CLINICAL, EPIDEMIOLOGICAL AND ELECTROPHYSIOLOGICAL ASPECTS OF ENVENOMING BY THE PAPUAN TAIPAN (*Oxyuranus scutellatus canni*).

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"And voices in me said, If you were a man
You would take a stick and break him now, and finish
him off.

But must I confess how I liked him,
How glad I was he had come like a guest in quiet, to
drink at my water-trough
And depart peaceful, pacified, and thankless,
Into the burning bowels of this earth?"

from "Snake". D.H.Lawrence

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PREFACE

The work which forms the basis of this thesis was carried out at Port Moresby General Hospital, Papua New Guinea between May 1991 and October 1993. During that period, all patients bitten by snakes were entered into a prospective clinical study begun by Dr David Lalloo in 1990. I held the post of visiting clinical lecturer in the Department of Medicine, funded by the Wellcome Trust of Great Britain. Between August 1992 and October 1993, I was clinical team leader of the University of Papua New Guinea-Oxford University medical research team based in Port Moresby, under the joint auspices of Professor Sirus Naraqi and Professor David Warrell. During the first year of the studies reported here, Dr David Lalloo and I worked together with the snakebite patients. In the second year of the studies, I was assisted by a research registrar, Dr Nneka Nwokolo and other Papua New Guinean registrars who worked under my supervision. Throughout the study, the nursing staff provided excellent skilled help in the management of patients. The immunoassays were done by Dr David Theakston and his staff in the Venom Unit at the Liverpool School of Tropical Medicine. Mark O'Shea, herpetologist, kindly permitted me to use his photographs, Plates 1.1 and 1.2 and figure 1.2. To all of these people I owe a considerable debt and I express my gratitude.

SUMMARY

The purpose of the work described in this thesis was to further knowledge about the circumstances and effects of envenoming by the Papuan taipan (*Oxyuranus scutellatus canni*) and to determine how morbidity and mortality can be reduced. My specific aims have included analysis of the epidemiology of taipan bite, of first aid treatment and of the efficacy of current antivenom treatment. I have also determined the *in vivo* electrophysiological effects of the neurotoxins in taipan venom and applied these observations to assess drugs of potential benefit in the treatment of envenomed patients.

Each year, approximately 160 patients are seen at Port Moresby General Hospital (PMGH), Papua New Guinea after having been bitten by a snake. Between 90 and 100 of these are significantly envenomed and up to 50% require intubation and ventilation despite treatment with antivenom. Snakebite is the commonest indication for admission to the intensive care unit at PMGH. In the two years of the study, a total of 169 snakebite patients were admitted to the intensive care unit for a total of 831 days, 28.5% of available ITU bed days. During the period of the study, at least 20 patients bitten in remote parts of the Province died before reaching hospital. In each of the last three years, the Papua New Guinea Department of Health has spent over £100,000 on antivenom, the majority of which is used in Central Province. Frequently however, rural health centres either run out of antivenom or have a type inappropriate to the snake species biting in their area.

The development of enzyme linked immunoassays (EIA) for the detection of snake venom antigen has enabled retrospective identification of biting species,

has provided the opportunity to make reliable species specific observations and has helped to define the epidemiology of snakebite. In this study, EIAs were used to identify definitive taipan bites from a prospective series of all snakebite victims admitted to PMGH between May 1991 and May 1993. All patients with signs of systemic envenoming were managed according to treatment guidelines contained in standard treatment books published by the Papua New Guinea Department of Health. The circumstances of the bite and all clinical details were recorded on a proforma.
Electrophysiological studies were done on selected patients with neurotoxicity who were believed on clinical grounds to have been bitten by a taipan. The results of these studies were compared with similar studies done in a group of volunteer Melanesian controls. A subset of the envenomed patients with established neurotoxicity was included in studies looking at the effects of edrophonium and 3,4-diaminopyridine. Patients were included in this study if taipan venom antigen was detected at a significant level in one or more of: an admission serum sample, a bite site aspirate or a swab of the bite site. One hundred and seventeen patients were included in the epidemiological analysis, 106 in the clinical study and smaller numbers in each of the electrophysiological studies.

The taipan was confirmed to be the species accounting for the majority of cases of significant envenoming in Central Province. Between 1991 and 1993, 89% of envenomed patients admitted to PMGH in whom a definitive identification of biting species was made, were bitten by a taipan. The Papuan black snake (*Pseudechis papuanus*), which is widely feared and believed by most lay people and many medical staff to

be the most numerous and dangerous species in Central Province, accounted for less than 1% of bites. This suggests a change in the herpetofauna of the Province over the past 30 years and has significant implications for the use of monovalent antivenoms. There is little role for the continued use of monovalent black snake antivenom in Papua New Guinea, polyvalent antivenom is a better option if a black snake bite is considered a possibility.

Virtually all taipan bites occurred during daylight hours in low lying coastal parts of the Province, predominantly in Eucalyptus savannah. Few patients were able to describe distinguishing features of the snake that bit them. The majority of patients envenomed by a taipan had non-clotting blood by the time they reached a medical facility and this sign has a predictive value of over 95% for taipan bite in Central Province. Despite the majority receiving antivenom,46.7% of patients progressed to require intubation and ventilation. Antivenom was of maximal value if given within 4 hours of envenoming but even early antivenom did not prevent progression of neurotoxicity in a high proportion of patients. Antivenom appeared to hasten the resolution of coagulopathy in envenomed patients but there was little evidence that this produced a significant clinical advantage.

Envenomed patients had normal sensory and motor conduction velocities but showed markedly reduced evoked compound muscle action potentials and slightly reduced sensory nerve action potentials. There was a decremental response on repetitive nerve stimulation testing, enhanced by exercise or tetanic stimulation. These findings are consistent with neuromuscular block localised to the synapse. The decline and recovery of Compound Muscle Action Potential (CMAP) amplitudes

closely mirrored recordings of peripheral grip strength. The rate of recovery suggests that reinnervation may be occurring. Motor unit architecture appeared relatively normal on electromyographic studies during the recovery phase suggesting that toxin damage is predominantly localised to the nerve terminal. Both edrophonium and 3,4-diaminopyridine produced an electrophysiological response in envenomed patients, maximal when the two drugs were used in combination. There was a minor increase in peripheral grip strength but insufficient improvement to be clinically useful.

The majority of deaths which occured from snakebite in Central Province during the study were attributable to delay in the patient reaching hospital. Amongst patients who reached PMGH, most morbidity and mortality was associated with complications of intubation and the management of ventilated patients, some of which were avoidable. Current antivenom treatment is expensive and appears to be of limited benefit. Patients who received antivenom within 4 hours of the bite fared significantly better. There is realisable potential for a far higher proportion of patients to be treated within this time. The use of pressure bandages as first aid treatment to delay absorption of venom may prolong the period in which existing antivenom produces maximum benefit. There is a however, a need for the development of better antivenoms for the treatment of snakebite victims in PNG. Possibilities include the development of species specific or subunit antivenoms directed against specific toxins. Electrophysiological studies provide an objective basis for studying the effects of pharmacological interventions in snakebite patients with neurotoxicity, but neither edrophonium or 3,4-diaminopyridine can be recommended for the treatment of patients envenomed by the Papuan taipan.

Plate 1.1 and 1.2 The Papuan Taipan (*Oxyuranus scutellatus canni*)

CHAPTER 1 - BACKGROUND

i. Background and aims

Snakebite is a problem predominantly of rural populations in the tropics. The effects of envenoming are often exacerbated by fear, ignorance, inappropriate first aid and by the inaccessibility of medical care. Papua New Guinea is no exception. It is impossible to be precise about the true incidence of snakebite in Papua New Guinea because of the remoteness of the terrain but an average of 160 patients a year presented to Port Moresby General Hospital (PMGH) between 1990 and 1993 reporting a bite, of whom approximately 60% had signs of envenoming and 30% required mechanical ventilation because of respiratory paralysis caused by venom neurotoxins. The majority of victims of snakebite are young, previously well adults, and children. The mortality rate is not known but there were at least 10 deaths from snakebite in Central Province in both 1991 and 1992, the majority occurring outside hospital. This is likely to be an underestimate. The majority of envenomed patients are treated with antivenom, either at a health centre or at PMGH. This is manufactured by Commonwealth Serum Laboratories in Melbourne, Australia and is prohibitively expensive. A single vial of polyvalent antivenom costs £594, a vial of monovalent taipan antivenom £545. Between January 1991 and March 1993, the Department of Health spent £394,000 on antivenom. Despite this, there were occasions during the course of this study when the rural health centres, and even PMGH, had no antivenom supplies with which to treat envenomed patients.

This study focuses on the Papuan taipan (*Oxyuranus scutellatus canni*). 87% of envenomed patients admitted to PMGH in the past three years in whom the biting species could be reliably identified were bitten by taipans. Taipan victims showed little response to antivenom and the majority of management problems and deaths were in patients bitten by this species. This study set out to determine how to reduce the morbidity and mortality resulting from taipan bite in Papua New Guinea. It involved analysing existing treatment in the community, in the hospital and the potential for developing new therapeutic approaches.

The clinical features of envenoming by the Papuan taipan have been recently described by Currie[1] and Lalloo[2] and clinical description is not the main focus of this thesis. Clinical details of the patients in this series are included where directly relevant or where original but I have attempted to avoid repetition of existing work. The specific aims of this study were:-

1. to define the epidemiology of taipan bite in Central Province.
2. to assess current pre-hospital treatment of taipan bite.
3. to describe the clinical features of envenoming by the Papuan taipan.
4. to assess the use and efficacy of antivenom in the management of envenoming following a taipan bite.
5. to determine the *in vivo* electrophysiological effects of envenoming by the Papuan taipan.
6. to use clinical and electrophysiological parameters to assess drugs of potential value in the treatment of neurotoxicity due to taipan venom.

7. to make recommendations which might improve the management of patients and diminish morbidity and mortality from snakebite in Central Province, Papua New Guinea.

PAPUA NEW GUINEA

i. Topography and geography

The island of Papua New Guinea sits at the junction of two global crust plates, the movement of which has created its dramatic topography. The interior of both Papua New Guinea and Irian Jaya is dominated by high mountains which rise to over 4000 metres in places and form a spine extending along the length of the island. To both north and south of the mountain ranges, huge river systems flow out to the sea forming extensive deltas and swampy floodplains, most notably the Fly, Sepik and Ramu rivers. Two arcs of islands fringe the mainland to the east, many of which have active or dormant volcanoes[3]. The tectonic activity of the last 100 million years continues today and much of the country is still prone to earthquakes and volcanic eruptions.

The geographic barriers which have influenced the migration of both man and animals have not remained constant. Within the past 50,000 years there have been extensive changes in sea level[4]. At times, Papua New Guinea has been linked by a landbridge to what is now Australia forming a more extensive southern land mass called Sahul. This land mass remained separate from the archipelagoes of Sunda (now Indonesia) to the west due to the depth of the oceans surrounding them, the so-called Wallace line. The mammalian fauna of Papua New Guinea is dominated by marsupials and the herpetofauna of the island today reflects that of the Australian

subcontinent. The venomous land snakes of Papua New Guinea are all elapids and are all related to similar Australian species. The distribution of venomous snakes within PNG is not uniform; the mountains appear to form a demarcation line with some species peculiar to either the north or the south. Even during the times of Sahul, there was very little low lying land connection between what are now the northern and southern halves of the island. The average temperatures were considerably lower than they are today, by perhaps 3-6°C. As the temperatures increased, the glaciers began to melt and the sea level rose until Papua New Guinea became a separate island.

The distribution and composition of the herpetofauna in Papua New Guinea today may be explained by earlier land connections and by the physical barriers of the mountains and rivers which have prevented free migration internally. Climatic differences within the land mass of Papua New Guinea and Irian Jaya may also have limited the distribution of species. In Australia, the taipan (*Oxyuranus scutellatus scutellatus)* is not found where the average maximum winter temperature is less than 18°C[5]. Much of the high ground in Papua New Guinea and Irian Jaya is uninhabitable. There is extensive local variation in rainfall ranging from an average of less than 1000mm/year along the southern Papuan coast to greater than 8000mm/year in the Star mountains of Western Province[6]. This produces wide variation in both flora and fauna and may well influence the distribution of snakes. There are also seasonal variations in rainfall throughout the country with distinct wet seasons which are usually, but not solely, in the months of January to April.


PAPUA
NEW GUINEA
Scale 1: 7,500,000
MILES
KILOMETRES
PAPUA
NEW GUINEA
NEW GUINEA
PAPUA
BISMARCK ARCHIPELAGO
Bismarck Sea
Solomon Sea
Coral Sea
Gulf of Papua
Huon Gulf
Vitiaz Strait
Torres Strait
Ysabel Channel
ADMIRALTY ISLANDS
ST.MATTHIAS GROUP
WITU ISLANDS
D'ENTRECASTEAUX ISLANDS
LOUISIADE ARCHIPELAGO
New Britain
New Ireland
Bougainville Island
Solomon Islands
CENTRAL RANGE
BISMARCK RANGE
FINISTERRE RANGE
KRATKE RANGE
OWEN STANLEY RANGE
HUON PENINSULA
WHITEMAN RANGE
Port Moresby
Lae
Madang
Rabaul
Wewak
Mount Hagen
Goroka
Kavieng
Kieta
Alotau
Popondetta
Daru
Thursday Island
GREAT BARRIER REEF

ii. History

The prehistory of Papua New Guinea is not well understood but it is believed that the country was first inhabited by seafaring migrants travelling from the east[7]. The first documented European contact with the land mass of New Guinea was in 1526 when a Portugese explorer, Jorge de Menenses landed on the north coast. The island was named New Guinea in 1545 by the Spaniard Ortiz Retes, the appearance of the Melanesian people reminded him of the people of the Guinea coast of Africa. "Papua" is believed to derive from the Malay word *papuwah*, meaning frizzy-haired. There was increasing European contact over the next three centuries resulting in the colonisation of the western half of the island by the Dutch in 1828. Germany and Britain divided the eastern half of the country, administering New Guinea in the north and the territory of Papua respectively. In 1906, Australia took over the administration of Papua from the British.

New Guinea was invaded by Australian forces during the first world war. In the 1920's, although significant German economic interests remained, it became a League of Nations mandated territory under Australian administration. In January 1942, the Japanese invaded Rabaul on the island of East New Britain and subsequently went on to occupy New Guinea and parts of Papua. Following the conclusion of the war, the administration of New Guinea and Papua was combined and the first legislative council for the combined territory was convened in 1951. The parliamentary system was gradually developed over the following 20 years with the establishment of a democratically elected House of Assembly which had the function of preparing the country for self government. Full independence was declared on September 16th, 1975

and Papua New Guinea is now a sovereign state within the British Commonwealth.

The new government of Papua New Guinea inherited an economy which was largely dependent on Australian aid. The small monetary sector was almost exclusively foreign owned. The major commodities which were sold abroad were coffee, copra, cocoa, rubber and palm oil, all of which were subject to wild fluctuations in price on the world market. The development of the country's extensive mineral resources has subsequently formed an increasingly important part of the economy. The aims of the new government in 1975, based on a report by the Overseas Development Group of the Institute of Development Studies at Sussex[8], involved increasing equity of income and services, increased economic independence and an emphasis on small scale enterprises. Unfortunately, geographic barriers, political instability and social unrest have all inhibited the achievement of these laudable aims. In 1975, over 80% of the population were rural dwelling subsistence farmers. By the late 1970's urban population numbers were increasing by around 12% each year. Although urban drift has continued, the rate has slowed considerably in most areas except Port Moresby[3]. Most of the larger cities have squatter settlements. The presence of a large, unemployed, landless population and the absence of a welfare net has resulted in an increase in crime, which, in turn, has discouraged investment and tourism.

iii. Central Province and National Capital District

The capital of Papua New Guinea is Port Moresby, named after Captain John Moresby, a British naval captain. The large urban area surrounding the

city had an estimated population of 195,197 in the 1990 national census[9]. National Capital District (NCD) is surrounded by Central Province which covers an area of 29,500km^2 and has a population of 141,197. The coastal belt of Central Province is relatively flat and consists typically of mangrove swamps and palm trees. Further inland, dry eucalyptus and grass savannahs stretch up to the rainforest covered foothills of the Owen Stanley mountain ranges which reach to a height of over 4000 metres (see Plate 1.3). The mean daytime temperatures in the coastal areas range from 27.8°C to 32.1°C throughout the year. The staple diet in the coastal areas is predominantly banana, yams and fish. In inland areas of the Province, sweet potato and taro are the main staple foods. In NCD, imported and processed foods such as rice and tinned fish now make up a significant part of the diet of the majority of the population. In Central Province however, only around 10% of the population were wage earning at the time of the 1980 census and traditional staples remain extremely important.

iv. Language

There are over 750 languages described in Papua New Guinea. Most are localised Austronesian languages which are peculiar to small, often geographically isolated, areas. The main *linguae francae* in coastal Papua are Hiri Motu and English. A form of pidgin English *tok pisin*, is spoken widely throughout much of the rest of the country including the islands.

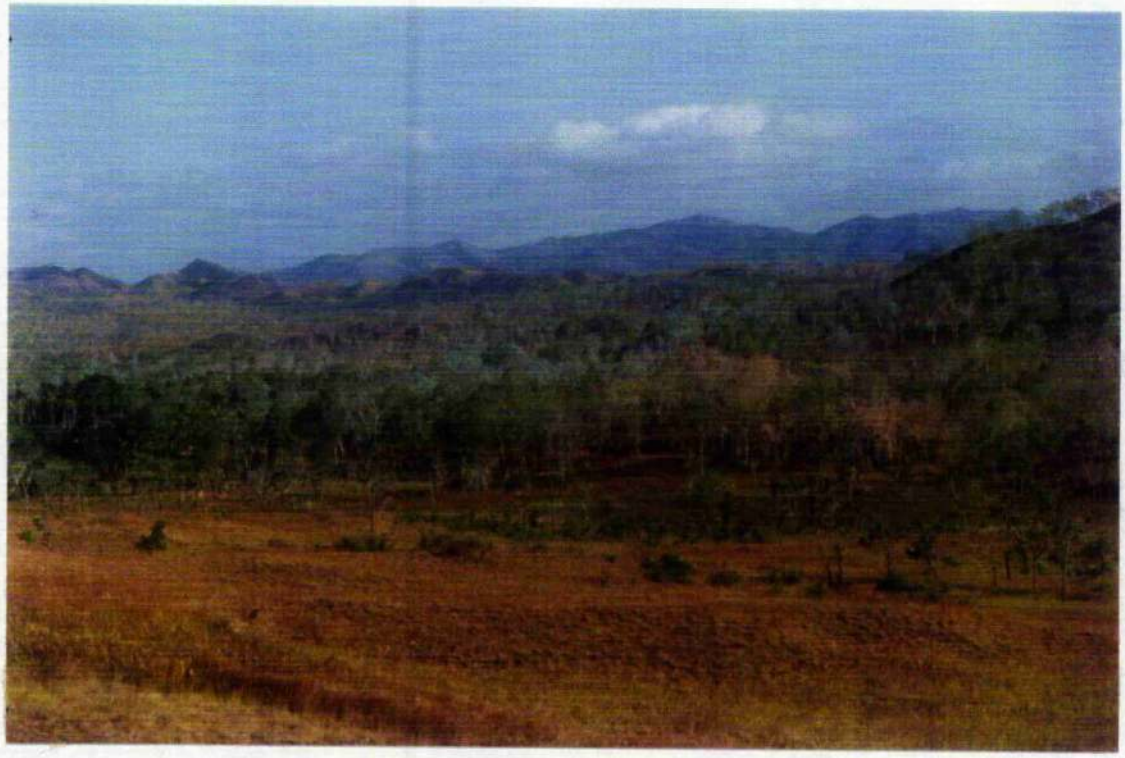

Plate 1.3 Eucalyptus savannah outside Port Moresby

v. Health

The majority of the population of Papua New Guinea are rural dwellers. Their first point of contact with health services is usually at a village health centre, staffed by a Health Extension Officer or qualified staff nurse, or at a village aid post. None of the 17 health centres in Central Province has a doctor. There is a small mission hospital at Veifa'a in the west of the Province which does not have a doctor at present. Health centre staff prescribe and manage the majority of non-surgical cases. Patients can be referred for out-patient appointments or for admission to Port Moresby General Hospital (PMGH) but it is often a long and arduous journey. PMGH is a teaching hospital with 600 beds, which receives patients from throughout the Province and is a tertiary referral centre for the whole country. It is staffed by a variety of national and expatriate doctors. All of the nursing staff in the hospital are Papua New Guinean. The hospital has a six bedded intensive care unit with facilities to ventilate patients. It also has laboratory and X-ray facilities.

The infant mortality rates in Central Province and National Capital District are below the national average of 72/1000[10] but still remain high. Hospital admissions due to diseases common in the western world such as ischaemic heart disease, hypertension and diabetes are all increasing, but the major causes of morbidity and mortality in both adults and children are infectious diseases.

SNAKES AND SNAKEBITE IN PAPUA NEW GUINEA

i. The venomous snakes of Papua New Guinea

Papua New Guinea has a rich herpetofauna which shares many similarities with that found in northern Australia. There are five families of land snakes, the Typhlopidae, Boidae, Acrochordidae, Colubridae and Elapidae. There are seven dangerous venomous land snakes which all belong to the family Elapidae. In Papua, the species responsible for the vast majority of bites is the Papuan taipan (*Oxyuranus scutellatus canni*). The other significantly venomous species are: death adders (*Acanthopis sp.*), Papuan whipsnakes (*Demansia sp.*), the Papuan black (*Pseudechis papuanus*), the small-eyed snake (*Micropechis ikaheka*), the eastern brown snake (*Pseudonaja textilis*) and the king brown snake (*Pseudechis australis*). Although the eastern brown snake has been found in a small area of Northern Province, the only venomous snakes found in most of northern Papua New Guinea and northern Irian Jaya are the death adder and small eyed snake.

ii. The Papuan Taipan

The Papuan Taipan, *Oxyuranus scutellatus canni* is a separate subspecies from the Australian taipan,(*Oxyuranus scutellatus scutellatus*) which is found in northern Queensland and the Northern Territory. The name "taipan" is said to derive from the aboriginal vernacular of the Cape York peninsular, where the Australian sub-species is common[11]. The distinguishing features of the Papuan species, described by Slater[12], include more pronounced scale keels, a distinctive dorsal red stripe and perhaps greater average size than its Australian counterpart.

The distribution of the snake in Papua New Guinea appears to be exclusively within what used to be Papua, south of the main mountain ranges which divide the island. It is found mainly in the coastal belt, in the dry arid savannah scrub land, but also in wetter areas to the west. Taipans are present on the edge of the Sogeri plateau at a height of about 400 metres, but it is unlikely that they inhabit areas at a much greater altitude. Taipans are common in Central Province, Western Province and the western edge of Milne Bay provinces. They are also known to occur around Malalaua and may have been responsible for two recent snakebite deaths in Kerema, also in Gulf Province. Taipans are also common in the southern plains of Irian Jaya extending at least as far west as Senggo. The species is particularly well documented in Central Province and it is clear that it is prevalent throughout the whole of the low lying coastal belt from Cape Rodney in the east to Bereina in the west. Figure 1.2 shows sites where live specimens have been collected in Papua New Guinea.

The Papuan taipan, is a long, slender, fast moving snake. Adult specimens vary from 1.5-3.5 metres in length. The head of the snake is long and coffin shaped, with a distinct neck. The eye is round and relatively large, with a red iris. The tail is thin and whip like. The fangs are sited anteriorly and average around 10mm in length. The colouring of the snake varies considerably. The dorsum may be olive, dark brown or grey with probable seasonal variation. The ventral surface is usually cream or off-white and may have orange red stippling. The most distinctive feature of the snake from a distance is a copper coloured stripe on the dorsum due to colouration of the interstitial skin of the vertebral scale rows. This colouration may become more evident as the snake

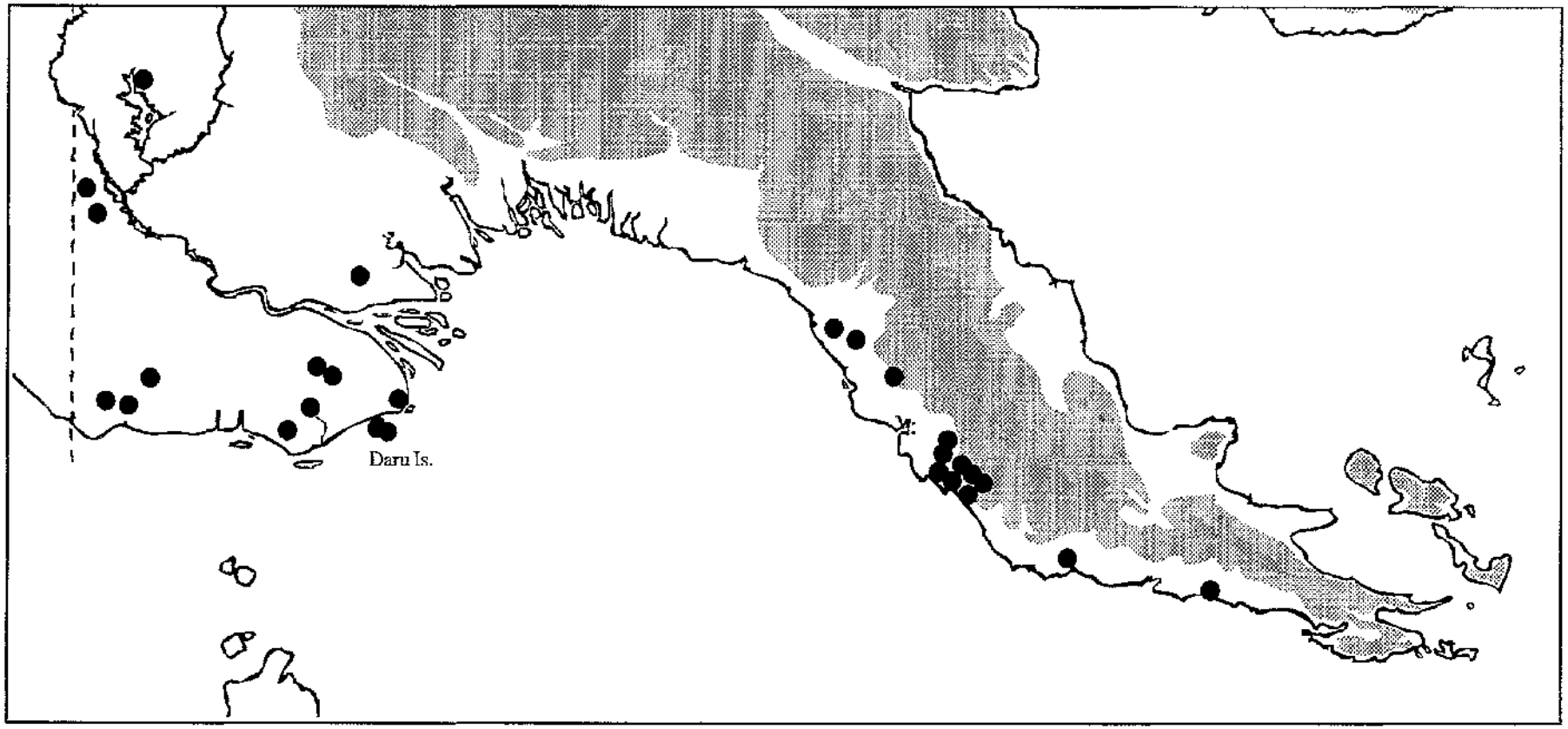

Fig.1.2: Distribution of the Papuan taipan, *Oxyuranus scutellatus canni*, with collection localities.
from "A Guide to the Snakes of Papua New Guinea." by M. O'Shea, Independent Publishing, Port Moresby.
Light shading - altitude 300-1500m; dark shading - altitude 1500+m.
Collection localities: ●.

prepares to strike, as more of the interstitial skin is exposed. The snake does not have a loreal or subocular scale, a morphological feature common to elapids. The supraocular scale shelves sharply giving the snake a frowning expression. At mid-body, there are 21 or 23 scale rows, with a total of between 220 and 250 ventral scales. The anal plate is single and there are 45-80 subcaudal scales[13-16].

The taipan is regarded as a shy snake. It has acute eyesight and sense of smell and generally avoids contact with man. If startled however, it becomes aggressive and commonly strikes repeatedly. The average venom yield from a single milking of an Australian taipan is 100-200mg with as much as 400mg recorded[17]. At the time of the strike, the snake flattens its head and lunges forwards and upwards. Because of the tendency to strike repeatedly, a large volume of venom may be injected. The length and position of the fangs allow the snake to inject venom even through relatively protective coverings such as canvas boots although by far the majority of bites are on exposed skin. The snake is oviparous, diurnal and crepuscular. It may be more active nocturnally during especially warm weather.

The snake usually feeds on small mammals, rats, bandicoots and possibly ground nesting birds. Taipans do not feed on amphibians. It appears that since the introduction of the cane toad *Bufo marinus*, to Central Province in the 1940's, the numbers of taipans have increased whilst the numbers of other elapids, notably the Papuan black snake *Pseudechis papuanus*, have declined. It has been suggested that the amphibian eating elapids have suffered as a result of eating the toxin containing cane toads creating an expanded ecological niche for the taipan. A similar

scenario may explain changes in the herpetofauna of Queensland over the past 50 years[18].

Papuan taipan venom contains multiple toxins some of which are discussed at greater length below. In animal experiments it has been shown to be extremely toxic with LD50's of between 2 and 64ug/kg in guinea pig and mouse respectively when given intravenously[19,20]. When the venom was given subcutaneously to mice, Trethewie found an LD50 of 120ug/kg[21].

iii. Snakebite in Papua New Guinea

The credit for the pioneering work on snake envenoming in Papua New Guinea goes almost entirely to C.H.Campbell who worked at Port Moresby General Hospital from 1960. He published extensively on snakebite and collated much of his work in an M.D.thesis[19]. He published detailed descriptions of the patterns of envenoming seen with different species, the effect and side effects of antivenom and on other aspects of treatment. Campbell was hindered in his conclusions by difficulty in the definitive identification of the biting species. A few patients brought a dead snake in with them, but the majority did not. In some cases, Campbell felt confident enough to ascribe a bite to a particular species on the basis of the patient's description of the snake. The majority of snakebite victims in Papua New Guinea however, are bitten by a fast moving snake in long grass and, as Campbell recognized, patients' descriptions were often scanty and unreliable.

The development of enzyme immunoassays to identify species specific venom antigen, has been a major step forward in the study of envenoming[22]. We are now able to identify with certainty the snake species responsible for many cases of envenoming. This allows

reliable distinction of the clinical syndromes of envenoming and offers a new insight into the epidemiology of snakebite in the area. At present this technology remains a research tool in Papua New Guinea but there is scope for the diagnostic application of rapid venom detection assays to indicate the appropriate antivenom to use. This is discussed in chapter 7. At a population level, assessment of biting species in different areas may help to direct the appropriate allocation of antivenom supplies and relevant ancillary pharmacological treatments such as anticholinesterases which are of value in the management of patients envenomed by death adders[23].

Campbell first published on Papuan elapid bites in 1961 when he described 15 patients, all of whom developed some form of neurotoxicity, ranging from slight ptosis to respiratory paralysis which required tracheotomy and ventilation[24]. All but one of the patients were bitten during daylight hours and all were bitten on the foot or the leg. Six patients were noted to have a bleeding tendency including one who had haematuria and two who had "haemoglobinuria". Antivenom was given to all patients, a combination of tiger snake and taipan antivenom to the majority. In addition, death adder antivenom was given if a patient described being bitten by a short snake and black snake antivenom if there was an excessive bleeding tendency. One patient who was given tiger snake and taipan antivenom had a rapid reversal of his ptosis and Campbell suggested that this patient had probably been bitten by a death adder. Fourteen of the 15 patients in the series survived and Campbell made the point that with adequate care of the airway and ventilatory support elapine snakebite victims reaching hospital should survive.

Campbell published further observations on envenomed patients seen in Port Moresby throughout the 1960's. In 1964, he described a series of 52 envenomed patients[25]. Of 52 bites, 50 occurred during the hours of daylight, the other two at dusk. 51 patients were bitten on the lower leg or foot. The age range of the patients was 3-45 years. 44 patients were male, 8 female. Five patients brought dead snakes with them, 3 death adders, 1 taipan and 1 Papuan black snake. Fang marks were only seen in 8 cases. Many patients had received local treatment before admission to hospital. This usually took the form of local incisions. An unspecified number had tourniquets applied. The earliest sign of envenoming was the appearance of local tender lymph nodes. Neurotoxicity initially manifested as ptosis, followed by external ophthalmoplegia, bulbar and respiratory paralysis. 39 of the 52 patients described (75%) developed ptosis, 21 of these developed complete ophthalmoplegia and 15 required tracheotomy and ventilation. Two patients in the series died(4%) although, as Campbell pointed out, it is probable that 15 would have died had advanced respiratory support not been available. There was evidence of bleeding in 11 patients with haematemesis or haemoptysis; 3 had haemoglobinuria.

In 1966 and 1967, Campbell published three further papers delineating the syndromes of envenoming by death adders, Papuan black snakes and taipans[26,17,27]. He described a series of 6 taipan bites of which two patients brought a dead snake to the hospital. The other four were identified on the basis of the victim's description of a bite from a black snake with a red back. Three patients developed signs of neurotoxicity of which two required tracheotomy and ventilation, one of whom had coexisting coagulopathy. One patient had coagulopathy in isolation. Of the two

patients who brought snakes to the hospital, one had local lymphadenopathy alone and one complained of abdominal pain but did not develop any signs of envenoming.

In the 5 years between 1967 and 1971, there were a further 192 admissions to Port Moresby General Hospital with snakebite due to a venomous snake, on the basis of either clinical signs of envenoming and/or a dead specimen. Price and Campbell suggest that of these admissions, all were unidentified except 22 death adders although they do not state how the death adder bites were identified definitively[28]. The case fatality rate during this period was 3.1%. Brian and Vince described 54 cases of children bitten between August 1981 and October 1984[29]. Forty nine of these had signs of neurotoxicity of which 38 (73%) also had coagulopathy. Two patients had coagulopathy alone. Sixteen patients required intubation including 11 who had already received antivenom. The case fatality rate was 7.7%. Currie et al. in their paper collated Papua New Guinea snake bite data from a variety of sources[30]. This included a prospective study over a period of 30 months at Port Moresby General Hospital in which 208 patients were seen and data from 48 patients admitted to Madang hospital during the same period. Fifty six of the patients seen in PMGH required ventilation. The mortality rate was 6.3%. Serum, urine and bite site swabs were taken from a total of 262 patients between December 1986 and June 1989. Taipan venom antigen was detected in 174 serum samples (73%), death adder venom antigen in 11 serum samples (5%) and eastern brown snake in 2 (1%). Unfortunately it was not possible to assay for Papuan black snake venom antigen due to difficulty in obtaining venom to raise the test antiserum against.

ENVENOMING BY THE TAIPAN

i. The effects of a bite by the Australian taipan

The Papuan taipan (*Oxyuranus scutellatus canni*) was identified as a separate subspecies from the Australian taipan (*Oxyuranus scutellatus scutellatus*) by Slater in the early 1950's[12]. The snakes are however closely related and it appears that there are many similarities between the clinical features of envenoming by the two subspecies. Bites by the Australian taipan are relatively rare. The snake is found predominantly in the Cape York peninsula, the Gulf of Carpentaria and in North West Australia which are all relatively sparsely populated areas. It is also found in eastern Queensland. The first definitive reports of taipan bite [31-33] and earlier reports of suspected cases[34] describe a variety of clinical features including vomiting, headache, abdominal pain, a bleeding tendency, ophthalmoplegia and peripheral paralysis resulting in death from respiratory failure in two of four cases. The first description of the use of the newly developed taipan antivenom to treat an envenomed patient was in 1957 although definitive identification of the snake was not made[33]. This patient, who had both coagulopathy and neurotoxicity, made a clear and distinct improvement within two hours of being given 9000 units of taipan antivenom. In 1980, there was a report of the rapid death of a 4 year old boy after a bite subsequently shown by immunoassay to be from a taipan, presumably due to a respiratory arrest[35]. In 1981, Brigden and Sutherland reported a case of a patient bitten by a taipan who had rhabdomyolysis and myoglobinuria[36]. Jamieson and Pearn found one definite taipan bite amongst 218 children admitted to hospital in south-east Queensland following snakebite between 1978 and 1987[37]. Morphological

changes in red blood cells were described in a patient envenomed by an Australian taipan and were demonstrated after exposing red cells to taipan venom *in vitro*[38]. In the same year, King and Smith reported the cases of a cane farmer from Mount Molloy admitted with coagulopathy and neurotoxicity following the bite of a snake later identified as a taipan by the use of a venom detection kit[39]. The venom detection kits do not however distinguish between the taipan and the inland taipan (*Oxyuranus microlepidotus)* which may also be present in the Mount Molloy area.

The difficulty in making a definitive diagnosis, the wealth of venomous snakes and the geographical and temporal spread of a relatively small number of taipan bites demands the collation of work from several observers to describe the clinical effects. It appears that the features of envenoming are similar to those produced by the bite of the Papuan taipan described by Campbell but there are important differences. Several of the features listed by Mirtschin et al. such as fixed dilated pupils, hypotension, convulsions, oliguria and local bite site swelling and necrosis are not typical of Papuan taipan bite[5]. Campbell and subsequent workers in Port Moresby did not observe the clear improvement seen by Lester in any patients following treatment with antivenom[33]. The clinical relevance of Broad's unpublished observation that the LD50 of *O.s.canni* (0.0505mg/kg) is significantly lower than that of *O.s.scutellatus* (0.099mg/kg) is unknown[40] as is the observation that there are some differences between the protein composition of the two venoms.

ii. Taipan venom

The mean venom yield at a single milking from an Australian taipan (*Oxyuranus scutellatus scutellatus*) has been estimated at 120 mg dry weight (maximum 400mg)[17]. The LD50 of whole venom in mice has been estimated at 0.064mg/kg with a certain lethal dose of 0.17mg/kg [20,41]. The combination of the high venom yield and toxicity of the venom makes the taipan one of the most dangerous venomous snakes in the world.

When dried crude *Oxyuranus scutellatus scutellatus* venom is separated on a Sephadex column, four protein and one non-protein fractions are seen. If the individual fractions are tested for LD50 in mice, the most toxic, with an LD50 of 2ug/kg, is fraction two. Its action is paralytic. Fraction four also causes respiratory paralysis but at a lower LD50 of 100ug/kg. Fohlman et al, proposed that this fraction contained a post synaptic curarimimetic toxin, similar to those which have been found in other elapid and hydrophid venoms[42]. Fraction one appears to contain the prothrombin activator whilst fraction five, which is predominantly non-protein, contains the pigment which gives the crude venom its yellow colour. Fraction three contains phospholipases which appear to be related to, but distinct from, the phospholipases of fraction two discussed below.

Taipoxin

Fraction two, which is the most lethal of the individual fractions and more lethal than the crude venom (LD50 12ug/kg) consists of four components when separated electrophoretically. Fohlman et al. isolated the major component of the fraction, and christened the toxin "Taipoxin"[42]. Taipoxin is a phospholipase A_2. It

has a molecular weight of 46,700 and consists of three distinct components, the alpha, beta and gamma fractions. There are two isoforms of the beta subunit. The stoichiometry of the taipoxin complex is not completely understood although the subunits are clearly present in a ratio of 1:1:1. Amino acid analysis of the terminal sequences shows considerable homology with the terminal sequences of notexin, from the Australian tiger snake (*Notechis scutatus*), with a basic phospholipase isolated from the venom of the black-necked spitting cobra (*Naja nigricollis*) and with porcine pancreatic phospholipase A_2.

Kamenskaya and Thesleff demonstrated that the major effect of taipoxin was to inhibit transmitter release from presynaptic cholinergic nerve terminals[43]. Both spontaneous and evoked transmitter release was affected. The rate of onset of inhibition of transmission in a nerve diaphragm preparation was increased by stimulation of the nerve and by increased temperature[43,44]. Removal of the nerve-diaphragm preparation to a toxin free bath failed to prevent progression of toxicity. Cull-Candy demonstrated similar findings and estimated the LD50 of the isolated toxin at 2ug/kg body weight[45]. He also demonstrated that the toxin produced ultrastructural abnormalities at the pre-junctional terminal, most notably a depletion in synaptic vesicles, omega shaped indentations on the presynaptic membrane and the presence of granular material in affected terminals.

Taipoxin hydrolyses phospholipids, predominantly at the presynaptic membrane and it is probable that it produces its effect through the destruction of molecules integral to normal neuromuscular transmission. The *in vitro* action of phospholipases is typically triphasic. There is an initial inhibition of transmitter release followed by a short period of

enhanced transmission, in turn followed by a further period of declining transmission and ultimately failure. The initial phase is unaffected by conditions which alter enzymatic activity (eg. temperature, concentration of calcium ions) and may be related to the binding of the toxin at a site distinct from the catalytic site[46]. The second phase may be due to hydrolysis of membrane lipids and subsequent depolarisation[47] although other workers have suggested that the inhibition of slow activating potassium channels and subsequent prolongation of opening of voltage sensitive Ca++ channels might be the mechanism of increasing transmitter release[48]. Calcium appears to be an essential cofactor for enzymatic activity. Most other divalent cations inhibit enzyme activity. It has been noted *in vitro* that even if the preparation is washed during stage 2 of the triphasic process, neuromuscular paralysis will progress, suggesting that the toxin is already tightly and irreversibly bound to the presynaptic membrane[49]. The structural changes which were described by Cull-Candy, are believed to be due to a failure in vesicular cycling[45]. It appears therefore that there are two stages of action of the toxin, the initial biphasic stage, which may correlate with binding, and the subsequent irreversible failure of transmission which may result from the catalytic action of the toxin on the membrane structure either at or close to the binding site.

The exact nature of the binding sites has not been elucidated. It appears that the effects of crotoxin, B-bungarotoxin and taipoxin, although similar in end result, do not involve binding at the same receptor site. Mutual potentiation has been observed *in vitro* when the toxins are given together, as has a lack of competition between them[49]. However, Degn and Seebart have shown that taipoxin inhibits the specific

binding of crotoxin to guinea pig synaptosomes[50]. Whatever the nature of the binding sites, it appears that they are highly specific and the authors suggest that this may be due to the toxin's affinity to protein rather than lipid domains. Binding is poorly reversible. It appears that the action of the toxin does not involve internalisation but may involve a conformational change following binding[51]. Other investigators have found that neurotoxic phospholipases bind and hydrolyse negatively charged phospholipids with a high degree of specificity[52].

A number of investigators have attempted to solve the questions of mechanism of block by looking at cell free systems such as synaptosomes and mitochondria. Harris discussed the problems of extrapolating these findings, highlighting the wide variability in uptake seen at different temperatures, toxin concentrations, sources of preparation and duration of incubation[53]. The reliability of extrapolating from observations in cell free systems is therefore open to doubt. Unfortunately the same problems recur in subcellular systems in which attempts have been made to look at transmitter release. There is little doubt, however, that the ultimate effect of taipoxin is to destroy nerve terminals. It may be that the omega shaped indentations and mitochondrial damage seen reflect the early stages of this destruction rather than a halt in endo or exocytosis.

Taipoxin is known to be myotoxic when injected subcutaneously into the hind limb of laboratory rats[54]. Only the alpha subunit is myotoxic in isolation but the effect is augmented by the presence of the other subunits. Ultrastructural changes occurred in the muscle as early as one hour after injection in both innervated and deinnervated muscle. There was rapid loss of membrane potential from the damaged myofibrils

with gradual recovery from around the third day to complete recovery around 20 days. Harris suggested that the myotoxicity of taipoxin was probably due to hydrolysis of the membrane lipids resulting in a non-specific increase in membrane permeability and a loss of cross membrane ionic gradients[55].

Other phospholipases

Taipan venom is known to contain a variety of other phospholipases, three of which have been purified and characterised[56]. The clinical significance of these single chain phospholipases is not known. The binding of one of them, iodine labelled "OS2" was inhibited by other toxic phospholipases and also by potassium channel blockers, an observation raising the possibility of specific interaction with a family of potassium channels.

Post synaptic neurotoxins

Fohlman postulated the presence of a curarimimetic neurotoxin in fraction 4 of the crude venom[42]. Mebs confirmed the presence of a post synaptic neurotoxin[57]. The clinical significance of this toxin is believed to be minor.

Taicatoxin

Possani and coworkers have recently identified a further toxin in the venom of *O.s.scutellatus* acting specifically on calcium channels[58,59]. This toxin, which they have christened "Taicatoxin" blocks the high threshold calcium channel current of excitable membranes in the mouse heart. It appears to act at an extracellular site with a high degree of specificity

and does not affect the low threshold calcium currents. The toxin consists of three molecules, an alpha-neurotoxin, a phospholipase enzyme and a hydrolytic enzyme inhibitor. The specificity of the toxin suggests that the mechanism of action of the toxin does not involve extensive hydrolysis of the membrane and also a recognition of the structural differences of the different calcium channels. It also appears that the effects of the toxin are not restricted to cardiac muscle, high threshold calcium channels in some muscle and neurosecretory cells were also affected. The clinical significance of taicatoxin is at present unknown.

Prothrombin activator

Kellaway and Williams demonstrated that crude taipan venom from *Oxyuranus scutellatus scutellatus* caused citrated plasma to coagulate[60]. Denson demonstrated the presence of a prothrombin activator in the venom with a very pronounced procoagulant action[61]. The prothrombin activator is a complex of a catalytic factor similar to factor Xa with a molecular weight of 57,000, and a co-factor similar to factor Va with a molecular weight of 220,000. The prothrombin activator has also recently been shown to activate factor VII[62].

Other venom components

Taipan venom is known to cause degranulation and agglutination of platelets *in vitro*[63]. It is also believed to have some thrombin like activity *in vitro* and weak fibrinolytic activity. The venom also has weak haemolytic activity although this is much less marked than that associated with many other Australasian elapid venoms[64]. The presence of a weak, heat stable

anticoagulant in taipan venom has been suggested[65] but no haemorrhagin activity has been demonstrated[66].

BACKGROUND TO INTERVENTION STUDIES

The potential therapeutic role for pharmacological agents in established neurotoxicity is to improve the clinical state of a patient by enhancing neuromuscular transmission once the toxin has bound and has inhibited transmitter release. There is no obvious mechanism whereby any existing drug would prevent the action of a circulating toxin more effectively than antivenom. The potential is therefore that of an adjunct to, rather than a replacement for, antivenom. Once neurotoxicity is established in taipan bite, presumably once the toxin has bound to the membrane, antivenom has little or no effect (discussed below). The clinical impression is that this situation is frequently reached before a patient receives antivenom. In these circumstances a drug that delayed or prevented the need for intubation and ventilation, would substantially reduce the risk of the most hazardous phase of envenoming.

The *in vitro* studies of taipan venom suggest that the predominant effect is presynaptic. The ability of the post synaptic receptors to respond to acetylcholine appears largely unaffected. A drug which increases the synaptic concentration of acetylcholine may have therapeutic value. There are two groups of drugs which appear to be possible candidates, drugs which inhibit acetylcholinesterase, slowing the degradation of released transmitter, and drugs which act presynaptically to increase transmitter release. In order for a drug to be useful in the management of patients with neurotoxicity, it must be safe, preferably available in both oral and intravenous preparations and produce a sustained increase in

peripheral muscle strength, most importantly in the respiratory and bulbar muscles. Anticholinesterases have filled all of these roles in the treatment of neurotoxicity due to post synaptic neurotoxins following envenoming by the Philippine cobra (*Naja naja philippensis*)[67], the Malayan krait (*Bungarus candidus*)[68] and the death adder (*Acanthopis spp.*)[23,69].

A second group of drugs which could produce a beneficial response in presynaptic neurotoxicity are the aminopyridines. These agents act by blocking voltage dependent potassium channels in the region of the nerve terminal[70]. Delay in the opening of potassium channels in this region in turn prolongs the opening of calcium channels through which calcium enters the presynaptic terminal. The release of acetylcholine is augmented by a high concentration of calcium in the pre-synaptic terminal. Aminopyridines appear to have little effect on miniature end plate potentials (mepps) but significantly increase the quantal content of the end plate potential (epp). Aminopyridines have been used in a variety of clinical situations[71] including reversal of non-depolarising neuro-muscular blockade[72], myasthenia gravis[73-75], congenital myasthenic syndrome[76], botulism[77-80] and Eaton-Lambert syndrome[81-83]. Watt et al showed some evidence of an improvement in the respiratory function of rabbits poisoned by both B-bungarotoxin and whole krait venom after administration of 3,4-diaminopyridine[84,85]. In the clinical situations in which aminopyridines have been used, their action has been found to be significantly augmented by co-administration of an anticholinesterase and the drugs appear to have a synergistic effect[86].

CHAPTER 2 - METHODS

1. Introduction

Epidemiological, demographic and clinical details of both envenomed and non-envenomed patients were recorded on standard proformas, examples of which are shown in appendices 1 and 2. All patients were examined in the casualty department of Port Moresby General Hospital (PMGH) on admission and managed according to the recommended treatment regimens detailed in the standard treatment books published by the Papua New Guinea Department of Health[87,88]. All envenomed patients were examined at a minimum of six hourly intervals for the first 36 hours after admission and at regular intervals thereafter until discharge. Patients were reviewed between one and two weeks after discharge. Selected patients, who gave informed consent, had a series of electrophysiological tests performed during their admission and some received pharmacological interventions. All of the studies which were carried out had the full approval of the National Ethics Committee of Papua New Guinea.

2. Criteria for inclusion

Patients were included in the study if they were admitted between May 1991 and May 1993, had clinical signs consistent with envenoming and if the biting species was definitively identified as a Papuan taipan by one or more of the following means:

i. Dead snake brought and identified as a taipan from an identification key[16] and subsequently confirmed by visiting herpetologist.

ii. Taipan venom antigen detected in at least one of :- serum, bite site swab, bite site aspirate or admission urine sample at a level of more than 18ng/ml*.

iii. Taipan venom antigen detected at level of less than 18ng/ml in at least one specimen, but with corroborative evidence that biting species was a taipan, either:

- Spontaneous unsolicited description of dark snake with red back by patient at admission,

or

- Positive CSL venom detection kit immunoassay for taipan venom from swab of bite site at admission.

*A level of 18ng/ml was taken as the cut-off for significance in the assay for venom antigen. This is explained below.

A total of 117 patients met these criteria. In addition, a prospective series of 100 patients claiming to have been bitten by a snake, but without clinical signs of envenoming, was collected from May 1991. These patients were included in order to compare the results of EIA venom antigen assays with those taken from envenomed patients.

3. Epidemiology of taipan bite

Information was sought to elucidate the epidemiology of taipan bite in Papua. The answers to a variety of questions concerning the circumstances and specific details of the bite were recorded on a proforma (see appendix 2). The proformas of all patients admitted to PMGH between May 1991 and May 1993, who were later confirmed to have been bitten by a taipan, were included in the analysis, a total of 117

patients. This included 11 patients who were admitted when the author was not in Port Moresby but who met the other diagnostic criteria. These patients were seen and managed by either Dr David Lalloo or Dr Nneka Nwokolo using the same protocol.

4. Clinical studies

i. Admission criteria

The clinical records of 106 confirmed envenomed taipan bites, seen and managed by the author between May 1991 and May 1993, form the basis of the clinical analysis.
Clinical signs of envenoming were regarded as unequivocal if they included one or more of the following : tender local lymphadenopathy in the bitten limb, incoagulable blood, spontaneous bleeding and evidence of neurotoxicity.

ii. Clinical management

All patients were examined at admission in the casualty department and managed according to approved regimens[87,88]. Envenomed patients were given one vial of antivenom, either polyvalent or monovalent taipan antivenom (Commonwealth Serum Laboratories, Melbourne), depending on whether the species was known, given as an infusion in 100ml of dextrose, over 20 minutes. 12.5mg or 25mg of promethazine was given intravenously as premedication according to body weight. One ml of tetanus toxoid, given subcutaneously and a single dose of 1MU penicillin given intramuscularly or intravenously depending on the presence or absence of coagulopathy, were also administered at admission. Penicillin was then given for a further five days.

Envenomed patients were admitted to the high dependency ward. Further management depended on the clinical course. Fresh frozen plasma was given to a small number of patients in whom bleeding persisted. Patients with pooling of secretions were nursed on their side and pharyngeal suction was used as required. Electrocardiograms were done at admission, 24 hours and at other times in some patients. All significantly envenomed patients had a urinary catheter inserted to monitor urine output.

Any additional unrelated pathology identified or developing during the course of admission was managed as considered appropriate.

iii. Intubation and ventilation

Patients were intubated if they had obvious pharyngeal pooling of secretions and were considered at risk of aspirating. They were ventilated, using a Bird Mark 7 pressure cycled ventilator, if respiratory function became inadequate. This usually manifested as diminishing chest expansion, falling tidal volume and minute ventilation and increasing respiratory rate. Unfortunately it was not possible to measure blood gases at PMGH during the course of the study. Patients were sedated using intravenous boluses of diazepam and morphine as required. A local pharyngeal anaesthetic spray was used prior to intubation. Neuromuscular blocking agents were not used. Patients were weaned from the ventilator when they were able to maintain an adequate tidal volume, had a respiratory rate of less than 25/minute, were able to cough effectively and could sustain hand grip and head lift for at least 5 seconds. They were subsequently extubated when adequate respiratory function was maintained. All patients who were intubated had a portable chest X-ray to check the

position of the endotracheal tube. Other patients were X-rayed if clinically indicated.

iv. Clinical progression and effect of antivenom

All patients were examined at regular intervals. For the first 36 hours they were examined at least once every six hours and subsequently a minimum of once every 12 hours until discharge. In order to compare the clinical course in different patients, specific stages of progression or resolution of neurotoxicity were identified. These included the onset and resolution of ptosis and ophthalmoplegia, times to intubation, ventilation, weaning and extubation, length of intubation and ventilation and time to discharge. The time taken after envenoming for complete resolution of ophthalmoplegia was estimated as the midpoint between the time of the last observation at which eye signs were definitely present and the first observation at which they were definitely absent. The time taken to initial improvement in ophthalmoplegia was estimated similarly. Successive grip strength measurements were recorded using a hand held dynamometer. A simple classification of neurotoxicity was used for comparative purposes (see appendix 3). The ability to discriminate the taste of sugar, salt, quinine (bitter) and vinegar was tested in 10 patients both during hospital admission and at follow up.

Patients were requested to attend for review 14 days after the day of their admission or one week after their discharge. Bus fares were given to those needing them to encourage attendance. At follow up, a clinical examination was performed, any necessary blood tests done (if abnormalities were present at discharge) and the patient's weight was measured.

v. Autonomic function

Full testing of autonomic function in significantly envenomed patients proved impossible. There was no tilt table for measuring responses to 60% tilt and testing of responses to sustained grip and Valsalva procedure did not prove possible. The following tests were done on 9 patients, all of whom were tested when at stage 3 neurotoxicity (see appendix 3) :-

1. Ciliospinal reflex - pupillary reponse to swift downward scratch with orange stick on skin overlying sternocleidomastoid muscle.
2. Heart rate response to intravenous atropine
3. Blood pressure and heart rate response to 30 degree tilt, from head up to head down (maximum attainable with available beds).
4. Variation in RR interval during carotid sinus massage.

Direct and consensual pupillary reflexes, were tested in all patients.

vi. Non-envenomed

Patients who gave a history of snakebite but who appeared to be non-envenomed were managed in short stay beds attached to the casualty department. They were examined at presentation and at regular intervals thereafter. All patients who were suspected to be non-envenomed, were observed in the department for a minimum of 12 hours before being discharged.

5. Laboratory studies

i. Identification of biting species

The following samples were tested for the presence of
venom antigen :

1. Bite site swab
2. Bite site aspirate
3. Serum
4. Urine

The basis for inclusion in the study was the detection of significant levels of taipan venom antigen either at the bite site, in serum or in urine. Blood samples were taken from all patients at admission and the serum separated. This was then frozen at -70°C and stored. No antivenom was given at PMGH until a blood sample had been taken but 20 patients had already received antivenom at a health centre before transfer. When a bite site could be identified and had not been obscured by topical traditional medications, it was swabbed with a cotton wool swab previously damped with sterile water. This swab was stored at -70°C. In some patients, the swab was tested for the presence of venom antigen using bedside venom detection kits (VDKs, CSL Diagnostics, Melbourne) prior to freezing. When fang marks could be identified unequivocally, 0.5ml of 1% lignocaine was infiltrated locally. A 25 gauge needle was then inserted perpendicularly to the skin along the estimated direction of the fang incisions. Tissue fluid was aspirated, The syringe was then flushed with 0.5ml of sterile water and the sample frozen at -70°C. A 2ml urine sample was collected as soon as possible after admission and stored.

Swab, aspirate, serum and urine samples were stored and later analysed by Dr David Theakston and his staff at the venom unit at the Liverpool School of Tropical Medicine. Swabs were eluted in 1ml normal saline and these together with the serum, aspirate and urine samples were tested for the presence of venom antigen against : *Oxyuranus scutellatus canni*, *Acanthopis sp.*, *Pseudechis papuanus*, *Pseudonaja textilis* and *Micropechis ikaheka* venom[22,89]. Microsorp F96 microtitre plates (Nunc Gibco Ltd, UK) were coated with 100ug/ml rabbit anti-whole venom IgG (protein A-derived) in coating buffer at 4°C overnight. At all stages 100ul volumes were used in each well.Following washing, plates were post-coated with 1% bovine serum albumin (Sigma Chemical Co.UK) for 1 hour as a blocking step, and subsequently rewashed. Test samples, together with a series of venom standards (0.1-500ng/ml) and appropriate positive and negative controls diluted 1:10 in incubation buffer were then added in duplicate to the wells. To each of these 1% normal rabbit serum (10ul/ml) incubation buffer was added to prevent non-specific reactions. Following incubation at room temperature for 4 hours, plates were rewashed. A 1:500 dilution of rabbit anti-venom IgG alkaline phosphatase conjugate (Sigma Ltd,UK) in incubation buffer was added and the plates were incubated at 4°C overnight. They were subsequently rewashed and the substrate (p-nitrophenyl phosphate disodium, Sigma Ltd.UK, 1mg/ml in diethanolamine buffer) was added. Following generation of colour, the optical densities were read on a Titertek Mulitskan ELISA "through the plate" reader (Flow Laboratories,UK) after 30 minutes substrate incubation.

Venom levels in the test samples were estimated by reference to a standard curve. The baseline for the venom assay was established by assaying control samples

from 102 patients who had never been bitten by a snake. The mean OD and SEM was calculated for these controls and this mean + 2SEM was subtracted from the venom levels measured in patients.

Based on immunoassays in controls (n=102).

Optical Density		Venom Ag. Levels (ng/ml)		
mean	SD	median	mean	cutoff
0.35	0.73	0.00	3.48	18.16

The cutoff was derived from the standard curve (mean +2SD of optical density). Venom antigen levels of less than 18ng/ml were not accepted as definitive evidence that the biting species was a taipan except in 4 patients in whom there was strong corroborative evidence (detailed above).

ii. Venom Detection Kits

Commonwealth Serum Laboratories (CSL), Melbourne, produce bedside rapid diagnostic kits for the detection of the venom of the major Australasian elapids, with the aim of indicating appropriate antivenom to use. The kits are designed to be used with either bite site swab, urine or serum sample with the first recommended. The test is based on an enzyme linked immunoassay and can detect levels of antigen as low as 10ng/ml[90-93]. Bite site swabs from 42 patients were tested immediately on admission. The swab was then frozen at -70°C and stored for later analysis using the laboratory method described above. When the VDK gave an

unequivocal positive result, monovalent antivenom was used.

iii. Biochemistry and routine haematology

The following investigations were carried out on patients during their admission. Additional samples were analysed in individual cases if deemed clinically appropriate.

1. Urea and electrolytes - Admission, days 2,3 and discharge.
2. Liver function tests - Admission and discharge.
3. Creatinine kinase - Admission, days 2,3 and discharge.
4. Full blood count and platelet count. - Admission, days 2,3 and discharge.
5. Blood slide for red cell morphology and differential white count - Admission.
6. Urine sample for urinalysis and microscopy - Admission.

The biochemistry was done in the hospital laboratory using Technicon RA-1000 and Technicon RA-XT autoanalysers. Hospital standard ranges were used. Routine haematology was done by Mrs Julie Black and Mr Asi Matuka using a Coulter counter model M450 (Coulter Electronics Ltd, Australia). Differential white cell counts were determined manually from slides fixed in methanol and stained with 50% Giemsa for 10 minutes. Platelet counts were determined manually in a modified Neubauer counting chamber. Ammonium oxalate was used as a diluent. Normal ranges for the white cell count and platelet count were determined from 100 Melanesian

controls by determining the mean +/- two standard deviations. Unspun urine samples were examined for casts, cells and crystals in the research laboratory. Urinalysis was done using Multistix (Boehringer Mannheim).

iv. Haemostasis

1. Tube clotting test - Admission and six hourly until clotting (some patients only).

The presence or absence of coagulopathy was determined using the 20 minute whole blood clotting test (20WBCT)[94]. 2mls of blood was put in a clean, unused glass tube and allowed to stand undisturbed. When a clot had failed to form after 20 minutes, the test was considered positive indicating significant defibrination. In some patients, sequential 6 hourly clotting tests were done to determine the time taken for the blood to clot after antivenom.

Analysis of the mechanism of the disturbances of haemostasis was not included as part of this study. Several patients included in this series did, however, have coagulation factor and fibrin(ogen) degradation product assays performed as part of a separate study and will be reported elsewhere.

v. Clinically non-envenomed

All patients who appeared to be non-envenomed had admission serum samples taken for venom antigen detection. When a bite site could be identified swabs and aspirates were also taken. 2ml of whole blood was taken for the whole blood clotting test[94].

6. Electrophysiological studies

i. Conduction studies

As a prelude to studies using pharmacological interventions, and to look in detail at the course of envenoming at the bedside, a series of baseline studies were performed on adult patients with suspected taipan bites and also on a number of healthy Melanesian controls, all of whom gave informed consent. Only patients who were later confirmed by immunoassay as taipan bites were included in the analysis of envenomed patients. All studies were done using a Medelec Neurostar MS92B EMG machine. All patients were studied either in the intensive care unit or the high dependency ward. Air temperatures varied from 25°C - 28°C. Control studies were done in the laboratory in the clinical sciences department, with air temperatures varying from 23°C - 27°C. Skin temperatures were recorded using a Temp/SC 200T thermistor (Lafayette,Indiana). No corrections were made for variations in skin temperature between individuals or between recordings in the same individual but patients with significant peripheral hypoperfusion were excluded from the analysis.

Sequential studies were performed on envenomed patients during the course of their hospital admission and when possible at follow up appointments after discharge. The position of recording electrodes was marked at each examination so that electrode positions were, as far as possible, consistent. The times of study varied between patients and for purposes of comparison, stages of clinical neurotoxicity (see Appendix 3) were used in addition to time elapsed post envenoming. This system was used because of the wide variation in severity and time course of neurotoxicity

between patients. A single study was performed in the control patients except for a small number in which repeat studies were done to check the reproducibility of results.

The following studies were done at rest:

1. Motor nerve conduction studies in ulnar and median nerves including:

   i. measurement of CMAP amplitude
   ii. measurement of F-wave latencies (minimum of 10 responses)
   iii. calculation of Motor conduction velocities

2. Sensory nerve conduction studies in ulnar and median nerves including:

   i. measurement of sensory nerve action potential amplitudes.
   ii. calculation of sensory nerve conduction velocities

Compound muscle action potentials (CMAPs) were recorded from abductor digiti minimi and abductor pollicis muscles after stimulation of the ulnar and median nerves respectively at the wrist. Ulnar and median nerves were chosen for the majority of studies because of their accessibility and the ease of performing consistent studies. In addition, the majority of patients admitted to PMGH are bitten on the lower limb and many have had emergency treatment with a tourniquet and local incisions which may cause transient or permanent nerve damage. A bipolar stimulator was used and a bipolar 3cm surface recording electrode positioned over the belly and tendon of the muscle. A wrap around ground electrode was placed

between the stimulating and recording electrodes. The threshold stimulus was determined and recorded as the stimulus intensity at which a maximal amplitude CMAP was obtained. A supramaximal stimulus intensity of 125% threshold was used for recording. For motor studies the stimulus duration was 0.1msec. The filter settings were: 2Hz-10kHz for motor studies, 20Hz-2kHz for sensory studies and 20Hz-10kHz for concentric needle EMG.

CMAP amplitudes were measured from the baseline to the peak of the negative deflection. Latencies were measured from the stimulus artefact to the onset of the negative deflection and duration was measured from the onset of the CMAP to the return to the baseline. Both were calculated using the machine's automated callipers. Motor nerve conduction velocities (MNCV) were calculated by using two stimulation points along the course of the appropriate nerve. For the median nerve, stimulation sites used were at the wrist and cubital fossa. For the ulnar nerve, the sites were the wrist and the medial aspect of the proximal third of the forearm. Surface distances were measured using a hand held tape marked in millimetres. F waves were recorded by supramaximally stimulating the ulnar nerve at the wrist and recording the shortest latency of 10 responses recorded over abductor digiti minimi.

Sensory amplitudes were measured by stimulating the digital branches of median and ulnar nerves in the palm at a distance of 8cm from recording electrodes placed over the mixed nerves at the wrist. Latencies were determined and conduction velocities calculated.

ii. Repetitive nerve stimulation studies

Repetitive nerve stimulation studies were performed by stimulating the ulnar nerve at the wrist and recording over abductor digiti minimi with surface pad electrodes. Stimulation intensities of 125% threshold were used and a train of nine stimuli given at a frequency of 3 Hz. The percentage change in CMAP amplitude was recorded between responses 1 and 4 and 1 and 9, using the automated callipers. Five second bursts of stimuli at 50 Hz were given to some patients and controls, followed by subsequent low frequency (3Hz) trains of nine stimuli at 5 seconds. Changes in the size of the first CMAP in the train and in the ratio of 1-4 and 1-9 were recorded.

iii. Electromyography

The majority of patients studied in this series had a coagulopathy which precluded extensive concentric needle EMG studies. A small number of patients with unusual clinical abnormalities were examined, notably patients with clinically evident fasciculation and three with pronounced difficulty with jaw opening. In all cases studies were not done until the 20WBCT was normal.

iv. Controls

Control studies were done on 26 people including relatives of envenomed patients and some members of staff at the hospital. All were fit Melanesians with no clinical evidence of neurological abnormality. Values were recorded from both right and left arms except in one patient with a previous wrist injury. F wave values were obtained in 23 patients and

compared both as a group with envenomed patients and as far as possible, height and age matched. Repetitive nerve stimulation studies were performed in 15 control subjects.

iv. Single fibre studies

Single fibre studies were done on four patients subsequent to the series reported here. The findings in these patients will be published separately. These studies were done with Dr Sean Connolly from the Regional Neurosciences Centre, Newcastle upon Tyne.

7. Intervention studies

i. Patient selection

Patients were included in one arm of the study if they filled the following criteria:

1. proven taipan bite - by wound swab venom detection kit or dead snake identification and subsequent comfirmation by ELISA.
2. adult (>14 years)
3. with significant neurotoxicity, minimum stage 2 and either static or progressing.
4. with a known and significant reduction in CMAP estimated as at least 50%.
5. having given informed consent.

Exclusion criteria:

1. pregnancy
2. past history of epilepsy.
3. inability to co-operate with testing eg., inability to tolerate electrophysiological testing or grip strength measurements, vomiting, agitation, unstable clinical state.

Patients were included at various times after envenoming and at various stages of neurotoxicity (stages 2-5) but only included once the presence of neurotoxicity was clearly established as both present and static or deteriorating.

ii. Interventions

Three intervention groups were selected:-

Group 1. Edrophonium 10mg intravenously or placebo.

Group 2. 3,4-diaminopyridine 10mg intravenously or placebo.

Group 3. 3,4-diaminopyridine 10mg followed by edrophonium 10mg at 7 minutes, both given intravenously.

A solution of 3,4-DAP was prepared by dissolving 10mg of powder (Small Scale Pharmaceuticals,Brighton) in 10 mls of sterile water under aseptic conditions. The solution was then filtered through a sterile millipore filter. The solution was administered by slow intravenous push over 1 minute into a peripheral vein. Each administration was preceded by injection of 0.6mg atropine 5 minutes before and followed by infusion of 100mls of either 5% Dextrose or N.Saline solution. Patients in groups 1 and 2 were given either active drug or a saline placebo. In group 1, given the short half life of edrophonium, this was conducted in a

double blind crossover fashion with a 30 minute recovery period between the two injections. No crossover procedure was attempted with 3,4- DAP due to the longer half life of the drug. Both active drug and placebo were drawn up into syringes labelled A and B by an assistant who recorded the code. This procedure was not carried out in Group 3 where only active drugs were given. 10 mg of edrophonium was given 7 minutes after the injection of 3,4-DAP. This time period was chosen to allow time to observe the initial effect of DAP and to fit in with the observations which were taken each 5 minutes.

iii. Assessment

The following tests were done in each patient:

1. CMAP amplitude in abductor digiti minimi at rest following 125% supramaximal threshold stimulus to ulnar nerve at the wrist.
2. muscle grip strength readings (MGS)
3. eye movements recorded as degrees of abduction.
4. grade of ptosis.
5. mouth opening recorded in millimetres interincisor distance.
6. sequential repetitive nerve stimulation response at 3 Hz. (some patients only)

Recordings were done using a Medelec Neurostar MS92B EMG machine. A 3cm bipolar stimulator was used fixed over the ulnar nerve and taped in place throughout the readings. Disc surface recording electrodes were taped over the abductor digiti minimi. The stimulus threshold was determined and a stimulus of 125% threshold used for baseline readings. This stimulus intensity was used throughout subsequent recordings. Three readings were

taken at each assessment and the maximum amplitude recorded from baseline to peak using the cursors and automated calculator. Repetitive nerve stimulation tests done in some patients were done using the electrodes and results were recorded as the percentage increment or decrement between 1 and 4 in a train of 9. A dynamometer was used to record maximal muscle grip strength. Three readings were taken at each assessment with the arm fully extended and the readings averaged. In all cases any electrophysiological recordings were made prior to grip strength readings to avoid any potential post exercise effects. Eye movements were assessed on the maximum lateral movement of the abducting eye ranging from zero to normal. Ptosis was assessed using the MRC scale and the extent (in mm) of iris uncovered on upward gaze with the head held facing horizontally. In non-intubated patients, the maximum inter-incisor distance was recorded using a hand held ruler.

Objective assessment of respiratory function proved difficult. Recording of maximal inspiratory pressure tidal volume and peak flow were initially attempted but proved highly variable and were abandoned from the analysis.

8. Data analysis

The epidemiological and clinical data were analysed using the "Epi Info" statistics package (USD,Inc.Georgia). Numerical data were stored using the Lotus R123 spreadsheet programme and analysed using the Instat biostatistics programme (GraphPad Software Inc.). Continuous variables which were not normally distributed were compared using the Kruskal-Wallis test. Continuous variables which were normally distributed were compared using the two tailed t test,

assuming equal or unequal variance as appropriate. Categorical data were compared using the Chi squared test with Yates correction or Fisher exact test if the expected value was less than 5.

CHAPTER 3 - THE EPIDEMIOLOGY OF TAIPAN BITE

1. Incidence and mortality rates

Table 3.1 shows the numbers of proven taipan bites and the total numbers of envenomed patients admitted to PMGH in the period May 1991 - May 1993. Estimated incidence and mortality rates for National Capital District (NCD) and for Central Province are shown based on a calculation from PMGH admissions and the national Papua New Guinea census of 1990[9]. The figures do not include envenomed patients who were not referred to, or did not arrive at PMGH.

Table 3.1 Taipan bites: incidence and mortality

	NCD	Central Province
Proven Taipan Bites	24	93
Incidence/100,000/yr	6.2	33
Mortality/100,000/yr	0	1.8
All envenomed patients	38	156
Incidence/100,000/yr	9.8	55
Mortality/100,000/yr	0.5	3.2

Between May 1991 and May 1992, 74 patients were seen in the casualty department at PMGH who reported having been bitten by a snake but who did not show any clinical signs of envenoming. 45 of these were bitten in National Capital District, which would give an estimated incidence of snake bite of 32.8/100,000 for the urban area and 75.8/100,000 for Central Province.

2. Biting species

There were a total of 194 patients with signs of systemic envenoming admitted to PMGH between the beginning of May 1991 and the beginning of May 1993. Definitive diagnoses of biting species were made on 130 of these on the basis of venom detection at a level >18ng/ml from serum, bite site swab or aspirate. Venom antigen was detected in 161 of the 194 patients and this distribution is also shown in Table 3.2.

Table 3.2: Biting species

SPECIES	Definitive		Venom detected	
Oxyuranus scutellatus canni	117	90%	143	88.8%
Acanthopis spp.	11	8.5%	15	9.3%
Pseudonaja textilis	2	1.5%	2	1.2%
Pseudechis papuanus	-	-	1	0.6%

3. The demography of taipan bites

i. Geographical location of bite

The geographical locations of the 117 confirmed taipan bites are plotted on the map **Figure 3.1**.

ii. Age and sex of victims

Figure 3.2 shows the number, age and sex of patients bitten by taipans during the period May 1991-May 1993.

Many patients did not know their date of birth and estimations of age had to be calculated from memorable events, such as the age of the patient at the

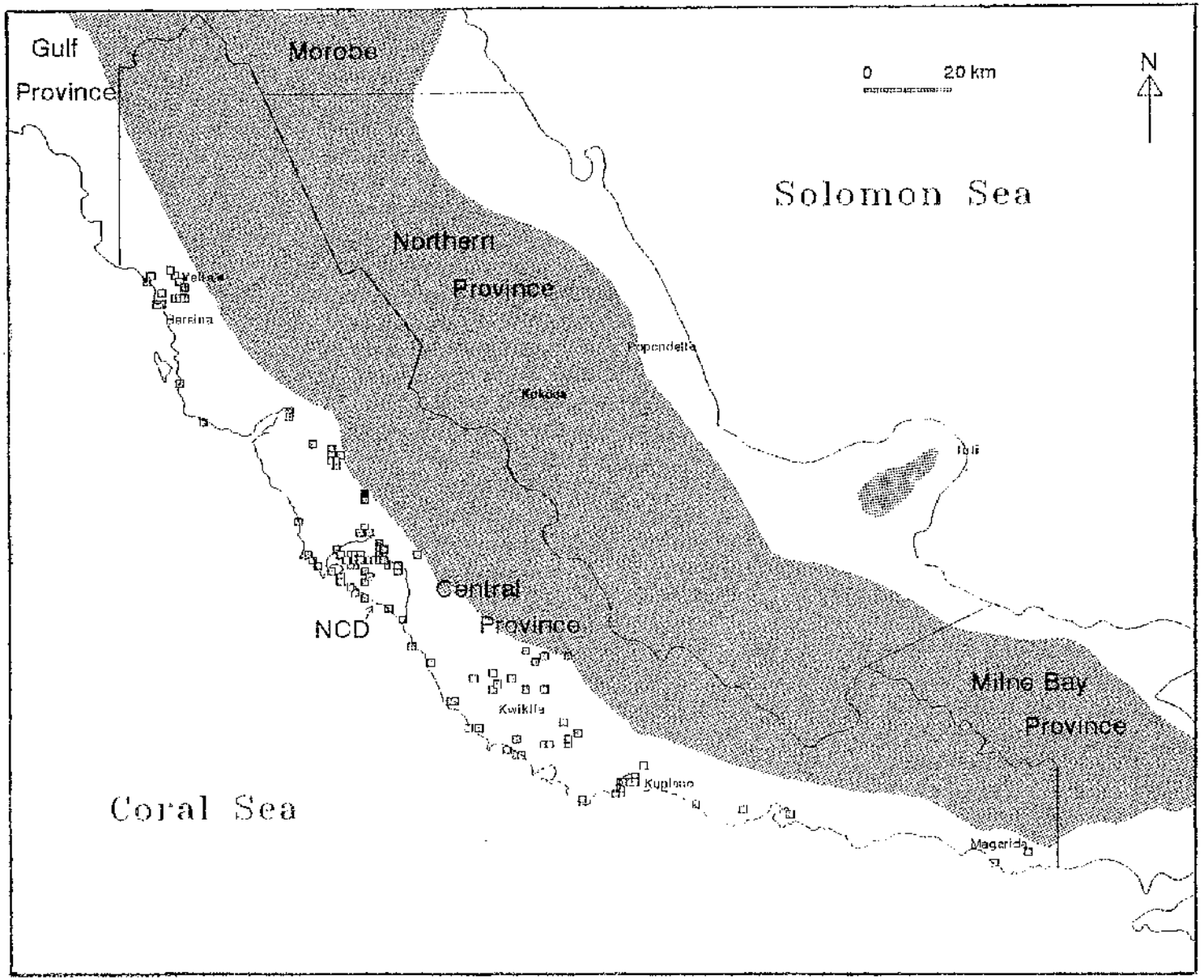

Fig.3.1 Geographical location of taipan bites in series.
(▒▒ Shaded area indicates altitude >300 metres)

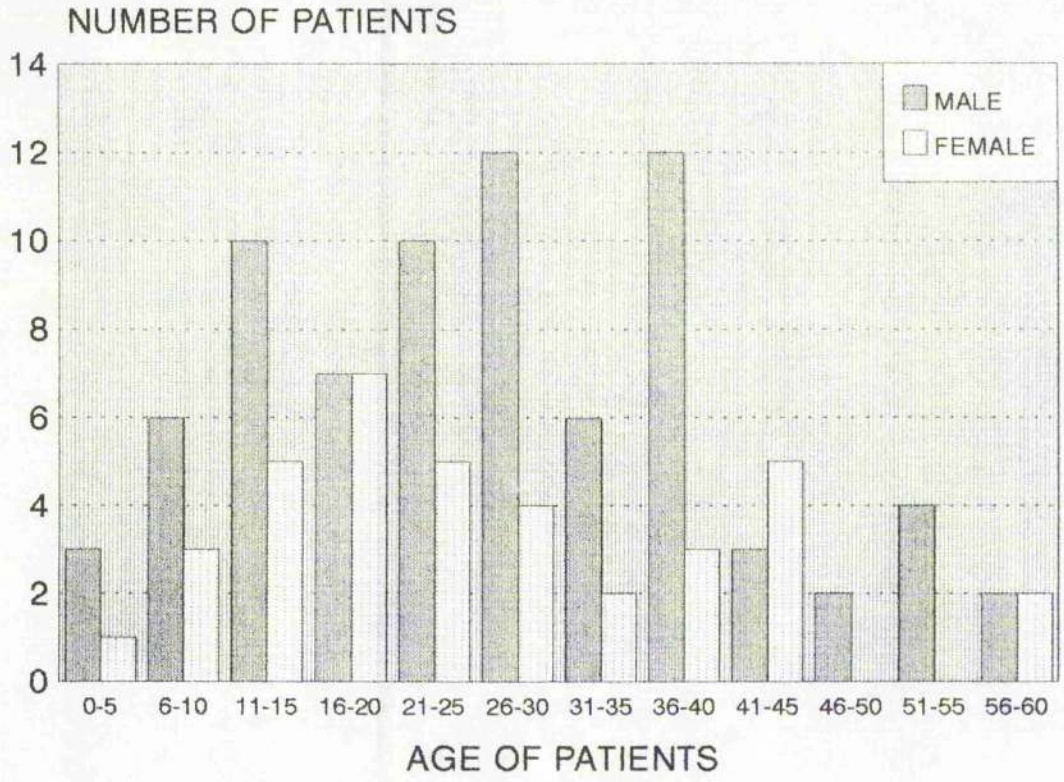

Figure 3:2 Age and sex distribution of patients envenomed following taipan bites between May 1991 and May 1993.

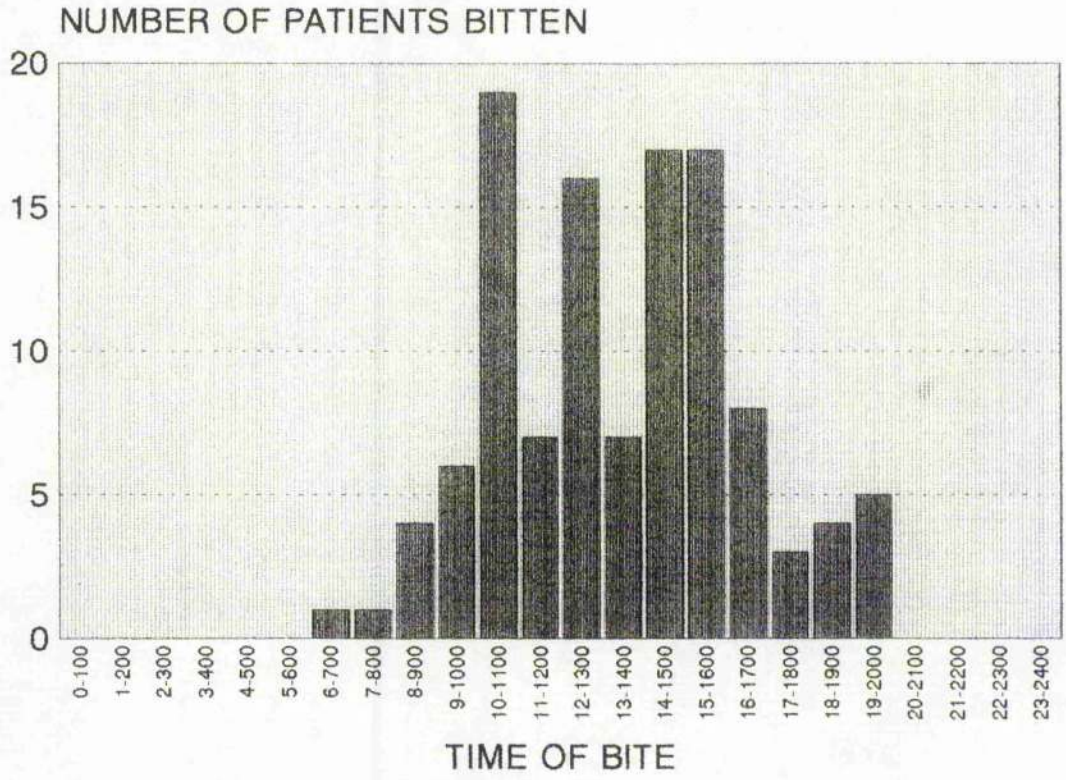

Figure 3:3 The time of the bite in cases of proven taipan envenoming between May 1991 and May1993.

declaration of independence in 1975. The data must be interpreted with this inherent inaccuracy borne in mind. Seventy seven of the envenomed patients were male (66%), 40 female (34%). The estimated mean age of males and females was 27.9 and 25.8 years respectively.

iii. Time of the bite

Figure 3.3 shows the distribution of confirmed taipan bites by time of bite. The bite time is based on the patient's estimate. Each division begins on the hour such that a bite at 1000 hours is included in the 1000-1100 group but not in the 0900-1000 hours group.

iv. Month of the bite

Figure 3.4 shows the number of confirmed taipan bites admitted to PMGH during each month of the study. Rainfall figures are shown which are calculated from the monthly rainfall averages from 3 centres, Port Moresby (Lat:09° 27', Long:147° 12', elevation 35 metres), Ou Ou Creek (Lat:08° 55', Long:146° 34') and Moreguina (Lat:10° 20', Long 148° 28'). Three centres were chosen in an attempt to compensate for the significant variations which occur between different areas of the Province from which the patients came. All are low altitude weather stations at less than 200 metres above sea level. Rainfall data from the four other weather stations in Central Province were not used, Kilakila and Loloata Island because they are geographically very close to the Port Moresby weather station and have almost identical monthly totals. The rainfall data from Kane were not used because the station is at high altitude (>1000 metres) in the Owen Stanley ranges, has a high rainfall all year round which is unrepresentative of Central Province and is

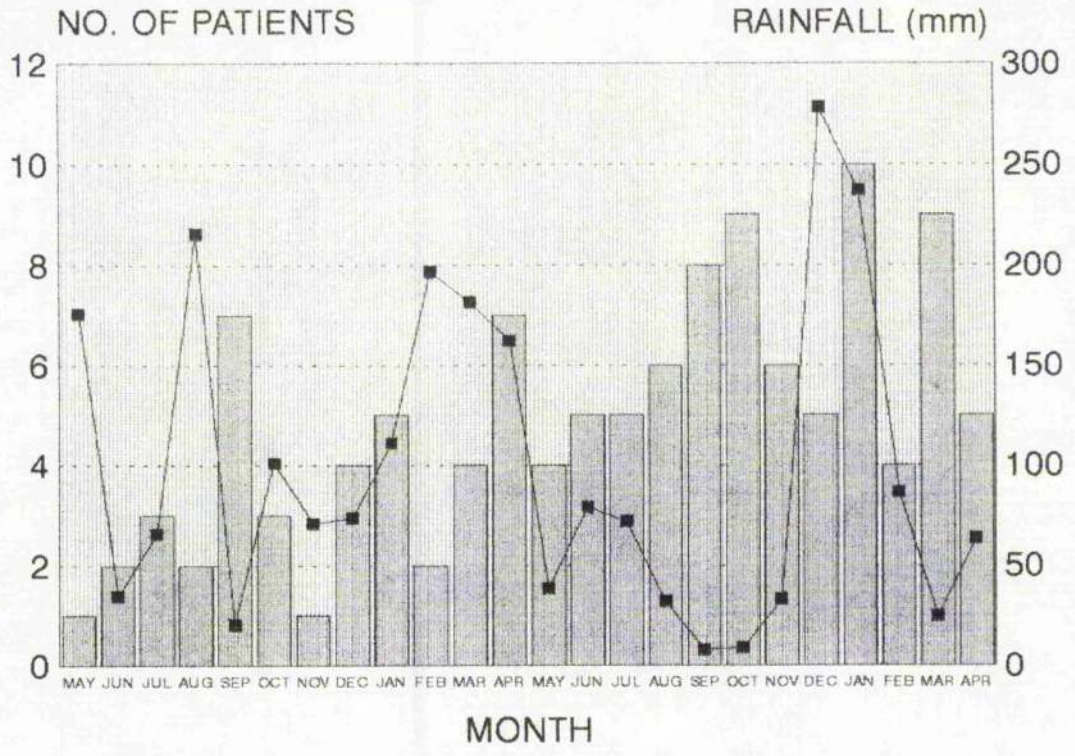

Figure 3:4 The number of patients admitted to PMGH envenomed following a taipan bite each month between May 1991 and May 1993. Averaged rainfall data shown as line.

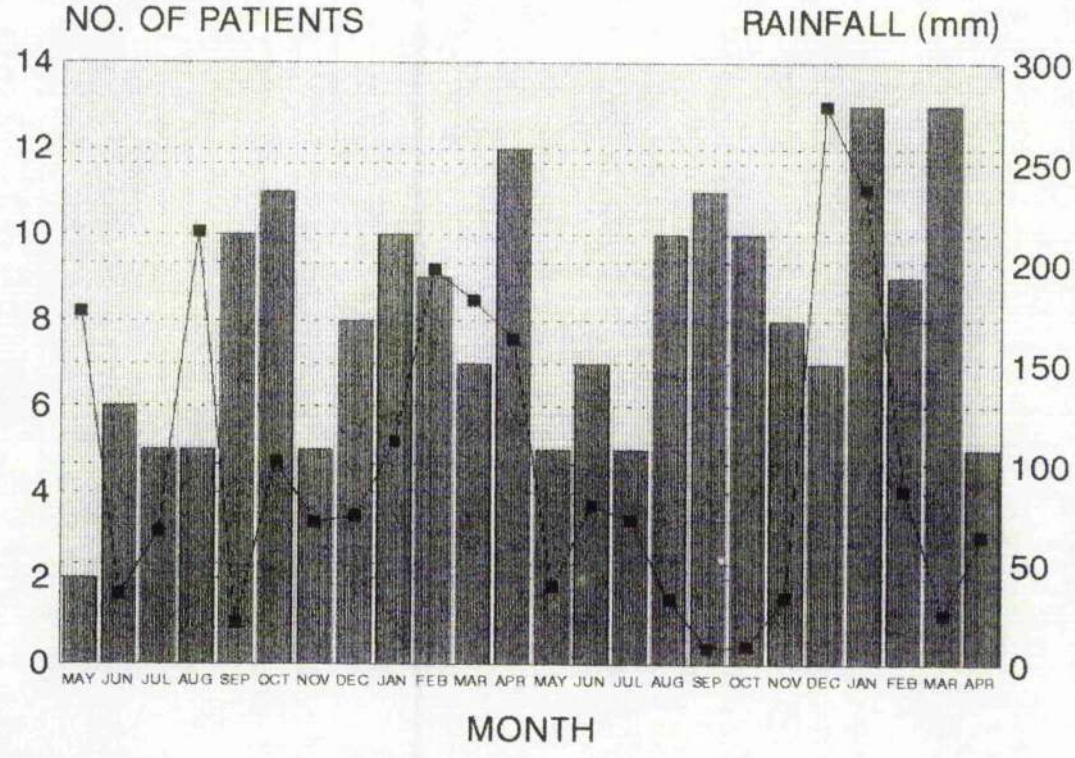

Figure 3:5 The number of envenomed patients admitted to PMGH each month between May 1991 and May 1993 (all snake species). Averaged rainfall from three centres shown as line.

not an area in which taipan bites occur. The rainfall data from the other station, Bereina, in the west of Central Province, was incomplete.

Figure 3.5 shows the total number of envenomed patients admitted to PMGH during each month including many who were suspected to have been bitten by taipans but who did not meet the criteria for definitive diagnosis (see chapter 2).

v. Circumstances of the bite

Table 3.3 shows the circumstances in which the 117 patients in the study were bitten. A distinction is made between those who were bitten on cleared bush tracks and those who were bitten in rough bush or kunai grass. The 52 patients who were bitten in overgrown areas were participating in a variety of activities at the time of being bitten, including hunting, collecting firewood, on route to a garden, playing and urinating.

Table 3.3: Circumstances of the bite

Activity when bitten	Number
Working in garden	32
On bush track	17
On road	3
Around house surrounds	3
In bush/kunai grass	52
Unknown	10

vi. Site of the bite wound

Table 3.4 shows the site of the bite wound in the 116 patients in whom it could be identified.

Table 3.4: Site of the bite wound

Site	Number
Foot	65
Ankle	17
Calf/shin	29
Thigh	3
Hand	2

Of the two patients who were bitten on the hand, the first attempted to pick up a live snake. The second was taking wood from a wood pile in which the snake was concealed. Of the patients bitten on the thigh, one was sitting beside the road when he was bitten, one was walking in long grass. The third patient was found lying in the road and was unable to give any explanation of the sequence of events.

vii. Victims' description of the snake

Very few patients could reliably identify the snake that bit them. Most described a black or dark snake but could not describe other identifying features. Several patients gave descriptions which gave no clue to the identity of the biting species. The majority of victims were also unable to estimate the length of the snake that bit them. Details are shown in **Table 3.5** and **Table 3.6**

Table 3.5 Victims'description of the snake colour

Description given	Number of patients
"Taipan"	1
Black	44
Black/red back	12
Black/brown back	5
Black/yellow, black/green, black/grey	4
Brown	7
Other	4
Unseen	40

Table 3.6: Victims' description of the snake length

Length of snake	Number of patients
1 metre or more	44
<1 metre	12
Unseen/unsure	61

4. Pre-hospital treatment

i. First aid

Table 3.7 shows the first aid treatment instituted by patients or their companions prior to reaching either a health centre or hospital.

Table 3.7: First aid treatment

First aid treatment	Number of patients
Tourniquets	40
Tourniquet + cuts	8
Razor cuts	11
Pressure bandage	1
None	57
Limb immobilisation	0

Tourniquets were made from a variety of materials including grass, cloth, a strip of rubber and rope. In general they were not tied sufficiently tightly to cause significant ischaemia although one patient, not included in this series, was seen who lost the use of his leg after prolonged use of a tight tourniquet[95]. Nineteen patients had razor cuts made around the site of the bite. Typically, these were in the pattern of multiple small,shallow, vertical incisions (Plates 3.1,3.2). No attempt was made to excise the bite site itself in any patient.

ii. First point of contact with medical help

Table 3.8 shows the first point of contact with medical help for the 117 patients in the series. Mean and median times of arrival after envenoming are shown for patients arriving at a health centre first, for those self-referring immediately to hospital and for those who were referred to hospital after being seen first at a health centre. The patients who presented initially at a health centre were predominantly those who were bitten in the geographical locations furthest from Port Moresby. 85% of this group

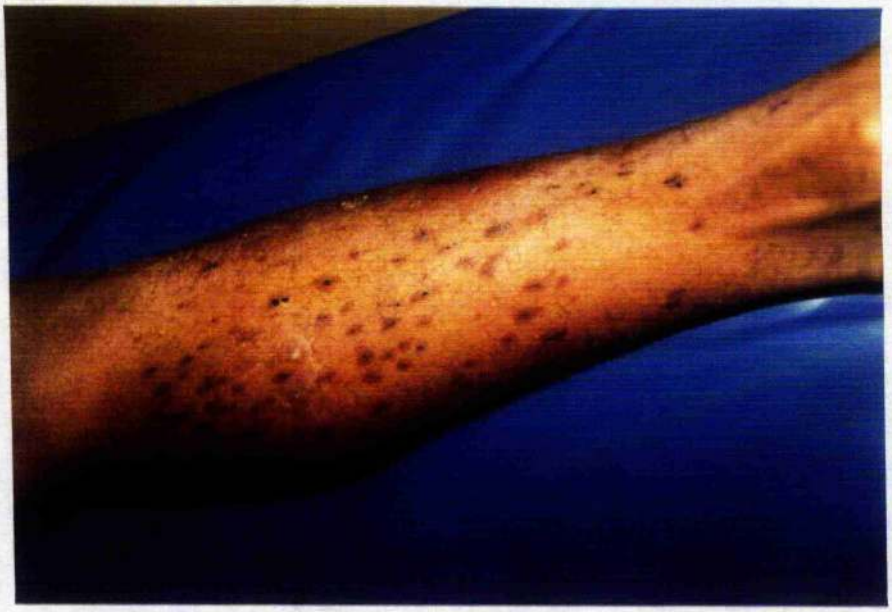

Plate 3.1 Scarification around bite site

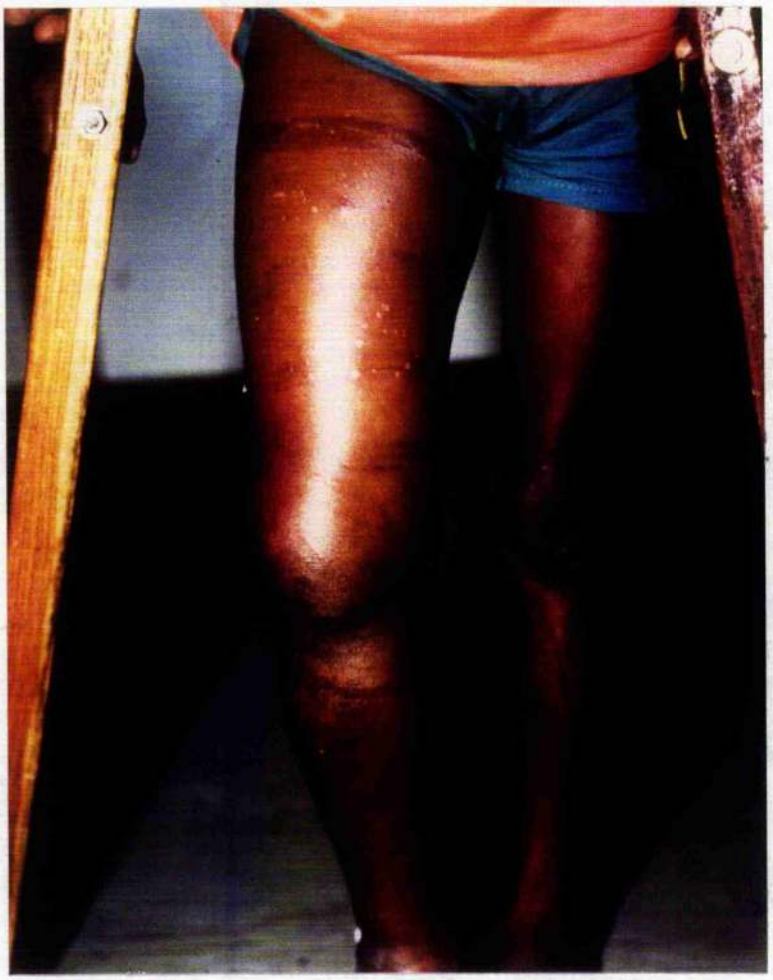

Plate 3.2 The consequences of a tight tourniquet

were referred from 4 health centres, Bereina, Vaifa'a, Kwikila and Kupiano (see map).

Table 3.8: First point of contact with help

	Health Centre	Hospital	Referred
Number of patients	55	62	-
Median arrival(hrs)	2.0	2.75	7.5
Range (hrs)	0.5-24	0.5-74	1.25-48

Twenty of the 55 patients who were referred from a health centre to Port Moresby were given antivenom prior to transfer. Eleven were given polyvalent antivenom, 6 were given taipan monovalent antivenom and 3 were given black snake antivenom. Despite a perceived need, 35 patients were not given antivenom at the health centre prior to referral. The usual reason for this was lack of stock of antivenom at the health centre. Seven patients had pressure bandages applied to the bitten limb by health centre staff prior to referral, 6 of these were treated by the same person. Only one patient who was given antivenom, also had a pressure bandage applied.

CHAPTER 4 - CLINICAL STUDIES : RESULTS

1. Basis for inclusion

i. Envenomed patients

A total of 106 patients with signs of systemic envenoming were included in the clinical series. The basis for inclusion was the presence of unequivocal signs of systemic envenoming together with subsequent detection by laboratory ElA of significant levels of taipan venom antigen Table 4.1.

Table 4.1: The source of venom antigen

	Patients with venom antigen level >18ng/ml
Serum alone	33
Swab alone	8
Aspirate alone	11
Serum and swab	19
Serum and aspirate	7
Aspirate and swab	9
Serum, swab and aspirate	15
Total	102

Four other patients with clinical signs of envenoming were included in the series. These were: three patients who gave spontaneous descriptions of having been bitten by a red backed, dark coloured snake, who also had detectable levels of taipan venom antigen at <18ng/ml in at least one of swab, aspirate

or admission serum sample. The fourth patient had a serum antigen level of 17ng/ml with a positive bedside VDK ELISA for taipan venom antigen from a bite site swab. Unfortunately this patient's wound swab was misplaced and a confirmatory laboratory EIA antigen test was not done. A bite wound is shown in Plate 4.1.

In several patients, the exact location of the bite site was not clear. In others, a variety of local topical traditional medications had been placed over the site, so swabs and aspirates were not done. The table does not give an indication of the sensitivity of each test. All but three patients had serum samples analysed. Taipan venom antigen was detected in a high proportion of admission urine samples of envenomed patients (21 of 39 in this series) but at levels <18ng/ml in all cases.

ii. Clinically non-envenomed patients

Patients were defined as clinically non-envenomed if they did not have any of:- tender lymphadenopathy, prolonged clotting, spontaneous bleeding or signs of neurotoxicity. One hundred consecutive patients reporting a snakebite, but not clinically envenomed, were studied from May 1991. Serum samples were analysed on all of these and aspirates and swabs were done when the bite site could be identified. Taipan venom antigen was detected in 23 patients and at levels greater than 18ng/ml in 15. Death adder venom antigen was detected in 6 patients and eastern brown snake venom antigen in one. Eighteen of the 100 patients in the series reported nausea and abdominal discomfort, symptoms consistent with envenoming. Eight of the 18 patients with symptoms did not have detectable venom antigen and 5 were shown to have levels of taipan venom antigen

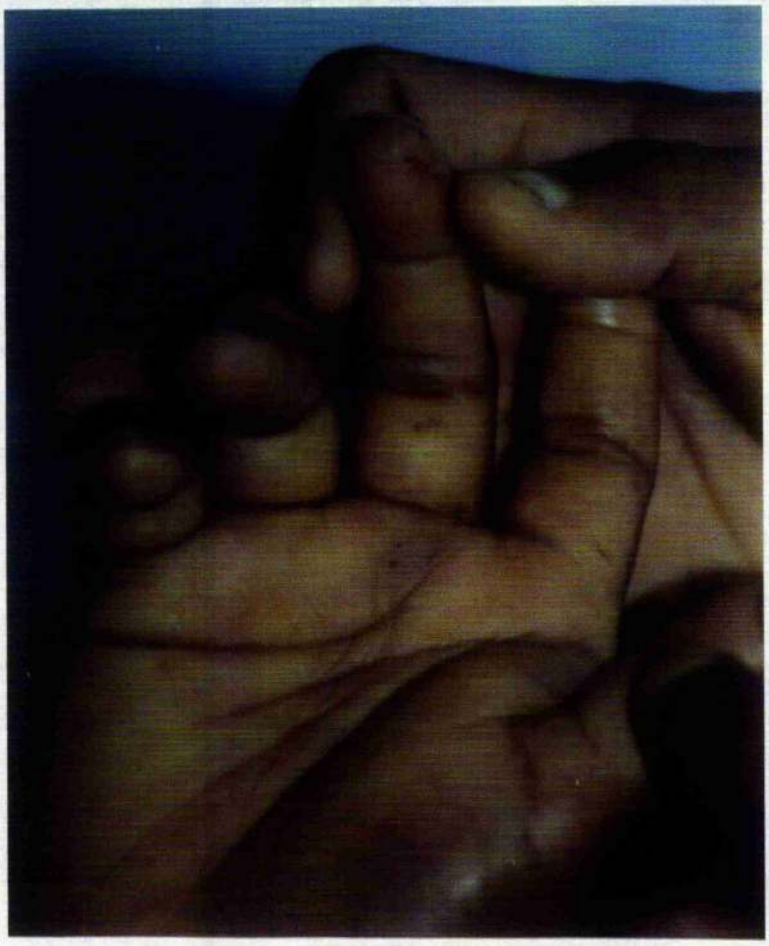

Plate 4.1 Fang marks at bite site

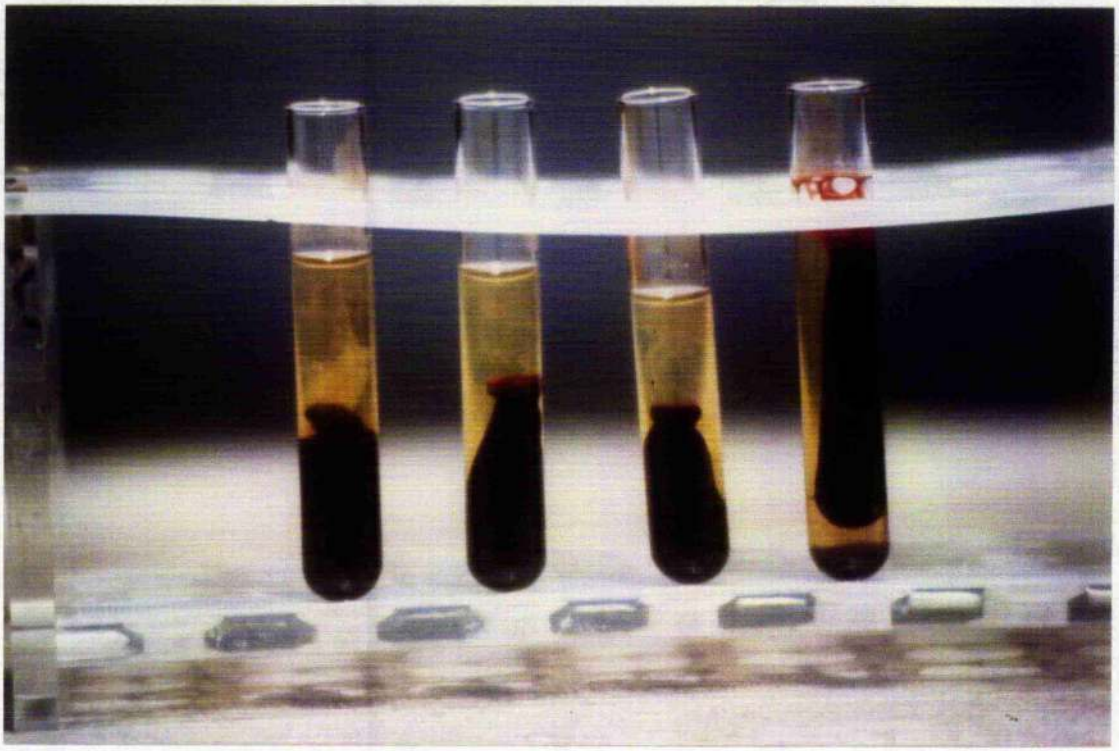

Plate 4.2 Non-clotting blood at admission and subsequent samples taken at 6 hourly intervals

greater than 18ng/ml. None of the patients with symptoms, developed any signs of envenoming.

2. Clinical course of envenoming

i. Early symptoms

Table 4.2. shows the symptoms occurring at or before admission in patients envenomed following a taipan bite. 89% of patients complained of pain in groups of lymph nodes draining the site of the bite, most commonly inguinal nodes. Abdominal pain was a feature in 67% of patients all of whom were bitten somewhere on the lower limb. The two patients in the series who were bitten on an upper limb did not complain of this symptom. Vomiting was common (64.1%) and occurred within minutes of the bite in all patients who were to experience this. 58% of patients complained of "heavy eyes" at the time of admission, including some patients who did not have clinical evidence of ptosis at the time but who subsequently developed the sign unequivocally. Headache was a common feature (57.6%) and developed within 1-2 hours of the bite and usually resolved within 10 hours. Typically the headache was frontal, bilateral and continous. 51.9% of patients complained of bleeding which was from a variety of sites, most commonly from gums and mouth, but including local incisions made at the site of the bite and less commonly in vomitus, from the nose or per rectum. All of the patients in this series who were to have bleeding manifestations had evidence of active bleeding at the time of admission to hospital. Drowsiness was reported by 44.3% of patients and this preceded the administration of antivenom and promethazine in the majority of these (77%). Dysphonia, dysphagia and diplopia were complained of by

approximately one third of patients at the time of admission. All three symptoms became much more common during the progression of the neurotoxicity. 17.6% of patients reported, or were witnessed, collapsing within minutes of the bite. One of them was subsequently witnessed to have a grand mal seizure, one was admitted with conjugate deviation of the eyes and appeared post ictal, one had fundal haemorrhages and one was admitted with apparent diffuse hypoxic brain damage. 15.5% of patients complained of dyspnoea at admission. A higher proportion had clinical evidence of impaired respiratory function (most commonly tachypnoea and reduced respiratory excursion) but it appeared that patients were not immediately aware of this. Seven patients complained of general myalgia although none of these had muscle tenderness on palpation or visible myoglobinuria.

ii. Early signs

Table 4.3 shows a summary of the clinical signs present at admission in envenomed patients and those developing during the course of the hospital stay. Tender lymphadenopathy localised to the nodes draining the bite site was the most common early sign of envenoming. All patients who had tender lymph nodes at admission either had, or developed prolonged clotting and/or neurotoxicity. Six patients did not have tender nodes but had other signs of systemic envenoming. 77.9% of envenomed patients had a coagulopathy on admission, defined as blood that did not clot in a clean, new glass tube after 20 minutes (see plate 4.2)[94]. Abdominal tenderness (57.8%) and evidence of spontaneous bleeding (51.9%) were the next most common early signs of envenoming (see Plate 4.3). Persistent bleeding from venepuncture sites was common amongst

SYMPTOM	N	%	SYMPTOM	N	%
Lymph node pain	89/100	89	Dysphonia	35/97	36.1
Abdominal pain	67/99	67.7	Dysphagia	34/97	35.1
Vomiting	66/103	64.1	Diplopia	33/97	34
Ptosis	58/100	58	Collapse	18/102	17.6
Headache	57/99	57.6	Dyspnoea	15/98	15.3
Bleeding	54/104	51.9	Myalgia	7/98	7.1
Drowsiness	43/97	44.3			

Table 4.2 showing symptoms present at or before admission in 106 patients envenomed following taipan bite. Incomplete data on some.

SIGN	ADMISSION (N)	%	PRESENT DURING HOSPITAL STAY	%
Tender lymph nodes	98/104	94.2	98/104	94.2
Abdominal tenderness	59/102	57.8	59/102	57.8
Bleeding	54/105	51.4	54/105	51.4
Ptosis	50/99	50.5	95/105	90.5
Restricted eye movements	45/98	45.9	89/105	85.0
Restricted jaw opening	34/91	37.4	67/86	77.9
Dysarthria	34/92	41.3	59/93	63.4
Hypotension (Systolic<80mmHg)	3/102	2.9	3/102	2.9
Non-clotting blood	81/104	77.9	81/104	77.9

Table 4.3 showing signs present at admission and developing during hospital stay in patients envenomed following taipan bite N=106, incomplete data in some.

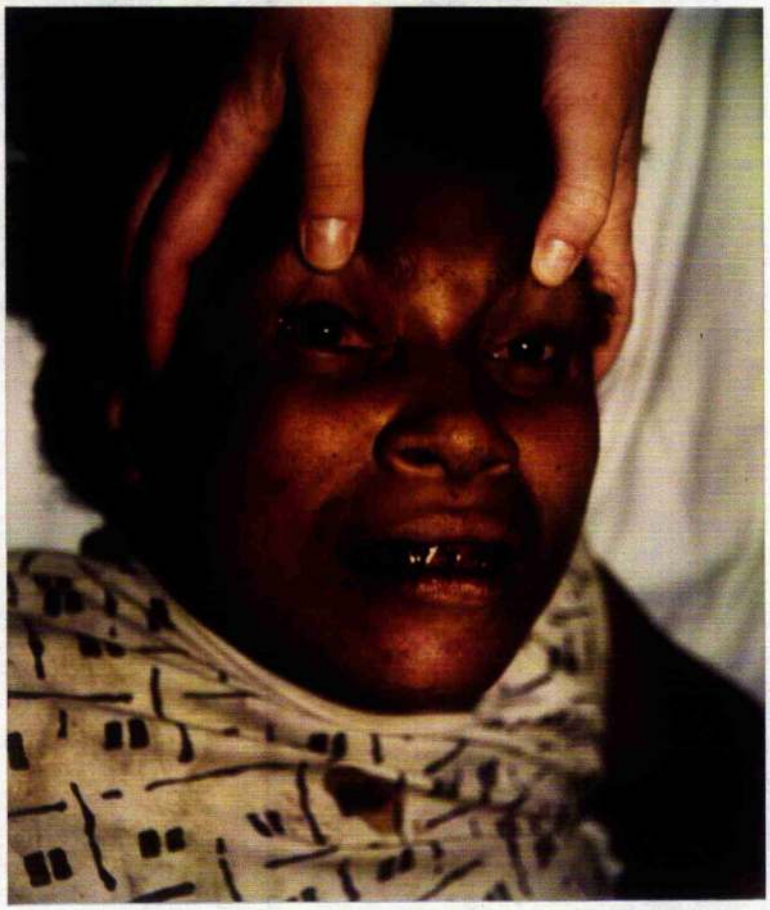

Plate 4.3 Gum bleeding

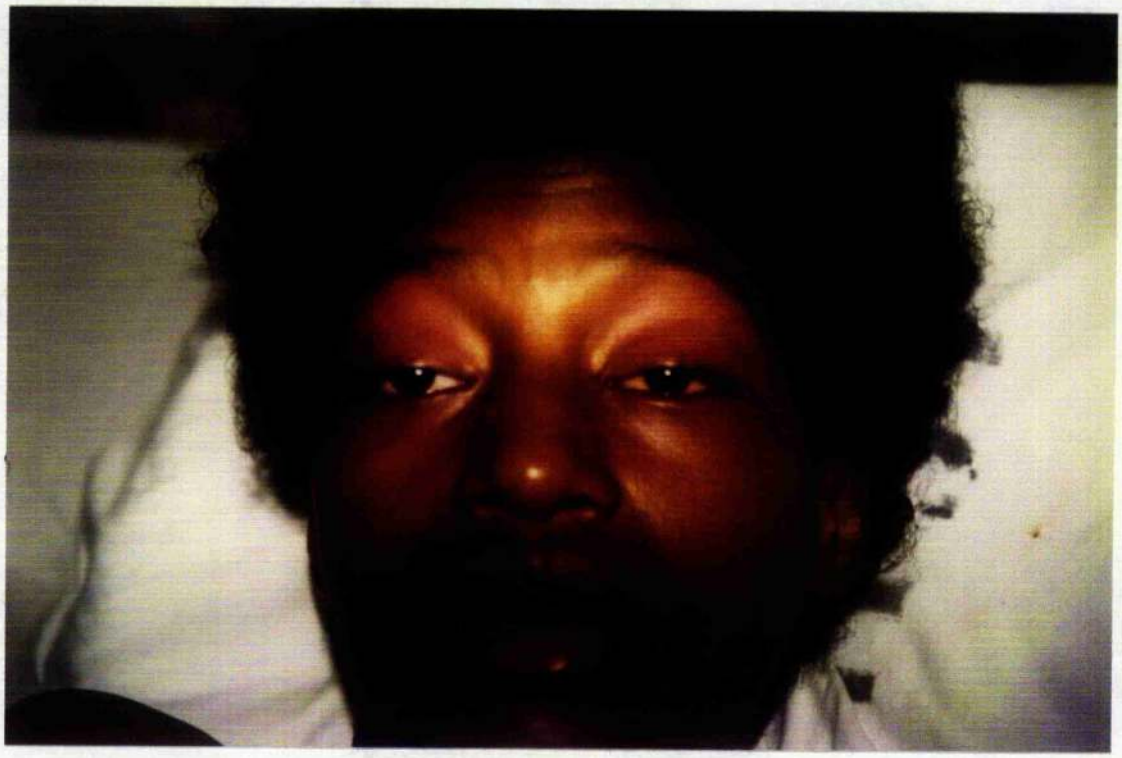

Plate 4.4 Ptosis and ophthalmoplegia

patients with a coagulopathy. The major sites of spontaneous bleeding are shown below in Table 4.4.

Table 4.4: Sites of bleeding

Site of bleeding	N
Gingival sulci	45
Cuts around bite site	14
Fang marks	8
Haematemesis	8
Grazes	6
Epistaxis	2

50.5% of envenomed patients had unequivocal ptosis at admission, and 90.5% had ptosis at some time during their hospital stay (Plate 4.4). Only half of the patients who eventually developed restriction of eye movements or jaw opening showed these signs on admission. Dysarthria was a common sign which typically appeared along with other features of neurotoxicity. It was not always easy to discern dysarthria objectively, particularly when the patient's first language was not pidgin English. Three patients were found to be hypotensive at admission before the administration of antivenom. None of these was in the group reporting collapse at the time of the bite. In all three cases, the blood pressure returned to normal following administration of intravenous fluids. Thirty five patients had a bradycardia of less than 60/minute during their admission, usually occurring at the same time as maximum neurotoxicity. Electrocardiograms which were done on some of these patients showed a sinus bradycardia often with septal T wave changes.

Fasciculation or vermicular movement was seen in 20 patients (19%), most commonly visible in the skin

overlying gastrocnemius but also overlying deltoids and pectoral muscles in some patients. This usually became evident around 10-20 hours after envenoming and lasted for up to 72 hours. Fasciculation always occurred in association with other signs of significant neurotoxicity, of sufficient severity in the majority of cases, for intubation and ventilation to be required.

iii. Duration of admission and outcome

Table 4.5 shows a summary of major clinical events and outcomes of the patients in the study.

Table 4.5 : Summary of clinical outcome

Survived	102/106	96.2%
Bled	54/105	51.4%
Developed neurotoxicity	95/105	90.4%
Required intubation	60/105	57.1%
Required ventilation	49/105	46.7%

The mean duration of admission was 6.49 days, (SD 3.53), median 6 days. Two patients, who were kept in hospital for treatment of previously undiagnosed pulmonary tuberculosis, were excluded from this analysis.

iv. Progression of neurotoxicity

The initial signs of neuromuscular paralysis were ptosis followed by external ophthalmoplegia, progressive peripheral weakness and involvement of bulbar muscles. The mean time of onset of signs of neurotoxicity was 8.2 hours (SD 6.6), range 0.5 to 22

hours, with a median time of onset of 6.5 hours. In 60 patients (57.1%), involvement of the bulbar musculature was sufficiently severe for them to require intubation. The median time of intubation amongst those requiring it, was 17 hours with a range of 5.3 to 55 hours. The median duration of intubation required was 88 hours with a range of 2 to 500 hours. Paralysis of respiratory musculature was sufficiently severe for 49 patients (46.7%) to require mechanical ventilation. The median duration of ventilation required was 62 hours with a range of 6 to 500 hours. The prolonged intubation and ventilation in one patient is detailed below in conjunction with details of antivenom treatment. No association was seen between the patient's weight and the severity of neurotoxicity.

Sequential grip strength readings showed a decline beginning around 10 hours and typically reaching nadir values at around 20-25 hours after envenoming. The rate of recovery was fastest in those with least severe clinical neurotoxicity. In patients who required intubation and ventilation, grip strength readings began to increase on the 4th or 5th day after envenoming and reached maximal attainable values for each individual between 2 to 3 weeks after envenoming. A typical trace is shown in figure 5.6 together with CMAP amplitude readings taken at the same time.

v. Autonomic function

All 105 patients in the series who were tested, had normal direct and consensual light reflexes and normal accommodation. All 9 tested had normal ciliospinal reflexes. Seven patients who received intravenous atropine had an increase in heart rate with a mean maximum increase of 28 beats per minute, range 6-46. Eight out of 9 patients showed an increase in systolic

blood pressure in response to head down tilt of 30 degrees with a mean increase of 8.8mmHg, range 4-10mmHg. The 9th patient showed a decrease in systolic blood pressure of 15mmHg. Eight out of nine patients showed a mean increase in diastolic blood pressure in response to head down tilt, with a mean increase of 8mmHg, range 3-10mmHg. One patient, whose systolic pressure had increased following head down tilt, showed a fall in diastolic blood pressure of 6mmHg. Changes in RR intervals were variable. RR intervals decreased in 5 patients after tilting with a mean decrease of 0.11 seconds. RR intervals increased in 4 patients with a mean increase of 0.05 seconds. Responses to carotid sinus massage were also variable. 3 patients showed an increase in RR interval varying from 0.04 to 1.28, 3 patients showed no change and 3 patients showed a decrease in RR interval ranging from 0.03 to 0.12 seconds.

vi. Complications and deaths

Six of the 60 patients who required intubation developed pneumonia during the course of their hospital stay. In two of these there were signs suggesting aspiration before admission. In all cases, signs resolved after treatment with antibiotics. Four patients had prolonged stridor after extubation. One of these patients required reintubation and later developed tracheal stenosis requiring a tracheostomy. This patient had been intubated with a rubber endotracheal tube at a time when no plastic tubes were available in the hospital. Two patients developed marked periorbital oedema. In one patient (see Plate 4.6) this appeared to be part of an antivenom reaction. Conjunctival oedema was relatively common in ventilated patients but did not produce lasting problems. One

patient developed thrombophlebitis from a drip site infection. This responded to antibiotic therapy.

Four patients died in this series of 106 patients (3.8%). A three year old boy died after a respiratory arrest during a difficult intubation. A 17 year old girl was moribund on arrival at the hospital after a respiratory arrest and could not be resuscitated. An elderly man with concomitant pulmonary tuberculosis, died suddenly in the ward after an apparent recovery from neurotoxicity requiring intubation and ventilation. The fourth patient, a 15 year old girl who did not receive antivenom because none was available at the time, could not be weaned from the ventilator, developed pneumonia and died 21 days after admission.

vii. Sequelae

Forty eight patients (47% of survivors) attended follow up appointments which were between 2 and 3 weeks after the time of the bite. Seventeen of them had clearly defined loss of sensation around the bite site, usually extending in all directions over an area of up to 100 square centimetres (see Plate 4.5). All modalities were affected. There was gradual recovery of sensation in the few patients who were followed up for longer intervals over the following weeks. No sensory abnormalities were detected distant to the bite site. Two patients had marked persistent muscle weakness at follow up and were unable to squat. Maximal grip strength was regained between 12 and 20 days in the majority of patients who had significant neurotoxicity (see Figure 5.5). Two patients had persistent minor swelling at the bite site. No patients were seen with serum sickness. No patients reported noticing any change in their sense of taste or smell following

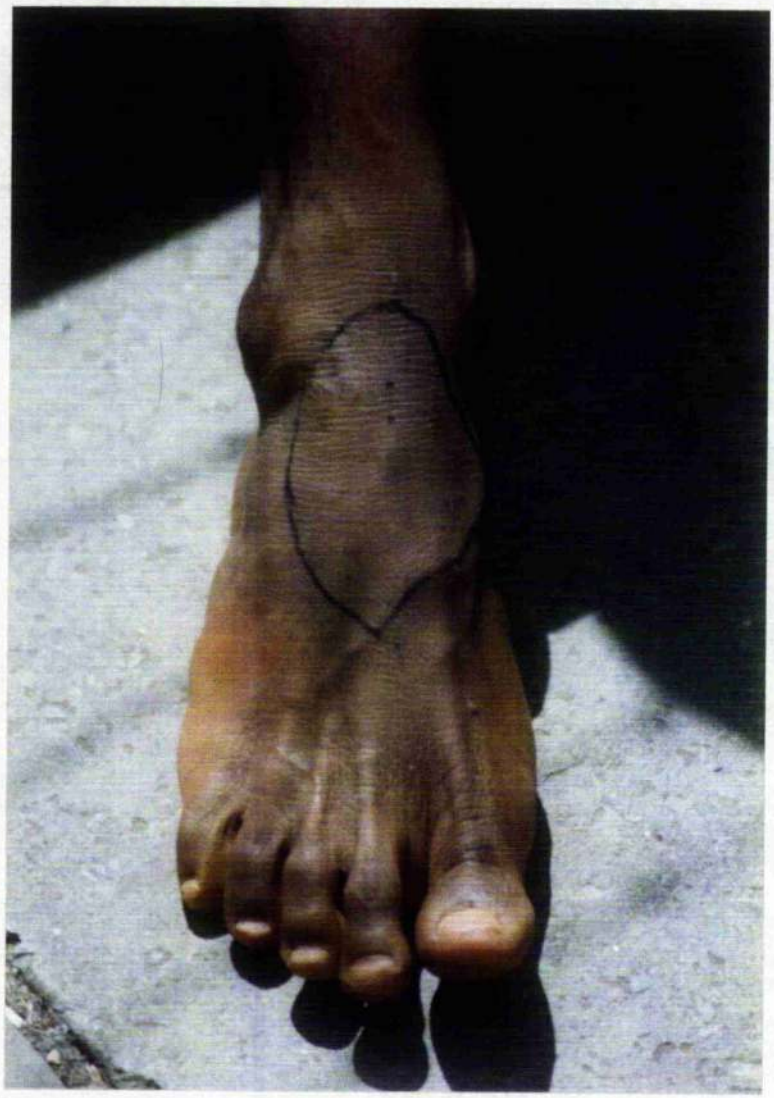

Plate 4.5 Local sensory loss around bite site.
(Site of fang marks indicated)

envenoming. Taste was specifically tested in 10 patients both during their admission and at follow up and all could discriminate between sweet (sugar), salt, bitter (quinine) and sour (vinegar).

3. Laboratory results

i. Biochemistry

The mean maximum creatinine recorded in 91 patients was 150umol/l with a median of 120 and a range of 80-999umol/l (normal range 50-120umol/l). Renal function returned to normal during the course of admission with conservative management in all patients. Sixty five patients had urinalysis performed on a sample of urine at admission. Fifty five of these had proteinuria (85%). Haematuria or haemoglobinuria was detected in 47 (72%). Microscopy was performed on 19 admission urine samples and 10 of these contained granular casts and white cells.

The mean creatinine kinase level recorded in 70 patients was 724 IU/l with a median of 369 and a range of 33-4330 IU/l (normal range 0-243IU/l). Lactate dehydrogenase levels were measured on 17 patients. The mean maximum recorded was 345IU/l, median 360, range 51-513IU/l, (normal range 120-230IU/l). Aspartate transaminase levels were measured on 83 patients. Mean levels were 69 U/l, median 49 U/l,range 11-433 U/l (normal range 5-50U/l).

ii. Haematology

The results of haematological investigations from 87 patients are shown below in Table 4.6

Table 4.6 : Routine haematology in envenomed patients

	Median	Mean	Range
Admission Hb (g/dl)	13.0	13.0	7.2-17.8
Nadir Hb (g/dl)	11.7	11.7	5.4-16.4
Admission WCC(10^9/l)	11.4	12.5	4.5-27.1
Admission Platelets(10^9/l)	169	178	47-466
Nadir Platelets (10^9/l)	149	160	71-272

iii. Coagulation studies

The twenty minute whole blood clotting test was abnormal in 81 of 104 clinically envenomed patients (77.9%). The test was normal in all 100 clinically non-envenomed patients.
Detailed coagulation studies were not carried out specifically as part of this study although some of the patients included in this series did have assays of clotting factors performed. The results of studies of mechanisms of coagulopathy in Papuan taipan bite, which include these patients, are presented elsewhere[2].

iv. Venom detection kits (VDKs)

VDK tests were done on a total of 42 patients from this series. The results are shown in Table 4.7 and refer to taipan venom unless specified.

Table 4.7 : Venom detection kit results

	LABORATORY VENOM ANTIGEN ASSAY		
	Negative	<18ng/ml	>18ng/ml
VDK Positive	4	2	31
VDK Negative	4	1	0
VDK Positive for different species	0	0	0

4. Antivenom treatment

i. Type of antivenom

Twenty patients were given antivenom at a health centre prior to referral to PMGH, of whom 17 received appropriate antivenom. Ten patients were given a second vial of antivenom at the hospital, either because the initial antivenom was considered inappropriate (1) or because signs of envenoming were progressing and adequate supplies were available to give a second vial (8). One patient was given a vial of death adder antivenom at the hospital because he had neurotoxic signs without coagulopathy and envenoming by a death adder was considered a clinical possibility which had not been covered by the initial treatment of taipan antivenom. No polyvalent antivenom was available in PMGH at the time. There were occasions during the study when no antivenom was available in the hospital, or when the clinically most appropriate antivenom was not available. The median time of administration of the first vial of antivenom at either hospital or health centre was 5.5 hours, range 0.75 to 76 hours. **Table 4.8** shows the antivenom treatment given to the 106 patients in the series.

Table 4.8 : Type of antivenom given

First AV	Second AV	No. Patients
Polyvalent	-	53
Polyvalent	Polyvalent	4
Polyvalent	Taipan	1
Taipan	-	26
Taipan	Taipan	3
Taipan	Death Adder	1
Black snake	Polyvalent	1
Black snake	-	4
Death Adder	-	1
No antivenom given		12

ii. Antivenom - adverse reactions and prophylaxis

The majority of patients receiving antivenom were given prophylaxis to minimise the risk of antivenom reactions. The regimes given varied and are detailed below. Minor early antivenom reactions occurred in 10 patients, 10.8% of those given antivenom. Seven of these patients had received polyvalent antivenom, 3 had been given taipan antivenom. Eight of the patients with antivenom reactions had urticaria and pruritus, one of whom developed facial and periorbital swelling (Plate 4.6). Two patients became agitated and developed a tachycardia during administration of antivenom but did not complain of pruritus. No severe anaphylactic reactions occurred. All patients with minor reactions were given intravenous hydrocortisone and all except one were able to complete antivenom treatment. No patients required adrenaline.

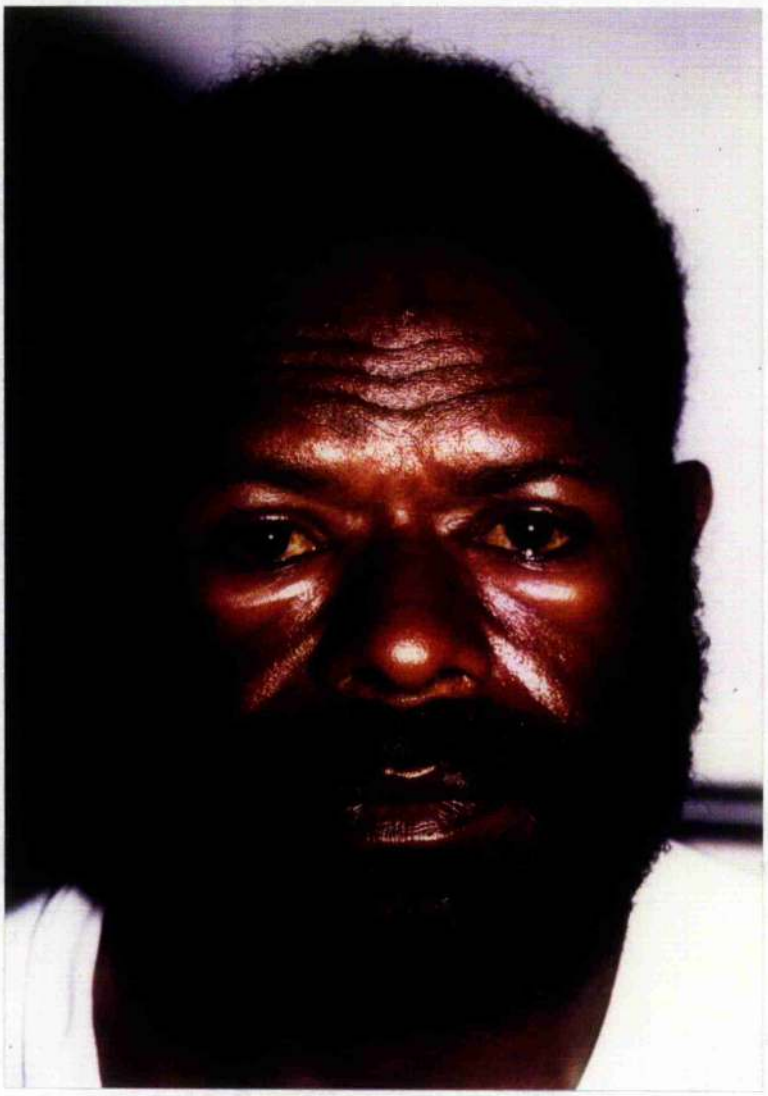

Plate 4.6 Conjunctival and periorbital oedema in early antivenom reaction

Table 4.9 shows the prophylaxis given to the 93 patients who were given antivenom, either at the health centre or at the hospital. The number of patients who had a minor antivenom reaction (AVR) is shown.

Table 4.9 : Antivenom reaction prophylaxis

Prophylaxis	No.Patients	AVR
Promethazine alone	74	8
Promethazine + hydrocortisone	10	1
Promethazine + adrenaline	1	0
Hydrocortisone alone	3	0
No prophylaxis	4	1
Unknown	1	0

5.The efficacy of antivenom

The following section analyses various measures of efficacy and the time post envenoming at which appropriate antivenom was given. It is assumed that the effect of one vial of polyvalent antivenom is equivalent to one vial of taipan antivenom, both contain 12,000 units of taipan antivenom.

i. Antivenom, survival and outcome

Table 4.10 shows the proportion of patients who survived, the proportion who required intubation and mechanical ventilation and the mean length of admission for patients receiving antivenom (AV) and those who received either no antivenom, or inappropriate antivenom (4).

Table 4.10 : Antivenom and outcome

	AV	No AV
Survival	88/89 (99%)	14/16(88%)
Intubated	54/89 (61%)	6/16(38%)
Ventilated	44/89 (49%)	5/16(31%)
Mean (median) length admission	6.6(6.0)	5.5(5.0)

Figure 4.1 shows plots of progressive grip strength recordings from 10 typical patients with advanced neurotoxicity, 5 of whom received antivenom and 5 of whom did not. Each point indicates the percentage of the maximum grip strength recorded in each patient, whether at admission or following recovery.

ii. Antivenom, coagulopathy and bleeding

Table 4.11 shows the proportion of patients in each group who had coagulopathy and bleeding manifestations at admission. It also shows the time after antivenom (or admission in those who did not receive antivenom) at which samples from patients with incoagulable blood at admission was noted to clot. Seven patients in the AV group were given fresh frozen plasma, in three cases this was due to persistent bleeding after admission, in the other four as a precaution because of significant bleeding which had occurred prior to admission. Two patients who had not received antivenom were given FFP. No patient in the group who did not receive antivenom had significant bleeding after admission.

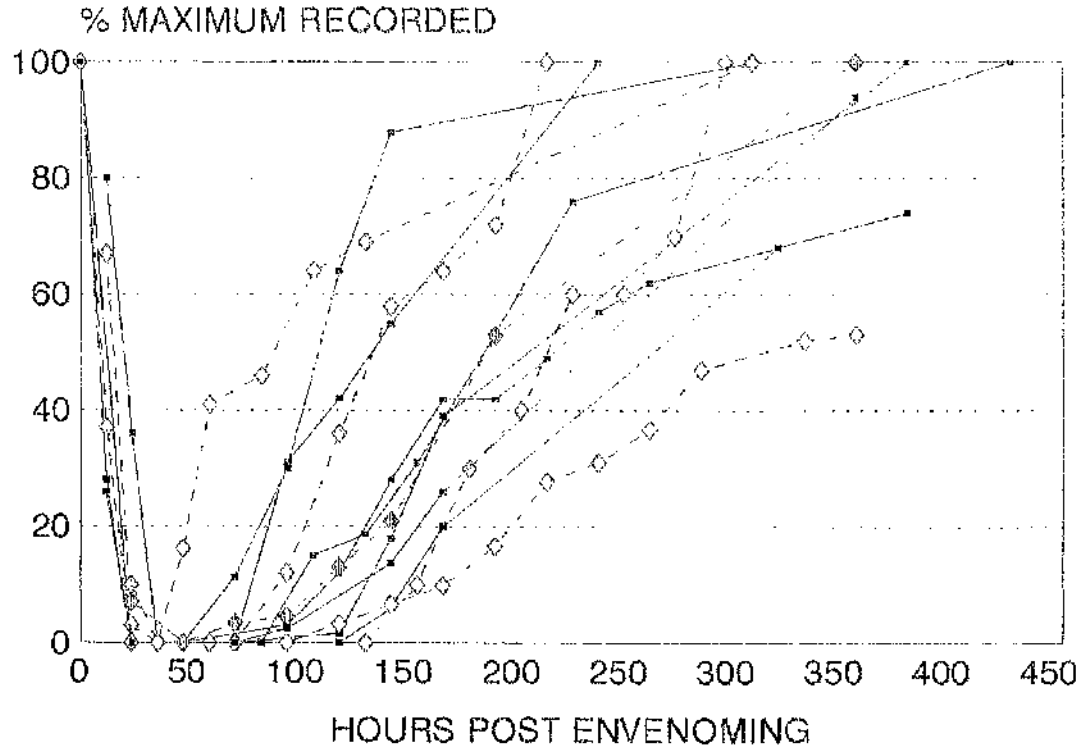

Fig.4.1 showing the recovery in peripheral grip strength in patients who did and did not (broken line) receive antivenom. All patients had neurotoxicity of stage IV or V.

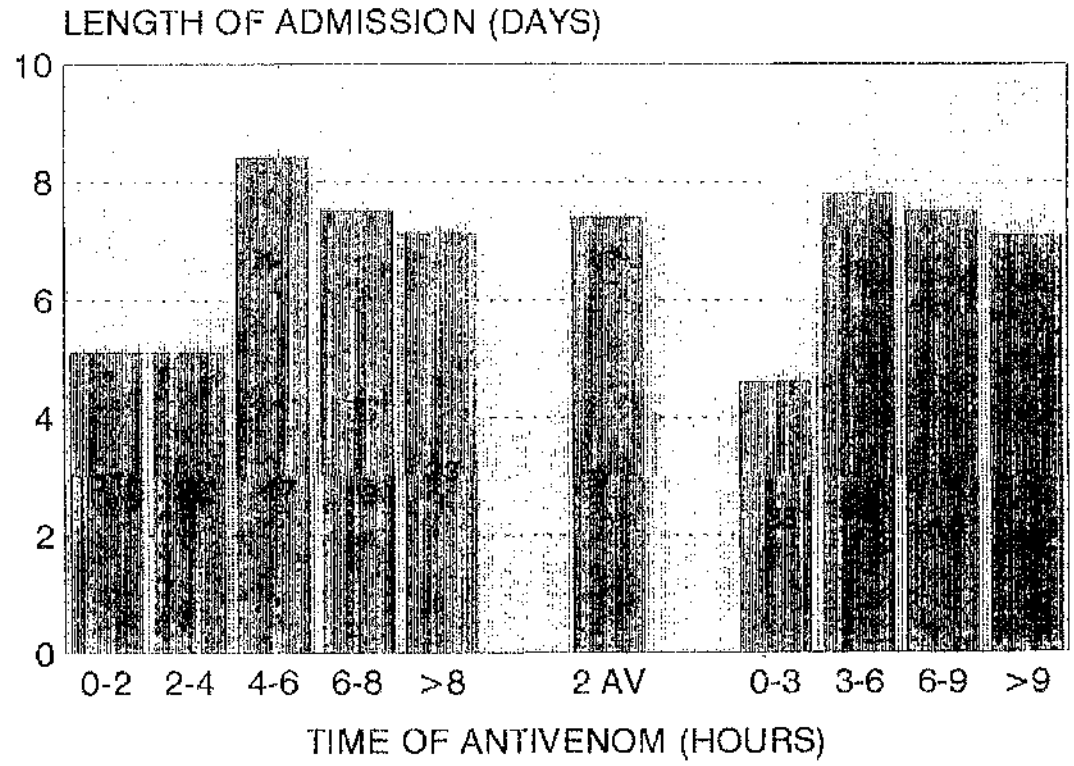

Fig. 4.2 Mean length of admission in patients grouped according to the time after envenoming at which antivenom was given. Two groupings are shown for comparison. Figures within columns indicate number of patients. Mean time of first AV in 2AV group 7.0 hours.

Table 4.11 Antivenom and coagulopathy

	AV	No AV
Non-clotting at adm.	76/88 (86%)	5/16 (31%)
Bleeding at or after adm.	51/89 (57%)	3/16(19%)
Clotting by 6 hours	17/29 (58.6%)	0/2
Clotting by 12 hours	26/29 (89.7%)	0/2

iii. Time of antivenom and outcome.

Figure 4.2 shows the number of patients receiving appropriate antivenom at different times after envenoming and the mean length of hospital admission.

Figure 4.3 shows the proportion of patients requiring intubation compared with the time after the bite at which they received antivenom. Two groupings of time of receiving antivenom are shown for comparison. Figures are also included for the small number of patients who received two vials of appropriate antivenom. In this group, the mean time of receiving the first of the two vials of antivenom was 7 hours. The median length of intubation is shown for those patients who required it. Of 16 patients who did not receive antivenom, 6 required intubation.

Table 4.12 shows the relationship between the time of receiving antivenom and either developing neurotoxicity for the first time or having signs of neurotoxicity which progressed after treatment. The proportion of

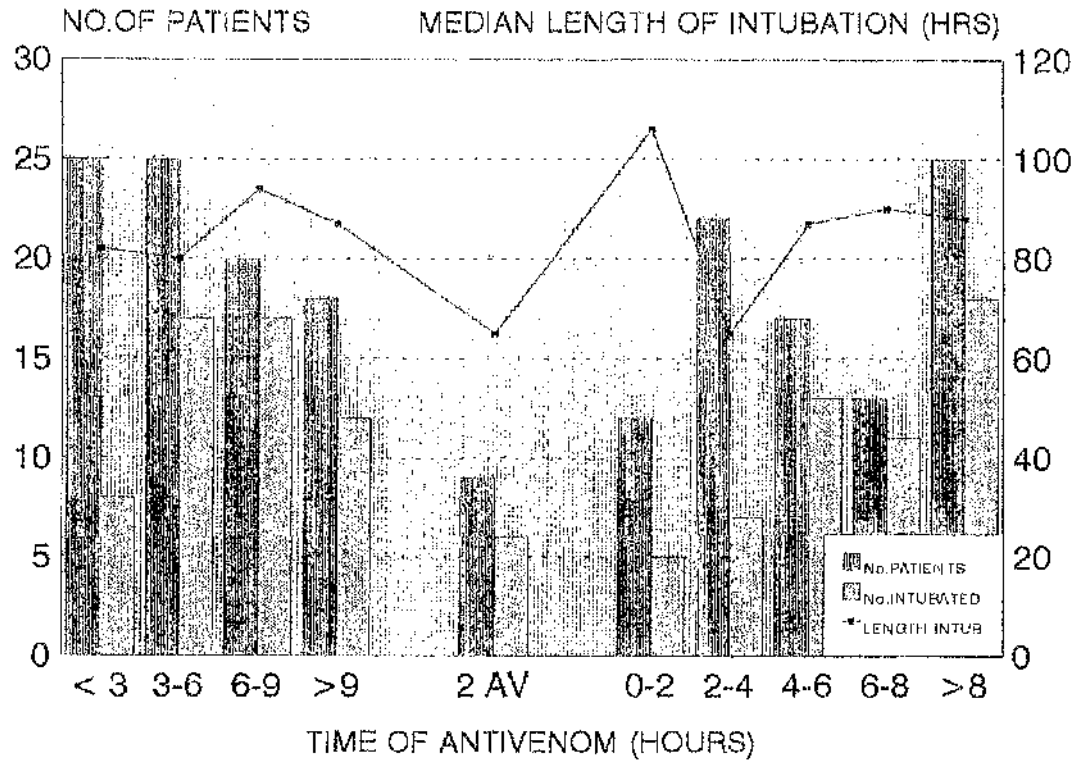

Fig 4.3 The proportion of patients requiring intubation in groups of patients receiving antivenom at different times post envenoming. Two separate groupings shown for comparison.

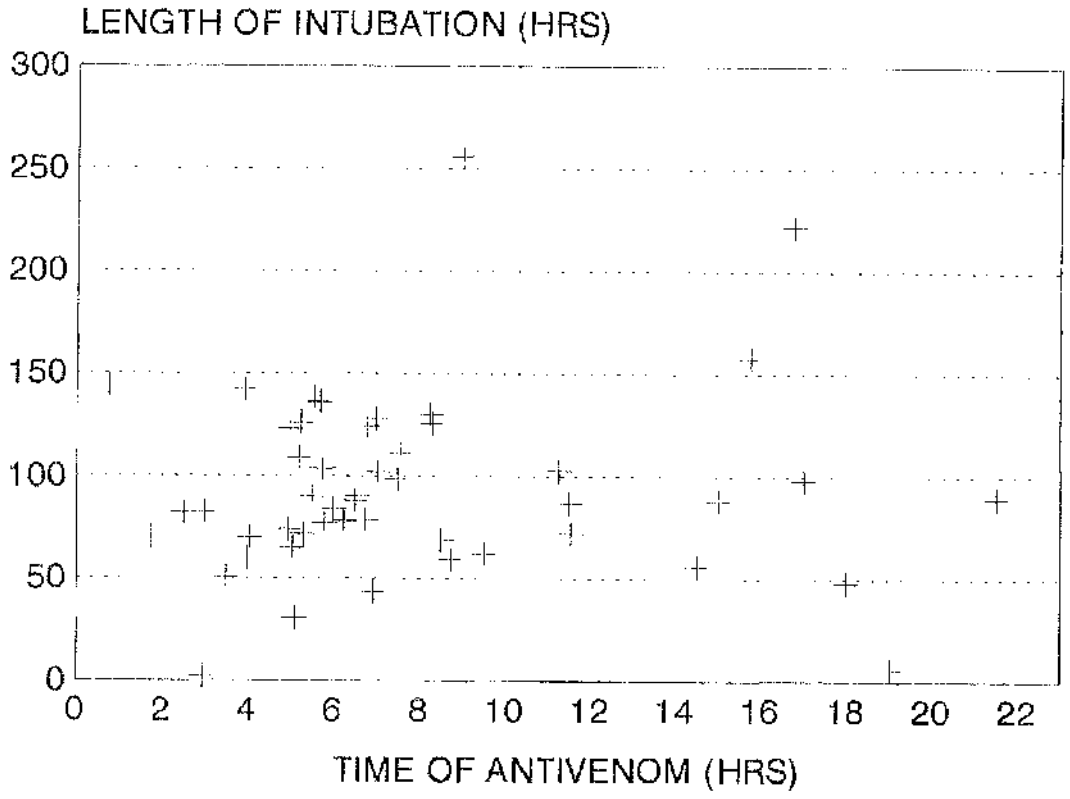

Fig.4.4 Scatter plot of time of receiving antivenom and the length of intubation required in those patients who progressed to stage IV or stage V neurotoxicity.

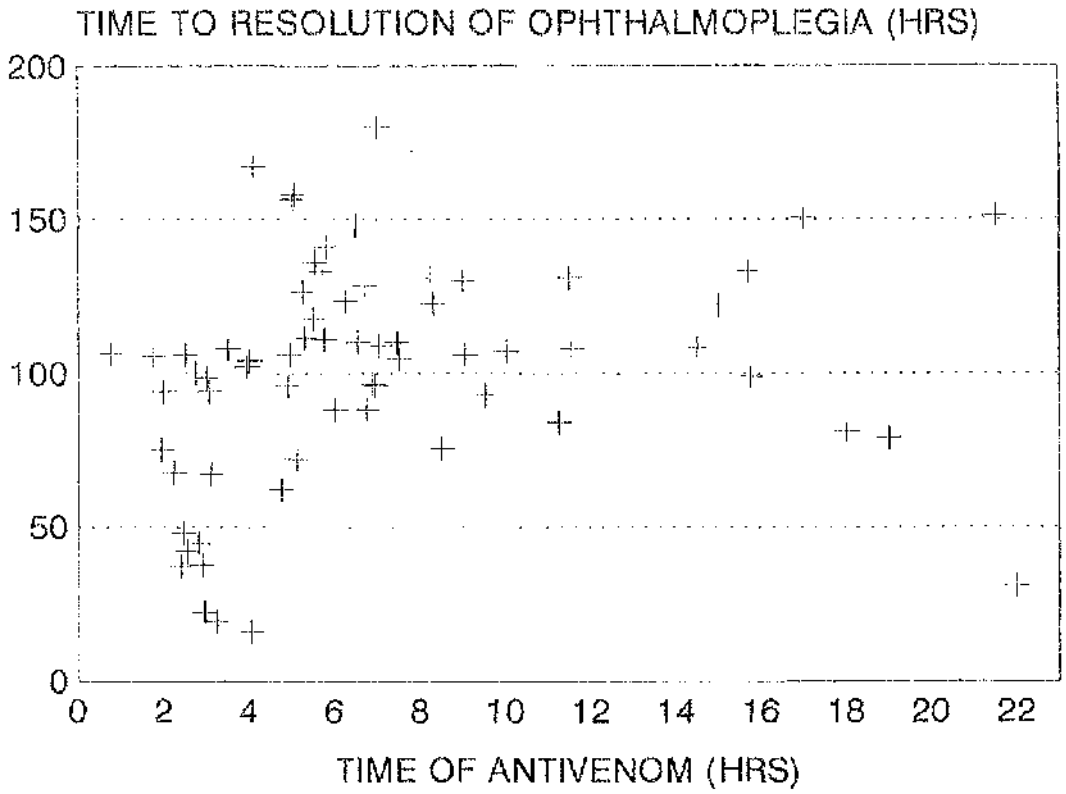

Fig. 4.5 Scatter plot showing time of receiving antivenom and the length of time after envenoming to complete resolution of eye signs of neurotoxicity.

TIME OF AV	NUMBER OF PATIENTS	DEVELOPING NEUROTOXICITY AFTER AV	DETERIORATING POST AV	NO PROGRESSION
0-2	11	0	7 (64%)	4 (36%)
2-4	21	11 (52%)	6 (29%)	4 (19%)
4-6	16	7 (44%)	6 (38%)	3 (18%)
6-8	13	5 (39%)	7 (54%)	1 (7%)
8-10	10	3 (30%)	5 (50%)	2 (20%)
>10	15	0	11 (73%)	4 (27%)

Table 4.12 showing the time of receiving antivenom and the proportion of patients who developed neurotoxic signs or showed a deterioration of existing neurotoxicity.

patients receiving antivenom in each time group who did not deteriorate is shown.

Figure 4.4 is a scatter plot showing the time of receiving antivenom and the length of intubation required.

Figure 4.5 is a scatter plot showing the time of receiving antivenom and the time to complete recovery of ophthalmoplegia.

Tables 4.13 and **4.14** shows the relationship between the time of receiving antivenom and the time to start recovery and to complete resolution of neurotoxic eye signs. For comparison, two groupings of time of receiving antivenom are shown.

TIME OF ANTIVENOM (HRS)	NUMBER OF PATIENTS	HOURS TO COMMENCE NEURORESOLUTION	HOURS TO COMPLETE NEURORESOLUTION
0-2	4	34.0 (31)	95.4 (94.5)
2-4	14	41.0 (44)	74.3 (70)
4-6	15	46.9 (48.3)	120.7 (116.8)
6-8	11	47.0 (43.9)	124.5 (116.9)
8-10	8	45.9 (41)	118.3 (116)
>10	14	51.8 (51)	122.9 (119)
NO ANTIVENOM	9	41.1 (37)	106 (102)
2 VIALS AV	9	49.9 (49)	103.2 (98.5)

Table 4.13 showing the time from envenoming to the onset of recovery and complete resolution of neurotoxicity in groups of patients given antivenom at different times. Only patients in whom accurate estimates could be made are included. Median times shown in brackets.

TIME OF ANTIVENOM (HRS)	NUMBER OF PATIENTS	HOURS TO COMMENCE NEURORESOLUTION	HOURS TO COMPLETE NEURORESOLUTION
0-3	12	37.3 (39)	71.6 (68)
3-6	20	46.4 (48)	106.6 (108)
6-9	17	46.6 (45)	116.2 (110)
9-12	5	56.1 (45)	115.2 (110)
>12	11	49.1 (42)	124.4 (118)

Table 4.14 showing similar data to Table 4.13 with time of antivenom divided into 3 hour brackets.

CHAPTER 5 - RESULTS OF ELECTROPHYSIOLOGICAL STUDIES.

The results of the electrophysiological studies in both control and clinically envenomed patients are shown in the form of tables and figures.

i. Control studies

Figure 5.1 shows the results of median and ulnar nerve conduction studies in fit Melanesian controls.

ii. Motor and sensory studies in envenomed patients

Figure 5.2 shows the results of median and ulnar nerve conduction studies from envenomed patients. All had obvious neurotoxicity, all were intubated and the majority were also ventilated. Sequential studies were done throughout the clinical course in all patients; the values used in this table are taken from studies performed at the stage of maximal clinical neurotoxicity.

iii. Sequential studies in envenomed patients

Figure 5.3 shows sequential compound muscle action potential (CMAP) amplitudes from six patients recorded after stimulation of the ulnar nerve at the wrist and recording over abductor digiti minimi. These are typical of the shapes of curve seen in all the envenomed patients studied.

Figure 5.4 shows sequential CMAP values from the same six patients in Fig 5.3 after stimulation of the median nerve at the wrist and recording over abductor pollicis. These traces are also typical of those seen in the rest of the patients studied.

	N=	MEAN CV (M/S)	2SEM	MEAN CMAP/ SNAP	2SEM
Median Nerve	51	60.0	1.0	9.8mV	0.7
Ulnar Nerve	51	63.8	1.3	8.7mV	0.8
Median Nerve (S)	50	58.2	1.6	43.0uV	1.6
Ulnar Nerve (S)	50	56.0	2.3	23.4uV	4.5
F Wave (Ulnar)	46	26.2	0.9		

Fig 5.1 showing values of motor and sensory nerve conduction velocities and amplitudes of Compound Muscle Action Potentials (CMAP) and Sensory Nerve Action Potentials (SNAP) in Melanesian controls (age range 14-60).

	N=	MEAN CV (M/S)	2SEM	MEAN CMAP or SNAP	2SEM
Median Nerve	24	59.0	2.2	2.1mV	0.6
Ulnar Nerve	24	58.8	2.4	2.5mV	0.7
Median Nerve (S)	16	55.4	3.1	30.7uV	4.7
Ulnar Nerve (S)	16	55.4	3.1	13.1uV	3.4
F Wave (Ulnar)	16	26.1	0.9		

Fig 5.2 Motor and sensory nerve conduction velocities and CMAP and SNAP amplitudes in envenomed patients with stage 4 or 5 neurotoxicity. (Age range 16-55)

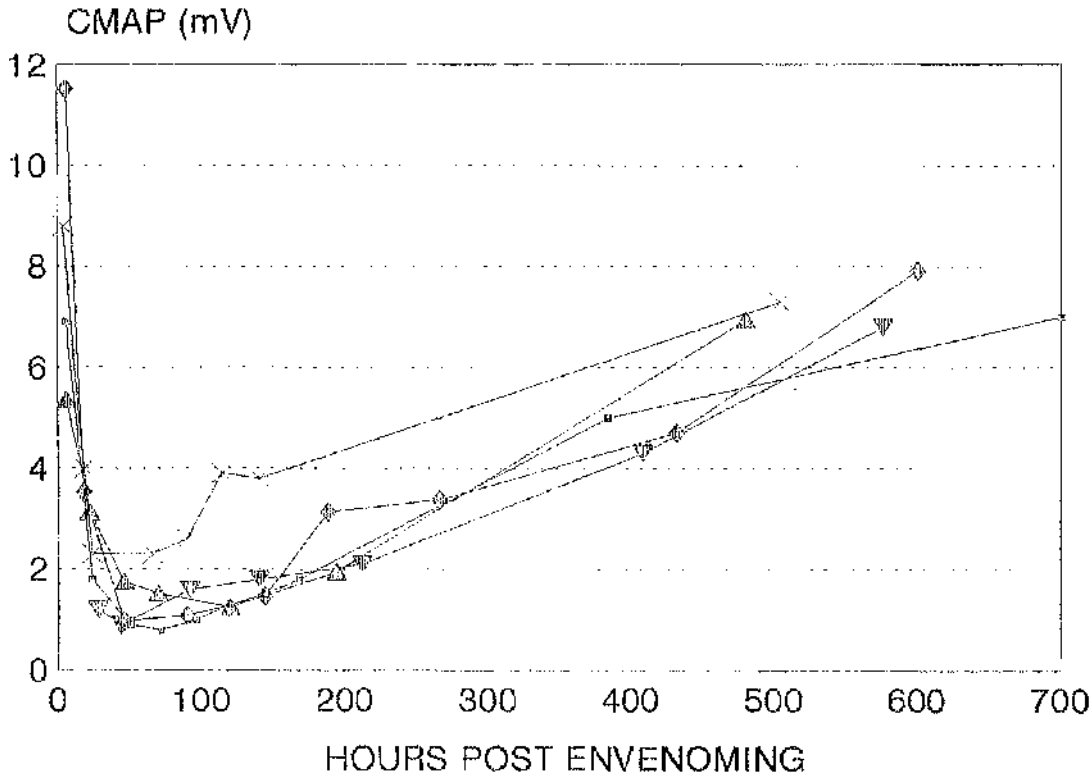

Fig 5.3 Sequential CMAP amplitudes recorded in abductor digiti minimi after stimulation of the ulnar nerve. All 5 patients required ventilation.

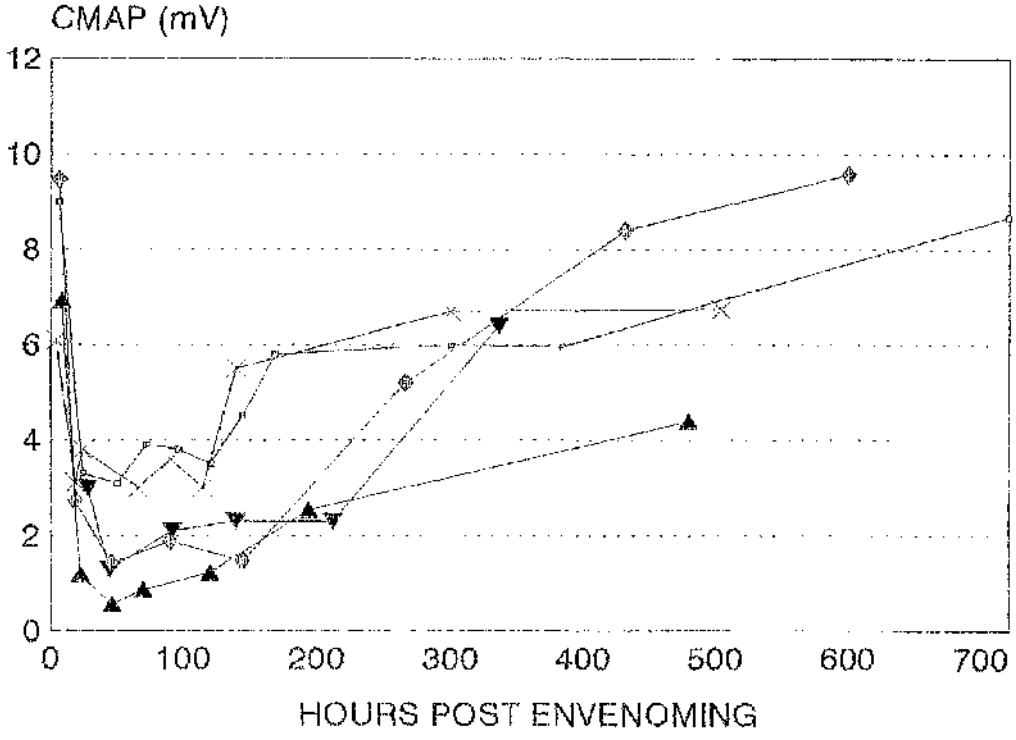

Fig 5.4 Sequential CMAP amplitudes recorded in abductor pollicis after stimulation of the median nerve. Studies from same patients as figure 5.3.

Figure 5.5 shows sequential grip strength readings from the same six patients. Each value is the mean of three measurements using a hand held dynamometer.

Figure 5.6 shows sequential studies of CMAP amplitudes recorded after stimulation of ulnar and median nerves from a single envenomed patient (S4193). Grip strength recordings are plotted on the same graph to show the correlation between the two.

Figure 5.7 shows the first section of Figure 5.6 in more detail with clinical events marked. At the time of discharge, the patient still had marked peripheral weakness and reduced CMAPs, both of which gradually improved over the following weeks.

iv. Repetitive nerve stimulation studies

Figure 5.8 shows the results of repetitive nerve stimulation tests in controls and envenomed patients with grade 4 or 5 neurotoxicity (ie. intubated +/- ventilated). Incremental or decremental changes are calculated by dividing the amplitude of the 4th or 9th CMAP in a chain of 9 by the amplitude of the first. This is expressed as a percentage and the table shows the mean values for control and envenomed groups. The significance of the observed differences between the two groups was calculated using the unpaired t-test.

Figure 5.9 shows the results of repetitive nerve stimulation studies performed after a high frequency stimulus of 50Hz for 5 seconds. Differences are shown between the calculated increment or decrement between the 1st and 9th CMAP in a train. In addition, the difference in the amplitudes of the 1st CMAP in the pre-tetanic train and the 1st CMAP in the post tetanic train are shown.

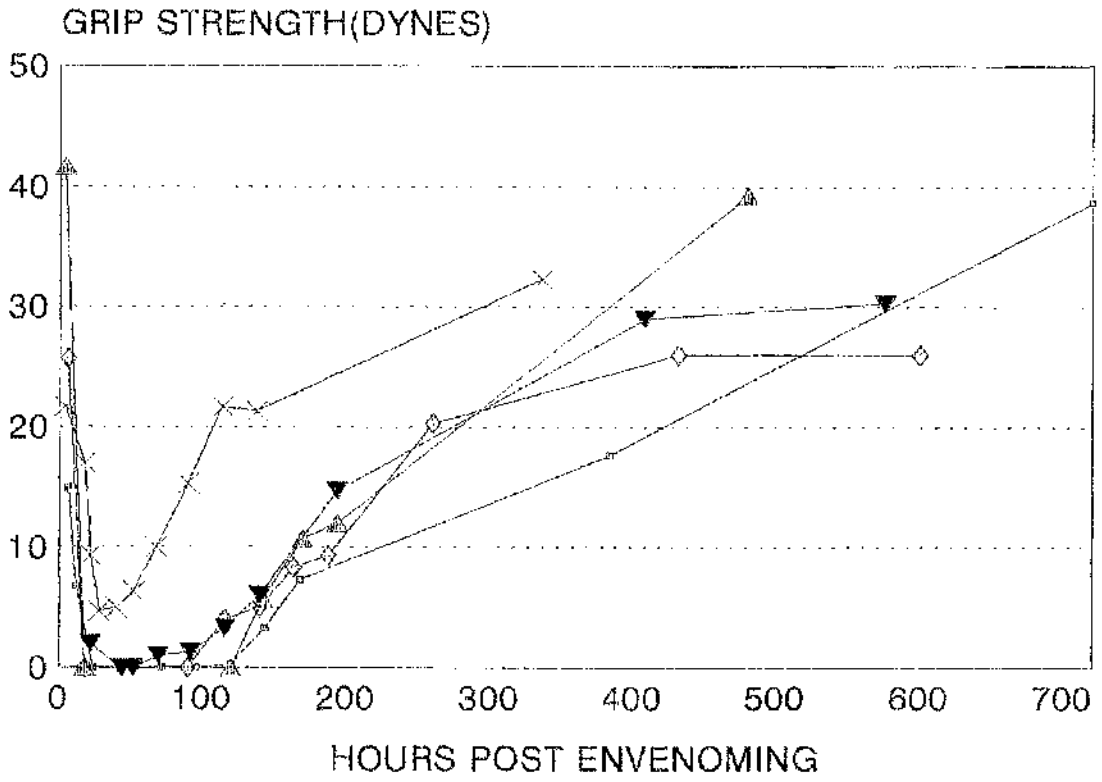

Fig 5.5 Sequential grip strength readings from patients shown in Fig.5.3 and Fig.5.4

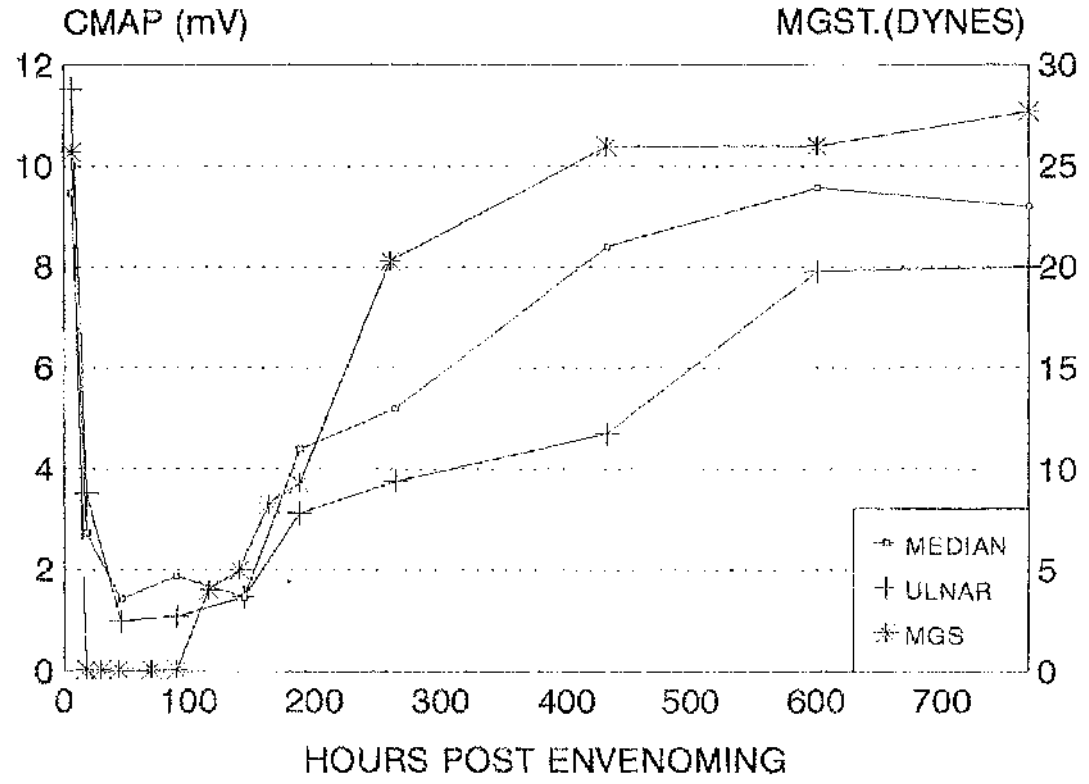

Fig 5.6 The relationship between CMAP amplitudes and grip strength readings in patient S4193 throughout clinical course.

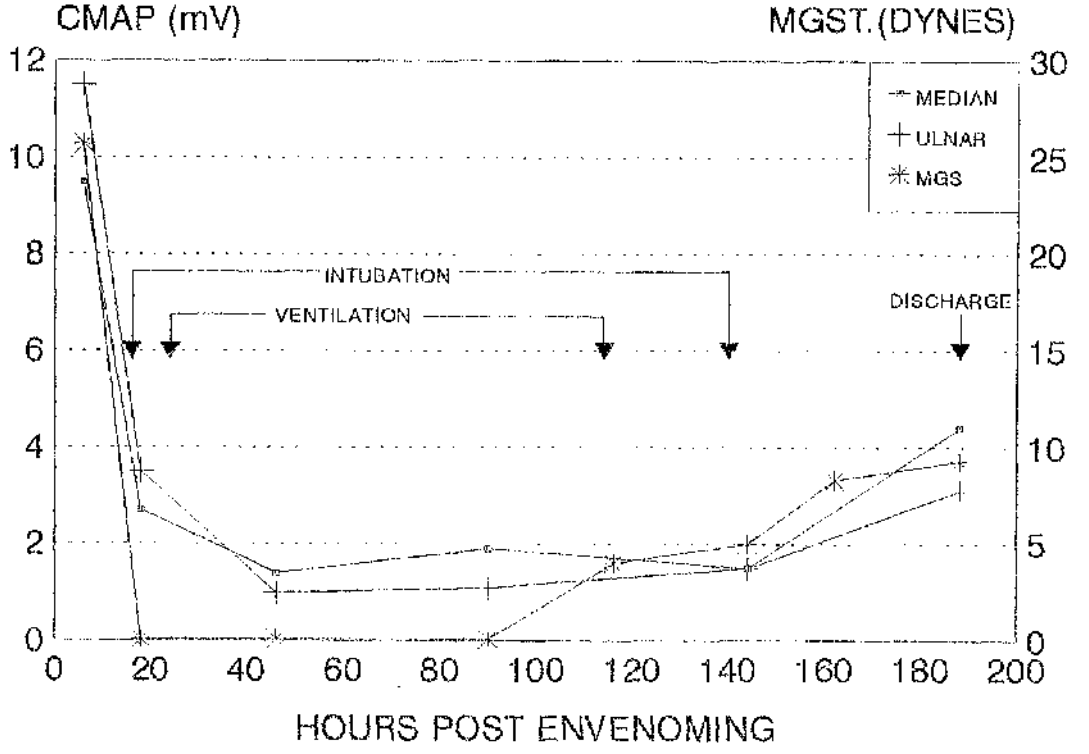

Fig 5.7 Sequential CMAP and grip strength readings in patient S4193. Patient admitted and antivenom given 6 hours after envenoming. Clinical events marked.

	MEAN CHANGE CMAP 9/1	95% Confidence limits for mean
CONTROLS N=22	104.2%	102.5-105.9%
ENVENOMED PATIENTS N=16 (STAGE IV/V)	92.5%	88.5-96.5%
	p<0.0001	

Fig.5.8 Changes in CMAP amplitude during a train of 9 stimuli at 3 Hz in control patients and in envenomed patients with significant neurotoxicity.

	POST-TETANIC CMAP 9th/1st	95% Confidence limits for mean	POST TETANIC.1st/ PRE-TETANIC.1st
CONTROLS N=15	106.5%	104.6-108.4%	103 %
ENVENOMED PATIENTS N=9 (STAGE 4/5)	66.7%	51.9-81.4%	174 %
	p<0.0001		p<0.0001

Fig.5.9 The effect of 5 seconds stimulation at 50hz on a subsequent train of 9 stimuli at 3Hz. and the relationship between the amplitude of the first post tetanic CMAP and the initial pre-tetanic CMAP

Figure 5.10 shows a typical decremental response at rest in abductor digiti minimi after repetitive stimulation of the ulnar nerve at 3 Hz. Subsequent recordings show the response following repetitive stimulation after sustained exercise of the muscle.

v. Needle electromyography

Insertional activity was normal in all patients studied. Recruitment was markedly reduced, maximally in patients at peak neurotoxicity with gradual improvement thereafter. Patients studied at follow up 10-14 days after envenoming still had reduced recruitment patterns but the morphology of the motor unit action potentials appeared normal. Spontaneous activity was seen in several muscle groups during the first 12-72 hours after envenoming. The most frequent abnormalities were fibrillation potentials and positive sharp waves and occasional fasciculation potentials were also seen. These abnormalities were most pronounced in association with clinically evident fasciculation which was seen in 20 patients, most commonly in gastocnemii but also occasionally in deltoids and pectoral muscles. Limitation of jaw opening was a common early sign seen in 37% of patients. Needle studies in the masseter muscles of 3 of these patients showed fibrillation potentials and positive sharp waves but no evidence of complex repetitive discharges.

INTERVENTIONS IN THE MANAGEMENT OF NEUROTOXICITY.

1. The effect of edrophonium in envenomed patients

Table 5.1 shows the mean and median percentage changes in CMAP amplitudes recorded in 11 patients following an intravenous dose of edrophonium or saline.

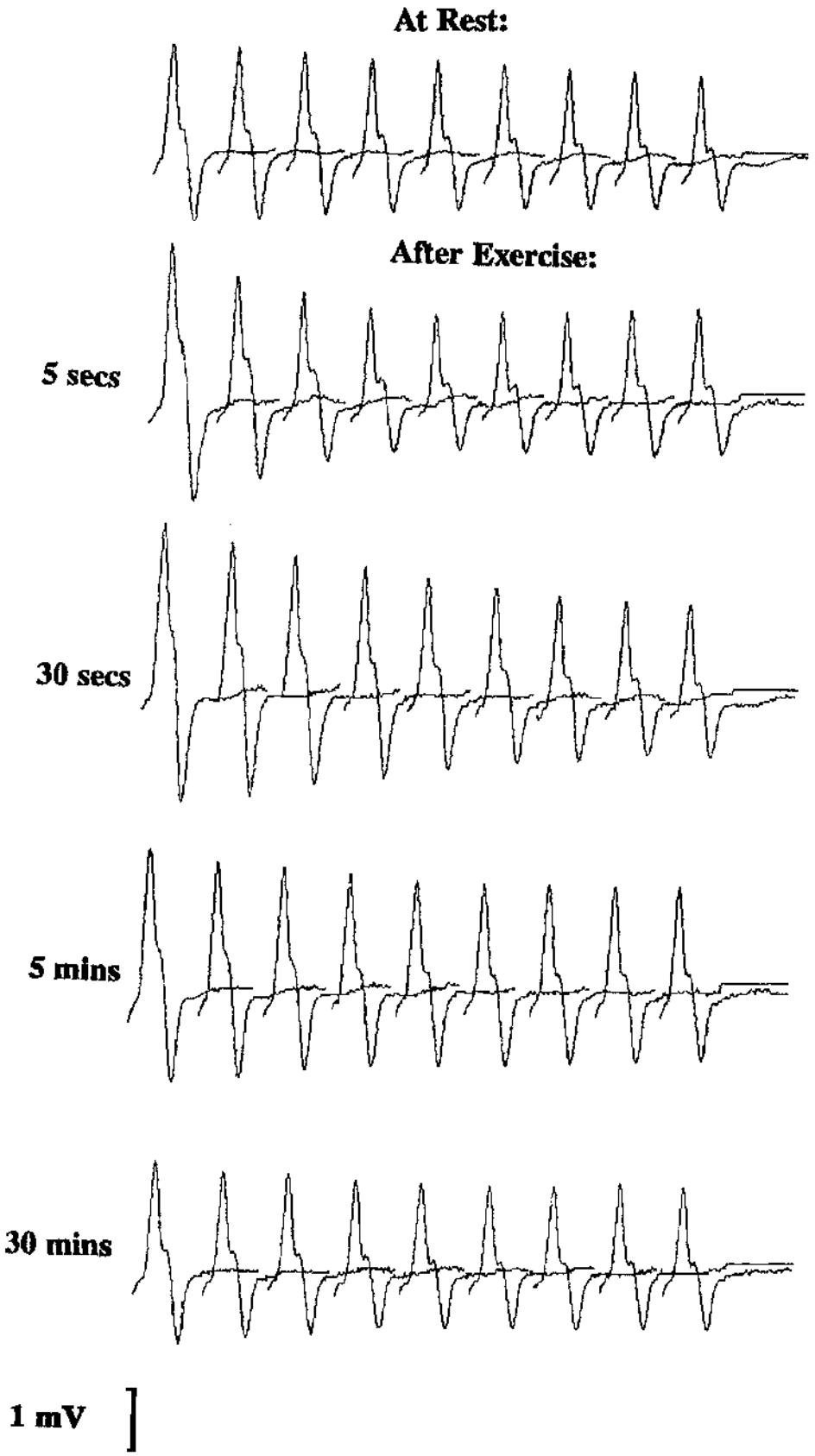

Fig. 5.10 showing CMAPs recorded in abductor digiti minimi after repetitive stimulation of the ulnar nerve at 3 Hz.

The probability values that the observed differences occurred by chance are shown. Table 5.2 shows similar data for grip strength recordings from the same patients. Figures 5.11 and 5.12. show the change in CMAP amplitude recorded after intravenous edrophonium and placebo. Figures 5.13 and 5.14 show the results of sequential grip strength measurements after edrophonium and placebo respectively.

Table 5.1 : Mean and median changes in CMAP amplitudes after edrophonium

Median values in brackets, n=11.

TIME (mins)	EDROPHONIUM	PLACEBO	p value*
T4	9.0% (8)	-0.5% (0)	0.001
T9	17.7% (15)	-0.5% (0)	0.001
T14	12.1% (5)	-0.5% (0)	0.004
T19	10.7% (8)	-0.4% (0)	0.001
T24	7.7% (4)	-1.0% (0)	0.005

Table 5.2 : Mean and median changes in grip strength after edrophonium

TIME	EDROPHONIUM	PLACEBO	p value*
T4	5% (0)	0.1% (0)	0.8
T9	2.8% (0)	-1.6% (0)	0.94
T14	6.9% (4)	-5.8% (0)	0.04
T19	2% (0)	-6.4% (0)	0.26
T24	2.2% (0)	-6.4% (0)	0.22

* p value calculated using paired t-test.

In the majority of patients edrophonium produced a small but measurable increase in the amplitude of the

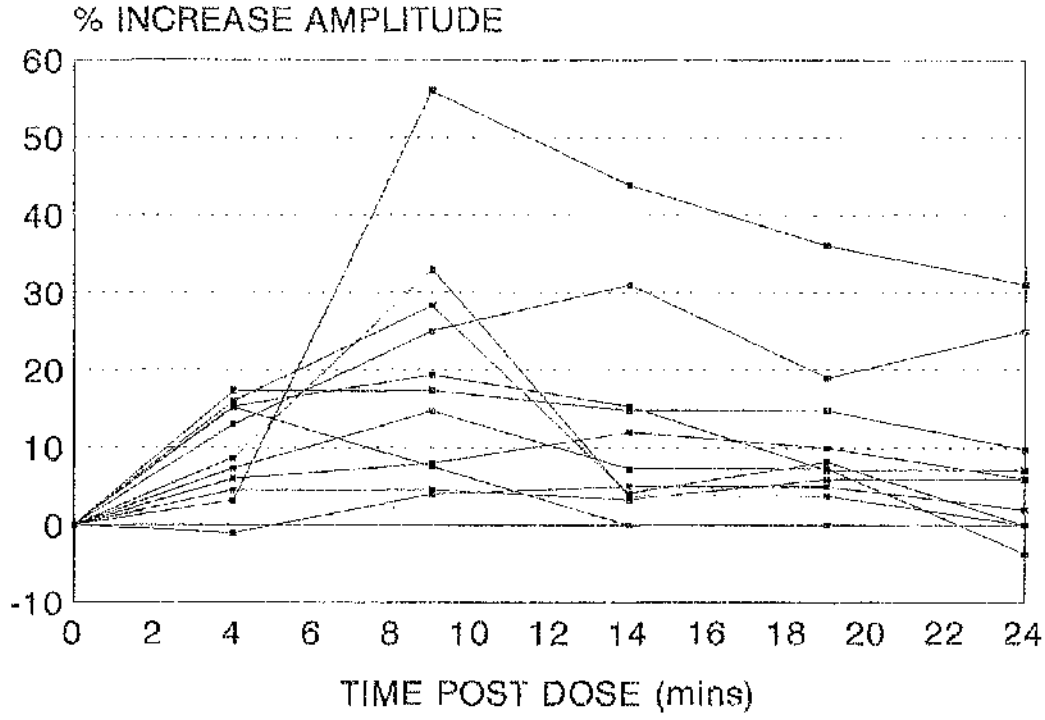

Fig 5.11 The effect of 10mg edrophonium on CMAP amplitudes recorded from abductor digiti minimi after stimulation of the ulnar nerve (n=11).

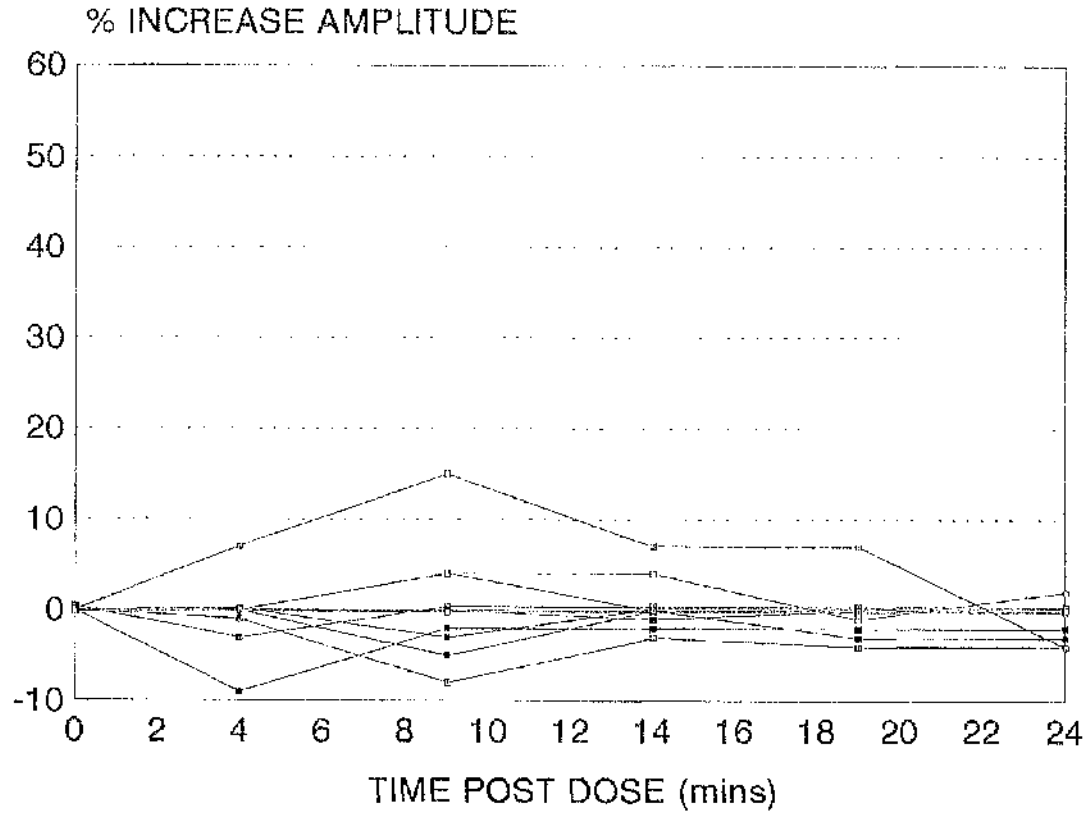

Fig 5.12 Sequential CMAP amplitudes after placebo.

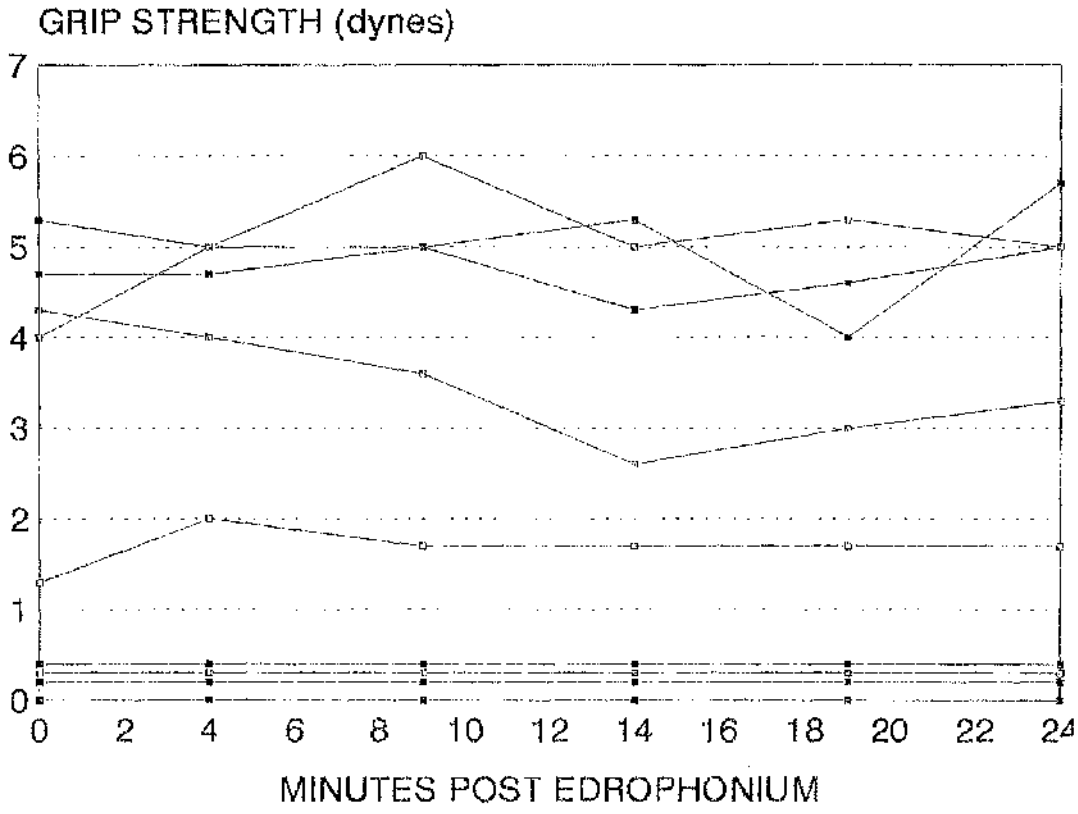

Fig 5.13 Grip strength following intravenous edrophonium (10mg).

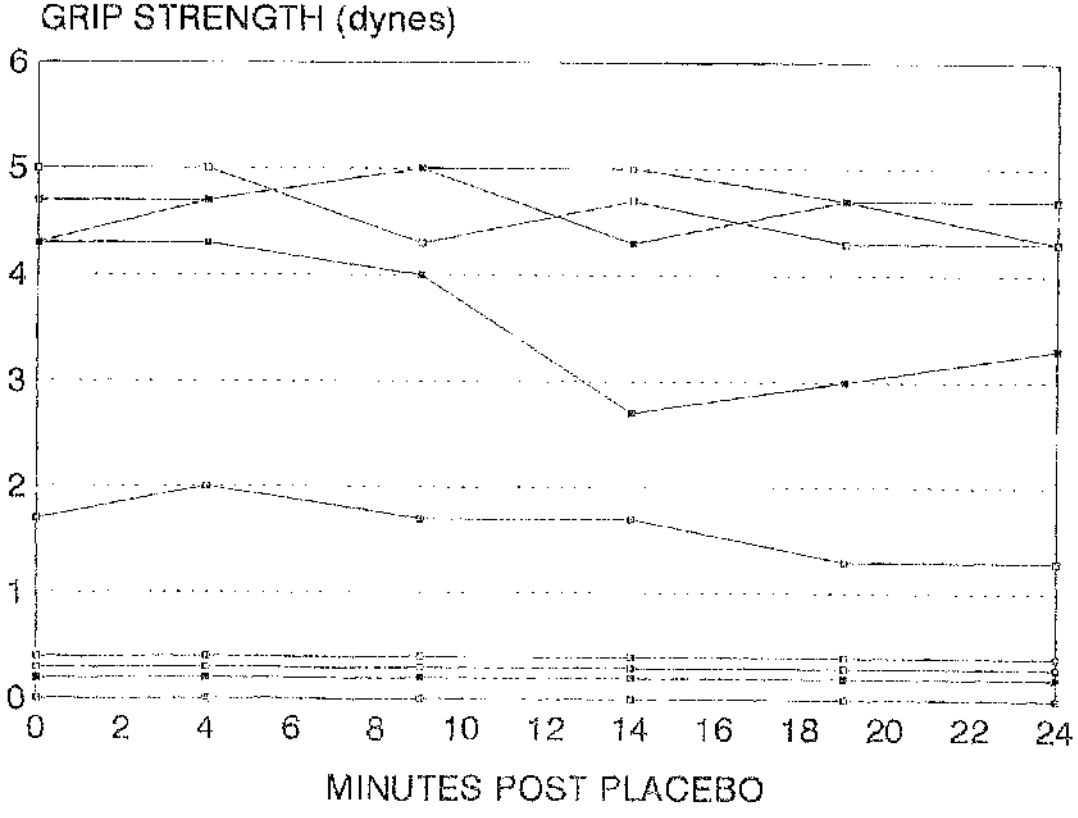

Fig 5.14 Grip strength after placebo.

CMAP measured over abductor digiti minimi. This was maximal around 9 minutes after the injection of the drug and was typically of the order of 15% (range -4% to 56%). Increases in CMAP amplitude were not generally seen in the placebo group. The difference between the active drug and placebo groups was significant up to and including T24. The majority of patients also showed a marginal increase in grip strength after edrophonium, mean increases are shown in Tables 5.2. Increases were also seen in some patients after receiving placebo and the difference between the two groups was not significant except at T14. In 3 patients there was a small increase in the range of eye movements after edrophonium. No change was seen in any patients after placebo. No improvement in the degree of ptosis or in the maximal extent of mouth opening was recorded in any of the 11 patients after either active drug or placebo.

Side effects

No side effects were observed or reported.

2. The effect of 3,4-diaminopyridine

Table 5.3 shows the mean and median changes in CMAP amplitude recorded after 10 mg 3,4-DAP and after placebo. **Table** 5.4 shows the mean and median changes in grip strength after active drug and placebo.

Table 5.3: Mean and median changes in CMAP amplitude after 3,4-DAP (n=12/6)

TIME	3,4-DAP	PLACEBO	p value*
T5	2.8% (3.5)	-0.8% (0)	0.28
T10	5.4% (4.8)	-3.7% (-4)	0.008
T15	6.5% (5)	-3.3% (-3.5)	0.005
T20£	6.4%	-3.6%	
T30	6.0% (9)	-4.7% (-5.5)	0.07
T45	2.0% (4)	-4.4% (-4)	0.04

£ only 6 and 5 readings respectively at T20.

Table 5.4 : Changes in grip strength after 3,4-DAP

TIME	3,4-DAP	PLACEBO	p value*
T5	5.5% (3)	3.0% (0)	0.58
T10	10.6% (0)	4.6% (0)	0.43
T15	12.1% (8)	4.6% (0)	0.09
T30	13.1% (6)	5.4% (0)	0.44
T45	10.6% (2)	-0.8% (0)	0.31

* p values calculated using unpaired t-test.

Figure 5.15 shows the percentage changes in CMAP amplitude after 10 mg of 3,4-diaminopyridine. **Figure** 5.16 shows the responses in the same patients after placebo. **Figure** 5.17 and **Figure** 5.18 show the results of sequential grip strength measurements.

The results of both CMAPs recorded in abductor digiti minimi and muscle grip strength after both 3,4-DAP and placebo were variable. The general trend was a small increase in amplitude recorded after active drug and also a small increase in grip strength. Neither was

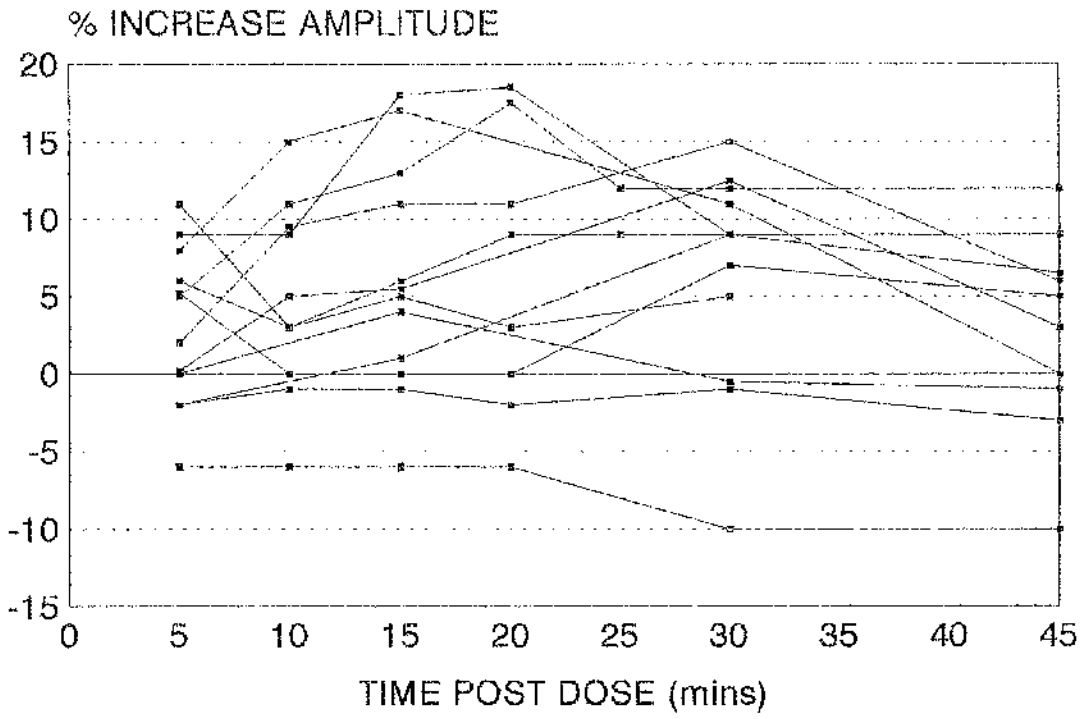

Fig 5.15 The effect of 10mg 3,4-diaminopyridine on CMAP amplitudes recorded from abductor digiti minimi.

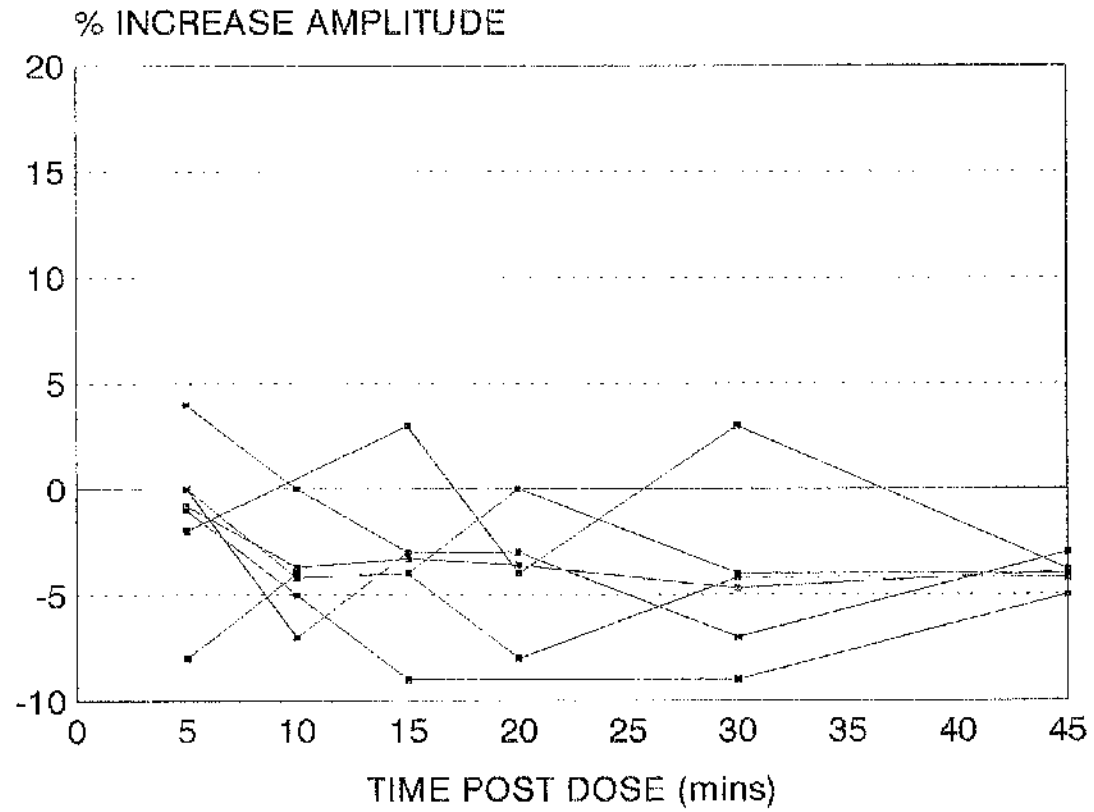

Fig 5.16 Sequential CMAP amplitudes after placebo

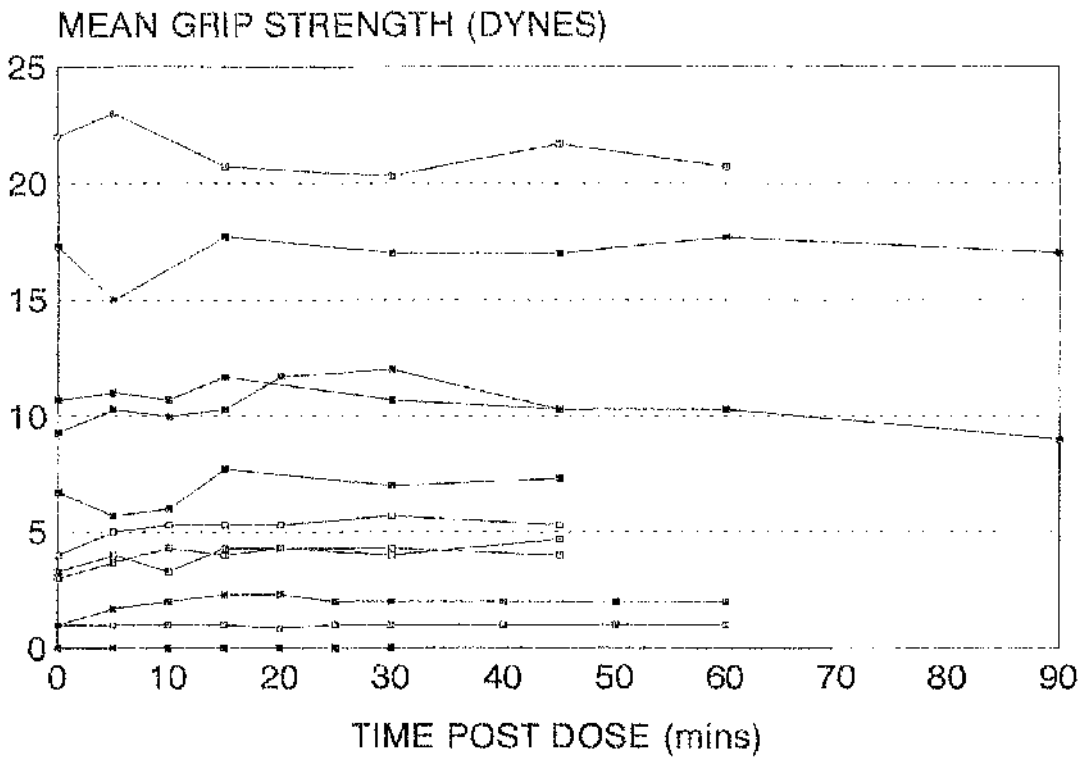

Fig 5.17 Grip strength after 10mg 3,4-DAP.

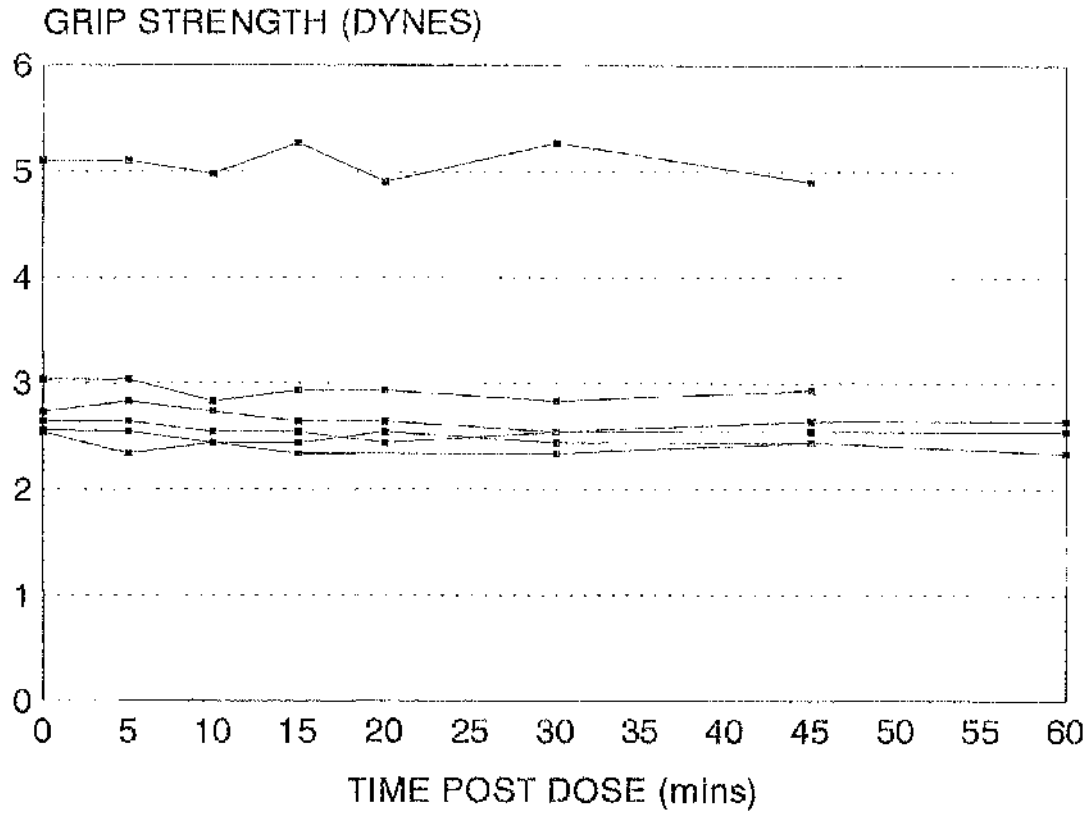

Fig 5.18 Grip strength after placebo.

impressive or uniform and the variation of the responses recorded was high. There was a difference in the amplitude response between the active drug and the placebo groups reaching significance between T10 and T30. There were patients in both groups whose grip strength improved and ranking of the responses showed no evidence of a significant difference between the two groups. In general, patients given placebo showed little change in either variable although one patient showed some improvement in grip strength in successive readings raising the suspicion of a learned response. It was noticeable however that those patients with initial unrecordable grip strength (ie. reading 0 dynes) did not show any improvement after either active drug or placebo. One patient showed a marginal increase in eye movements 10 minutes after injection of 3,4-DAP but in the other 11 there was no change in either degree of limitation of eye movements, degree of ptosis or jaw opening. Maximal inspiratory pressures were recorded in one patient who was intubated and ventilated. No changes were noted after either active drug or placebo.

Side effects

At the end of each test, the patient was asked about any symptoms occurring following injection of "the drug". Several patients reported transient discomfort along the site of the vein and all of these had been given active drug. Three patients reported perioral and peripheral paraesthesia around 5 minutes after injection, and several patients reported flushing, increased salivation and increased sweating. In 2 patients this was visible to the investigator. All 3 patients had been given active drug. No symptoms were reported from patients given placebo. One patient

needed to pass urine within minutes of receiving 3,4-DAP on two occasions. This did not occur on the occasion when she was given placebo. Widespread fasciculation was seen in one male patient, predominantly involving deltoids, pectoral muscles and gastocnemii. No ECG changes were detected after administration of the drug except for an increase in heart rate which was attributed to the administration of atropine.

3. The effect of edrophonium and 3,4-diaminopyridine in combination.

Table 5.5 shows the mean and median increases in CMAP amplitude after administration of 10 mg 3,4-DAP and 10mg edrophonium. Controls quoted for comparison are the patients given saline in the second section of the trial. The percentage changes in CMAP amplitude measured in patients given the combination of 3,4-DAP and edrophonium are shown in **Figure 5.19.**

Table 5.5 : Mean and median changes in CMAP amplitude after 3,4-DAP and edrophonium. (n=10/6)

	3,4-DAP/Edro	PLACEBO	p value*
T5	4.6% (4)	-0.8% (0)	0.13
T10	10.2% (5)	-3.7% (-4)	0.05
T15	19.3% (9)	-3.3% (-3.5)	0.002
T20	16.8% (13)	-3.6% (-3)	0.013
T30	17.6% (13)	-4.7% (-5.5)	0.002
T45	15.9% (13)	-3.8% (-4)	0.04
T60	16.7%		
T90	15.5%		

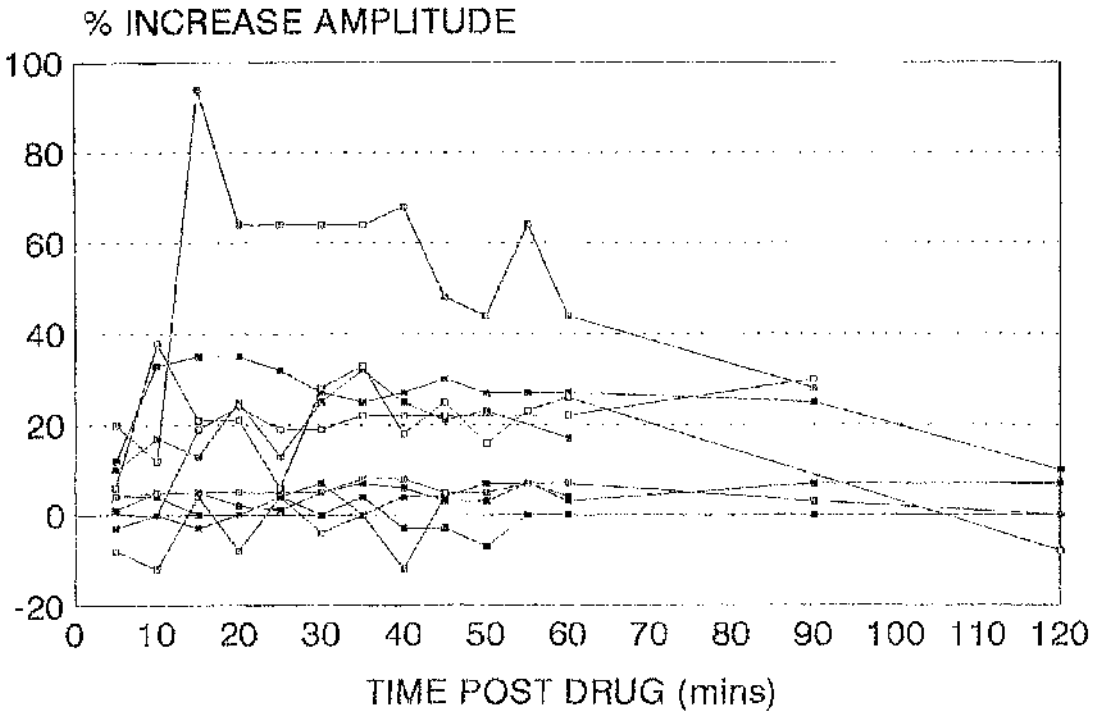

Fig. 5.19 Sequential CMAP amplitude measurements after 10mg 3,4-DAP followed by 10mg edrophonium at 7 minutes (n=10).

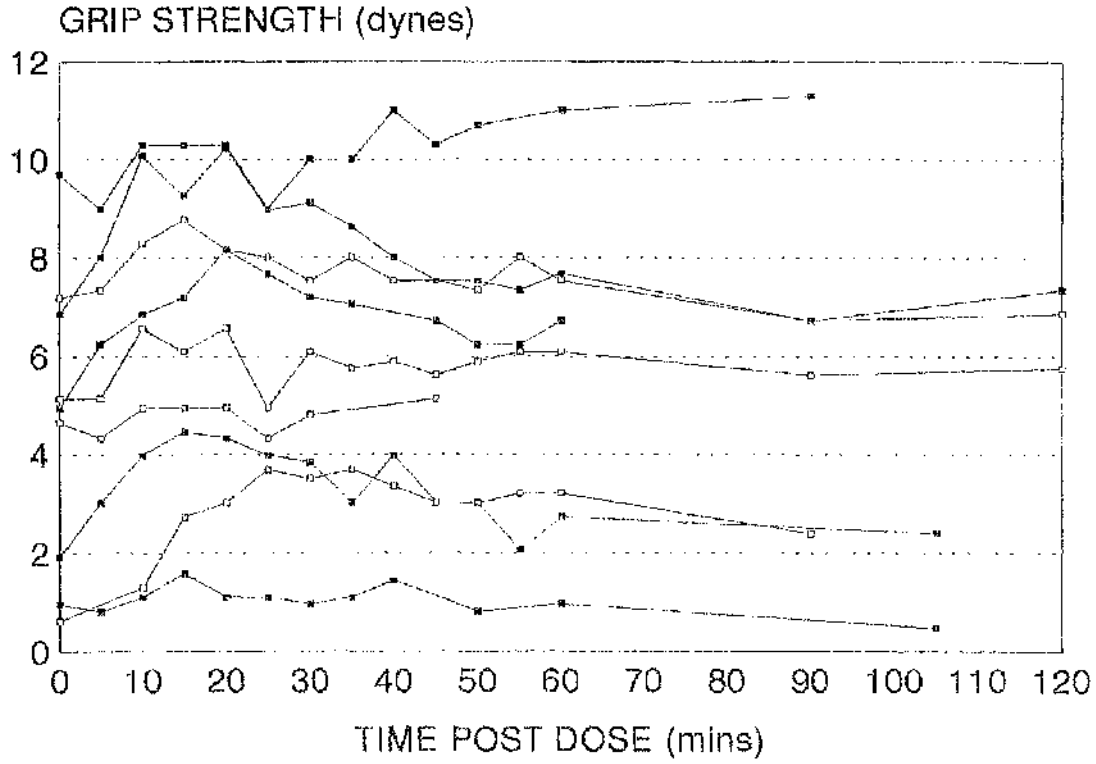

Fig 5.20 Sequential grip strength measurements after 10mg 3,4-DAP followed by 10mg edrophonium at 7 minutes.

Table 5.6 shows the mean and median changes in grip strength after administration of 10mg 3,4-DAP and 10mg edrophonium. **Figure 5.20** shows the results of sequential grip strength recordings in the 9 patients.

Table 5.6 : Changes in grip strength after 3,4-DAP and edrophonium (n=9˜/6)

	3,4 DAP/Edro.	PLACEBO	p value*
T5	5.5% (1)	-0.8%(0)	0.94
T10	33.8% (22.5)	-3.7%(-4)	0.01
T15	38.8% (27.5)	-3.3%(-3.5)	0.01
T20	39.5% (22.5)	-3.6%(-3)	0.01
T30	28.5% (21.5)	-5.1%(-7)	0.04
T45	20% (10)	-3.7%(-3.5)	0.03
T60	18%		
T90	8%		

* p values calculated using unpaired t-test.

˜ The calculated mean values in table 5.6 excludes one set of grip strength readings from a patient who showed a profound increase in grip strength from a very low initial average of 1.3 dynes to a maximum average of 7.7 dynes, an increase of over 400% after receiving 3,4-DAP and edrophonium. Whilst this increase appeared real, it would clearly bias the numerical values of the averaged data dramatically. A second dose of both drugs in the same patient also produced an impressive increase but to a lesser degree, from an initial average of 4 dynes to a maximum of 9.7 dynes. The results of CMAP and grip strength measurements made after doses of 3,4- DAP and edrophonium 27 and 46 hours after envenoming in this patient are shown in **Figure 5.21** and **Figure 5.22**. These were the largest percentage responses seen in any patient.

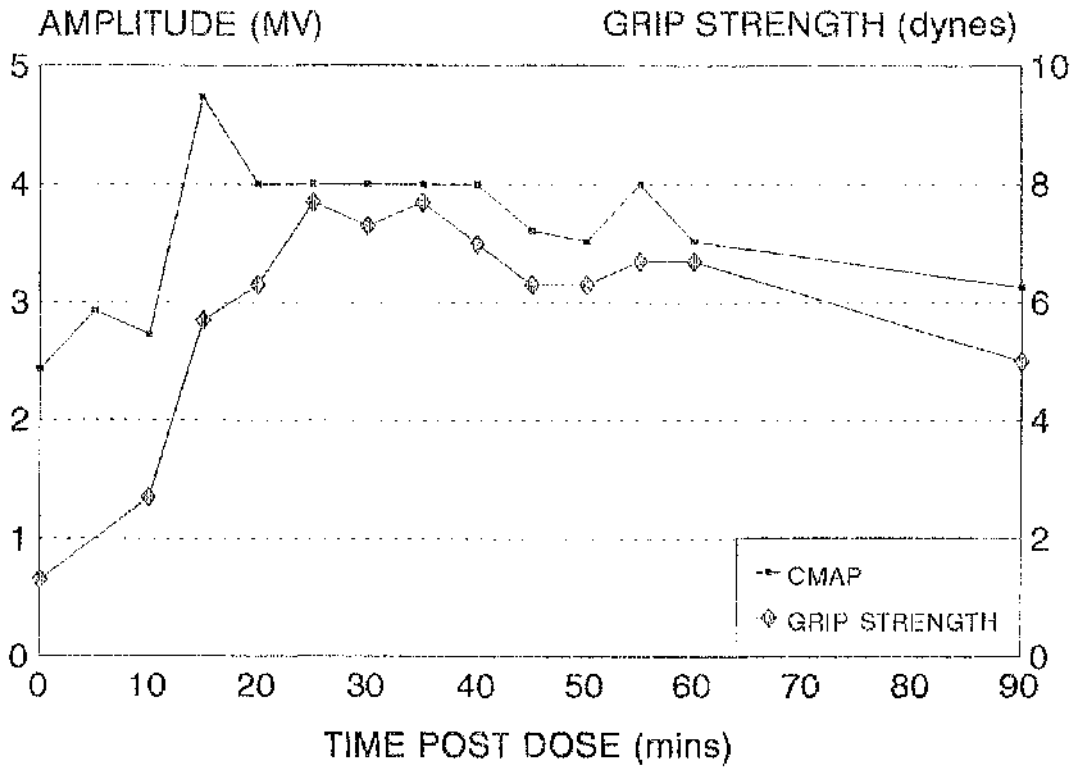

Fig 5.21 Sequential measurements of CMAP amplitude and muscle grip strength in patient S3693 after 10mg 3,4-DAP and edrophonium at 7 minutes. Study performed 27 hours after envenoming, clinical stage IV.

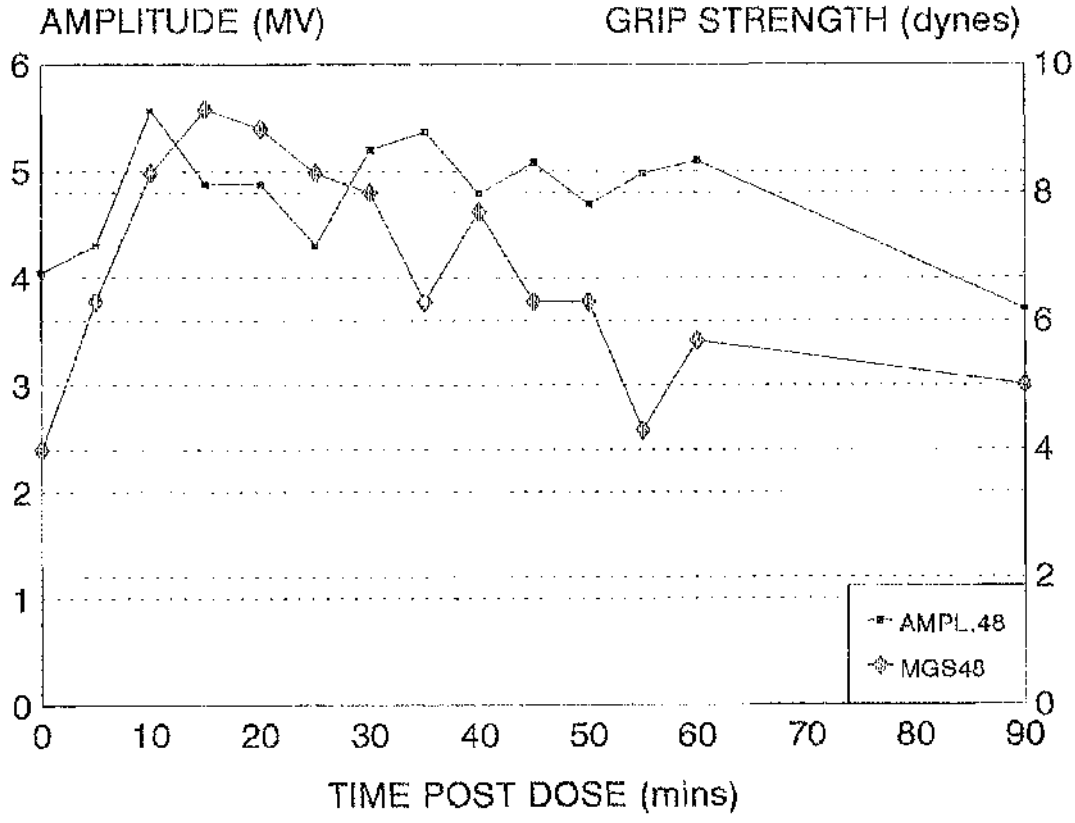

Fig 5.22 Similar recordings in same patient 46 hours after envenoming

Table 5.7 shows a comparison of the responses to a combination of 3,4-DAP and edrophonium and to 3,4-DAP alone from the studies reported above.

Table 5.7 : Mean change in grip strength and CMAP amplitude after 3,4-DAP with and without edrophonium

	GRIP STRENGTH			AMPLITUDE		
	DAP/Edro	3,4-DAP	p	DAP/Edro	3,4-DAP	p
T5	5.5%	5.5%	0.74	5.1%	3.2%	0.65
T10	33.8%	10.6%	0.06	10.2%	5.4%	0.6
T15	38.8%	12.1%	0.02	19.3%	6.7%	0.3
T30	28.5%	13.1%	0.28	17.6%	6.0%	0.29
T45	20.3%	10.6%	0.2	15.9%	2.1%	0.07

The combination of 3,4-DAP and edrophonium produced a measurable increase in both CMAP and muscle grip strength in the majority of patients to whom they were given. There was a correlation between the two responses, demonstrated in **Figures 5.19** and **5.20**. This response was significantly different from the responses seen in the saline control group at all times after T5. The size of the increases in both amplitude and grip strength in those patients who showed the biggest response was greater than in those who received 3,4-DAP alone. However when the two groups were specifically compared and ranked, there was only a significant difference in the grip strength responses at T15. In the majority of the study group, the maximal response in both variables was at around 10-20 minutes after the injection of the 3,4-DAP (8-13 minutes after the injection of edrophonium). One patient showed a very marginal improvement in eye movements from 10-30 minutes after the injection of the drug. Another

patient showed an improvement in the degree of ptosis from grade 3 to grade 2 between 10 and 45 minutes after the drug. Both of these patients showed an impressive increase in grip strength and CMAP amplitude at the same time. One other patient showed a convincing improvement in mouth opening, from a maximum inter-incisor distance of 22mm initially to a peak of 31mm corresponding with the maximal grip strength improvement (20-25 minutes). No other patient showed greater than a 3mm variation in mouth opening at any time. Maximal inspiratory pressures recorded in one intubated patient appeared to show a minor improvement around 20 minutes. Repetitive stimulation tests were performed on 5 of the 10 patients. Stimuli were given every 5 minutes at 125% threshold at a frequency of 3 Hz. There were minor variations between recordings but no trend emerged to show a significant change in the decremental response.

Side effects

There was a marked increase in oral secretions in 6 of the 10 tests requiring additional suction of the endotracheal tube or pharynx. Six patients felt hot and had a visible increase in sweating between 5 and 10 minutes after the 3,4-DAP. Five patients complained of pain along the vein proximal to the injection site. One patient complained of perioral paraesthesia after each of three injections of the drug. Fasciculation was seen in 5 patients most obviously in deltoids, pectorals and gastrocnemii, but also involving other muscle groups. One patient needed to pass urine within minutes of the injection, another catheterised patient showed a marked increase in urine production soon after the active drugs were given.

The patients included in the various intervention studies described in this chapter were studied at various clinical stages and at various periods of time post envenoming. The rate of progression of neurotoxicity varied considerably between patients. **Figure 5.23** and **Figure 5.24** show the maximal increases in amplitude recorded in each patient given an active drug and the time post envenoming (5.23) and the clinical stage at the time (5.24).

RESPONSE BY TIME POST ENVENOMING

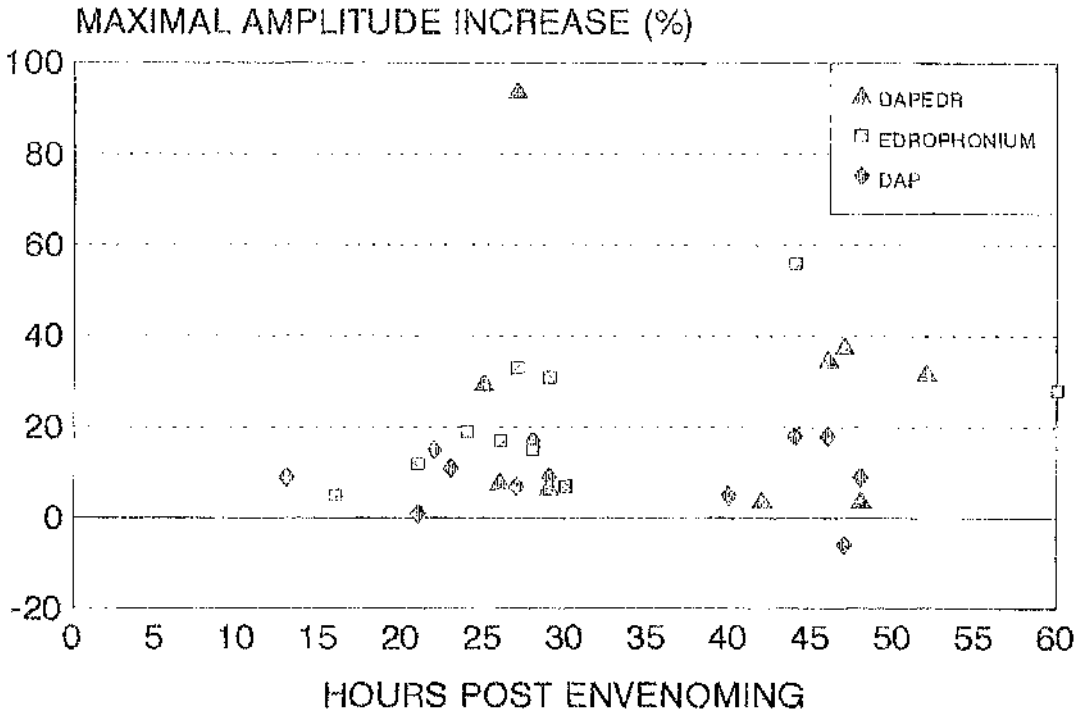

Fig 5.23 showing the maximal recorded amplitude increase with each intervention and the time post envenoming at which study performed.

RESPONSE BY CLINICAL STAGE

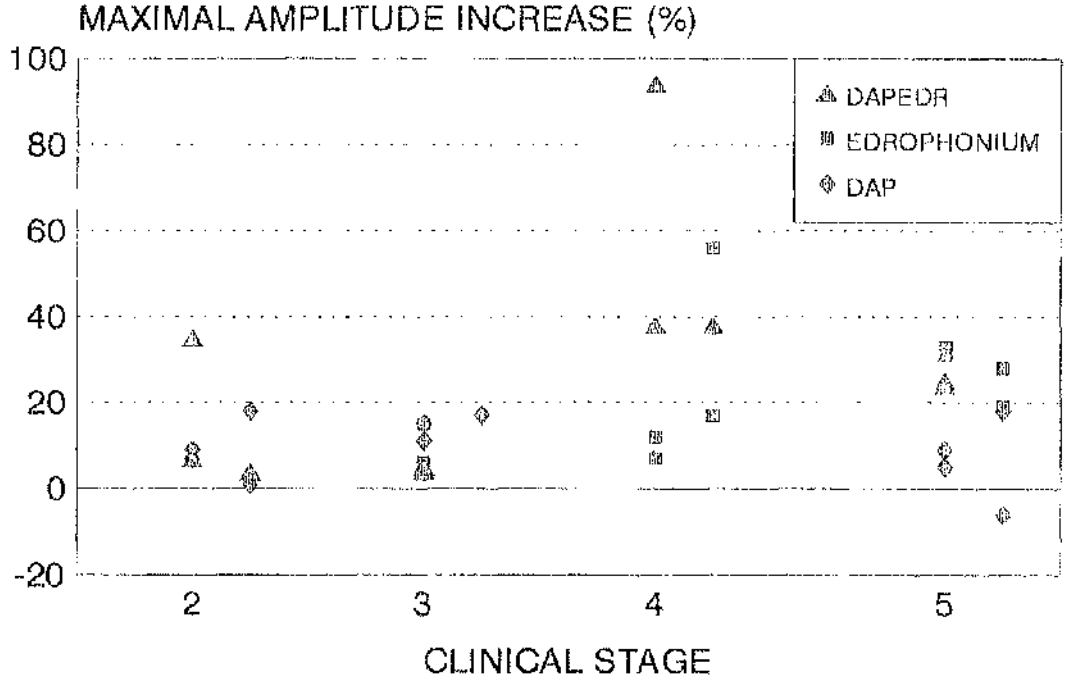

Fig 5.24 showing the maximal recorded amplitude increase with each intervention plotted against clinical stage.
See appendix 3 for clinical staging.

CHAPTER 6 - THE EPIDEMIOLOGY OF TAIPAN BITE

1. Who gets bitten? When, where and why?

i. Incidence and mortality rates

Incidence rates of snakebite are calculated in different ways by different authors and it is difficult to compare the results of studies, both within Papua New Guinea and between different areas of the tropics. This study distinguishes between incidence rates of definitive envenoming and incidence of reported snakebite. Envenoming is defined as the presence of one or more unequivocal signs of systemic envenoming witnessed by the study team, including lymphadenitis, prolonged clotting, bleeding and neurotoxicity. The estimated incidence of envenoming and mortality rates for Central Province (55 and 3.2/100,000) are underestimates of the true figures because they do not account for envenomed patients who were managed at health centres without referral to Port Moresby General Hospital (PMGH), or who died before or during referral. There were at least 8 patients who died before arrival at PMGH during the study period. If these patients are included in the calculation, the estimated rural mortality rate is almost doubled to 6.1/100,000/year. By comparison, the mortality rate for snake bite deaths in the whole of Sri Lanka between 1975 and 1979 was 5.3/100,000[96] and in Myanmar was greater than 3.3/100,000/year in 1985[97].

All patients with suspected snakebite in the urban area of National Capital District (NCD) are referred to PMGH so the mortality and incidence figures (0.5 and 9.8/100,000/year respectively) are likely to be more reliable than those quoted for Central

Province. These values are lower than those calculated by Currie (2.1 and 21.8/100,000)[30] and lower than the calculated urban rate of envenoming found by Campbell (30/100,000)[19] and Lalloo (15.5/100,000)[2]. The trend towards a fall in the number of reported snake bites in NCD over the past 30 years is almost certainly explained by the increased urbanisation of NCD and the disappearance of habitat. The estimated incidence rates quoted for all reported snake bites (envenomed and non-envenomed) in the year May 1991-May 1992 are likely to be reasonably accurate for urban NCD but significant underestimates for Central Province. Relatively few non-envenomed patients are referred to Port Moresby from distant health centres and none of the patients managed at health centres alone are counted in the estimates. An analysis of health centre records suggested that the actual total number of bites from Central Province reported to the medical services between May 1991 and May 1992 was in the order of 200, an incidence of 140/100,000.

Figure 3.1 shows that there were also marked differences in the number of bites from different geographical areas of rural Central Province. No attempt has been made to estimate more localised incidence figures from these areas because there is insufficient information on variables such as local population numbers, population movements and locally reported bites to be able to calculate such figures with any accuracy.

ii. Biting species and area of bite

The areas where the 117 patients in this series were bitten are shown in **Figure 3.1**. With the exception of a single bite from the western edge of the Sogeri plateau (1100 feet above sea level), all the bites were

from the low lying coastal belt of Central Province. Slater saw a live taipan at Sogeri[12] and it is interesting to note the presence of a confirmed bite from this area in this series. It is clear that the Papuan taipan is by far the most important venomous species in Central Province and this may well have changed since the studies of Campbell in the early 1960's when he believed the Papuan black snake to be the most common venomous snake in the area[27]. Currie et al. drew attention to the fact that taipan bites had become much more common[30,98] and this is borne out by the findings of this study. During the two year study period, taipan bites accounted for 90% of all envenomed patients in whom a definitive diagnosis of biting species was made and a similar proportion of envenomed patients in whom venom antigen was detected. Papuan black bites are now extremely rare amongst snake bite admissions to PMGH, causing no definitive bites and only one "probable" bite, during the two years of the study. Because of the difficulty in establishing definitive diagnoses in earlier studies it is impossible to say with certainty to what extent the relative frequencies of bites by different species have changed, but the evidence certainly suggests that they have. In Campbell's paper on patients envenomed by the Papuan black snake, only one of the 13 snakes responsible was definitively identified[27]. His assertion that the characteristic red back of the taipan was sufficiently distinctive to be identified and described by the victim was not borne out by this series (see below).

One possible explanation for the apparent rarity of the Papuan black snake is that the decline in numbers is associated with the introduction of the cane toad (*Bufo marinus*) to Central Province in the 1940's. The toad was initially introduced to Queensland from

South America in an attempt at biological control of the Grey Cane beetle whose larvae damaged the sugar cane crop. The toad secretes toxins from its neck glands which are toxic to snakes. Papuan black snakes and death adders both feed on amphibians whilst taipans do not[99] and it may be that the decline in numbers of the former may have created an expanded ecological niche for the latter. The herpetofauna of Queensland appears to have changed significantly over the past 40 years for the same reason[18]. Four freshly killed specimens of *Pseudechis papuanus* were identified during the course of this study, two from Vaifa'a in the west of the province, one from Moreguina in the east and one from Wasur in eastern Irian Jaya. It is interesting that these are areas where the cane toad is currently either rare or absent. Whether this is coincidence or supporting evidence for the cane toad theory is open to conjecture. Other environmental changes such as deforestation and changes in population distribution may also have influenced the distribution of snake populations.

The Papuan black snake continues to have great mythological and cultural significance among the Mekeo[100], Motu[101,102] and other coastal groups of Central Province. Taipans are usually dark in colour and both it and the whipsnake (*Demansia spp.*) are commonly mis-identified as the Papuan black snake. Only one patient out of 117 in this series confidently identified the snake that bit him as a taipan. The majority of patients described a long black or brown snake and identified this as a Papuan black snake. Thirteen patients who noticed red colouration in the snake still believed that they had been bitten by a Papuan black. Even dead taipans, shown to groups of both rural and urban dwellers, were frequently mis-identified as Papuan black snakes. Some communities in

Papua New Guinea and Irian Jaya make no distinction between the two species, either regarding them all as black snakes or as different sexes of the same snake, a belief reported from Western Province[103] and also related to me in two villages in south-eastern Irian Jaya. For the health worker treating the victim of a bite, the distinction between the two species is important and a patient's description of the biting snake as a Papuan black must be regarded with caution when selecting antivenom.

The use of immunoassays to identify retrospectively biting species allows a more rational approach to the use of antivenom than has previously been possible. At the time of writing, the Papua New Guinea Department of Health continues to buy black snake antivenom for distribution throughout Central Province (91 vials were purchased between January 1991 and the end of March 1993, at a total cost of £36,832). From the results of this study, black snake antivenom appears to have no therapeutic role in the treatment of snakebite in Papua New Guinea. There is no evidence that black snake antivenom is of any value in the treatment of bites by other elapid snakes. The use of black snake antivenom may prevent patients receiving appropriate antivenom with potentially detrimental consequences. The misconception about the perceived over-riding importance of *Pseudechis papuanus* is likely to persist for some time despite attempts at education of health staff. If polyvalent antivenom, which contains both taipan and black snake antivenom, is always used in cases of suspected Papuan black bite instead of black snake antivenom, that misconception will be less dangerous. The simplest way to achieve this would be to stop distributing black snake antivenom and this recommendation will be made to the Papua New Guinea Department of Health[104].

iii. Age and sex of victim

There was a preponderance of male patients bitten in this series (64%), very similar to the figure of 62% found by Currie in his nationwide study of envenoming between 1987 and 1989[30]. Campbell found a male to female ratio of 4:1 in the 1960's[19]. Currie suggested that the proportional increase in the numbers of females bitten has been due to the change in the demography of patients admitted to PMGH, from a predominantly urban population, with male predominance, to a predominantly rural one with much less marked differences between the sexes. Males still seem to be at greater risk in both populations. The most obvious explanation for the greater frequency of bites in males is that it reflects the greater exposure of the male population to snake ridden areas, but there may be other factors. Some high risk activities such as hunting are almost exclusively male activities but others such as gardening and collecting wood are predominantly done by women. 46% of the women were bitten in the garden compared with only 16% of the men. Gardening is usually a collective activity for women and children and it may be that snakes are more likely to be disturbed and to flee from a noisy group than from a lone male walking through the bush. Snakes are more likely to be seen on well worn paths and in cultivated gardens than on overgrown bush tracks. Males may be more likely to display bravado when they see a snake and by attempting to kill or capture it, expose themselves to an increased risk of being bitten. At least 2 of the males in this series were bitten avoidably, whilst attempting to kill the snake. This study suggests that a combination of differential exposure and behavioural factors are relevant.

The age distribution of patients was similar to that found by Currie[30], 24% of the patients were under the age of 15 compared with a figure of 25.9% in Currie's study. Over 80% of bites occured in patients between the ages of 11 and 45 during what might be considered as their independent, active and productive years. Currie found that the female:male ratio increased in rural patients aged 30 years old and over, a result which he attributed to older women spending a greater proportion of their time working in the gardens. This was not borne out by the results of this study which, if anything, showed the opposite.

iv. Month and time of bite

Few Melanesians in rural areas wear watches so the times of bites given by patients must be regarded as loose approximations. Despite this, two reliable observations can be made, first that few bites occur outside daylight hours and those that do occur soon after nightfall; secondly, that the majority of bites occur in the middle of the day. The two factors which influence the distribution of the bite times are the activity of the human population and the activity of the snakes themselves. Taipans are typically diurnal and crepuscular[12,13]. In this series, only 4.5% of bites occurred during the hours of darkness and all of these were just after dusk. In Madang Province, where the two major venomous land snakes (*Micropechis ikaheka* and *Acanthopis spp*) are more active at night, Hudson reported 33% of bites occurring after nightfall[105]. The majority of taipan bites occurred while the victim was engaged in activities related to subsistence farming. These included: working in a vegetable garden (a "garden" is typically a piece of cleared land which may be some distance from the village and often surrounded

by uncleared bush); en route to or from a garden; collecting wood; hunting or even urinating in long grass. The majority of these are daytime activities. It is no surprise that the incidence of bites in the rural population was considerably higher than that of the semi-urban population of NCD, and appears to reflect the degree to which a given population is involved in "at risk" activities.

Rainfall may influence the number of patients bitten by snakes by affecting both human and snake behaviour. After rain, subsistence farmers work in their gardens while during long, dry spells they may do relatively little. The movement and activity of both snakes and the prey upon which they are feeding are also likely to be influenced by the availability of water sources, which could either increase or decrease their proximity to humans. There is a distinct wet season in Central Province, typically between December and March. Bell commented that taipans appear to be most active at the beginning of this period[106]. The data shown graphically in Figure 3:4 suggests that there was little seasonal variation in numbers of bites during the two years of the study. Although there appear to be more taipan bites in the second year of the study, the total number of clinically envenomed patients was very similar. This suggests that this trend is due to a higher pick-up rate in venom antigen testing, perhaps due to better technique with taking swabs and aspirates or with the assay. There is some suggestion that periods of exceptionally high rainfall at any time of year were followed by higher bite rates. In 1992 however, after several exceptionally dry months, the incidence of all bites began to climb despite the continuing absence of rain. It is possible to hypothesize that this reflects the increasing necessity for snakes to proximate to human populations

to find food and water. Although an anecdotal and unsubstantiated observation, we noticed that a heavy rainfall after a dry spell was commonly associated with the admission of several envenomed patients within a few hours. It seems likely that rain did cause an increase in the number of bites for the two reasons given above, but that there was little difference overall between wet and dry seasons. It is unclear whether other facets of snake behaviour which may be seasonal, such as breeding habits, have a significant influence on movement, feeding and the incidence of human bites.

v. Circumstances of the bite

The vast majority of victims of taipan bite are bitten below the knee whilst walking or working in long grass or an overgrown garden. Almost all victims were unshod, those who were wearing shoes were bitten above rather than through them. These observations are not surprising and were the same as the conclusions reached by Campbell[19] and Currie[30]. Although envenoming by an Australian taipan has been described which occurred following the penetration of fangs through a boot[31], it is probable that if the rural population of Central Province were supplied with, and wore, calf length boots in which to work, the number of envenomings would fall dramatically. Whilst this is an impractical proposition for the majority of the population, it would not be unreasonable to expect employers to supply boots to employees facing extensive exposure, such as those working on rubber plantations, coconut plantations and cattle ranches for example.

The vast majority of bites resulted from the victim inadvertently disturbing the snake at close quarters with one case where a patient was bitten after

attempting to pick up the snake. This is consistent with Slater's description of the Papuan taipan as "a shy and retiring snake, preferring flight when encountered but if interfered with, defending itself readily"[12]. Twelve of the 51 patients in whom the number of bite site puncture wounds could be clearly distinguished, had more than two. It is impossible to say whether these wounds represent multiple bites. Campbell pointed out that some apparent "fang marks" may be wounds caused by the snake's palatine teeth or teeth of the lower jaw[19]. There was no apparent association between the number of puncture wounds at the bite site in this series and the level of venom antigen subsequently detected in serum, swab or aspirate.

2. What happens after a bite?

i. Pre-hospital treatment

The delay which many patients experienced before arrival at a health centre and the further delay which occured at the health centre and during transfer to PMGH emphasises the importance of first aid measures. In the bush, potentially harmful practices are still widespread. Tourniquets were used by nearly a third of the patients in this series, made from a variety of materials including grass, rope, pieces of rubber and cloth. The majority of these were not arterial tourniquets and did little damage but one 10 year old boy was seen during the study period who lost the use of a leg due to prolonged ischaemic injury (Plate 3.2)[95]. Nineteen patients had incisions made around the site of the bite, usually with a razor blade, stone or piece of glass. Several patients with venom induced coagulopathy had prolonged bleeding from these cuts

requiring treatment with pressure bandages after admission to hospital. Eight patients were admitted to hospital with pressure bandages applied to delay absorption of venom. Seven of these were referred from a health centre (6 referred by the same person), one patient had the bandage applied by a companion at the time of the bite. The majority of health centres did not apply pressure bandages, perhaps on the assumption that having given antivenom, there is no benefit in doing so. It might be argued that for patients who face a protracted journey to hospital, a pressure bandage should be applied whether or not antivenom has been given, in an attempt to continue to delay the absorption of venom. The value of pressure bandages in delaying the absorption of venom is still open to debate but the consensus seems to be to encourage their use at present[107-109]. For patients bitten in the rural tropics, where any increase in the "safety period" for a patient to reach hospital may be life saving, I believe that there is a good rationale for continuing their use. Clearly further efforts need to be made to disseminate this message in Papua.

Only 20 of the 52 patients who presented initially to a health centre were given antivenom before referral to PMGH. The health centres frequently did not have antivenom in stock and this was why so few received it. Three of the 20 patients who were treated were given inappropriate antivenom. All were given black snake antivenom, an error which can be prevented by further education of health centre staff and by halting the supply of black snake antivenom. It is recommended that promethazine and adrenaline should be administered with antivenom[87,88], but this was not always done. The necessity of prophylactic adrenaline may be questioned[110] but at present, health centres administering antivenom should follow the guidelines

issued in the standard treatment books which advocates its use[87,88]. Several patients were admitted heavily sedated after inappropriately high doses of promethazine, a potentially dangerous situation in the presence of compromised gag reflexes and pharyngeal pooling of secretions[111]. The recommended doses, accounting for body weight, should be adhered to.

ii. First point of contact

Slightly fewer than 50% of patients presented initially to a health centre before referral to PMGH and the majority of these were seen at one of only four health centres. The median time of arrival after the bite was slightly longer in patients presenting directly to hospital (2.75 hours) than it was in patients who presented at a health centre (2 hours). More significantly, the median time of arrival at PMGH of patients who had been seen initially at a health centre was 7.5 hours. It is believed that the earlier that antivenom is given, the greater the benefit the patient receives from it and the evidence from this study (see Chapter 7) suggests that antivenom is of maximum value if received within 4 hours of envenoming. It may not always be possible for all health centres in Central Province to stock antivenom, because of the cost of the drug and the necessity for a reliable cold chain. The four centres who see the majority of the patients however, (Bereina, Vaifa'a, Kwikila and Kupiano) clearly must have appropriate antivenom in stock at all times. These health centres should be targeted to improve early management of victims in their area and could be encouraged to exchange information with the hospital. This should include informing each health centre of the details of species biting in their catchment area, of the appropriate

antivenoms to use and which patients to refer to Port Moresby[112]. Simplification of antivenom prescribing policies will make this easier to achieve[104]. Staff at all health centres should be encouraged to utilise existing radio links with PMGH to discuss the management of patients about whom any uncertainty exists.

CHAPTER 7 - CLINICAL ASPECTS OF TAIPAN ENVENOMING AND ANTIVENOM TREATMENT

1.Inclusion criteria

Admission to this study was on the basis of unequivocal clinical evidence of envenoming subsequently confirmed to be due to a taipan bite by the detection of irrefutable levels of taipan venom antigen. Levels of venom antigen in serum are very labile and were influenced by prior administration of antivenom and the time elapsed since envenoming. There was no clear association between levels of antigen detected and clinical outcome. Some patients had relatively high circulating levels of taipan venom antigen in their blood without clinical signs of envenoming including 15 of the clinically non-envenomed patients who had levels of serum venom antigen >18ng/ml. One possible explanation for the lack of correlation between the level of venom antigen and clinical signs of envenoming is that the main antigenic component of the venom is not one of the most clinically significant toxins. A second factor which may be relevant is that it is bound rather than circulating toxin which produces many of the clinical effects. The detection of venom at the bite site does not necessarily imply systemic envenoming, a factor of considerable importance for those using venom detection kits.

The main aim of the criteria used for inclusion of patients in this study was to guarantee unequivocal clinical envenoming by a taipan. Some background activity was detected in immunoassays in the serum of control subjects. This is taken into account with the cut-off levels chosen at greater than two standard deviations from the control mean to exclude false

positives. Background activity is less likely to be a problem with swabs, aspirates and urine samples. Lower concentrations of venom antigen could probably be safely regarded as significant in these specimens, but in the absence of adequate control data to prove this, 18ng/ml was used as a cut-off for all samples. The use of stringent defining criteria is likely to have produced an underestimate of the total number of taipan bites admitted to PMGH during the two years of the study but guarantees that those patients studied were bitten by taipans.

2. Clinical course of envenoming

i. Early symptoms and signs

The clinical findings of this series of taipan bites are consistent with the findings of Currie et al. and of those reported elsewhere[1,2,113]. Currie also identified his taipan bites on the basis of antigen detection in serum, bite site swab, aspirate or urine sample. He used an assay cut-off venom antigen level of 5ng/ml[1]. A higher cut-off level has been used in this series based on findings in the control series (details above). Lymph node pain was present in 95% of envenomed patients seen by Currie, abdominal pain was reported by 80%, vomiting by 60% and headache by 75%. Clinical bleeding and/or prolonged clotting was seen in 44% of patients and neurotoxicity in 81%. The main difference from Currie's series is that a higher proportion of envenomed patients had prolonged clotting (78% v.44%). It is interesting to note that the median time of onset of neurotoxic signs in both series was 6.5 hours after envenoming, considerably later than that described by Senanayake et. al in their discussion of other elapid neurotoxins[114,115]. This emphasises the

need to observe patients who claim to have been bitten by a snake, for a minimum of 12 hours before dismissing the possibility of envenoming.

17.6% of patients in this series collapsed within minutes of the bite, a similar proportion to the 24% reported by Currie. The mechanism of collapse is not clear. Fourteen out of 18 patients appeared to have recovered full consciousness by the time of admission to hospital with no indication of the cause of collapse, 3 others had evidence of cerebral insult and one evidence of a fundal haemorrhage. Possible explanations for the collapse include: vasovagal attacks, hypotension secondary to the effects of vasoactive components of venom or released endogenous mediators or seizures secondary to cerebral bleeding or thrombosis. Grand mal seizures were witnessed in one patient in this series and have been described following envenoming by other elapids[116]. It is quite possible that all three mechanisms might occur. In June 1993, after the end of this series, a 14 year old boy was admitted to PMGH who had a cerebrovascular accident involving the middle cerebral artery which occurred within minutes of a taipan bite[117]. This may have been a thrombotic episode secondary to the action of the venom procoagulants or it may have been a spontaneous bleed.

Headache was a common and early feature of taipan envenoming in this series and has been described as a feature of envenoming by many Australasian elapids[116]. The mechanism of the headache is intriguing and unexplained. The nature of the headache, the speed of onset and relatively short duration, suggest that the pathogenesis is related to a circulating venom component, perhaps through a direct or indirect vasoactive effect. The release of endogenous vasoactive substances by venom components has been described in

association with other venoms[116] but whether taipan venom produces similar effects is unknown.

Limitation of jaw opening has been described by several authors in association with taipan bite[2,19,31]. Typically the jaw rests in a closed position and there is considerable resistance to passive opening. The explanation for this consistent finding is unclear. It is possible that it reflects an imbalance between the opposing actions of the masseter and pterygoid muscles. Alternatively, weakness in the lateral pterygoids may prevent protraction of the mandible which is necessary before depression. The temporo-mandibular is a ginglymo-arthrodial joint and opening involves both gliding and hinge movements. In support of this explanation, the jaw of affected patients can be opened much more easily if the mandible is first pulled anteriorly. Concentric needle studies of the masseter in 3 patients with limitation of mouth opening showed minimal sporadic fibrillation potentials and positive sharp waves but no evidence of myokymia or other regular electrical activity as one might expect to see in trismus. It seems most likely that the inability of envenomed patients to open their mouth is due to muscle weakness rather than increased muscle activity.

The mechanism of the bradycardia and electrocardiographic abnormalities seen in many envenomed patients is not clear. ECG abnormalities have been described following envenoming by an eastern brown snake and have been observed after experimental envenoming of dogs[119,120]. It is possible that the abnormalities may be due to the local effects of intravascular coagulation and microthrombi compromising the coronary circulation. Taipan venom is known to contain a selective calcium channel blocker, taicatoxin, which may act selectively on cardiac conducting tissue. It is intriguing that

electrocardiographic abnormalities were common in this series of taipan bites but have rarely been described with other elapids whose venoms also contain powerful prothrombin activators. The electrocardiographic abnormalities which were observed were transient and there was no evidence of persisting cardiac damage in any of the patients.

In patients with clinically evident fasciculation, concentric needle EMG studies of involved muscles showed fasciculation potentials, fibrillation potentials and positive sharp waves. No repetitive discharges were seen although myokymia has been reported in association with a bite by the timber rattle snake[121] and repetitive discharges from damaged endplates have been described in association with B-bungarotoxin[122]. Occasional fibrillation potentials and positive sharp waves were also seen when concentric needle studies were done on other muscles in envenomed patients. Abnormal impulse generation occurring somewhere in the terminal arborisation might result in propagation of an action potential throughout the motor unit and produce the clinical fasciculation seen. Toxin induced damage of the nerve terminal itself could result in fibrillation potentials and positive sharp waves but does not explain why clinically apparent fasciculation should occur. On the other hand, it is difficult to reconcile extensive pre-terminal axonal damage with the observations that conduction velocities remain normal and that the architecture of motor units in recovering patients appeared relatively normal. An alternative possibility is that the abnormal muscle activity results from the effect of a toxin which alters the electrical stability of the resting muscle membrane. Non-depolarising muscle relaxants might help to clarify whether the mechanism of fasciculation is of neural or muscle origin. Neuromuscular blocking agents

were not used routinely in patients in this study, but fasciculation was not seen in 2 patients who received pancuronium. The presence of fasciculation or vermicular movement is a sign that should be looked for specifically in patients envenomed by other snakes with neurotoxic venoms and the mechanism requires further study.

ii. Progression of neurotoxicity

The progression of neurotoxicity, from ptosis, to external ophthalmoplegia, bulbar paralysis, respiratory paralysis and ultimately peripheral paralysis, has been described by several authors[1,2,19,113]. *In vitro* observations have shown that the speed of onset of neuromuscular block in nerve muscle preparations, is enhanced by repetitive stimulation of the nerve[43]. The distribution of muscle involvement seen with both pre-and post-synaptic elapid neurotoxins is similar to that seen in myasthenia gravis. In myasthenics, weakness typically becomes apparent after prolonged use of a muscle. It is possible that the sequence of progression of muscle involvement in victims of taipan bite similarly reflects usage. An alternative explanation is that the muscles that are affected first by circulating toxins are those with the smallest motor units and lowest "safety factor" for neuromuscular transmission. The extrinsic eye muscles are known to have innervation ratios as low as 3:1[123] and this may make them more susceptible to the effects of neurotoxins than for example limb muscles with ratios of perhaps 120:1[124].

The progression of recovery from neurotoxicity is the reverse of the onset, with the first signs of improvement occurring in extraocular muscles. Although there was considerable variation between patients,

discussed further below, a "typical" seriously envenomed stage V patient could be weaned from the ventilator by the 4th or 5th hospital day and could be discharged home around day 7-8. At the time of discharge, eye movements appeared normal to the observer but patients frequently complained of diplopia at the extremes of gaze. Full recovery of peripheral muscle power took around 2-3 weeks by which time vision was normal. The time course of full recovery suggests that re-innervation is occurring at the nerve terminals. Concentric needle EMG studies performed at follow up showed essentially normal motor unit architecture. This suggests that the toxin damage is localised to the nerve terminals and that the terminal arborisation is relatively unaffected.

iii. Autonomic function

Adequate testing of the autonomic system was not achieved in significantly envenomed patients. Several of the most useful tests such as response to sustained grip, Valsalva or 60 degree tilt were not possible. The limited studies which were performed suggest that baroreceptor reflexes function normally in envenomed patients and the responses were similar to those recorded in controls. Postural hypotension was not seen, suggesting normal function of sympathetic constrictor fibres. The response to intravenous atropine was comparable to that reported in normal subjects by Ewing[125] suggesting that the vagal supply to the heart is unaffected by envenoming. The pupil reflexes were normal in all patients and resting pupil size was unremarkable suggesting no action of venom on either sympathetic or parasympathetic fibres controlling pupillary size. Ciliospinal reflex pathways were also intact.

iv. Complications

The management of patients requiring intubation and ventilation is difficult where both facilities and expertise are limited. One young child died as a result of a respiratory arrest during an emergency intubation and it is possible that in different circumstances this death might have been avoided. It is imperative that the impending need for intubation is appreciated as early as possible and the help of the most skilled person available should be obtained. Many of the middle-grade staff in PMGH are highly skilled at procedures such as intubation and ventilation. Advanced airway and respiratory support should however still be deemed a specialist area and the advice and help of an anaesthetist should be sought whenever possible. Aspiration in a small number of patients prior to arrival at hospital is probably unavoidable but several patients may have aspirated after admission, either as a result of unwarranted delay before intubation or past a deflated cuff. The laryngeal stridor which occurred in 4 patients emphasises the need for optimal management of patients with endotracheal tubes in situ. Tracheal mucosal damage may have resulted from traumatic intubation, from failure periodically to deflate pressure cuffs, from over-enthusiastic suctioning and from prolonged use of endotracheal tubes. Ideally endotracheal tubes should be changed every 48 hours. With limited resources this was rarely possible but this economy exposes patients to increased risk of infection and tube blockage. Airway management is probably the single most important aspect in the care of victims of taipan bite and an area where important complications and unnecessary deaths can be avoided by attention to detail.

v. Sequelae

A localised sensory deficit around the site of the bite was a common finding and persisted for several weeks. The absence of other sensory abnormalities suggests that the mechanism is local damage of cutaneous nerves, possibly by the action of phospholipases in the venom deposited locally. Other sequelae were rare. Recovery of motor function typically took 2-3 weeks in severely envenomed patients but complete recovery appeared to occur in all cases. One patient, who collapsed at the time of the bite, had a persistent neurological deficit after what appeared to have been diffuse hypoxic brain damage. One patient required a tracheostomy after developing tracheal stenosis following prolonged intubation with a rubber tube. This unfortunate series of events occurred when there were no plastic endotracheal tubes in the hospital and highlights the need to ensure that appropriate equipment is available.

3. Laboratory results

i. Biochemistry

The majority of the patients included in this series had evidence of muscle damage with both elevated creatinine kinase and aspartate transaminase levels. Clinical evidence of significant systemic myotoxicity was not seen however. Taipoxin and crude taipan venom have both been shown to be myotoxic when applied to skeletal muscle[54] and there are reports of myotoxicity following a bite by the Australian taipan[36,116]. Myoglobinuria has been reported following a bite by the Papuan taipan[1]. It appears that the myotoxic effect of circulating venom is not usually clinically

significant. Myoglobinuria is only visible at concentrations approaching 1mg/ml[126,127] and was not detected visually in any patients included in this series. It was witnessed however in one boy with ischaemic muscle damage secondary to prolonged use of a tourniquet, reported elsewhere[95]. The creatinine kinase level in this boy was greater than 10,000 IU/l. Typically, symptomatic rhabdomyolysis is associated with creatinine kinase levels of this order[126] which were not seen in patients in this series.

Renal impairment has been described in association with envenoming following bites by several elapid species including the Australian taipan[116]. Several mechanisms have been proposed for renal impairment in snakebite including a direct toxic effect of venom, hypotension, the deposition of microvascular thrombi and a toxic effect of constituents released following extensive muscle damage. A high proportion of the patients in this series had proteinuria and granular casts in their urine. None of the patients with impaired renal function had hypotension, hypovolaemia or evidence of significant myotoxicity. It seems most likely that the abnormalities in renal function which were seen were caused either by a direct toxic effect of circulating venom or by deposition of micro-emboli in the renal vascular bed. Significant renal tubular damage was seen in one boy with ischaemic muscle damage following the use of a tourniquet[95]. In addition to the toxic effect of myoglobin itself, release of potassium and other metabolites from damaged muscle cells may produce both renal and cardiac toxicity[128]. Whether an effect of the toxin or of first aid measures, if significant muscle damage has occurred active measures may be required to limit the consequences[95]. Adequate hydration is a simple and important first step[129,130].

ii. Haematology

42.5% of patients had an admission white cell count above the normal range for PMGH (4-12x10^9/l). This was due to neutrophilia in all cases in which a differential count was performed and appears to be a reaction to envenoming. This was reported by Campbell and has also been described in association with envenoming by the Australian taipan[131,132]. The increase in white cell count does not depend on administration of antivenom. Neutrophilia was not seen in snakebite victims without signs of systemic envenoming. The absence of significant local effects of the venom suggest that local release of inflammatory mediators is unlikely to be the cause but it may be that cytokines are released in response to circulating venom. In turn, they may stimulate the release of marginated white cells. Lymphocytopenia has been described in association with envenoming by the Australian taipan[133]. In this series, lymphopenia (absolute count <1.0 x10^9/l) was seen in 11 out of 66 patients who had differential white counts, but in only one patient was the count below 0.5 x10^9/l. The gross sphero-echinocytic transformation described by Arthur[38] was not seen in any patients but increased red cell crenation was seen in 28/66 admission films. Arthur postulated that the change in morphology was due to a direct effect of venom phospholipases on red cell membranes and it is possible that the increased crenation seen in this series was due to the same mechanism, operating to a lesser degree.

Mean and median haemoglobin levels fell marginally in envenomed patients by 1.3g/dl below admission levels. The nadir was usually reached on day 2 or 3. This is probably explained predominantly by the effects of haemodilution in patients treated with

intravenous fluids. There was no evidence of significant haemolysis and overt blood loss from bleeding sites was usually trivial. Sutherland found little evidence of haemolysis in monkeys given taipan venom[131]. Mean and median platelet counts also fell by 18 $x10^9/l$ and 20 $x10^9/l$ respectively. There was no association between admission levels or changes in haematological parameters and the presence of coagulopathy or severe neurotoxicity. This is somewhat surprising. One might have anticipated that platelet levels might have fallen more significantly in patients with coagulopathy of sufficient severity to be clinically evident. At least two mechanisms may contribute to the fall in platelet count:- thrombocytopenia is common in defibrination[134] and taipan venom is known to cause agglutination of platelets *in vitro*[63]. Thrombocytopenia has been described in association with envenoming by the Australian taipan[135].

iii. Haemostasis

The 20 minute whole blood clotting test (WBCT) is a simple and sensitive indicator of envenoming. It does not help to distinguish between the various elapid species in Central Province which cause coagulopathy, but an abnormal test excludes a bite by a death adder, the species responsible for the majority of non-taipan bites[2]. Prolongation of clotting was an early sign, occurring as soon as 30 minutes after the bite in 2 patients, and was present by the time of admission in all patients in whom it was to occur. Lalloo demonstrated a close correlation between an abnormal WBCT and a fibrinogen level below normal limits[2]. The main mechanism of coagulopathy in taipan envenoming is the action of a potent prothrombin activator. This

produces a significant fall in levels of fibrinogen, and a reduction in levels of other clotting factors including factors II,V,VIII,,IX,XI and XII. The mechanisms of the action of the venom on the clotting system are discussed in detail by Lalloo[2,136].

iv. Venom detection kits

The VDKs, used with bite site swabs as recommended by the manufacturers, detected venom antigen in 37 out of the 42 clinically envenomed patients in which they were used (88%). Laboratory enzyme immunoassay (EIA) detected venom antigen in 33 of these swabs. It is possible that the lesser sensitivity of the laboratory assay may be due to the effects of prolonged storage of the swabs and the use of a second diluent prior to analysis. Only 1 swab was positive on laboratory assay but negative on VDK, in which venom antigen was detected at 2ng/ml, below the manufacturer's claimed lower limit of sensitivity. In all patients in which venom antigen was detected on both VDK and laboratory immunoassay, the type of venom detected correlated.

VDKs currently cost £104 for a three test kit. Their potential value in Papua New Guinea is to allow the confident use of appropriate monovalent antivenom by identifying the biting species. This could help diminish the administration of inappropriate antivenom, decrease the frequency of side effects which may be more common with polyvalent antivenoms with a higher protein content[137,138] and potentially produce financial savings. The current cost of a vial of monovalent taipan antivenom is £545 compared with £594 for a vial of polyvalent. It would be ideal to use VDKs in all patients admitted to PMGH with signs of systemic envenoming but the cost of this is not clearly

justified. 90% of patients admitted to PMGH have been bitten by taipans and the majority of these have non-clotting blood at admission. The presence of non-clotting blood has a high predictive value (0.96) for taipan bite and it seems reasonable and pragmatic to give all patients with non-clotting blood monovalent taipan antivenom. Each year one or 2 patients who have been bitten by a Papuan black or eastern brown snake may be given inappropriate antivenom but retrospective analysis of admissions between 1990 and 1993 suggests that these patients have not had adverse clinical outcomes. The most useful potential role of VDKs in PMGH appears to be to help to discriminate between patients bitten by a taipan who do not have non-clotting blood (22.1% in this series) and patients who have been bitten by a death adder. A positive VDK does not necessarily imply systemic envenoming and their use is not indicated unless this is suspected. The use of VDKs is discussed more fully elsewhere[104].

4. Antivenom treatment

i. Type of antivenom

Polyvalent antivenom is used more commonly than monovalent antivenom by both health centre and hospital staff. It has the advantage that it covers most of the possible local venomous snakes and consequently there is no need to make a specific diagnosis of the biting species. The disadvantages of this policy are that polyvalent antivenom is considerably more expensive than monovalent antivenom and that there may be a higher risk of antivenom reactions[137,138]. The patient's description of the biting snake is usually of little value in the identification of the biting species (see chapter 3) but the presence of non-

clotting blood is a useful discriminant. In 96% of snakebite cases with non-clotting blood, where a definitive species diagnosis was made by immunoassay, a taipan was incriminated. and, as discussed above, a case can be made for giving all patients with non-clotting blood taipan antivenom. Papuan black bites are now extremely rare. There are no clear distinguishing clinical features of Papuan black envenoming[139] and there seems to be little argument for continuing the purchase and use of monovalent black snake antivenom.

ii. Antivenom reaction prophylaxis

Early antivenom reactions were seen or reported in 10.8% of patients given antivenom, considerably higher than the 4.8% reported by Sutherland in a review of returned reports of antivenom use from around Australia[138]. In a review of all patients given antivenom at PMGH between 1990 and early 1993, early reactions were seen in 6 of 134 patients, a rate of 4.5%[110]. It is interesting to note that 4 early reactions were seen in the space of a month at the end of this study (3 in patients who received polyvalent and 1 who received taipan antivenom) and it is conceivable that this was associated with the use of a new batch of antivenom which arrived just prior to this. The numbers in this series are too small to confirm or refute the suggestion that antivenom reactions may be more common after polyvalent antivenom. Late "serum sickness" antivenom reactions appear to be most common in patients who have received multiple doses of antivenom. The absence of any late reactions amongst the 48% of patients who attended for follow up may be due to the fact that few patients in this series received more than one vial of antivenom. Unlike doses of antivenom the doses of drugs used for

the prophylaxis or treatment of antivenom reactions should be calculated on the basis of body weight. This point did not appear to be universally understood amongst health staff. One child was seen whose condition had been exacerbated by an excessively high and sedating dose of promethazine given with antivenom at a health centre. This was exceptional, but highlights the point that drugs which may sedate, should be used judiciously, in patients such as this one, who have impaired gag reflexes[111].

The question of whether prophylaxis for antivenom reactions should consist of an antihistamine used alone or in combination with adrenaline, as recommended by Sutherland[140,141], is not answered by this study. While there were no serious adverse reactions in the course of this series, there is clearly a potential risk of anaphylaxis. The prophylactic use of at least an antihistamine should be recommended in all patients and all health workers who are giving antivenom must have the knowledge, drugs and equipment to treat an anaphylactic reaction.

5. The efficacy of antivenom

Antivenom has been an integral and established part of the therapy for Australasian taipan bite since 1957 and yet there are few published data assessing its efficacy. There is only a single published report of a patient who survived without antivenom treatment after being envenomed by an Australian taipan[31] but this cannot be used as evidence of the efficacy of the drug. Fourteen patients in this study made a full recovery after significant envenoming by the Papuan taipan despite not receiving antivenom. At all times during the course of this study patients were treated with antivenom in accordance with the treatment regimes

recommended by the Papua New Guinea Department of Health[87,88]. For economic and logistic reasons however, there were times during the study when there was no antivenom available presenting an important opportunity to evaluate its role. As Campbell pointed out, there are innumerable factors which make analysis of antivenom therapy extremely difficult, for example no two patients can be assumed to have received or absorbed the same quantity of venom and we know little about the extent of variation of venom constituents between taipans. Nonetheless, there is some information in this study which allows some critical assessment of the following generally accepted principles:

1. antivenom is significantly beneficial to a patients outcome.
2. antivenom is most beneficial if given soon after envenoming.

In addition, if there is an optimal time at which to give antivenom, is there a time beyond which antivenom is of no value? Is the single vial of antivenom given to most patients in Papua New Guinea enough? Should Sutherland's assertion that "on first principles..it is never too late to administer antivenom, except when the patient is dead"[142] be applied in Papua New Guinea where antivenom supplies are finite and exhaustible?

i.Antivenom and survival

Table 4.10 shows that mortality was higher in the group of patients not receiving appropriate antivenom. Of the 2 deaths in this group, one was a 3 year old boy treated with black snake antivenom, who died following a respiratory arrest during a delayed intubation. His clinical course to that time had been unremarkable and the death can be attributed to delay rather than absence of correct antivenom. The second death was that

of a 15 year old girl (S1892) who was admitted when there was no antivenom available, had prolonged neuromuscular paralysis and could not be weaned from the ventilator. She developed pneumonia in the second week of her admission, became septicaemic and ultimately died. The protracted clinical course, and consequent complications, appear to be attributable to the absence of antivenom although there were other factors which may have contributed, including malnutrition, hypomagnesaemia, ventilator dependency and relatively low body weight. There were avoidable delays in commencing parenteral nutrition and in performing a tracheostomy, both of which may have exacerbated the situation. The possibility that this death occurred due to failure to receive antivenom is a powerful indication to adopt Sutherland's maxim quoted above but does not remove the need for a dispassionate appraisal of the use of a limited resource.

ii. Antivenom, intubation and ventilation

Surprisingly, the proportion of patients requiring intubation and ventilation was higher in the group of patients receiving antivenom than in the heterogeneous group who did not, although this did not reach statistical significance. There are significant sources of bias amongst what is a small number of patients in the no-antivenom group. Nine of the 16 patients who did not receive antivenom presented to hospital more than 20 hours after the bite. Four of these patients were not given antivenom because they only had mild neurotoxic signs but 4 others did require intubation and ventilation suggesting that the group was not solely selected from mild cases. It is possible that the 4 patients who received black snake antivenom in error, and the patient who received death adder

antivenom, may have received some therapeutic benefit from these medications although several investigators have demonstrated little if any cross protection between antivenoms[40,41]. The other 7 patients failed to receive antivenom solely because of lack of supplies. The mean length of hospital admission was not significantly different between the two groups.

The mean duration of intubation and ventilation in patients not receiving appropriate antivenom was longer than for those receiving it although this was not statistically significant. This is due to the very prolonged ventilation of patient S1892 (see above) and the small numbers in the group. The median duration of intubation was comparable to the group who received antivenom. The start of recovery and the completion of resolution of ophthalmoplegia in the group of patients not receiving antivenom appears to have been as quick as in those patients who were given antivenom later than 3 hours after the bite. Recovery of peripheral power (**Fig.4.1**) occurred at a similar rate in antivenom and no-antivenom patients. There was considerable variation in the rate of recovery of grip strength, with one patient in each group in Fig 4.1 particularly slow:- the slowest of whom was a patient who did not receive antivenom. It is impossible to know whether this patient would have recovered faster had he received antivenom.

iii. Antivenom, coagulopathy and bleeding

In this study, when bleeding manifestations occurred, they did so soon after envenoming. Significant bleeding after hospital admission was rare in both groups and was not influenced by administration of antivenom. The time after envenoming at which previously incoagulable blood began to clot appeared to

be shorter in patients receiving antivenom but numbers in the non-antivenom group were too small to assess the significance of this observation. Only one of 9 patients admitted greater than 20 hours after envenoming in the no-antivenom group still had non-clotting blood. The recovery of fibrinogen levels and normalisation of prothrombin time occurred at a similar rate in a single patient with severe coagulopathy who did not receive antivenom as in 8 patients who did[2].

iv. Time of antivenom and outcome

The mean and median length of hospital admission was significantly shorter in patients who received antivenom fewer than 4 hours after envenoming ($p<0.002$) than it was in patients who received antivenom later than this. There was little variation in either variable when compared with various times of receiving antivenom greater than 4 hours after envenoming (Fig.4.2). This observation suggests a significant beneficial effect of early antivenom. Patients who received antivenom more than 4 hours after envenoming were significantly more likely to require intubation than those who received antivenom less than 4 hours after envenoming ($p<0.001$, RR 2.04, 95% confidence limits 1.3-3.23). The proportion of patients who did not develop neurotoxicity of sufficient severity to require intubation was highest in those given antivenom fewer than 2 hours after envenoming. The period of time to complete resolution of eye signs was significantly shorter in the group of patients given antivenom within 4 hours of envenoming ($p<0.0001$), the scatter plot, Fig.4.5 also shows this trend. The time to onset of neuroresolution was not significantly different between the two groups. It has been demonstrated *in vitro* that the effect of bound taipoxin persists even after a

nerve muscle preparation has been washed and moved to a toxin free bath[51]. This situation may be analogous to the situation where a patient is given antivenom after the majority of the toxin has already bound. It emphasises the potential importance of first aid measures such as the use of pressure bandages which may delay the absorption of venom[143,144].

The mean and median lengths of intubation were shorter in the small group of patients given antivenom between 2 and 4 hours after the bite than in those given antivenom before 2 hours although this trend did not reach statistical significance ($p<0.09$). It is relatively consistent amongst the other groups (Fig.4.3). This observation may be an artefact created by the small numbers in both groups, but it is conceivable that it reflects an optimal window in which to give antivenom. It is possible that the neutralising capacity of antivenom given too early may be limited by clearance from the circulation before the majority of venom has been absorbed from the depot deposited by the snake. There was however no significant difference between the time to onset or completion of neuroresolution between the 0-2 and 2-4 hour groups.

Too few patients were given two vials of antivenom to make much assessment of the possible benefit of larger volumes of the drug. The median time of administration of the first vial in those who received 2 was 5.25 hours (mean 7 hours). There is no evidence to suggest that these patients received any benefit from receiving a second vial of antivenom at this late stage.

v. Antivenom treatment - summary and conclusions

It is impossible to prove the significance of the relationship between time of antivenom and clinical

outcome shown in this small series, but the absence of a clear benefit of antivenom given more than 4 hours after envenoming has important implications. There is no evidence that antivenom had any effect in lessening established neurotoxicity and to be effective probably must neutralise circulating neurotoxin before it becomes bound to the presynaptic membrane. In the majority of patients, even those who received antivenom early, appropriate antivenom at best only partly achieved this. There is limited evidence to suggest that antivenom promotes faster recovery from coagulopathy but the absence of significant bleeding episodes in any patients after the first few hours after envenoming, suggests that this may only be of limited clinical value

The best chance of producing maximal benefit with antivenom appears to be to give it within 4 hours of envenoming. Seventy five per cent of patients arrived at a health centre or the hospital early enough for this to have been possible. It is impossible to say from this study whether a higher volume of antivenom given this early would produce a greater benefit and this question still needs to be answered. Campbell described two cases of taipan bite treated with multiple doses of antivenom, in one patient there was no reversal, in the other no development of neurotoxicity[145]. Sutherland treated two envenomed laboratory monkeys with 11 times the required *in vitro* quantity of taipan antivenom. One monkey made a slow recovery whilst the other died[144]. A simple randomised trial could be devised in PMGH to assess the comparative benefits more than 1 vial of antivenom but large numbers of patients would be required to overcome the confounding variations between patients. The expense at present would be prohibitive.

The overriding message from this analysis is that the current antivenom treatment in Central Province fails to achieve its objective in the majority of patients. Most patients who die from taipan bite in Papua New Guinea do so because of respiratory failure or because of a complication associated with managing respiratory failure. There is no evidence to suggest that a single vial of antivenom prevents this if it is given more than 4 hours after envenoming but limited evidence that it does so if given earlier. Only 30% of the 106 patients in this series were given antivenom before 4 hours, but this could have been achieved in 75%. It is possible that delaying absorption of venom from the bite site, by the use of pressure bandages, may increase the period within which antivenom is most useful.

The taipan antivenom used in Papua New Guinea is made using Australian taipan venom. Papuan taipan venom (*O.s.canni*) is known to have a lower LD50 in mice than *O.s.scutellatus* and there also appear to be differences in its protein composition[40]. Antivenom made using *O.s.canni* venom might be more effective for treating envenoming due to a bite by the Papuan taipan. There is scope to develop subunit antivenoms more specifically directed against particular venom components. It is likely, however, that even a specific anti-taipoxin antivenom would be of little value once the toxin has bound to the presynaptic membrane, and this binding time is probably what is reflected by the apparent watershed around 4 hours. Improving management in the immediate future depends on delaying the absorption of venom and by increasing the proportion of patients who are treated early. In practical terms this means that envenomed patients must be treated with the appropriate antivenom immediately after reaching the health centre or hospital - an obvious statement, but a message that

CHAPTER 8 - ELECTROPHYSIOLOGICAL STUDIES

1. The effects of envenoming

i. Control data

The control data for sensory and motor conduction studies in ulnar and median nerves are similar to those quoted in Caucasian patients[124], suggesting that the range of normal values in Melanesians is comparable. There were, however, minor differences. The mean amplitude of compound muscle action potentials (CMAP) recorded following stimulation of both median and ulnar nerves, were of greater amplitude in the Melanesian series although the standard deviations of the distributions were almost identical. There was also some variation between the two series in the size of the sensory amplitudes recorded. In particular, ulnar sensory amplitudes were generally lower in the Melanesian series. The majority of the Melanesian control group were rural dwellers and subsistence farmers. It is possible that there might be significant physical differences, for example in skin thickness (and hence resistance) or recurrent minor trauma to peripheral sensory nerves, between this predominantly rural group, and a Caucasian control group. Both might significantly influence the amplitudes of the sensory potentials. The most likely explanation for these differences however, is inter-observer variation in technique.

ii. Motor and sensory studies in envenomed patients

CMAP amplitudes were dramatically and unequivocally reduced in envenomed patients with clinical evidence of neurotoxicity. Amplitudes

typically reached their nadir at around 24 hours after the time of envenoming and were usually 10-20% of the admission values (when the majority were stage 1 or 2) in patients who required ventilation. Smaller reductions were seen in patients with less severe clinical neurotoxicity. Motor nerve conduction velocities in envenomed patients were comparable to those found in the control series. Sequential measurements of motor conduction velocities showed little variation throughout the clinical course of neurotoxicity. The changes in motor amplitudes are consistent with profound neuromuscular blockade. The normal conduction velocity results suggests that the effect of the venom is localised to the synapse, with no evidence of any effect on the peripheral nerve itself. This is in keeping with the experimental evidence that suggests that the predominant effect of taipoxin is localised to the neuromuscular junction[43,49,146,147]. The F wave responses in the envenomed patients in which they could be recorded (impossible where CMAP amplitudes were very markedly reduced) were not significantly different from those obtained in controls considered as a group and when matched for age and height. This suggests that the integrity of the motor nerves from anterior horn cell to neuromuscular junction is preserved and that conduction is normal. There was no significant difference between the mean stimulus threshold in envenomed and non-envenomed patients.

Mean sensory conduction velocities were marginally slower at the peak of neurotoxicity than at admission or discharge but the difference was not significant. The lowest values in the series correlated with the lowest recorded skin temperatures in patients with decreased peripheral perfusion, and can probably be explained as a temperature effect. Sensory

amplitudes were lower in envenomed patients than in controls ($P<0.01$). There was definite clinical evidence of local sensory neuropathy around the site of the bite but no evidence of abnormal sensation elsewhere. Loss of taste and smell has been described following envenoming by the Australian taipan[31,34] but was not detected or reported in any patients in this series. The local neuropathy around the bite site appears to be a direct toxic effect of venom on superficial cutaneous nerves. The findings of minor sensory abnormalities on electrophysiological studies raise the possibility that some sensory fibres are affected by circulating venom components. There are however several potential sources of error in recording low amplitude sensory potentials. Extrinsic factors such as variation in skin temperature and minor degrees of oedema which was occasionally seen in hypostatic limbs in paralysed patients could have influenced the amplitude of sensory nerve action potentials. Sequential recordings in some patients showed considerable variation in the size of the sensory amplitudes although the values were lower at peak neurotoxicity than at admission or following recovery. These studies suggest, but do not prove, that sensory amplitudes are diminished in severely envenomed patients; more tightly controlled studies are required to clarify this. If sensory conduction is impaired by taipan venom, the effect is a minor one.

Sequential grip strength readings showed a rapid decline and slow recovery phase in envenomed patients. The sequential studies shown in Fig 5.1-5.7 demonstrate a close correlation between the changes in CMAP amplitude and grip strength. The rapid decline in CMAP and grip strength mirrors the clinical deterioration of the patient and the values of both are approaching their nadir at the time that the patient required intubation. The administration of antivenom to the

patient in Fig 5.6 and Fig. 5.7, 6.5 hours after envenoming, at which time the only clinical evidence of neurotoxicity was an equivocal diminution in lateral eye movements, did not prevent the deterioration. This suggests one of two things; either that the neurotoxins were already irreversibly bound to the presynaptic membrane at this time or that the volume of antivenom given failed to neutralise circulating neurotoxin. If the former is the case, there is a considerable delay between binding of the neurotoxin and maximal inhibitory effect on transmitter release. *In vitro* work on the effect of taipoxin suggests that the rate of onset of the effect of taipoxin is influenced by the rate of firing of the motor neurone and a delay has been recorded between binding and blockade[43,45]. It may be that that the period of maximal therapeutic opportunity for using antivenom is considerably earlier than suggested by the onset of clinical signs of neurotoxicity, a hypothesis supported by the clinical outcome of patients given antivenom at different times, discussed in chapter 7.

The recovery in CMAP amplitudes is slow and mirrors clinical recovery. The length of time for the amplitudes to regain maximal values varied between patients but was typically of the order of two to three weeks. Muscle grip strength readings also reached their maximum at about the same time. Ultrastructural studies of the effect of taipoxin on the nerve terminal *in vitro* demonstrate clear morphological changes[45] and it appears that the effect of the toxin is irreversible. The time taken for full recovery probably reflects the time taken for resprouting of nerve terminals, which may take some weeks. Complete recovery from the myotoxic effects of taipan venom *in vitro*, also took around 3 weeks[54], although clinically, direct myotoxicity appears to be of much less significance.

iii. Repetitive nerve stimulation studies

The decremental responses on repetitive nerve stimulation (RNS) are indicative of a deficiency in synaptic transmission but do not distinguish between a pre-synaptic and post-synaptic problem. Both pre- and post-synaptic neurotoxins are known to occur in taipan venom[42] although the predominant effect on neuromuscular transmission is believed to be pre-synaptic. The findings of low CMAP amplitudes and decremental responses at both low and high frequency seen in envenomed patients, are similar to those seen in myasthenia gravis. The classic RNS appearance of pre-synaptic dysfunction is seen in the Lambert Eaton myasthenic syndrome (LEMS) where a low initial CMAP amplitude is followed by a decremental response at low frequency stimulation, but an incremental response at high frequency repetitive stimulation (HRS). Stimulation at higher frequencies in envenomed patients in this study, produced more profound decremental responses but no incremental responses were seen. In LEMS, two patterns of response are seen following HRS, either an immediate incremental response or an initial decremental response which is then followed by an increment[148]. Oh suggests that the latter pattern may reflect pre-synaptic block of a more severe degree. In botulism, the size of the incremental response to HRS is inversely proportional to the severity of the neurotoxicity[148,149]. The clinical severity of the neurotoxicity in patients envenomed following a taipan bite is significantly more extreme than that usually seen in LEMS. Although the clinical consequences share some common features, the mechanism of presynaptic block is entirely different. It would therefore be simplistic to expect a similar "LEMS-type" response to

HRS. No incremental responses were seen in any envenomed patient in this series.

After a short burst of high frequency stimulation, the proportional increase in amplitude of the CMAP recorded in envenomed patients was significantly greater than that seen in controls. In the controls with normal neuromuscular function, the end plate potential (EPP) is already exceeded for each muscle fibre and an increase in acetylcholine (ACh) makes little difference to the size of the CMAP. In envenomed patients however, the diminished ACh release produces low amplitude EPPs which may be sub-threshold. It may be that the marked initial increase in the amplitude of the CMAP following tetanic stimulation results from the proportionate increase in the quantal release of ACh[150]. Kamenskaya and Thesleff, however, failed to demonstrate post tetanic facilitation in envenomed preparations *in vitro*[43]. The proportional increase in post-tetanic CMAP, recorded in envenomed patients probably reflects increased quantal release from unaffected nerve terminals rather than any effect overcoming the toxin inhibition of transmitter release. The decremental response seen at low frequency repetitive stimulation (LFRS) was significantly increased following tetanic stimulation or exercise (**Fig** 5.10). Initial post-tetanic facilitation, which often lasted for up to 30 minutes, was followed by a period of post-tetanic exhaustion in which the amplitude of the recorded CMAP was less than that of the pre-tetanic CMAP. Repetitive high frequency stimulation of envenomed *in vitro* preparations caused a rapid decline in EPP amplitude, and the *in vivo* observations may reflect this. The rate of onset of neuromuscular block in envenomed *in vitro* preparations was accelerated by stimulation of the nerve[43]. In the clinical setting, this is reflected by the use of a

muscle and this may be a factor in the progression of muscle involvement in envenoming.

iv. Concentric needle electromyography

The observation of reduced recruitment patterns is consistent with significant neuromuscular blockade and is not unexpected. Recruitment patterns gradually recovered over 2-3 weeks after envenoming in keeping with clinical recovery. Motor unit morphology appeared relatively normal during this recovery phase suggesting that the toxin damage may be restricted to the nerve terminals, if more proximal damage had occurred, extensive resprouting might have been expected to produce more eccentric re-innervation. Considerable spontaneous electrical activity was seen in several muscle groups. The observation of irregular fibrillation potentials and positive sharp waves may be due to spontaneous erratic transmitter release from damaged nerve terminals. However, both abnormalities also occur in association with axonal damage and the electrophysiological findings do not distinguish the source[124]. An alternative and perhaps more plausible explanation for the spontaneous activity is that it represents the effect of a toxin acting on the muscle membrane itself and perhaps altering the resting membrane potential.

v. Single fibre studies

It was not possible to do single fibre electromyography on any patients included in this series although studies were done subsequently on four patients envenomed following a taipan bite. The results of these studies are presented elsewhere[151].

vi. Summary of abnormalities

In summary, the main electrophysiological abnormalities found in patients envenomed following a taipan bite are :

1. reduced CMAP at rest.
2. decremental response on low frequency repetitive stimulation.
3. decremental response on high frequency repetitive stimulation.
4. post-tetanic and post-exercise facilitation.
5. post-tetanic exhaustion.

Oh describes similar findings in antibiotic induced myasthenic syndrome and in procainamide induced paralysis[148]. In both of these, there are pre- and post- synaptic mechanisms affecting synaptic function. It is possible that both pre- and post- synaptic neurotoxins play a significant role in the paralysis which follows a taipan bite but the absence of a clinical response to antivenom or pharmacological interventions suggests that the predominant effect is pre-synaptic.

2. Intervention studies

i. The effect of edrophonium and 3,4 diaminopyridine

The results of the studies on the effect of edrophonium and 3,4-DAP detailed above, suggest that both produce a small electrophysiological response and that 3,4- DAP produces a small physiological response in envenomed patients with profound neurotoxicity. The two drugs appear to have a synergistic effect although there is insufficient data to prove unequivocally that

the response to both drugs is significantly greater than that to 3,4-DAP alone (Table 5.7). A response was measurable by an increase in CMAP amplitude together with an increase in muscle grip strength but was not normally accompanied by a change in respiratory function, the degree of ophthalmoplegia, ptosis or restriction of jaw opening. Neither effect was dramatic and there was no evidence of a difference in the degree of the response at different stages of neurotoxicity or at different time intervals after envenoming (Fig 5.23 and 5.24). It seems likely that the increase in amplitude of the CMAP produced by the drugs is in proportion to the existing diminution of transmitter release. This suggests that the main effect of the drugs is on relatively unaffected nerve terminals with little effect on transmitter release already inhibited by toxin. Unfortunately the response one might expect in a non-envenomed patient is not known. 3,4-DAP appears to produce a greater increase in the amplitude of the CMAP than edrophonium. This is probably due to the different mechanisms of action. 3,4- DAP augments release of acetylchloline. Edrophonium increases the bioavailability of existing acetylcholine but does not affect release. Logically, the effect of the two drugs on the facilitation of neuromuscular transmission might be expected to be additive and this appears to be the case.

There is some evidence that there are isolated "responders" who show a more pronounced response to the drugs. The patient whose studies are shown in Figures 5.21 and 5.22, was a 16 year old girl who did not receive antivenom until 7 hours after envenoming by which time she had complete ophthalmoplegia and weakness of her bulbar muscles. She was intubated 13 hours after the bite but did not require ventilation and was discharged after 6 days. Taipan venom antigen

was detected in a bite site swab and an admission serum sample. The explanation as to why this patient showed a more marked response to the combination of 3,4-DAP and edrophonium is obscure, with no apparent link to the stage of neurotoxicity, to the period of time which had elapsed since envenoming or to the time of receiving antivenom. It is conceivable that the proportions of pre- and post-synaptic toxins in venom from different taipans may vary, or that there may be intrinsic differences in neurophysiological architecture between patients which affect resilience to neurotoxins but these and other possible explanations are purely speculative.

ii. Dosage and timing

A variety of stages of clinical neurotoxicity were chosen for the trials of the drugs in an attempt to find a period of maximal effect. No differences in response were apparent. In the absence of a clear beneficial clinical effect I did not believe that it was justifiable to give repeated courses of drugs to any patients, the maximum number of doses received by any one patient was 4. The doses of edrophonium and 3,4-DAP used in the study are the standard doses used in the "tensilon" test and in the treatment of Lambert Eaton syndrome respectively. Higher intravenous doses of 3,4-DAP have occasionally been given in LEMS[82] but were not attempted in this study. In addition to the frequent occurrence of minor side effects at the doses used, the potential proconvulsant effect of high doses of aminopyridines was considered a reason for circumspection, especially in view of limited clinical data that envenoming by the Australian taipan may on occasions itself cause convulsions[116].

iii. Side effects

Side effects of the drugs given were seen in several patients and are detailed in the results section. The unwanted muscarinic effects of increasing cholinergic activity after 3,4-DAP are a potential problem, in particular the stimulation of oral and pharyngeal secretions. In the regulated setting of the intensive care unit, it was easy to use pharyngeal suction to prevent aspiration. Clearly in a rural setting, or in transit, a patient with an impaired gag reflex and with diminished pharyngeal muscle activity could be compromised. Premedication with atropine helped to minimise the problem of excess secretion, but did not abolish it. Transient local thrombophlebitis was seen in several patients and dilution of the intravenous preparation of 3,4-DAP preparation to a minimum volume of 10ml is essential. As a precaution, a minimum of 100ml of a crystalloid solution was run through the intravenous cannula after the 3,4-DAP was given. This may have helped to prevent any persisting problems.

iv. Study Design

There are significant limitations and potential sources of error in the design of these studies. It is conceivable that bias, for example in interpreting eye movements, could have been introduced because of the difficulty experienced in maintaining true "blinding" in all cases. Patients who had noticeably increased secretions after an injection had clearly received active drug. The analysis is based predominantly on hand grip and CMAP, the most objective of the tests. Obtaining reliable readings of CMAP depended on religious attention to detail with technique, in

particular with fixation of electrodes, but with cooperative patients this did not prove unduly difficult. I attempted to maximise the objectivity of assessments of eye movements and degrees of ptosis by measuring the change in proportion of iris covered and by measuring the maximum angle of lateral gaze from the midline with the head facing directly forward. Typically, however, eye movements could not be sustained and precision was difficult. This is a potential source of erroneous observations and did not prove a sensitive test for detecting minor changes.

Ideally, one would wish to measure the effect of the pharmacological interventions on respiratory function. This did not prove possible. Patients with neurotoxicity who were not intubated, usually had some weakness of facial muscles and limited jaw opening and were unable to form a tight seal around the mouthpiece of a vitalograph or spirometer. Patients who were intubated +/- ventilated, did not tolerate repeated measurements of their maximal inspiratory pressure and the few readings obtained were very variable even in control patients. For these reasons, sequential assessment of respiratory function did not prove satisfactory.

v. Concluding remarks

There is insufficient evidence from these studies to advocate the use of anticholinesterases or 3,4-DAP in the management of patients with neurotoxicity secondary to taipan venom. The presence of a measurable response does however suggest that there may be value in looking more closely at the effect of higher doses of these drugs on *in vitro* preparations poisoned with taipoxin. There is also scope to look more closely at the effects of these

drugs on preparations poisoned with other neurotoxins and their potential value as adjuncts in the management of neurotoxic snakebite should not be discarded completely. Anticholinesterases have been shown to be of value both as a diagnostic aid and as therapy for patients bitten by snakes with post-synaptic neurotoxins[23,67,68,69,85]. Their effect in patients bitten by snakes with pre-synaptic neurotoxins appears to be much less dramatic. The absence of a response to edrophonium may be diagnostically useful. The absence of a coagulopathy does not itself distinguish patients who have been bitten by a death adder and those who have been bitten by other species. If a clinician sees a clear improvement in eye signs of neurotoxicity following a dose of edrophonium, he or she may give monovalent death adder antivenom with a degree of confidence.

These studies demonstrate that simple electrophysiological measurements provide a reproducible and well tolerated means of monitoring neurotoxicity at the bedside. The objectivity and repeatability of the measurements provides a means of assessing interventions in neurotoxicity. The need for an effective intervention remains.

CHAPTER 9

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

1. There appears to have been a significant change in the distribution of venomous snakes in Central Province during the past 30 years. The Papuan taipan is now the major species causing snakebite morbidity and mortality in Central Province, PNG. Over 99% of these bites occurred at altitudes of less than 300 metres. The predominance of taipan bites is not universally recognised amongst health staff and has significant implications for antivenom treatment.

2. Patients can rarely describe distinguishing features of the snake that bit them. In some areas the Papuan black snake and taipan are not recognised as separate species. This frequently leads to mis-identification of the biting snake and in some cases subsequent use of incorrect antivenom which is avoidable.

3. Papuan black snakes are now extremely rare in Central Province and appear to be limited to the extreme west and east. Distinguishing clinically between a taipan bite and a black snake bite is impossible and if a black snake bite is suspected, polyvalent antivenom should be given. The role of black snake antivenom in PNG is now extremely limited and the health department should stop purchasing it.

4. Over 95% of taipan bites occurred during daylight hours. There was no evidence of significant seasonal trends or associations with rainfall during the two

years of this study.

5. The majority of victims of taipan bite are bitten on the lower limb below the knee whilst walking in overgrown bush. Provision of calf length boots to workers at particularly high risk, such as plantation and cattle ranch workers, would provide significant protection and be feasible.

6. Tourniquets and local incisions are still widely used as first aid treatment throughout Central Province with occasional disastrous results. Further efforts at education about safe first aid measures are necessary.

7. Delay in referral is the most significant avoidable factor in mortality from snakebite and could be reduced by further education of health centre staff and better use of existing communication, in particular, radio links. Health centre staff should be encouraged to use pressure bandages to delay absorption of venom, even in patients who have been given antivenom. This may increase the safe period for envenomed patients to get to hospital before the onset of life-threatening neurotoxicity.

8. The majority of patients bitten by a taipan have non-clotting blood at admission to hospital. Over 97% of snakebite victims admitted to PMGH in the past two and a half years with coagulopathy, where the species was identified, had been bitten by a taipan. The predictive value of non-clotting blood for taipan bite is so high that it would be reasonable to give monovalent taipan antivenom to all such patients.

9. There are important differences between the clinical features of patients bitten by the Papuan and Australian taipans, notably, spontaneous systemic bleeding is significantly more common in victims of the Papuan subspecies. It also appears that the response of Papuan victims to antivenom is less favourable than that of Australian victims. The pattern of neurotoxicity appears to be similar in envenoming by both species.

10. No evidence of autonomic dysfunction was found in any envenomed patients. Sensory abnormalities were limited to an area local to the bite site.

11. Current antivenom treatment did not reverse and in many cases failed to inhibit the progression of neurotoxicity. Antivenom appeared to speed the resolution of coagulopathy but this did not appear to be of great clinical significance. There is a need for improved antivenom, either specific to the Papuan subspecies or a subunit antivenom.

12. Antivenom was of maximal benefit if given within 4 hours of envenoming. The proportion of patients who receive antivenom in this time span could be significantly increased by maintaining antivenom supplies to four health centres, Veifa'a, Kwikila, Kupiano and Bereina, and further education of the health staff working there. Over 75% of envenomed patients in this series could have been given antivenom within 4 hours of envenoming.

13. There was no difference between monovalent taipan and polyvalent antivenom either in efficacy or in the incidence of side effects. The optimal volume of antivenom required to treat the victim of a

taipan bite remains unknown.

14. Early antivenom reactions occurred in 7% of all patients given antivenom despite the use of prophylaxis. All reactions were mild and not life threatening. No late antivenom reactions were seen, possibly because no patients received more than two vials of antivenom. The optimal prophylaxis to prevent antivenom reactions is unknown but all patients should be given at least a parenteral antihistamine. All staff giving antivenom must know how to treat an anaphylactic reaction and have the requisite drugs and equipment.

15. Most morbidity and mortality in snake bite victims in hospital was associated with difficulties with intubation and the management of ventilated patients. Many of these problems were avoidable.

16. Venom detection kits proved a sensitive means of detecting venom in bite site swabs. They should be used on selected patients at admission to help distinguish biting species. There are potential benefits both in terms of better use of antivenom and by producing financial savings. The discriminatory value of VDKs is potentially most useful in identifying the biting species in patients who do not have a coagulopathy.

17. Envenomed patients had markedly reduced evoked CMAP amplitudes, normal motor conduction velocities and normal F wave responses. The decline and recovery of CMAP amplitudes correlated closely with the clinical stage of neurotoxicity including measurements of peripheral grip strength.

18. Repetitive nerve stimulation studies showed decremental responses in envenomed patients consistent with abnormal synaptic transmission. Transient post-tetanic and post-exercise facilitation occured followed by post-tetanic exhaustion.

19. Sensory nerve action potentials were reduced in envenomed patients. Sensory nerve conduction velocities were also marginally reduced. Clinical sensory abnormalities were restricted to the area of the bite site.

20. Spontaneous electrical activity was frequently detectable in the form of positive sharp waves, fibrillation potentials and fasciculation potentials in the muscles of envenomed patients. Clinically evident fasciculation was visible in some patients, most notable in gastocnemii and deltoid muscles. This may reflect spontaneous transmitter release or a direct action of a toxin on the muscle membrane.

21. Edrophonium and 3,4-DAP both produced an electrophysiological response in envenomed patients suggesting a minor improvement in neuromuscular transmission. The effect was maximal when the two drugs were used in combination. The improvement in peripheral strength was minor and not sufficient to be clinically useful.

22. Both edrophonium and 3,4-DAP produced a significant increase in oral secretions in envenomed patients which was diminished but not abolished by atropine. Caution must be exercised if these drugs are used in patients with compromised pharyngeal musculature

who are at risk of aspiration.

Campbell and Young wrote in 1961 "..we believe that with modern treatment, the elapine snake victim should not die if he lives long enough to reach a hospital"[24]. In 1994, with the improvements in communications, transport and rural health facilities of the past 30 years, victims of elapine snake bite in Central Province should not die.

CHAPTER 10 - REFERENCES

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APPENDIX 1

Proforma for clinically envenomed patients

SNAKEBITE STUDY

1. First Name.....
2. Surname.....

3. Study Number.....
   Hospital Number.....

4. Age.....

5. Sex (m/f)

6. Village.....

7. Sub-province.....
8. Province.....

9. Address in NCD.....

10. Date of bite / /
11. Time of bite.....
12. Date of admission / /
13. Time of arrival in hospital
14. Date seen / /
15. Time seen.....

16. Date of discharge/death

17. Area of Province where bitten.
18. Pre-hospital treatment details

Description of snake.....
Circumstances of bite.....

Vegetation.....
Distance from water...

SYMPTOMS	Time of onset	Progression
19. Part of body bitten.....		
20. Pain around bite.....(Y/N)		
21 Pain in limb.....(Y/N)		
22. Pain in lymph nodes.....(Y/N)		
23. Headache.....(Y/N)		
24. Abdominal pain.....(Y/N)		
25. Vomiting.....(Y/N)		
26. Collapse.....(Y/N)		
27. Bleeding.....(Y/N)		
From where.....		
28. Ptosis.....(Y/N)		
29. Double vision.....(Y/N)		
30. Dysphagia.....(Y/N)		
31. Dysarthria.....(Y/N)		
32. Limb weakness.....(Y/N)		
33. Respiratory symptoms.....(Y/N)		
34. Drowsiness.....(Y/N)		
35. Muscle pain.....(Y/N)		
36. Passing urine.....(Y/N)		

37. Previous history of snakebite....(Y/N) When.....
What treatment.....

38. Other significant medical condition(Y/N)

Name..... Study number.....

SIGNS Date time													
39. Local swelling													
40. Local tenderness													
41. Lymph nodes													
42. Tender nodes													
43. Muscle tenderness													
44. Bleeding gums													
45. Nose bleed													
46. Haemoptysis/emesis													
47. Bleeding bite site													
48. Other bleeding													
49. Abdo tenderness													
50. Liver (cm)													
51. Spleen (cm)													
52. Lungs													
53. Heart													
54. Conscious (grade)													
55. Fundi (Findings)													
56. Ptosis (+ - ++++)													
57. Up gaze (N/dec/abs)													
58. Down gaze (" " ")													
59. Lateral gaze(" ")													
60. Pupil size (mm)													
61. Interdental dist(mm)													
62 Slurred speech													
63 Facial weakness													
64 Lat jaw movement													
65 Passive jaw opening													
66 Tongue protrusion													
67. Respiratory pattern **													
68. Peak flow													
69. Gag (Y/N)													
70. Cough reflex													
71. Pooling secretions													
72. Grip (grade)													
73. Moving limbs													
74. Reflexes (+/++++)													
75. Intubated (Y/N)													
76. Ventilated (-/A/T)*													
77. Pulse													
78. BP													

* A/T auto/triggered

** N/shallow/diaphr/ven

Patient name..... Study number...

76. Exact site of bite.....
77. Drawing of bite..... 78. Inter-fang distance cm

79. Snake available.....(Y/N)
Length
Description

Extra Clinical information

Date Time

Patient name..... Study number.....

TREATMENT

80. Antivenom given.....(Y/N)
Time commenced....
Time completed....

Type given....

81. Second ampoule given.(Y/N)
Time commenced....
Time completed....

82. Intubated.....(Y/N)
Date
Time

Date extubated
Time extubated

83. Ventilated.....(Y/N)
Date commenced
Time commenced

Date discontinued
Time discontinued

84. FFP given.....(Y/N)
Number of units
At what times

Drugs given with polyvalent

85.	Promethazine	mg	IM/IV	at	am/pm
86.	Adrenaline	mg	SC/IM	at	am/pm
87.	Hydrocortisone	mg	IM/IV	at	am/pm
88.	Tet toxoid	ml	IM	at	am/pm
89.	Procillin	ml	IM	at	am/pm

Other drugs

Drug	Dose	Route	Frequency	Commenced	Ended

IV fluids:- Vol in 1st 24 hrs...
Vol in 2nd 24 hrs...
Vol in 3rd 24 hrs...

Urine:- Vol in 1st 24 hrs...
Vol in 2nd 24 hrs...
Vol in 3rd 24 hrs...

LABORATORY INVESTIGATIONS- Snakebite

Patient name..... Study number..

Date													
Time													
Clotting time													
WBC													
RBC													
Hb													
HCT													
MCV													
MCH													
MCHC													
Dif													
Plat													
Glucose													
Na													
K													
HCO^3													
Cl													
Urea													
Creat													
AST (ALT)													
Bil (Dir)													
ALP													
Prot													
Alb													
CK													
LDH													
Urine Analysis													

Patient name..... Study number...

	ADM	6	12	18	24	36	48	72	96	DIS
Date Time										
TEST										
Exam	+	+	+	+	+	+	+	+	+	+
EDTA FBC, Plat	+				+		+	+	+	+
Citrate	+		+		+		+	+	+	+
Serum (Ag, clotting.	+	+	+	+	+	+	+	+	+	+
FDP	+				+					+
BTG (only if non-clotting)	+				+					+
Hep plasma (Hb, Myo)	+		+		+		+	+	+	+
Lab for U/E, CK, AST	+				+		+	+	+	+
TNF	+				+					+
Wound Aspirate/swab	+									
Urine Ag	+									
Urine Stix, myoglobin	+				+		+	+	+	+

SUMMARY

1. Systemic symptoms.....(Y/N)
2. Local signs of envenoming.....(Y/N)
3. Neurotoxic signs.....(Y/N)
4. Prolonged blood clotting.....(Y/N)
5. Clinical bleeding.....(Y/N)
6. Signs of myotoxicity.....(Y/N)

7. Neurotoxicity development post antivenom.....(Y/N)
8. Neurotoxicity deterioration post antivenom.....(Y/N)
9. Need for intubation.....(Y/N)
10. Need for ventilation.....(Y/N)
11 Survived.....(Y/N)
12. Referral from other centre.....(Y/N)
13. Length of stay in hospital..... days

From time of bite:-

14. Time to arrival in hospital..... hrs
15 Time to symptoms of envenoming.... hrs
16. Time to neurotoxic symptoms..... hrs
17. Time to clinical bleeding..... hrs

18. Time to onset of incoaguable blood hrs
19. Time to observed neurotoxicity.... hrs
20. Time to peak of neurotoxicity..... hrs
21. Time to intubation..... hrs
22. Time to giving of antivenom..... hrs
23. Time to resolution of neurotoxicity hrs

Following antivenom:-

24. Time to normal clotting time..... hrs
25. Time to commence neuroresolution... hrs
26. Time to neurotoxicity resolution... hrs
27. Length of time intubated..... hrs
28. Length of time ventilated..... hrs

Complications

29. Antivenom reaction.....(Y/N)
30. Local tissue damage.....(Y/N)
31. Local infection.....(Y/N)
32. Renal impairment.....(Y/N)
33. Pneumonia.....(Y/N)
34. Serum sickness.....(Y/N)
35. Other.....(Y/N)

SNAKEBITE - Follow up

1. First name..... 3. Study number.....
2. Surname..... Date.....

4. Number of days since admission
5. Specific complaints

6. Episodes of fever..... (Y/N)
7. Intercurrent medical problems.(Y/N)

8. Temp Oral..... C 9. Pulse ..... /min
10. B/P Sitting..... / Weight..... kg

11. Bite site.....
12. Regional lymph nodes.....
13. Auscultation.....
14. Liver..... cms below costal margin
15. Spleen..... cms below costal margin

16. Eye movements.....
17. Fundi.....
18. Limb Tone.....Increased/Decreased/Normal
19. Reflexes.....Increased/Decreased/Normal

20. Other Signs.....
21. .....
22 .....

Stored

Serum	x
Hep	x
Citrate	x
Urine	x

WCC		Na		AST	
Hb		K		Bil	
Platelets		HCO3		ALP	
		Cl		Prot	
Clotting		Urea		Alb	
		Creat		CK	

Urine

APPENDIX 2

Proforma for clinically non-envenomed patients

SNAKEBITE STUDY (non envenomed)

First Name..... Study Number.....
Surname.....

Age..... Sex (m/f)

Village..... Sub-province..... Province.....

Address in NCD.....

Date of bite / / Time of bite.....
Date seen / / Time seen.....

Description of snake.....
Circumstances of bite.....

Area of Province where bitten...
Any pre-hospital treatment.....

SYMPTOMS SIGNS

Part of body bitten..... Description of bite.....

Local symptoms Local swelling/signs
Systemic symptoms Lymphadenopathy
Symptoms of bleeding Neurological signs
Neurotoxic symptoms Evidence of clotting disturbance

Previous history of snakebite....(Y/N)
Other significant medical condition(Y/N)

EDTA	x
Serum	x
FDP	x
CK only	x
Swab	x
Aspirate	x

Time	
Clotting time	

Hb	
WCC	
Plat	

Na	
K	
Cl	
HCO3	
Urea	
Creat	

AST	
Bil	
ALP	
Prot	
Alb	
CK	
LDH	

APPENDIX 3

Neurotoxicity grading scale

A simple grading system was used in order to compare patients. This was necessary because the time course and rate of progression of envenoming varied extensively. The sequence of progression of neurotoxicity was similar in patients who developed signs, with involvement of external ocular muscles, bulbar and respiratory muscles, in that order. Comparative assessment of peripheral muscle weakness between patients was more subjective and therefore not included in the grading. This system, although crude, proved workable. The suffix "A" was used to denote patients of grade 2-4 who were deteriorating, "B" to denote those who were recovering. All the patients included in the intervention studies were during the deteriorating phase or stage 5.

Grade 1	Envenomed but no neurotoxicity
Grade 2	Ptosis +/- partial opthamoplegia
Grade 3	Complete opthalmoplegia
Grade 4	Pooling of secretions requiring intubation or intubated but not ventilated
Grade 5	Ventilated

APPENDIX 4

Hospital snakebite observation chart

SNAKE BITE OBSERVATION

NAME _____ NUMBER _____ DATE _____

Time	Pulse	Respiration	Blood Pressure	Temperature	Headache?	Gland Tender?	Abdominal Pain?	Ptosis	Eyeballs Fixed?	Speech Thick?	Mouth — Open more than 1"?	Secretions pooling in Pharynx	Resps. — Intercostal	Resps. — Diaphragm	Bleeding Bite	Spitting Blood	Tracheotomy	Urine — Albumen Blood

[Govt Print.—1245-8 000.—9.85]

APPENDIX 5

RELATED PUBLICATIONS AND PAPERS SUBMITTED

Lalloo D.,Trevett A.,Kevau I.,Warrell D. Antivenom use in Australia (lett) M.J.Aust.1993;159:68

Lalloo D.,Trevett A.,Black J.et al. Neurotoxicity and haemostatic disturbances in patients envenomed by the Papuan black snake (*Pseudechis papuanus*). Toxicon.1994;32:927-936.

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Trevett A.J.,Lalloo D.G.,Nwokolo N.C.,Naraqi S.,Theakston R.D.G.,Warrell D.A. Pharmacological interventions in the treatment of neurotoxicity caused by taipan bite. (submitted Trans.Roy.Soc.Trop.Med.Hyg)

Trevett A.J.,Lalloo D.G.,Nwokolo N.C.,Naraqi S.,Kevau I.H.,Theakston R.D.G.,Warrell D.A. Antivenom in the treatment of Papuan taipan bite. An analysis of time of treatment and outcome. (submitted Trans.Roy.Soc. Trop.Med. Hyg.)

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