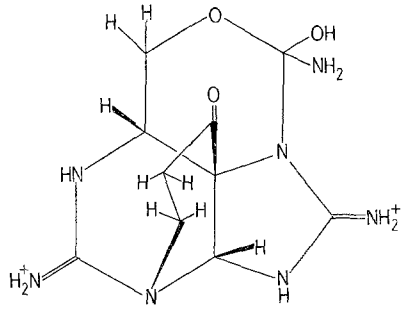

Fig. 4. The structure of saxitoxin.

The structure of tetrodotoxin (Figure 5) has been elucidated independently by 4 research groups[99–102]. It is an aminoperhydroquinazoline compound with a guanidino group. It has 2 cationic forms and forms a zwitterion by dissociation of the OH group at C10. One cationic form (pK_a 8.76) is shown in Figure 6 and is in equilibrium with a lactone form (no bridge between C10 and C5, C = 0 instead of C–OH at position 10, OH at C5, and a pK_a of 8.84). The toxin will then have a positive charge at neutral pH. The blocking action is also much stronger at pH 7 than at pH 9, indicating that one or both of the cationic forms are active. Saxitoxin has an OH group of pK_a 8.24 and it is then also in a cationic form at neutral pH. It thus seems likely that a guanidino group is essential in both toxins. Chemical modifications of tetrodotoxin at C4, OH replaced by H, OMe, OEt, or NH_2 severely diminish or abolish the activity. The low (1–13%) residual activities observed in these derivatives might be due to contamination with small amounts of unmodified toxin. Modifications involving a change in the ring structures (anhydrotetrodotoxin, tetrodonic acid, etc.) result in a complete loss of activity.

The tetrodotoxin receptor, which might be the molecule directly involved in the transient sodium influx, is a protein which loses its ability to bind the toxin below pH 4[103].

Batrachotoxin (Figure 6)[104] is a 20α ester of batrachotoxinin A (a derivative of the steroid pregnane) and 2,4-dimethylpyrrole-3-carboxylic acid. It is weakly basic with a pK_a of 7.45 and the LD_{50} (s.c.) is 2 µg/kg mouse. Several related substances have been synthesized and tested for toxicity (Table I). The 2,4,5-trimethylpyrrole-3-carboxylate is more stable and twice as toxic as batrachotoxin, whereas fully methylated N,2,3,4-tetramethyl-3-carboxylate is inactive, indicating that the free pyrrole NH group is essential.

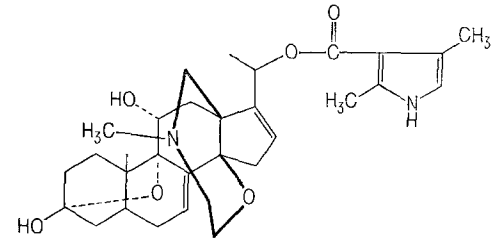

Fig. 6. The structure of batrachotoxin.

The batrachotoxin receptor is probably a protein, but whether it is identical with the tetrodotoxin receptor is uncertain. Denervated muscles become insensitive to tetrodotoxin but remain sensitive to batrachotoxin. This suggests that the toxins act on different substances.

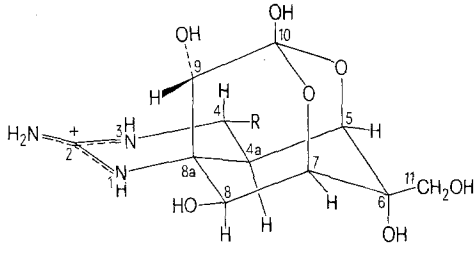

Fig. 5. The structures of tetrodotoxin and some of its derivatives. R = OH, Tetrodotoxin; R = H, Deoxytetrodotoxin, residual act. 13%; R = OMe, Methoxytetrodotoxin, residual act. 4%; R = OEt, Etoxytetrodotoxin, residual act. 2%; R = NH_2, Tetrodaminotoxin, residual act. 1%.

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The action of batrachotoxin resembles that of the scorpion toxins, which therefore might be substitutes for batrachotoxin. If so, they should be more easily available, and being proteins, labelled derivatives could easily be prepared, they could easily be coupled to insoluble matrices for affinity chromatography, etc.

Palytoxin[105]

This toxin has been isolated from polyps of the genus *Palythoa* (*phylum Coelenterata*). It has a molecular weight of 3,300 and an LD_{50} (i.v.) of only 0.15 μg/kg mouse. Palytoxin is thus one of the most potent animal toxins known (Table II). It is not a peptide as its nitrogen content is only 1.7%. The toxin is not adsorbed to an anion exchanger (DEAE-cellulose) at pH 7, but retarded on a cation exchanger (CM-Sephadex) in 0.02 *M* NaH_2PO_4 (pH 4.6) and it is probably a cation at neutral pH. Symptoms in mice are paralysis in hind limbs, diarrhea, severe convulsions, dyspnea, and death from respiratory failure. Thus, it appears to have a neurotoxic action, but being several magnitudes more potent than the curarimimetic toxins, it seems unlikely that it would have a postsynaptic type of action.

Conclusion

I have discussed in this article only the most active toxins, with the result that many interesting substances have been omitted, e.g. the toxins from bee and wasp venoms (apamin, melittin, etc.), of many amphibians (bufotoxins, etc.), ciguatoxins, and many more. Poisons are found in every phylum except birds. Shrews exemplify venomous mammals. One gets a good illustration of the number of poisonous animals by studying the monumental and impressive work by HALSTEAD[106] which consequently excludes terrestrial animals. An interesting fact in this connection is that there are about 20,000 species of spiders, most of which are poisonous.

A toxin ranking list has to be included in an article of this kind. The list is, of course, far from complete. Data on molecular weights, mouse lethal doses, etc. are lacking for many potent toxins, such as the dysentery toxin, a neurotoxin with a toxicity comparable to that of the botulinus toxins[107], the toxins from the jelly fish *Chironex fleckeri*[106].

A comparison on molar basis gives a better notion of the toxicities. Curare has about 1/30 of the toxicity of the curarimimetic snake venom neurotoxins, clearly indicating that curare has a much lower affinity for the acetylcholine receptor.

Toxic organisms have developed during millions of years more and more refined toxins, and this evolution has probably brought into existence toxins against every physiological function. Neurochemistry is to a great extent unexplored. Progress in this field will in the nearest future depend on specific toxins from various natural sources. Toxins from spiders, scorpions, snakes, frogs, and fishes are therefore not mere curiosities but valuable tools for research on the molecular mechanisms of neural function and synaptic transmission.

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ACTUALITAS

International Cell Research Organization (ICRO)

1. *Training Courses.* One of the main activities of ICRO is the organization of training courses on topics of high novelty and on modern techniques in cellular and molecular biology: Principles and techniques of tissue and organ culture; Genetics and Physiology of Bacterial viruses; Energy transducing systems on the sub-cellular level; Methods in mammalian cytogenetics; Membrane Biophysics; DNA-RNA Hybridization; Biogenesis of Mitochondria; Embryology and Epigenetics; Interaction between Animal Viruses and host cells, application of computers to experimental work in biology and chemistry; Methods in molecular biology, etc. The courses generally last 3–5 weeks, and include 16–20 young participants (sometimes more). The ICRO courses are fully international, both the teaching staff and the participants coming from the largest possible number of countries.

2. *The Problem of Developing Countries.* Most of the past ICRO courses have been organizing in European countries – east and west – but the demand from developing countries is increasing steadily. ICRO activities in developing countries may tend to give preference to topics of potential economic usefulness, such as applied microbiology, microbial protein production, fermentation industries, soil microbiology, plant genetics, etc.

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