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REPORTS AND ABSTRACTS

BRITISH VENOM GROUP

The British Venom Group held its Christmas meeting, its fourth annual meeting, at the Orion Hotel and later at the Founder's Hall, London EC1, on 15 December 1995. This group brings together British scientists interested in all aspects of natural toxins. It is sponsored by Therapeutic Antibodies (London) Ltd.

Snake venom phospholipases: separating the phospholipase activity from neurotoxicity. George G. Lunt (School of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, U.K.)

Caudoxin and crotoxin are both presynaptic neurotoxic phospholipases of the A_2 class (PLA_2). The phospholipase activity is Ca^{2+} dependent but may not be the sole component of neurotoxicity. Crotoxin binds to synaptic membranes independently of Ca^{2+} and also blocks the uptake of amine neurotransmitters in Ca^{2+}-free conditions.

The membrane damage resulting from PLA_2 activity has been measured by determining the release of LDH from synaptosomes. Caudoxin releases LDH in a dose-dependent manner and shows also Ca^{2+} dependence. The PLA_2 inhibitors quinacrine and 4-bromophenacylbromide had no effect on the LDH release provoked by caudoxin. Filipin, an agent believed to decrease membrane fluidity, unexpectedly increased the LDH-releasing effect of caudoxin.

Caudoxin blocked the uptake of dopamine by synaptosomes under Ca^{2+}-free conditions. Dopamine release from pre-loaded synaptosomes was enhanced by caudoxin under Ca^{2+}-free conditions. Under these conditions no PLA_2 activity was detectable.

These preliminary results are in agreement with proposals that the PLA_2 activity of the neurotoxins may well have a role in neurotoxicity but that it is not solely responsible for the neurotoxic effects.

Myotoxic toxins from snake venoms: implications for treatment. R. Dixon and J. Harris (Muscular Dystrophy Group Laboratories, Regional Neurosciences Centre, Newcastle General Hospital, Newcastle upon Tyne NE4 6BE, U.K.)

The management of patients envenomed by species whose venoms contain toxic phospholipases is extremely difficult. Antivenom therapy does not appear to be beneficial and recovery can take a long time. One significant problem with devising a therapeutic approach is that rather little is known of the pathological consequences of poisoning by the toxic phospholipases.

Our recent work with notexin (a myotoxic/neurotoxic phospholipase A_2 from the venom of the Australian tiger snake) and β-bungarotoxin (a neurotoxic phospholipase A_2 from the venom of the Taiwanese krait) has shown that muscle damage is precipitated by the binding of the toxin to the muscle membrane, and the production of small lesions in the plasma membrane of the muscle cell. These lead to hypercontractions of muscle fibres. The

investigated in the rat isolated anococcygeus muscle (Acm). BSV produced contractile responses of the Acm mediated by noradrenaline in the venom acting directly on postjunctional alpha-adrenoceptors; RSV produced alpha-agonist actions (contractile/excitatory responses) via some prejunctional mechanism(s), causing the release of transmitter noradrenaline; LQV produced relaxant (inhibitory) responses of the carbachol-precontracted Acm involving the L-arginine-nitric oxide synthase–nitric oxide pathway.

From neuropeptide to unstable aromatic pyruvate derivative: an unexplained enzymic trail in cobra venom. Lois Anderson and Mark J. Dufton (Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, U.K.)

Taiwan cobra (*Naja atra*) venom contains a peptidase activity which has received little investigation since its discovery in the 1930s. The specificity is for tripeptides and oligopeptides containing hydrophobic/aromatic residues (e.g. tyrosine, phenylalanine, leucine) and glycine. Previously tested substrate peptides were hydrolysed slowly, but we have discovered that methionine encephalin (YGGFM) is degraded rapidly. It is the best substrate we have tested to date (K_m approx. 0.90 mM; V_{max} approx. 26.70 mM min^{-1}), and its fate in the presence of whole venom reveals a probable interplay between several enzymes. Two actions are demonstrable on the neuropeptide. One is an aminopeptidase action, removing tyrosine, and the other is an endopeptidase action which cleaves the internal GF bond to release YGG and FM. The YGG is also quickly acted upon by an aminopeptidase to release the tyrosine (K_m approx. 0.96 mM; V_{max} approx. 9.75 μM min^{-1}). The free tyrosine formed in these reactions does not accumulate because it is, in turn, the most favoured substrate of the L-amino acid oxidase in the venom (K_m 0.37 mM; V_{max} 15.70 μM min^{-1}). The significant products from the latter enzyme are, in principle, 4-hydroxyphenylpyruvate and hydrogen peroxide, which are supposed to react together to produce 4-hydroxyphenylacetic acid. However, this is a major oversimplification because of the environmentally sensitive tautomeric equilibrium and chemical reactivity of the pyruvate in aqueous physiological media, even in the absence of peroxide. The reaction product equilibrium from tyrosine and the oxidase varies according to the enzyme/substrate concentration, buffer type, slight changes in pH and temperature, reaction quenching methods and the nature of measuring techniques. The 4-hydroxyphenylacetic acid product does not accumulate rapidly in solution, even without a peroxide scavenger being present; however, after conventional attempts to obtain solid product, it is found to be the major identifiable component of the residue. We are currently endeavouring to analyse and account for the complicated solution chemistry that follows the action of the oxidase.

In overview, it seems unlikely that these connected enzyme activities and their preference for encephalin-related peptides and amino acids is a coincidental by-product of a nondescript digestive process. At one end of this enzyme sequence, important neuropeptides that are released as a defensive response to envenoming are being degraded preferentially. At the other end of the sequence, aromatic amino acids removed from these peptides are being converted into a structurally unstable and reactive mixture of pyruvate-based compounds. Past experience shows that tyrosine metabolites often possess major pharmacological activities, so it is possible that a short-lived and reactive aromatic ligand is being produced *in situ* to assist the objectives of the poisoning syndrome. Even if the peptidases are not intended to exert a major toxic effect through their actions on enkephalins, they are evidently 'feeders' for the L-amino acid oxidase, an enzyme whose

widespread occurrence in snake venom has, surprisingly, never been satisfactorily explained.

The actions of philanthotoxin and its structural analogues on ionotropic glutamate and nicotinic acetylcholine receptors. Ian R. Mellor, Koji Nakanishi[1] and Peter N. R. Usherwood (Department of Life Science, University of Nottingham, University Park, Nottingham NG7 2RD, U.K.; [1]Department of Chemistry, Colombia University, New York, NY 10027, U.S.A.)

The venoms of many wasps and spiders contain small cationic polyamine–amide toxins, some of which are potent noncompetitive inhibitors of ionotropic glutamate receptors (GluR) and nicotinic acetylcholine receptors (nAChR), and others which are blockers of calcium channels. Philanthotoxin-433 (PhTX-433) has been isolated from the venom of the parasitic wasp *Philanthus triangulum* (Spanjer *et al.*, 1982) and subsequently shown to be an inhibitor of both ionotropic GluR and nAChR. PhTX-343 is an equipotent synthetic analogue of the natural compound (Fig. 1) which has been used extensively in studies with ionotropic GluR and nAChR.

The PhTX-343 molecule can be divided into four regions (Fig. 1): A, polyamine (spermine) chain, B, tyrosylmoiety, C, butyryl moiety, D, terminal amine group. Structural modification in all of these regions has resulted in both increased and decreased toxin potency.

A combination of structure–activity and electrophysiological observations has led to the the possible identification of four different sites of action of PhTX: a channel blocking site, an internal inhibitory site, an external potentiating site and a membrane site.

Analogues carrying photoaffinity labels have also been studied. These irreversibly bind to the receptor following activation by UV light and may be useful in determining the sites of action of PhTX.

Spanjer, W., May, T. E., Piek, T. and De Hann, N. (1982) *Comp. Biochem. Physiol.* **71C**, 149–157.

Fig. 1. Structure and regions of PhTX-343.

Interaction of polyamide toxin Philanthotoxin-343 with cloned and mutant glutamate receptors expressed in Xenopus *oocytes.* J. Harris, M. Mundey, S. Tomlinson, I. Mellor, K. Nakanishi, D. Bell[1] and P. N. R. Usherwood (Department of Life Science, University of Nottingham, University Park, Nottingham NG7 2RD, U.K.; [1]Department of Chemistry, Colombia University, New York, NY 10027, U.S.A.)

Electrophysiological properties of ionotropic glutamate (AMPA) receptors are thought to be dictated by a single amino acid residue in the putative channel forming TM2 region. GluR1, GluR3 and GluR4 possess a neutral glutamine (Q) residue at this site and homomeric expression of these clones produces channels with high calcium permeability and inwardly rectifying I–V relationships. The equivalent position in GluR2 is occupied

by a positively charged arginine (R) residue and co-expression of GluR2 with either GluR1, GluR3 or GluR4 leads to heteromeric channels with a low permeability to calcium and outwardly rectifying I–V curves. It is thought that the 'Q/R site' may also be important in determining the sensitivity of the receptor to open channel block by polyamine amide toxins. One such toxin Philanthotoxin-433 (PhTX- 433), isolated from the Egyptian digger wasp *Philanthus triangulum*, is a non-competitive antagonist of excitatory amino acid receptors. The synthetic analogue PhTX-343 has been used to provide further insight into AMPA sensitive glutamate receptor subunit composition using the *Xenopus* oocyte expression system.

Expression of GluR1 or GluR3 mRNA formed kainate sensitive homomeric receptors which were potently antagonised by PhTX-343. Injection of GluR2 mRNA alone failed to form functional homomeric channels, however, when coexpressed with GluR1 or GluR3, formed heteromeric receptors which were very insensitive to PhTX-343.

Mutation of glutamine to arginine in GluR1(Q582R) produced non-functional homomeric receptors but when coexpressed with GluR1 or GluR3 the presence of GluR1(Q582R) led to heteromeric receptors with a decreased sensitivity to PhTX-343 implicating that GluR2 and GluR1(Q582R) are similar but not identical.

We have demonstrated that the Q/R site not only controls calcium permeability and current–voltage characteristics but also determines sensitivity to PhTX-343. It is proposed that the subunit stoichiometry of the hetero-oligomeric receptors is the governing factor. Increasing the proportion of GluR2 subunits increases the number of positive charges in the channel, thus the sensitivity to toxin decreases. It is suggested that hydrogen bonds between the polyamine toxins and the hydrophilic residues in TM2 may account for the open channel block caused by these toxins. Two such residues (serines) in GluR1 subunits have been mutated to alanine (S572A, S576A). These mutants form functional homomeric receptors but do not appear to alter PhTX-343 sensitivity; hence further site directed mutagenesis is anticipated.

(Supported by a grant from the Wellcome Trust.)

Disruption of haemostasis by jararhagin, a haemorrhagic metalloproteinase from Bothrops jararaca *venom*. A. S. Kamiguti,[1] C. R. M. Hay,[1] R. D. G. Theakston[2] and M. Zuzel[1] ([1]Department of Haematology, Royal Liverpool University Hospital, U.K.; [2]Venom Research Unit, Liverpool School of Tropical Medicine, U.K.)

Local and systemic haemorrhage are consequences of crotaline and viperine envenoming. Fatal cases of systemic bleeding (intracranial) have been reported following envenoming. In some cases, systemic haemorrhage is associated with consumption coagulopathy; however, in others bleeding occurs in the absence of clotting defects. We have been investigating the effects of a haemorrhagic metalloproteinase from the venom of a South American pit viper *B. jararaca* on platelet and plasma components involved in haemostasis. This enzyme, jararhagin, causes systemic bleeding in animals. It also inhibits platelet aggregation induced by either collagen or ristocetin. It was found that in plasma, despite interaction with $\alpha_2$macro globulin, jararhagin still degraded high molecular weight substrates such as fibrinogen and hide powder azure. Jararhagin degraded the C-terminal region of fibrinogen Aα chains; however, this did not affect fibrinogen-dependent platelet responses. The remnant fibrinogen molecule (285,000 mol. wt) was still clottable by thrombin but showed a defective fibrin polymerization due to the loss of one polymerization site. Jararhagin rapidly and selectively degraded the primary collagen

receptor (gpIa/IIa or $\alpha_2\beta_1$ integrin) on the platelet surface, as demonstrated by flow cytometry and SDS–PAGE analysis of immunoprecipitated gpIa/IIa. No effects could be detected on gpIb, but jararhagin caused proteolysis of N-terminal region of von Willebrand factor (vWF). Since this region of vWF contains the platelet binding site, this could explain the effects of jararhagin on ristocetin-induced platelet aggregation. Therefore, due to the ineffectiveness of jararhagin inhibition in plasma, degradation of the principal platelet collagen receptor, degradation of vWF and the enzyme effects on fibrin polymerization, jararhagin is most likely to contribute to the systemic bleeding which occurs in envenomed patients.

The use of synthetic peptides for the inhibition of snake venoms. G. D. Laing[1] and V. Politi[2] ([1]Venom Research Unit, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, U.K.; [2]Polifarma S.p.a., Via Tor Sapienza, 138, 00155 Rome, Italy.)

Members of the Viperidae kill more humans than any other group of snakes. Their venoms contain haemorrhagins, the main lethal toxins, which often cause local and systemic bleeding, sometimes leading to intracranial haemorrhage and death. Current treatment involves intravenous injection of antivenoms, which are usually refined equine immunoglobulin fragments. Viperine venom haemorrhagins are high molecular weight metalloproteinase enzymes with disintegrin-like domains and cysteine-rich carboxy-terminal regions. Other members of the zinc metalloendoprotease family include the collagenases; the design of inhibitors to these enzymes is based on the synthesis of analogues of substrates in which the peptide bond normally cleaved is replaced by a non-hydrolysable chemical group. Collaboration with Polifarma S.p.a. of Rome has resulted in the assessment of synthetic peptido-mimetic inhibitors of haemorrhagic factors using both *in vitro* and *in vivo* methods. A group of synthetic peptido-mimetic compounds was assessed for their efficacy in inhibiting the pathogenic effects of the metallproteinases present in *Bothrops jararaca* (Jararaca) and *Echis pyramidum leakeyi* (Kenyan carpet viper) venom. One inhibitor (POL 647) completely neutralised a minimum haemorrhagic dose (MHD) of *E.p. leakeyi* venom as well as that of a haemorrhagin isolated by FPLC. The same inhibitor did not prevent procoagulant activity. In standard mouse protection assays, POL 647 compared favourably with equine antivenoms in neutralising *E. p. leakeyi* venom lethality. However, none of the compounds tested was capable of neutralising the haemorrhagic activity of whole *B. jararaca* venom or the isolated haemorrhagin jararhagin. These preliminary experiments suggest that as more information on the structure of venom metalloproteinase active sites and inhibitor interaction becomes available using crystallography, it may be possible to design more effective inhibitors which could compliment existing antivenom therapy.

An alternative assay to the standard rodent skin test for anti-haemorrhagic agents, using hen's eggs. P. G. Sells, A. M. Richards and R. D. G. Theakston (Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, U.K.)

Venoms consist of a variety of pathogenic constituents which cause haemorrhage, incoagulable blood, neurotoxicity, necrosis, etc., and, in order to evaluate antivenoms, we have to induce these conditions in animal models. The Home Office constantly seeks to reduce the amount of animal experimentation and it is towards this objective that we have developed a non-mammalian (hen's egg) assay for anti-haemorrhagic agents as an alternative to the standard rodent skin test. In the standard assay, mice are injected i.d. with a minimum haemorrhagic dose (MHD) of venom sufficient to cause a 10 mm diameter

lesion. Anti-haemorrhagic agents under test are incubated with the MHD of venom for 30 min at 37°C and the mixture injected i.d. Mice are killed 24 hr later and the extent of skin haemorrhage is recorded. Our alternative assay uses fertile hens' eggs, which have a highly vascularised yolk sac membrane at a very early developmental stage. Although the embryonic heart is beating, the blood vessel system develops prior to the appearance of intact reflex arcs and the onset of pain sensitivity. Eggs are cracked out of the shell into cling film 'hammocks' on Day 5 (after laying) and incubated at 35°C. On Day 7, filter paper discs impregnated with venom or anti-venom or a mixture of both, are placed on the yolk sac membrane. Two hours later, the degree of haemorrhage is assessed and the results are photographed. A measurable corona of haemorrhage surrounding the disc is induced by as little as 0.5 μg venom (*Echis leucogaster*) and remains stable, together with a heart beating embryo, for 24 hr. Systemic toxicity may also be assessed by the viability of the embryo. So far, the eggs have provided clear cut results, which parallel the mouse skin assay, using equine antivenom, mouse mAb ascites and plant extracts and are very cost-effective (11 p/egg compared with £2.00/mouse).

Systematics, venom variation and toxinology: bridging gaps between evolutionary biology and biomedical science. Wolfgang Wüster (School of Biological Sciences, University of Wales, Bangor, Gwynedd LL57 2UW, Wales, U.K.)

Venom variation at low taxonomic levels (between closely related species or within species) can seriously complicate both toxinological research and snakebite treatment. This is of particular importance because many of the world's most medically important snakes belong to complex groups in which species definitions and identification are either poorly understood or in a state of flux. In some recently revised groups (e.g. Asiatic *Naja*, *Echis* spp.), venom variation appears to be at least partly related to species affinities. This emphasises the importance of a sound taxonomic framework for toxinological studies. In other species, geographic variation in venom composition appears unrelated to taxonomic affinities (e.g. *Daboia russelli*), and in such cases, details of locality of origin of specimens or venoms are of crucial importance. Finally, the status of some species groups (e.g. *Bothrops atrox* species group) remain unresolved or in a state of flux, and this presents particular problems. There is a need for toxinologists to obtain, and for venom producers to supply, a maximum of information on identification and locality of origin of specimens, and for close collaboration between snake systematists and biomedical researchers in general.

The clinical problems caused by saw-scaled or carpet vipers (*genus* Echis) *in Africa and Asia.* David A. Warrell (Centre for Tropical Medicine, University of Oxford, Oxford, U.K.)

Snakes of the *Echis* complex are distributed throughout northern Africa from Senegal to Kenya, through the Middle East and western Asia and the Indian subcontinent including Sri Lanka. A recent controversial taxonomic revision suggested 12 species in three subgenera (Cherlin, V.A., U.S.S.R. Academy of Science, Tropical Zoology Institute Leningrad **207**, pp. 193–223, 1990).

The *Echis* complex is responsible for much snake bite morbidity and mortality, especially in West Africa (*E. ocellatus*), East Africa (*E. pyramidum*) and the Indian subcontinent (*E. sochureki* and *E. carinatus*). In parts of West Africa, the incidence and mortality of bites is as high as 120 and 8, respectively, per 100,000 population per year (Warrell, D.A. and Arnett. C. (1976) *Acta Tropica* (*Basel*) **33**, 307–341). More than 90% of bitten patients

are envenomed and develop local swelling, blistering and necrosis (9%) (Warrell, D.A. *et al.* (1977) *QJM* **46,** 3–62), whereas in northern India local blistering was common but necrosis was rare (Bhat (1974) *J. Indian Med. Assn* **63,** 383–392). Death is usually attributable to bleeding into the brain or elsewhere. *E. coloratus* envenoming may be more likely to cause thrombocytopenia and renal dysfunction than other *Echis* species, but the case fatality is low. Geographical variation in venom effects is likely but there have been few detailed clinical studies which might have revealed these differences. *Echis* venoms from seven different countries showed different coagulant properties (Theakston, R.D.G. and Reid, H.A. (1983) *Bull WHO* **61,** 949–956). *Echis* antivenoms have been clinically unreliable in neutralizing venoms from other geographical regions (Gillissen, A. *et al.* (1994) *Toxicon* **32,** 937–944). Currently, hope for improved *Echis* antivenoms rests on a new ovine Fab monospecific antivenom ('Echitab') which is undergoing clinical trials in Nigeria.

Early clinical experience with a new ovine Fab echis ocellatus *antivenom in Nigeria.* R. D. G. Theakston,[1] P. Meyer,[2] A. G. Habib,[3] A. A. Onayade,[4] A. Yakubu[5] and D. A. Warrell[6] ([1]Venom Research Unit, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, U.K.; [2]Department of Clinical Pathology, St Bartholomew's Hospital Medical College, Charterhouse Square, London, U.K.; [3]Department of Medicine, Ahmadu Bello University Hospital, Zaria, Nigeria; [4]Department of Community Health, Obafemi Awolowo University Teaching Hospital, Ile-Ife, Nigeria; [5]Epidemiology Unit, Ministry of Health, Bauchi, Nigeria; [6]Centre for Tropical Medicine, John Radcliffe Hospital, Oxford, U.K.)

Over the past decade, the availability of effective antivenom in northern Nigeria has decreased alarmingly. As a result, many seriously envenomed victims of *Echis ocellatus*, which is responsible for over 95% of bites in the region, did not receive effective treatment. This has resulted in increases in mortality and morbidity in affected communities. Because of this crisis, the Nigerian Federal Ministry of Health in collaboration with Therapeutic Antibodies Inc., the Venom Research Unit, Liverpool School of Tropical Medicine, and the Centre for Tropical Medicine, University of Oxford, have developed a new monospecific ovine Fab antivenom (Echitab) against the venom of this species. The theoretical advantages of such an antivenom over the normal equine $F(ab')_2$ antivenom are its more rapid tissue penetration and larger volume of distribution.

In a preliminary study, two ampoules (20 ml; 1.0 g) of Echitab (50 g/l protein) rapidly and permanently restored blood coagulability and cleared venom antigenaemia (measured by enzyme immunoassay) in 7 patients with proved systemic envenoming by *E. ocellatus* in Kaltungo, Bauchi State, Nigeria. Four of seven of these patients had early anaphylactic reactions which responded to adrenaline. In a randomised comparative trial of a lower dose (10 ml; 0.5 g) of Echitab or four ampoules (40 ml; 2.12 g) of Pasteur Ipser Africa (53 g/l protein) in 39 patients with systemic envenoming, only 36 and 35%, respectively, showed permanent clearance of venom and restoration of blood coagulability; the remaining two-thirds (64 and 65%, respectively) required up to three more doses of either antivenom and, exceptionally, 6 and 8 additional doses in two individuals initially treated with Ipser Africa antivenom. This suggested that (1) one ampoule of this batch of Echitab and four ampoules of Isper Africa are inadequate initial doses for most patients, and (2) one ampoule of Echitab is approximately equivalent in neutralising activity to four ampoules of Ipser Africa. These results were also supported by rodent studies (Laing *et al.* (1995) *Toxicon* **33,** 307–313). The problem of serum-induced reactions and the

need for production of an ovine Fab polyspecific antivenom for the region will be discussed.

Herbal treatment of snakebite in Papua New Guinea. Dietrich Mebs (Zentrum der Rechtsmedizin, University of Frankfurt, Kennedyallee 104, D-60596 Frankfurt, Germany.)

Snakebite is a serious problem in Papua New Guinea. Antivenom is rarely available particularly in rural areas. Instead herbal remedies are often applied by local healers or the medicine man. However, with the loss of cultural tradition, knowledge on medicine plants is also disappearing. In a village of East-Sepik and in two areas of Madang Province information about plants used in the treatment of snakebite were collected. Six plant species were indentified: *Maclura* sp. (Moraceae), *Melanolepis multiglandulosa* (Euphorbiaceae), *Osmoxylon micranthum* (Araliaceae), *Alphitonia incanea* (Rhamnaceae), *Cerbera floribunda* (Apocynaceae), *Mangifer minor* (Anacardiaceae, wild mango). The sap or bark extract of the plants are either applied onto the bite area or ingested. Chemical analyses of the plant ingredients are currently performed.

Neurotoxicity, anticoagulant activity and evidence of rhabdomyolysis in patients bitten by death adders (Acanthophis *sp.*) *in Southern Papua New Guinea.* David G. Lalloo,[1,2] Andrew J. Trevett,[1,2] Julie Black,[1] James Mapao,[1] Adolf Saweri,[1] Sirus Naraqi,[1] Dale Owens,[3] Aura S. Kamiguti,[4] Ronald A. Hutton,[3] R. David G. Theakston[5] and David A. Warrell[2] ([1]Department of Clinical Sciences, University of Papua New Guinea, Port Moresby, Papua New Guinea; [2]The Centre for Tropical Medicine, University of Oxford, U.K.; [3]The Katherine Dormandy Haemophilia Centre, Royal Free Hospital, London, U.K.; [4]Department of Haematology, University of Liverpool, U.K.; [5]The Alistair Reid Venom Research Unit, Liverpool School of Tropical Medicine, U.K.)

Thirty-two patients with enzyme immunoassay-proven death adder (*Acanthophis* sp.) bites were studied in Port Moresby, Papua New Guinea. Eighteen were envenomed; local signs were rare and none had incoagulable blood, but all except one had signs of neurotoxicity. Five (27.7%) envenomed patients required intubation and ventilation. One patient developed renal failure, previously undescribed following death adder bites. Laboratory investigations showed mild prolongation of prothrombin and partial thromboplastin times in some patients. *In vitro* studies showed that the venom contains anticoagulant activity, but does not cause fibrinogenolysis. In contrast to taipan envenoming, neurotoxicity did not progress after antivenom administration and there was reversal of neurotoxicity, evident within 6 hr, in three severely envenomed patients treated less than 12 hr after the bite. One patient treated with antivenom and anticholinesterases had the most dramatic response to treatment; the optimum management of bites by this species may include prompt treatment with both antivenom and anticholinesterases in addition to effective first aid.

Venom from Brown snakes (Pseudonaja *ssp.*) *induces a potent post-synaptic neurotoxicity which is rapidly reversed by a Fab based antivenom.* Russell G. A. Jones (Therapeutic Antibodies Ltd, The Medical College of St. Bartholomew's Hospital, Charterhouse Square, London EC1M 6BQ, U.K.)

Brown snakes (*Pseudonaja* ssp.) are amongst the most deadly found in Australia, due in part, to the pre- (textilotoxin) and post-synaptic (pseudonajatoxin a and b) neurotoxins in their venom. Studies using the murine phrenic nerve hemidiaphragm preparation have

shown the venom to produce rapid neurotoxic effects with no myotoxicity. We have developed a new ovine Fab based antivenom which, when pre-mixed with the venom neutralises, completely, all neurotoxins under all test conditions. We have also demonstrated, possibly for the first time, that an antivenom is capable of achieving a full and rapid (< 1 hr), reversal of the dominant post-synaptic neurotoxic effects *in vitro*. Only under conditions favouring pre-synaptic phospholipase type neurotoxins, with increased temperature and frequency of nerve stimulation, was it possible to demonstrate the minor but essentially irreversible presynaptic effects of textilotoxin. From these results we concluded that venom neurotoxicity was due, predominantly, to a high affinity post-synaptic component which can be reversed rapidly by a high affinity specific antivenom.