# Progress in the Characterization of Venoms and Standardization of Antivenoms

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## PREFACE

This account of recent progress in the characterization of venoms and the standardization of antivenoms is based on the report of a WHO Coordination Meeting on Venoms and Antivenoms, which was held in Zurich, Switzerland, from 24 to 27 September 1979. The participants in that meeting are listed in Annex 9. The remaining eight annexes provide much useful supplementary information, including detailed instructions for the processing and characterization of venoms and for the processing and standardization of antivenoms.

For many years WHO has had an interest in the treatment of bites and stings from venomous creatures and, although there have been informal meetings from time to time, none has specifically attempted to collect data on the clinical effects of snake and scorpion bites and stings and experience in their treatment. Furthermore, there is an urgent need to correlate such experience with the laboratory tests being applied to the antivenoms in attempts to measure the potency of these materials. One important advance that could be made in such standardization is to make available venoms that have been fully characterized and to establish international standards for antivenoms.

WHO has taken the first step by designating the Liverpool School of Tropical Diseases as the WHO Collaborating Centre for the Control of Antivenoms.

For use in English, "venom" and "antivenom" are considered to be the preferred names rather than "venin/antivenin" or "venene/antivenene".

## EPIDEMIOLOGY

### Incidence and Mortality of Snake Bites, Scorpion Stings, and Spider Bites

Injuries and death due to snake and spider bites as well as scorpion stings occur in most parts of the world, and especially in the tropics, where they may represent a major health problem. Unfortunately, knowledge of their epidemiology is fragmentary, mainly owing to the lack of reliable statistical data.

In the United States of America, approximately 8000 bites by venomous snakes are reported each year. About 12 deaths occur in untreated, undertreated, or mistreated children or in members of snake-handling cults. Approximately 1000 scorpion stings are reported each year; the last death was in 1968. About 3000 spider bites (usually Latrodectus or Loxosceles spp.) occur each year. Marine animal stings range into several hundred thousand each year but deaths are extremely rare.

Scorpion stings are a major health problem in Mexico, where there are an estimated 300 000 cases each year, with about 1000 deaths. Scorpion stings are also important in Trinidad and South America. Spider bites are mainly common in South America and Australia.

In Costa Rica, hospital admissions for snake bite have been estimated as 22.4 per 100 000 population per year, with 5 deaths per 100 000 (mostly due to bites by Bothrops atrox asper). In South America, 90% of snake bites are caused by Bothrops species. Mortality has been estimated as 2.4% but may be as high as 8% when no antivenom is given. After rattlesnake bites (Crotalus durissus terrificus) about 74% of the untreated victims die, but in patients receiving antivenom, mortality falls to 12%.

In North Africa, scorpion stings are medically more important than snake bites. For example in southern areas of the Libyan Arab Jamahiriya during 1979 there were 900 scorpion stings per 100 000 population and 7 deaths per 100 000 (most deaths being in children under 2 years old). In mid-Africa, the incidence of snake bite has been greatly underestimated. In savanna regions of West Africa, the carpet viper (Echis carinatus) is the most important cause of snake-bite morbidity and mortality. In one area in north-eastern Nigeria, there are about 120 bites and 8 deaths per 100 000 population each year; in northern Ghana, the incidence of bites is 86 per 100 000, with 24 deaths per 100 000.

In Europe, snake bite is relatively rare. Only 14 deaths due to adder bites (Vipera berus) have occurred in Britain during the last 100 years. In England and Wales only one death from adder bite was recorded in 1950-72, but there were 61 deaths from bee or wasp stings. The last adder bite death in Germany was in 1959. In Finland, there were 21 deaths from adder bite during 1936-60, and an incidence of 163 proven bites during the summer of 1961. In Europe, bites by imported venomous snakes are sometimes fatal

In south-eastern Asia, over 2500 deaths due to snake bite are reported annually (see Annex 1). The mortality is high in Burma, India, the Philippines, Sri Lanka, and Thailand; in the Maharashtra state of India, more than 1000 deaths per year due to snake bite have been recorded.

In Australia, about 3000 suspected cases of snake bite are reported each year and 600 victims are treated with antivenom. Between 5 and 14 patients used to die each year, but recently the mortality rate has fallen as a result of better treatment.

It is to be expected that the use of immunodiagnostic methods for assessing venom antigen and antibody and collaboration with anthropologists and traditional healers will greatly improve the epidemiological data on venomous bites and stings. The data should be reported using the ICD classification.[1]

## MEDICALLY IMPORTANT SPECIES

Medically important snakes are listed in Annex 2. The list is not definitive; it is compiled from published medical reports of bites by identified species. Scorpions of importance include species of Centruroides (Mexico, North and Central America), Tityus (South America), Androctonus, Buthus, and Leiurus (North Africa to South-East Asia). Spiders of the species Latrodectus occur in warm areas throughout the world. Loxosceles species can cause severe necrosis (mainly in the Americas). Spiders of the Phoneutria and Atrax genera are medically important in South America and Australia respectively.

## CLINICALLY IMPORTANT FEATURES OF ENVENOMING

### Systemic Envenoming

Snake bite envenoming produces changes that, specifically, may not be systemic or local and it is important for the clinician to assess all symptoms and signs, both local and systemic, in determining suitable treatment. In crotalid venom poisoning, systemic manifestations include hypotension or shock, bleeding, blood cell changes, and sometimes neurological effects. In viper bites involving defibrinogenation, such as bites by Echis carinatus or Agkistrodon rhodostoma, the diagnosis and to some extent the degree of severity can be assessed from the observation of spontaneous haemorrhage and non-clotting blood. Systemic poisoning from elapid bites principally involves the neuromuscular junction but other systems may be affected, including the heart, kidneys, and so on. Sea-snake venoms are primarily myotoxic in man, affecting skeletal muscle. At present these clinical manifestations appear to be the best guide for assessing the degree of systemic poisoning and for deciding on appropriate treatment. Certain laboratory procedures may improve the assessment.

### Local Envenoming

Local effects are negligible despite the presence of serious systemic envenoming in bites by sea snakes and certain elapids, such as kraits and mambas. On the other hand, local envenoming may be the major and often the only clinically observed feature in many viper bites. Local swelling is due to capillary exudation of plasma or whole blood, presumably from a cytotoxic action on vascular endothelium. With some viper envenomings, the severity of local swelling can be correlated with the severity of systemic poisoning; but sometimes (in Echis bites, for example) serious systemic poisoning can occur with only minimal local effects. In other cases, local swelling can be massive and very extensive. In nearly all cases of massive extravasation, provided there is no underlying local necrosis, conservative treatment results in complete recovery, emphasizing how ill-advised are disabling procedures such as routine fasciotomy.

Local necrosis is the most serious local manifestation because it can cause prolonged and sometimes permanent disability. Local necrosis can develop after bites by some (though not all species) of the vipers, by African spitting cobras (such as Naja nigricollis), by Asian cobras, and by some spiders such as Loxosceles. Local necrosis is often preceded by local blistering. The necrosis may develop slowly, appearing like "dry gangrene" over a matter of weeks and presumably being mainly ischaemic in origin. But in cobra bites, local necrosis

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[1] Manual of the international statistical classification of diseases, injuries, and causes of death, 1975 revision, Geneva, World Health Organization, 1977, vol. 1, p. 594, category E905.

appears in a matter of days and appears more like "wet gangrene"; presumably there is a direct cytolytic venom effect involving mainly the superficial subcutaneous tissues. Other factors may also be concerned in local necrosis, including locally applied chemicals, local incisions, tourniquet ischaemia, and so on. Bacteria are always found in mouths of snakes after capture, although flora and quantity differ in different species. Such differences, however, do not appear to be of prime importance in snake bite; lymphadenopathy, so commonly seen after snake bite, develops much too quickly to be caused by bacterial growth. Tetanus and gas gangrene following snake bite have occasionally been reported but appear to be exceedingly rare.

### Autopharmacological Features

An autopharmacological reaction is one that is produced by the release of substances from normal tissue caused by the stimulation of a foreign substance. Such released substances result in physiopharmacological reactions that may be deleterious to specific organs or tissues. Among the more important autopharmacological substances related to venom poisoning are histamine, 5-hydroxytryptamine, kinin, and adenosine, although a number of other tissue components may be involved in such reactions.

The role of these substances in snake venom poisoning is questionable. In vitro studies are difficult to correlate with in vivo studies, and much more so with clinical observations. The present evidence indicates that these reactions may play a minor role in most clinical cases. There are some species of snakes, however, whose venom is more prone to induce autopharmacological or unusual reactions. These include Vipera berus, Vipera xanthina palaestinae and Atractaspis engaddensis.

In some cases the Australian elapids appear to produce a transient rather than an immediate reaction, which appears to be autopharmacological in nature, but the etiology of these reactions is not understood.

In all cases of venom poisoning, the physician must be aware of the possibility of an autopharmacological reaction and be prepared to give specific treatment. In any anaphylactic shock or a severe anaphylactic reaction immediate therapeutic measures must be taken. In addition, there may be some other reactions to venoms that today remain ill-defined.

## THE CHARACTERIZATION OF VENOMS AND STANDARDIZATION OF ANTIVENOMS

### The Provision of Venoms

Venoms from snakes of the same species collected from different areas may have different pharmacological properties. Thus the venom of Echis carinatus collected from the snakes of West Africa is different from the venom of the same species collected from Iran. Although the provision of venoms from every snake species would be impracticable, it should be possible to obtain venoms from the species causing major health problems.

Differences in pharmacological activities of the snake venoms recognized by clinical observation need to be recorded and if possible correlated with the results of laboratory work. It is important, therefore, to provide a number of countries with reference reagents of venoms representative of a particular species. In order to implement this, international reference reagents of venoms are to be established, making a start with those from seven important species: Naja naja; Notechis scutatus; Echis carinatus (West Africa and Iran); Vipera russelii; Crotalus adamanteus; Bothrops atrox asper (Atlantic); and Trimeresurus flavoviridis (see Annex 3).

It is important to have the venom collected from the snakes of the same species in a given area and to have sufficient to freeze-dry at least 1000 ampoules for an international reference reagent. In addition, some of each venom should be set aside for the preparation of antivenom.

## The Characterization of Venoms

Several laboratories have agreed to take part in a collaborative study, coordinated by WHO, to measure the lethal, defibrinating, haemorrhagic, and necrotizing activities. Although these studies will not cover other activities, such as the neurological activity, they will provide useful data for the partial characterization of the venoms. The details of the collection and the requirements of the suitability of the venoms to be recognized as international reference reagents are shown in Annex 3. There is also a need to adopt a common method of testing lethal and other biological activities of the venoms (see Annex 3). Each dried venom will be characterized by an international collaborative study according to an agreed protocol and the findings will be presented to the WHO Expert Committee on Biological Standardization for adoption as an international reference reagent of venom from a specified species of venomous creature.

## The Provision of Antivenoms

A list of currently available commercial antivenoms is given in Annex 4.

In order to develop and establish international standard antivenoms, it is suggested that antivenoms should be raised in horses, using the same venoms as those proposed as the international reference reagents of venoms. Each antivenom will be monospecific and it is anticipated that two or three horses may be needed for each antivenom to provide a suitably potent material.

Since the international standard antivenoms will be monospecific and raised using the appropriate international reference venom, the antivenoms will not be available commercially and must be prepared specially. Furthermore, it must be emphasized that the ultimate test of efficacy is in clinical trials, the aim of which will be to correlate the activities of these monospecific antivenoms with laboratory tests. Laboratories that have tentatively agreed to prepare the antivenoms are listed in Annex 5.

## Immunization Schedules

The schedules used for the immunization of horses depend on so many factors related to the particular venom and the horse being immunized that no single schedule for all venoms can be established. With some mainly neurotoxic venoms (such as coral-snake venoms) the schedule could be relatively short and the total amount of venom given could be as small as 200 mg. With more complex venoms, however, such as those of the Crotalidae family, an intensive immunization course is necessary, sometimes lasting longer than 250 days, in order to build up a satisfactory immunological response towards the components that are present in low concentration or to those with a low molecular weight. The final doses of venom may be in the range of 500-1000 mg.

It is considered advisable to include some adjuvant to decrease the time required and to increase the immunogenicity of the venom used. It is also advisable to sterilize the venom by membrane filtration before the inoculation, in order to minimize abscess formation at the site of the injection. For venoms not giving rise to necrosis there has been no difficulty in using crude venom. When there are marked necrotizing effects, however, venoiding with glutaraldehyde or formaldehyde has been shown to be successful. This method of venoiding, which has given venoids protecting small animals against massive challenges with venom (tiger-snake venom), is worthy of further investigation with other venoms.

For the production of a large quantity of antivenom, healthy horses more than 5 years and usually less than 8 years old and under veterinary supervision are required. Their antivenom titre is built up by regular subcutaneous injections of gradually increasing doses of venom.

It is not known whether sufficient attention is being paid to the avoidance of the IgM peak of production when spacing the doses of immunization or whether the use of capsules slowly releasing antigen would be useful. Nor is there any explanation of "saturation" of hyperimmune animals.

In the laboratory multisite injection of rabbits with venom in complete Freund's adjuvant has given good antisera in the precipitin test.

For raising antivenoms to scorpions it is important to use the venom obtained by electrical stimulation rather than by maceration of the telson.

## The Refining of Antivenoms

There is a need to refine the proposed international standards for antivenoms and to produce them under such conditions that they could be administered to man.

Antibodies against venoms in plasma from immunized horses may be isolated and concentrated by different methods. However, enzyme-refined sera are to be preferred because they are less prone to cause serum reactions.

It is suggested that investigations should be made to determine whether the removal of the portion of the horse globulins in any way inhibits the capacity of the immunogloblins to diffuse in tissues. This is important because such a phenomenon would not be detected in a mouse protection test in which the venom and antivenom were mixed before injection.

It is important that all the antivenoms satisfy the Requirements for Immune Sera of Animal Origin formulated by the WHO Expert Committee on Biological Standardization.[1]

## The Potency Assay of Antivenoms

Many laboratories throughout the world are neutralizing venoms with antivenoms and calculating the 50% endpoint of neutralization by the Reed & Muench method. One of the fundamental requirements for this method to be appropriate is that the median point of the dilution series of the antivenom falls in the middle of the dose-response curve; when this is not the case, errors occur in the calculation. A much more acceptable method of calculating the median points is by probit analysis, or the method of Spearman-Kärber may be used.

There are many different methods in use in several laboratories for the assay of potency of antivenoms. A real problem arises in the assay of the antivenom in the laboratory using as an endpoint of neutralization a pharmacological property different from that known from clinical observation to be important in man. Thus, mice inoculated with Echis carinatus venom die rapidly with massive intravascular coagulation whereas human victims die after 1-2 weeks with bleeding exaggerated by defibrination. Mice inoculated with Naja nigricollis venom die rapidly with neurotoxic signs (convulsion) whereas human victims usually suffer local necrosis with severe, persisting disability, without neurotoxic symptoms.

Several methods for the expression of activity of antivenoms have been proposed, but once an international unit of activity has been assigned to an antivenom with respect to its ability to neutralize a given quantity of venom then standardization of other similar antivenoms will be possible by direct comparison with the international standard, using the parallel-line assay method. This is the only way in which antivenoms could be standardized in the future. The use of an appropriate test toxin is of particular importance in the assay of antivenom potency. For the discussion on this point, see the next section.

In order to effect this standardization, it is important to reach an agreement on the methods used and it is to be hoped that the details of such tests can be rapidly established.

## Standardization of Antivenoms

The standardization of antivenoms is not simple because of the antigenic complexity of snake venoms. As venoms possess different pharmacological activities, the antivenom should ideally be titrated against each important activity. As an example, venom of Trimeresurus flavoviridis contains two haemorrhagic principles that may stimulate different antibody titres in different antivenoms. Antihaemorrhagic potency of an antivenom relative to a standard can

[1] WHO Technical Report Series, No. 413, 1969.

only be determined when the two immunologically distinct haemorrhagic principles are used separately as test venoms instead of crude venom. For a complete evaluation of antitoxic potency of an antivenom, therefore, all the important venom components should preferably be separated and each used as a test venom.

The potency of any national or laboratory antivenom should be determined by reference to that of a stable standard antivenom. For this reason, there is a need to establish international standard antivenoms against venoms that have been extensively studied with respect to their pharmacological activities important to man. In view of the diversity in toxic components of venoms of the same snake species collected from different areas, the snakes from which the venom was collected must be identified geographically. The availability of characterized venoms and standardized antivenoms would greatly assist collaboration among laboratories in different countries.

Although the mouse protection test is not always reliable in predicting the clinical effectiveness of an antivenom, it is the most widely used assay procedure. Animal assays of venom and antivenom interaction are expensive and time-consuming and there remains some uncertainty as to what activities are being measured. Efforts should be encouraged to develop in vitro biochemical and immunological methods of assay to be used in conjunction with animal tests. A suggested method for the standardization of antivenoms is shown in Annex 5.

## THE DESCRIPTION, STORAGE, AND DISTRIBUTION OF ANTIVENOMS

### Labelling

The WHO Expert Committee on Biological Standardization, in its twenty-third report,[1] laid down some simple requirements for labelling antivenoms, including the potency of the antivenom; nature of the preparation; method of reconstitution; restrictions, if any, of its use in a particular country or area; the identity of each reference venom against which the potency has been expressed; and a list of snakes for which cross-protection may be expected. Unfortunately, some makers omit the potency and information as to the species of snakes from which the venoms are used for manufacture.

The instructions for use in any leaflet accompanying an antivenom should be clear, simple and printed in letters large enough to be able to be read under restricted light conditions. The best instruction pamphlets available are large and able to be enclosed in a plastic box, which has the additional advantage of protecting the ampoule from breakage. The labelling of the ampoule itself should be screen-printed as paper labels can become separated by repeated handling in high humidity and thus the ampoule may become unidentifiable.

The leaflet should contain instructions on first-aid in the field, on the recommended human dosage according to the clinical presentation, and on the route of administration as well as on adverse reactions, their prevention and treatment.

It is desirable that the instruction pamphlet should be accompanied by an inquiry sheet to be returned to the manufacturer of the antivenom so that the responses and reactions to antivenoms may be assessed.

Training clinicians through instruction pamphlets alone has not been found to be satisfactory. Education of the lay public and of medical students should therefore be supported. Although much has already been done in some countries and there is already a greater awareness of the problem of snake bite in other areas, there is a need for active promotion of such education.

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[1] WHO Technical Report Series, No. 463, 1971.

## Distribution

In most tropical countries the supply system is deficient and in many areas it is impossible to obtain antivenoms. On the other hand, in the developed countries distribution is easy and rapid. In the Federal Republic of Germany, for instance, there are 10 different depots from which the antivenoms are distributed. Even in some developing countries (such as in Costa Rica) depots holding antivenoms sufficient to treat a severe case are located in strategic places, which can be reached by the patient in no more than 2 hours under conditions of difficult transportation. In Nigeria, health auxiliaries in a rural clinic have been trained in the definitive management, including intravenous antivenom infusion, of most snake-bite victims.

In the countries in the Middle East, the distribution of antivenoms is usually in the hands of governments. The antivenoms are distributed to hospitals and medical stations free of charge. It is important, however, for those responsible for the purchase of antivenoms to ask advice concerning the suitability of the products available.

When transporting antivenoms in the liquid state to countries with high ambient temperatures, it is essential to ensure adequate refrigeration and insulation. Appropriate technology for the transportation and storage is a prerequisite for maintaining high-quality antivenoms. It is therefore important that practical principles should be developed at the international level for efficient snake and scorpion bite control programmes suitable for incorporation in national primary health care systems.

## Storage

The facilities for storage of antivenoms and vaccines are inadequate in many developing countries. Although antivenoms arrive in tropical countries with satisfactory potency, their storage at suitable temperatures, until they reach their final destination, is often unsatisfactory; antivenoms are sometimes kept at temperatures as high as 38 °C for long periods of time.

There is evidence that antisera maintained at 30 °C retain their potency for at least 6 months and possibly 1 year, but this does not obviate the necessity for the provision of a satisfactory cold chain (see Annex 6).

Antivenoms are supplied either in the liquid state or lyophilized. It is recommended that antivenoms for use in the tropics should be lyophilized rather than in the liquid form, since the former are more stable at high temperatures.

## Expiry Date

The period throughout which an antivenom may be expected to retain its activity when stored in the liquid state is 2 years after the date of issue (some control authorities allow 3 years or more), but its stability depends on the pH of the serum and the conditions under which it is kept. One important point is the date of manufacture, which is the date of passing the potency test.

The effects of some properties of polyspecific antivenoms on the stability are shown in Annex 6, Tables 2 and 3. The optimal range of temperature for storing antivenom is 5 °C ± 3 °C. If the antivenom is in the liquid form and kept at 5 °C, no change in potency occurs within 6 years, but after 8-10 years a 10-20% loss of neutralizing ability may occur against some (though not all) venoms.

The expiry date for a freeze-dried preparation, when sealed by fusion of the glass ampoule, should be many years, but for preparations in vials with a rubber stopper and metal crimped caps the declared expiry date should not be more than 5 years because of the possibility of an increase in the moisture content of the product, which would diminish the potency in some of the vials. Other factors that increase the stability of the antivenoms are high protein content and high pH.

## THE CLINICAL USE OF ANTIVENOMS

### Clinical Efficacy

There are considerable differences in the efficacies of the various antivenoms. Dosage is an important factor, and the protection provided against one particularly deleterious biological property may vary both qualitatively and quantitatively from one antivenom to another. In crotalid venom poisoning, most available antivenoms can be very effective in the hypotensive patient or during the shock state; late in the shock state they are less effective, but even in such cases, antivenoms should be tried. The haemorrhagic and coagulation effects of the venoms of Agkistrodon rhodostoma and Echis carinatus can be dramatically reversed. In certain elapid bites, the antivenom blocks or can reverse the neurological deficit. Sea-snake antivenom has proved highly successful, even when given as late as 2 days after the bite. Similar reversals of sytemic symptoms or signs have been reported in other snake venom poisoning cases and can be cited as examples of the effectiveness of antivenom.

The effectiveness of antivenoms in preventing or minimizing the local effects of venoms, especially the development of local necrosis, is uncertain and urgently requires clinical investigation.

### Dosage, Time-factor, and Monitoring Problems

The initial dose of antivenom is often calculated from mouse protection tests by scaling the antivenom dose up to take account of average venom yields. These calculations ignore the wide variability in the amounts of venom injected into man by biting snakes (often little or no venom is injected), the differences in responses of various animal species to venoms, and the different modes of death in small mammals compared with human victims of snake bites.

The variable time interval between the bite and the patient's presentation for treatment (there is sometimes a long delay) introduces yet another difficulty into the clinical study of antivenom. Experimentally, delay in administering antivenom results in a steep increase in the median effective neutralizing dose. For example, in monkeys injected with Australian venoms, if antivenom is withheld until early signs of poisoning develop, then from 10 to 50 times the quantity of antivenom sufficient for in vitro neutralization must be given to arrest progress (although first-aid with pressure dressings and splint can reduce the antivenom requirement). However, in man, sea-snake antivenom, as already mentioned, has successfully combated life-threatening poisoning, even when not given until 2 days after the bite. Patients apparently moribund from elapid bite poisoning have been dramatically saved by antivenom. In viperine poisoning (for example, A. rhodostoma and E. carinatus envenoming) antivenom has successfully rectified coagulation and bleeding effects several days after the bite. Experimentally, in monkeys injected subcutaneously with Vipera berus venom, intravenous antivenom (Zagreb) can greatly reduce local effects, even when administered as late as 4 hours after venom injection. But in humans there is so far little clinical evidence that antivenoms ameliorate local envenoming effects such as necrosis, although apparent failures may in some cases be due to a combination of the antivenom being given too late, in too small a dose, or by the wrong route, or even to the wrong antivenom being given.

In some cases the clinician aims to reverse a dramatic clinical effect, such as unconsciousness, paralysis, or hypotension. In a few cases, the antivenom requirement may be titrated against a venom effect that is easily measured, such as the non-clotting of blood in A. rhodostoma and E. carinatus envenoming. In most parts of the world, however, antivenom is usually given in an arbitrary dose, and it is not possible to check whether the various venom components have been neutralized. One of the greatest problems is that there are no tests that can give a reliable indication of a suitable antivenom dose for treating individual patients.

It is believed that for bites by the Australian snake species, the initial dose of antivenom can be judged adequately from the symptoms and signs of the patient. For example, the presence of paralytic features or coagulation defects may be taken as indications for doubling the initial dose of antivenom. For North American crotalids, it is suggested that, with some

exceptions, the dose of antivenom can be judged from the speed of spread of local oedema in the bitten limb. No reliable laboratory test is yet available to measure the level of venom in the body fluids after antivenom treatment as a means of checking that an adequate neutralizing dose of antivenom has been given. Clinical judgement remains paramount in this area. However, there are immunodiagnostic tests that can identify the venom and thereby indicate treatment by monospecific rather than polyspecific antivenom.

## Paraspecific Activity of Antivenoms

Immunodiffusion tests have shown widespread sharing of venom antigens among snakes. While there is a rough correlation with taxonomic relationships, there are many instances of common antigens in venoms of snakes taxonomically unrelated. For example, Notechis antivenom gives precipitin lines with venoms from Crotalus adamanteus, C. durissus terrificus, Agkistrodon piscivorus, A. rhodostoma, Bothrops atrox asper, and Cerastes cerastes. But there is no significant cross-protection against these venoms.

Cross-protection in animal tests is more frequently seen with closely related species. Thus, Crotalus atrox antivenom neutralizes venoms of 5 other rattlesnakes but not that from C. durissus terrificus. North American pit viper polyspecific antivenom shows some neutralization of venoms of Vipera xanthina palaestinae, V. ammodytes, Bitis gabonica, and Cerastes, but does not neutralize those of V. russelii or Echis. It neutralizes venoms of all North American pit vipers to some degree, as well as those of numerous pit vipers of tropical America and some from Asia. Antivenom to Vipera ammodytes neutralizes venom from V. berus and is used clinically for this purpose. Considerable cross-neutralization is seen among cobra venoms from both Asia and Africa, although venoms of some species, notably Naja nigricollis, are only imperfectly neutralized.

Antivenoms against Notechis, Acanthophis, and Oxyuranus neutralize 9 Naja venoms from Asian and African sources, as well as venoms of 6 other species of elapids in the mouse protection tests. However, only Notechis antivenom neutralizes Micrurus fulvius venom. Dendroaspis venoms are not significantly neutralized by these antivenoms. On the other hand, Notechis antivenom neutralizes venoms of 8 of 9 sea snakes better than the sea-snake (Enhydrina) antivenom.

Although current potency assays can be a guide to the quantity of antibody that may be effective in man, caution should be observed in applying their results (including results of paraspecific protection) too literally to the treatment of envenomed patients.

## Preferred Routes of Administration

Intravenous administration is considered to be the most effective route, and administration of antivenom intramuscularly or subcutaneously or by local infiltration should be discouraged. The injection of antivenom subcutaneously or intramuscularly has the added disadvantage that large haematomata may form at the site of injection in patients with incoagulable blood. The topical application of antivenom to the eye in injuries caused by spitting snakes needs to be evaluated, but in conducting research for this purpose the ethics of inducing pain in an experimental animal need to be considered. For intravenous infusion, dilution of the antivenom 1 in 5 or 1 in 10 in Hartmann's solution or physiological saline seems to reduce the incidence of reactions and gives better control over the rate of administration.

Slow intravenous injection of undiluted antivenom at a rate not exceeding 2 ml per minute has also been successfully used and has the advantage of requiring less equipment and avoiding pyrogenic reactions resulting from contamination of the infusion set. This method, however, has disadvantages when large volumes have to be injected. Such a method of administration of antivenom links the administrator with the patient through the most critical time of giving the antivenom. This is particularly important under tropical conditions.

## Clinical Trials

Antivenoms are among the few pharmacological agents in widespread use today whose therapeutic value remains largely untested by clinical trials. Experience with antivenom is still reported mainly in single case-reports, which often lack the identification of the biting species. There have been very few attempts to conduct controlled or randomized comparative clinical trials with antivenoms in groups of patients. The problems preventing adequate trials are:

(1) The highest incidence of snake bites is usually in rural areas where hospital and dispensary staff have neither the time nor the scientific training to undertake clinical trials. Useful information could be salvaged, however, from these areas if there were sufficient encouragement from the academic centres and if simple protocols were designed to obtain a minimum of essential information.

(2) Comparison of antivenom treatment with the natural course of untreated envenoming is usually ethically or legally unacceptable. Advantage can be taken, however, of those occasions, all too frequently experienced in some developing countries, when supplies of antivenoms are temporarily not available.

(3) In most tropical communities snake-bite victims first seek the help of traditional practitioners and go to hospitals only if the traditional remedies seem to have failed. These customs delay the start of antivenom treatment and introduce further confusing clinical factors (such as vomiting caused by emetic herbs). The increasing scientific interest in herbal remedies in many developing countries, which is being encouraged by WHO, might make it possible, however, to involve traditional healers in scientific efforts to establish the best treatment for snake bites.

(4) In most tropical communities only a minority of patients are able to bring irrefutable evidence of the biting species - in the form of the dead snake. This has deprived potential antivenom trials of many possible subjects, but the new and highly sensitive immunodiagnostic methods such as ELISA (enzyme-linked immunosorbent assay) could salvage a proportion of these "lost" cases.

In Mexico, patients stung by scorpions may refuse antivenom treatment because of the incidence of reactions. These patients could be used as clinical controls. The possibility of acquired protection from previous stings should be taken into account in assessing results of antivenom in this region; ELISA could be used in investigating serum venom-antibody levels. In the Libyan Arab Jamahiriya, two antivenoms are used in treating patients with scorpion stings (Lister Institute, England, and Pasteur Institute, Algeria). A clinical trial comparing their efficacy would be valuable.

In countries such as the United States of America, antivenom treatment appears to be firmly established so that it would not be possible to carry out clinical trials. However, there is still an opportunity to test supportive treatment, such as plasma expanders, in the controlled manner.

# REACTIONS TO ANTIVENOMS

## Early Reactions

Antivenom reactions are often classified as "immediate" and "delayed", but these terms have very specific meanings in immunology and it is therefore preferable to classify antivenom reactions as "early" and "later" reactions. Early reactions occur within 24 hours of antivenom administration and vary in severity from minor to lethal. Severe early reactions are often termed "anaphylactoid" when there is hypotension with or without collapse or airflow obstruction; anaphylactoid reactions start within a few hours of intramuscular antivenom and much sooner with intravenous antivenom. Early reactions may occur in from none to over 40% of all people treated with antivenoms (see Annex 7) depending on various factors, such as the type of antivenom, dose, route and method of administration, nature of the populations,

previous exposure to sensitizing substances, and so on. It is not known what fractions or properties of the antivenom are responsible for the reactions, nor is it known if the sensitizing factors are or are not related to one of the protecting antibodies.

It is generally understood that the more "refined" the antivenom, the less likely the probability of reactions following its injection. Clinical experiences in Malaysia support this contention (see Annex 7). However, the term "refinement" has broad and different meanings and it is difficult to compare results when the basic antibodies are complex, as in polyspecific antivenoms, or more simple, as in monospecific ones.

A survey of the immunochemical purity of commercially available antivenoms has disclosed a wide variety of composition. Only a few are nearly pure $F(ab)_2$ immunoglobulins and some are even crude unpurified hyperimmune horse serum. Heterogeneity of commercial preparations should be borne in mind when reaction rates are considered.

Over a 12-month period (July 1978 - June 1979), most patients who received antivenom in Australia were followed up in regard to antivenom reactions. All patients received immunologically nearly pure equine $F(ab)_2$ proteins. The reaction rate was related to the quantity of antivenom given, the manner of infusion and, to a lesser extent, the age of the patient. Two hundred patients received Latrodectus antivenom (1.0 ml of a 6% protein) by the intramuscular route. Only 1 immediate reaction occurred and 7 delayed reactions. Of 200 cases of snake bite in which antivenom was used, follow-up details from 189 cases were obtained. Immediate or delayed reactions were rare when low volumes of diluted antivenoms were used. When polyspecific antivenom was used the reaction rate was high. An average quantity of 70 ml (17% protein) of polyspecific antivenom was infused and 19 of 86 patients had significant reactions; 10% developed a debilitating serum sickness. These findings should add impetus to the development of a rapid ELISA procedure to increase the use of monospecific antivenoms.

The question of pyrogen reactions should not be overlooked, and attention should be given to the production of the least pyrogenic product.

## Later Antivenom Reactions

The common clinical features of these reactions are that between 5 and 24 days after antivenom the patients develop fever, urticaria, arthralgia, lymphadenopathy, proteinuria, or neuropathy.

The incidence of such cases may have been underestimated in the past because patients may not have bothered to report mild late reactions. A follow-up study of 150 patients treated with Wyeth Crotalidae antivenom in the United States of America showed a 75% incidence of later reactions, half of which were clinically significant. The minimum dose of antivenom given was 3 vials (equivalent to 6 g of protein). In most studies the incidence of later reactions increased and the interval before onset of symptoms decreased as the dose of antivenom was increased.

As with the earlier type of antivenom reactions, it has been assumed that these reactions were due to hypersensitivity to equine serum. A number of mechanisms are possible, however, including complement activation and venom-antivenom immune complex formation.

## Anticomplementary Activity

Patients sometimes suffer severe anaphylactoid reactions when infused with antivenom, even though they may never have had prior exposure to equine proteins. By *in vitro* tests, all antivenoms and antitoxins have variable degrees of anticomplementary activity. The immediate reactions that occur *de novo* when snake-bite victims receive concentrated antivenom intravenously may be due to a sudden binding of circulating complement by the antivenom. In this respect, antivenoms are of variable quality. Some contain a number of equine proteins other than the antibody moiety and thus are potentially more allergenic. At the Port Moresby General Hospital in Papua New Guinea, it was found that 3% of the patients treated developed anaphylaxis and another 5% a less severe general reaction. In Nigeria, treating the local

population with antivenoms has given rise to a rate of anaphylaxis of at least 6%. It is unlikely that any of the local population could have been exposed previously to equine proteins. These observations suggest that mechanisms other than allergy might be responsible, in a proportion of cases, for the immediate reactions.

Venoms and venom-antivenom complexes may also activate complement. Recent research confirms complement depletion in Nigerian patients with viperine envenoming, and further complement depletion immediately after intravenous antivenom. The complement depletion correlated neither with the incidence nor with the severity of early reactions. Nevertheless, slow infusion of antivenom, suitably diluted, is advisable.

## Prediction and Prevention of Reactions

True anaphylactic (IgE-mediated) hypersensitivity can be detected in some cases by skin tests; this procedure is extensively used in the United States of America. Detection of IgE specific for horse protein by the radioallergosorbent test (RAST) is possible in theory but not in normal practice. A history of previous administration of horse serum or previous reactions to serum injection may be obtained and should be a warning of possible trouble.

It is impossible to predict which patients will have anaphylactoid reactions from complement activation and which will have serum sickness or even other forms of complex disease following antivenom administration. Dilution of the antivenom is thought to minimize the chance of anaphylactoid reactions.

In Australia the use of skin testing for sensitivity to equine protein has been discouraged because the tests are misleading and delay treatment. The "damping down" of reactions may be achieved by dilution of antivenom and prior dosage of the patient with a non-sedating antihistamine as well as a small dose of adrenaline (0.1 mg for an adult given by the subcutaneous route). Patients with a known or suspected history of allergy to equine protein also receive intravenous steroids.

In Nigeria, intradermal and conjunctival hypersensitivity tests were found to be of no predictive value. Hypersensitivity to equine serum is very unlikely to be responsible for the high incidence (20%) of earlier antivenom reactions in that community.

## Treatment of Reactions

Since the predictive value of recommended tests for hypersensitivity, such as skin tests and conjunctival tests, is questionable, all patients who are to be given antivenom should be regarded as potential "reactors". All drugs and equipment required for dealing with reactions, therefore, must be available before antivenom is administered.

The routine administration of epinephrine (adrenaline) and antihistamines before antivenom has been advocated, but the side-effects of epinephrine need to be considered.

When the established immediate reactions occur, the administration of the antivenom should be stopped and epinephrine 1:1000 (0.5-1 ml) should be promptly injected subcutaneously or, if the patient is in shock, intramuscularly. This may have to be repeated or an intravenous or intracardiac injection given if shock persists or cardiac arrest occurs. An antihistamine may be given intramuscularly and steroids intravenously, though these take second place to epinephrine. Supportive therapy, including maintenance of the airway and plasma expanders or whole blood, may be needed. In many cases, it has been possible to continue antivenom administration after recovery from a reaction.

The delayed reactions may require treatment with steroids.

## ACTIVE IMMUNIZATION

### Vaccines against Snake Bite

Results of clinical analysis of severe bites by Habu snakes in the Amami and Okinawa Islands of Japan suggest that the severe cases are not due to delayed or inadequate medical treatment but to the introduction of a large quantity of venom into the victims. In such cases of heavy envenoming, the development of the lesion is so rapid that the antivenom alone is insufficient to neutralize the large amount of venom injected. In such cases, however, if the victims are immune to the venom even to a small degree, they are able to delay the onset of symptoms and can therefore be treated more successfully.

In connexion with these studies, the haemorrhagic factors (HR1 and HR2) have been separated to increase antigenicity; they were inactivated by 1% formalin to make APF venoid (alcohol-precipitated venoid) or mixed venoid (purified HR1 and HR2, mixed). Both venoids were inoculated with alum as an adjuvant formed by mixing 0.2 mol/litre $AlCl_3.6H_2O$ and 0.2 mol/litre $Na_3PO_4.12H_2O$ in the presence of the venoid. Standardization of the venoid was also investigated. Since 1970, field trials of vaccination with the Habu venoids have been carried out on Amami Island (see Annex 8).

Human volunteers received 3 or more injections of either 0.5 ml or 0.1 ml of the venoids at intervals of between 4 weeks and 6 months. Most persons who received 3 injections of 0.5 ml of both venoids attained anti-HR1 titres of 1 unit or higher after the third injection. Since there is evidence that the administration of 6000 units of the Habu antivenom is effective in the treatment of the Habu bites, it can be assumed that any person having a circulating antivenom titre of about 1.3 µg/ml should be protected against a bite. Anti-HR2 titres of persons who received 0.5 ml of both venoids attained on an average about 5 µg/ml. The clinical effectiveness of the vaccination is still under investigation. The main reactions were pain and swelling, which appeared at the point of injection. It was found also that reactions in persons who received 0.1 ml of the venoid were less severe than in those having received 0.5 ml. Seven persons who had received DHTA venoid 1-5 times, and 5 months to 2 years before they were injected with 0.1 ml of the venoid intradermally in the forearm, gave an immediate wheal with a pseudopodium and erythema accompanied by oppressive pain which occurred at the site of injection in 6 of the persons. The reaction in 1 person who had received the venoid before and 2 persons who had not received the venoid was negative. The positive reaction was apparent within 24-48 hours and disappeared in a few days.

### Vaccines against Scorpion Stings

According to a Mexican report, modification of a purified toxic fraction from the venom of the scorpion Centruroides noxius Hoffmann by treatment with glutaraldehyde has yielded a detoxified fraction that was shown to be immunogenic in rabbits.

## CONCLUSIONS

1. The terms "venom" and "antivenom" should be used in preference to "venin/antivenin" or "venene/antivenene".

2. Countries should make greater efforts to collect reliable data on the morbidity and mortality due to bites and stings. The ICD classification of injuries due to bites and stings[1] should be used for reporting the cases.

3. More widespread use should be made of immunodiagnostic tests such as ELISA (enzyme-linked immunosorbent assay) to assist and improve the reporting of epidemiological and clinical data.

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[1] Manual of the international statistical classification of diseases, injuries, and causes of death, 1975 revision, Geneva, World Health Organization, 1977, vol. 1, p. 594, category E905.

4. Greater attention should be paid to identifying the species of snake or scorpion causing the injury. This is particularly important in elucidating the envenoming pattern caused by different species. The wider application of ELISA could also help solve this problem.

5. More attention needs to be devoted to determining whether the snake or scorpion had injected any venom and, if so, to quantifying the envenoming.

6. Not enough information is available on the usefulness of immunodiagnostic tests in establishing the diagnosis and selecting the treatment of a bitten or stung person. More work on this question could facilitate identification of the venom injected and would also be helpful for studying the absorption and persistence of the venom and the quantity of antivenom required.

7. There is a need for international reference reagents of venoms, both for research purposes and for the preparation of international standards for antivenoms. International collaborative studies will have to be arranged for the characterization of the venoms.

8. There is also a need for international standards for antivenoms. International collaborative studies will be required for the calibration of the antivenoms.

9. The assay methods used for the measurement of antivenom potency (the ability to neutralize the lethal, defibrinating, haemorrhagic, and necrotizing activities of the venom) should be standardized. Where neurotoxicity and neuromuscular activity are important, tests to measure the ability of the antivenom to neutralize these properties should also be developed. The potency of any antivenom should be determined relative to that of a standard antivenom. The availability of a standard antivenom would greatly assist collaboration among laboratories in different countries.

10. Governments should be alerted to the correct storage of antivenoms and to the need to ensure that potent antivenom is more readily available, especially in the rural areas.

11. Greater emphasis should be placed on clinical trials of antivenoms, rather than relying on efficacy shown only in animal models.

12. There is a need for a detailed and comprehensive manual on the clinical indications for the use of antivenom and on the treatment of snake bites and scorpion stings.

13. The timing of the administration of antivenom may be critical and many more data need to be collected to confirm this.

14. More information is needed on the paraspecific effects of antivenoms, particularly where no monospecific antivenom is available.

15. Wherever feasible, the antivenom should be administered by intravenous infusion. The physician should remain with the patient throughout the early stages of administration of the antivenom so that he can take immediate measures to counteract any serum reactions.

16. There is a need for more accurate information on both the early and late reactions to envenoming. To assist governments in the collection of such data, an agreed classification using an agreed terminology should be developed.

17. The tests used to predict whether a patient is likely to have a reaction to the antivenom have proved unreliable in many areas, though the intradermal test is used routinely in the United States of America. Application of more recent tests, such as the radioallergosorbent test (RAST) should be investigated. It is important that health personnel anticipate the reactions that may occur in any patient given antivenom.

18. Education of physicians, medical students, health auxiliaries, and the lay public in regard to snake bites and other venomous bites and stings should be encouraged.

Annex 1

SNAKE-BITE MORTALITY IN SOUTH-EASTERN ASIA

| Country or area | Average No. of bites per year | Average No. of deaths per year | Yearly mortality per 100 000 population |
|---|---|---|---|
| Burma | 8 508 | 75.9 | 2.700 |
| China (Province of Taiwan) | | 36 | 0.270 |
| Hong Kong | 203 | 1.3 | 0.090 |
| India (Maharashtra State) | | 1 093 | 2.100 |
| Japan | 610 | 5.6 | 0.570 |
| Malaysia | 2 480 | 16 | 0.180 |
| Philippines | | 294 | 0.770 |
| Sri Lanka | | 104 | 0.820 |
| Thailand | 3 989 | 302 | 0.860 |

Annex 2

MEDICALLY IMPORTANT SNAKE SPECIES

Definition

Species are considered to be of medical importance if (from published medical reports of bites by identified species) they fall into one of three categories:

(1) commonly cause death or serious disability;

(2) uncommonly cause bites but are recorded to cause serious effects (death or local necrosis);

(3) commonly cause bites but serious effects are very uncommon.

Geographical areas

| Area | Category 1 | Category 2 | Category 3 |
|---|---|---|---|
| North America | Agkistrodon piscivorus<br>Crotalus adamanteus<br>C. atrox<br>C. viridis | Crotalus scutulatus<br>Micrurus fulvius | Agkistrodon contortrix<br>Crotalus horridus<br>Sistrurus miliarius |
| Mexico and Central America | Bothrops atrox asper<br>Crotalus atrox<br>C. basiliscus<br>C. durissus | Agkistrodon bilineatus<br>Crotalus molossus<br>C. triseriatus<br>C. polystictus<br>C. scutulatus<br>Lachesis muta<br>Micrurus nigrocinctus | Bothrops schlegeli<br>B. lateralis |
| South America | Bothrops atrox atrox<br>B. jararaca<br>B. neuwiedi<br>Crotalus durissus<br>C. durissus terrificus | Bothrops alternatus<br>B. jararacussu<br>Lachesis muta<br>Micrurus corallinus<br>M. lemniscatus<br>M. mipartitus | Bothrops bilineatus<br>B. schlegeli |
| North Africa | Bitis arietans<br>Echis carinatus<br>Naja nigricollis | Atractaspis sp.<br>Naja haje | Cerastes sp. |
| Mid-Africa | Bitis arietans<br>Echis carinatus<br>Naja mossambica<br>N. nigricollis | Atractaspis sp.<br>Bitis gabonica<br>Dendroaspis sp. (mainly D. polylepis)<br>Dispholidus typus<br>Naja haje<br>Thelotornis kirtlandii | Causus sp. |

Annex 2 (continued)

| Area | Category 1 | Category 2 | Category 3 |
|---|---|---|---|
| Southern Africa | Bitis arietans<br>Naja nigricollis | Atractaspis sp.<br>Dendroaspis sp.<br>Dispholidus typus<br>Naja haje<br>N. nivea<br>Thelotornis kirtlandii | Causus sp. |
| Europe | | Vipera lebetina | Vipera ammodytes<br>V. aspis<br>V. berus |
| Near and Middle East | Bitis arietans<br>Echis carinatus<br>Naja naja<br>Vipera lebetina<br>V. xanthina | Atractaspis sp.<br>Echis coloratus<br>Naja haje | Agkistrodon halys<br>Cerastes sp.<br>Vipera ammodytes |
| South-eastern Asia (Pakistan to Sulawesi) | Agkistrodon rhodostoma<br>Echis carinatus<br>Enhydrina schistosa<br>Naja naja<br>Vipera russelii | Bungarus caeruleus<br>Hydrophis cyanocinctus<br>Lapemis hardwicki<br>Ophiophagus hannah<br>Trimeresurus purpureomaculatus | Trimeresurus albolabris<br>T. wagleri |
| Far East | Naja naja<br>Trimeresurus flavoviridis (Ryukyu)<br>T. mucrosquamatus | Agkistrodon acutus<br>Bungarus multicinctus (China, Province of Taiwan)<br>Hydrophis cyanocinctus<br>Lapemis hardwicki<br>Ophiophagus hannah | Agkistrodon blomhoffi<br>A. caliginosus<br>A. halys group<br>Trimeresurus albolabris<br>T. stejnegeri (China, Province of Taiwan) |
| Australia and Pacific Islands | Acanthophis antarcticus<br>Notechis scutatus<br>Pseudonaja textilis | Austrelaps superba<br>Oxyuranus scutellatus<br>Pseudechis australis<br>Pseudechis papuanus<br>Tropidechis carinatus | Pseudechis porphyriacus |

Annex 3

# PROVISION, PROCESSING, AND CHARACTERIZATION OF VENOMS

## Provision of Venoms

The most appropriate sources of venoms appear to be the following:

| Snake species | Country of origin |
|---|---|
| Naja naja | Thailand |
| Notechis scutatus | Australia |
| Echis carinatus | Nigeria |
| Echis carinatus | Iran |
| Vipera russelii | Thailand |
| Crotalus adamanteus | United States of America |
| Bothrops atrox asper (Atlantic) | Costa Rica |
| Trimeresurus flavoviridis | Japan |

The venoms are required in 5-g, 10-g, or 50-g quantities, depending on the quantities to be filled into ampoules and the quantity required for the production of antivenom (see later).

The venoms of the snakes must be collected from one geographical area and pooled. In the event of there being several areas in which the snakes live, samples of venoms from at least two areas should be collected but not mixed.

The appropriate dry weights of the venoms in the ampoules would be:

10 mg for Bothrops atrox asper (Atlantic)
Trimeresurus flavoviridis
Crotalus adamanteus

2 mg for Vipera russelii
Echis carinatus

1 mg for Naja naja
Notechis scutatus

At least 1000 ampoules of each venom should be set aside as a proposed international reference reagent. These would be available after the characterization studies.

## Processing of Venoms

The venoms intended as proposed international reference reagents should be processed by:

(1) centrifugation;

(2) filtration through Millipore membranes;

(3) testing for sterility;

(4) ampouling accurately (within 1%) into all-glass ampoules;

(5) freeze-drying after freezing in liquid nitrogen;

(6) sealing by fusion of the glass;

(7) testing for stability by accelerated degradation tests;

(8) testing for accuracy of fill by weighing the quantities in 10 ampoules.

The activity of the venoms must be determined in several laboratories. The one test that must be done by each laboratory is the determination of the $LD_{50}$ by the following method.

Determination of Median Lethal Dose ($LD_{50}$)

The details of the test are as follows:

| | | |
|---|---|---|
| Animals | : | mice[1] |
| Route of injection | : | intravenous |
| Volume of injection | : | 0.2 ml |
| Rate of injection | : | the 0.2 ml given in 15 seconds |
| Expression of results | : | the 50% lethal dose ($LD_{50}$) is expressed in micrograms of venom per mouse |
| Dilution of venom | : | the dilution series of the venom will be such that at least 3 dilutions fall on the steep part of the dose-response curve |
| Number of animals | : | at least 5 animals are inoculated with each dilution of venom |
| Period of observation | : | the mice will be observed for 48 hours |
| Controls | : | 5 mice injected with saline as controls must survive the injection. |

The method of calculating the $LD_{50}$ should be by a statistically sound method, such as probit analysis or the Spearman-Kärber method.

Calculation of $LD_{50}$

The $LD_{50}$ for each venom may be calculated by the Spearman-Kärber method, which is valid provided:

- d, the log dose interval is constant;
- the full response range from 0 to 100% is covered; and
- the response distribution is nearly symmetrical.

[1] There is no difference between the results obtained by injecting venom doses exactly corresponding to the individual body-weight of each mouse and those produced by inoculating mice with a common dose corresponding to the average weight of the specimens in the lot (Schöttler, W. H. A. (1958) Bulletin of the World Health Organization, 19: 341).

Then, $\underline{m} = \underline{x}_{100} \pm \frac{\underline{d}}{\underline{n}} (\underline{\Sigma r} - \underline{n}/2)$, where:

$\underline{m}$ = log $LD_{50}$;

$\underline{x}_{100}$ = log dose giving 100% deaths and having 100% deaths for all higher doses;

$\underline{n}$ = number of mice used at each dose level;

$\underline{r}$ = number of mice dying at each dose level; and

$\underline{\Sigma}$ = summation over all doses between and including $\underline{x}_{100}$ and $\underline{x}_o$ ($\underline{x}_o$ being defined as the log dose giving 0 deaths and having only 0 deaths for all lower doses).

Calculation of fiducial limits for the $LD_{50}$

$$\underline{V}(\underline{m}) = \frac{\underline{d}^2}{\underline{n}^2 (\underline{n}-1)} \quad \underline{\Sigma} \left[ \underline{r} (\underline{n}-\underline{r}) \right]$$

where $\underline{\Sigma}$ denotes summation over the same range as in the previous paragraph.

The 95% fiducial limits to $\underline{m}$ are taken to be approximately $\underline{m} \pm \underline{t}_{0.05} \sqrt{\underline{V}(\underline{m})}$ where $\underline{t}_{0.05}$ has the value appropriate for $\underline{\Sigma} (\underline{n}-1)$ degrees of freedom, the summation in this case extending only over those dose levels giving death rates other than 0 and 100%.

Example

| Dose of venom (µg) | 0.28 | 0.35 | 0.44 | 0.55 | 0.69 | 0.86 | 1.07 |
|---|---|---|---|---|---|---|---|
| Deaths within 48 hours | 0/4 | 2/4 | 0/4 | 3/4 | 3/4 | 4/4 | 4/4 |

Here, $\underline{d}$ = log 1.25 = 0.097

$\underline{x}_{100}$ = log 0.86 = -0.065

$\underline{x}_o$ = log 0.28

$\underline{n}$ = 4

$\underline{t}_{0.05}$ = 2.20 for 4+4+4+4-1 = 11 degrees of freedom.

Thus, $\underline{m} = 0.065 \pm \frac{0.097}{4} (0+2+0+3+3+4-\lfloor 4/2 \rfloor)$

$= 0.065 \pm 0.243$

and $LD_{50}$ = antilog (-0.065 - 0.243) (rejecting the obviously inappropriate value antilog (-0.065 + 0.243))

= antilog (-0.308)

= 0.49 µg.

Also, $\underline{V}(\underline{m}) = \frac{0.097^2}{4^2 (4-1)} (0x4 + 2x2 + 0x4 + 3x1 + 3x1 + 4x0)$

$= \frac{0.009409}{48} (10)$

= 0.00196.

Hence, the 95% fiducial limits are antilog $(-0.308 \pm 2.20 \sqrt{0.00196})$

= antilog (-0.308 ± 0.097), i.e., 0.39 µg and 0.62 µg.

Annex 3 (continued)

Other Tests

The other tests to be applied to the venoms are outlined below:

Defibrinating activity:

1. Rats are injected intravenously with 1.0 ml of venom solution. After 3 hours, blood is collected by decapitation, citrated, centrifuged, and the fibrinogen assayed in the plasma according to the method of Blombäck & Blombäck.[1] The defibrinating unit is defined as the amount of venom necessary to decrease the fibrinogen level to 10% of the normal value.

2. A simpler and cheaper method is to inject mice intravenously with 0.1 ml of venom solution. After 1 hour venous blood is taken from the tail. The Minimum Defibrinating Dose is the least amount of venom producing non-clotting blood.[2] This method is less sensitive, however, than the first one. It is a good screening test, but less suitable for unit assessment.

Haemorrhagic activity:

This is assayed in rabbits, according to the method of Kondo et al.[3]

Necrotizing activity:

This is generally assayed in the same way as haemorrhagic activity, i.e., rabbit or rat skin test, except that the observation time is prolonged to 72 hours. The necrotizing unit is defined as the amount of venom necessary to produce a necrotic area 10 mm in diameter.

It is important that agreement should be reached as soon as possible on the details of the tests and for each laboratory taking part in the characterization of the venoms to gain experience in using the agreed method.

The following time schedule of the test programme for these international reference reagents is suggested:

(1) Collection of the venom, setting aside a part for immunization and sending another part to the test institutions.

(2) Testing of the material, according to the procedures outlined, within 4-6 months.

(3) Preparation of the international reference reagents by designated laboratories.

(4) Testing of the reference reagents within 3 months.

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[1] Blombäck, B. & Blombäck, M. (1956) Arkiv för kemi, 10: 415.

[2] Reid, H. A. (1967) In: Russell, F. E. & Saunders, P. R., ed. Animal toxins, Oxford, Pergamon Press, p. 323.

[3] Kondo, H. et al. (1960) Japanese journal of medical science and biology, 13: 43.

TABLE OF POISONOUS ANIMALS AND AVAILABLE ANTIVENOMS

I. SNAKES

| Producer or distributor | Venoms used in preparation | Trade or common name | Common name of snake | Additional venoms neutralized* | Comments |
|---|---|---|---|---|---|
| NORTH AMERICA | | | | | |
| Wyeth Laboratories, Box 8299, Philadelphia, Pennsylvania, USA | Crotalus durissus terrificus<br>Bothrops atrox asper<br>Crotalus adamanteus<br>Crotalus atrox | Antivenin (Crotalidae) Polyvalent | South American rattlesnake<br>Barba amarilla<br>Eastern diamondback rattlesnake<br>Western diamondback rattlesnake | Crotalus sp.<br>Sistrurus sp.<br>Agkistrodon sp. (Old & New World)<br>Bothrops sp.<br>Lachesis sp.<br>Trimeresurus sp. | Precipitated with ammonium sulfate, and lyophilized. |
| | Micrurus fulvius fulvius | Antivenin (Micrurus fulvius) | Eastern coral snake | Micrurus fulvius tenere | |
| Laboratorios "MYN", S.A., Av. Coyoacan 1707, Mexico City 12, D.F., Mexico | Bothrops atrox asper | Monovalent bothrops | Barba amarilla | | Enzyme digested, precipitated with ammonium sulfate, and lyophilized. |
| | Crotalus atrox<br>Crotalus d. terrificus<br>Crotalus tigris | Polyvalent Crotalus | Western diamondback rattlesnake<br>South American rattlesnake<br>Tiger rattlesnake | All Mexican crotalids | |
| | Bothrops atrox asper<br>Crotalus d. terrificus<br>Crotalus tigris<br>Crotalus atrox | Polyvalent Mexico | Barba amarilla<br>South American rattlesnake<br>Tiger rattlesnake<br>Western diamondback rattlesnake | All Mexican crotalids | |
| Instituto Nacional de Higiene, Av. M. Escobedo No. 20, Mexico City, D.F., Mexico | Bothrops atrox asper | Anti-Bothrops | Barba amarilla | | Pepsin digestion, and ammonium sulfate precipitation. (No recent confirmation.) |
| | Crotalus b. basiliscus<br>Crotalus d. terrificus | Anti-Crotalus | Mexican rattlesnake<br>South American rattlesnake | | |
| | Bothrops atrox asper<br>Crotalus b. basiliscus<br>Crotalus d. terrificus | Polyvalent | Barba amarilla<br>Mexican rattlesnake<br>South American rattlesnake | | |
| CENTRAL AND SOUTH AMERICA | | | | | |
| Universidad de Costa Rica, Ciudad Universitaria, Rodrigo Facio, San José, Costa Rica | Lachesis muta stenophrys | Anti-Laquesico | Bushmaster | Lachesis muta muta<br>Lachesis muta noctiyaga | Precipitated with ammonium sulfate. Freeze-dried or liquid. |
| | Bothrops atrox asper<br>Crotalus durissus durissus<br>Lachesis muta stenophrys | Polyvalent | Terciopelo<br>Central American rattlesnake<br>Bushmaster | Lachesis muta muta<br>Lachesis muta noctiyaga<br>Agkistrodon bilineatus<br>Bothrops nummifer<br>Bothrops picadoi<br>Bothrops nasutus<br>Bothrops ophryomegas<br>Bothrops godmani<br>Bothrops lateralis<br>Bothrops schlegeli<br>Bothrops nigroviridis | |
| | Micrurus nigrocinctus nigrocinctus<br>Micrurus nigrocinctus mosquitensis | Anti-coral (Central America) | | Micrurus carinicaudus dumerili<br>Micrurus fulvius fulvius | |

TABLE OF POISONOUS ANIMALS AND AVAILABLE ANTIVENOMS (continued)

| Producer or distributor | Venoms used in preparation | Trade or common name | Common name of snake | Additional venoms neutralized* | Comments |
|---|---|---|---|---|---|
| CENTRAL AND SOUTH AMERICA (continued) | | | | | |
| Instituto Nacional de Higiene, Guayaquil, Ecuador | Bothrops atrox asper | Anti-Bothrops | Barba amarilla | | Precipitated with ammonium sulfate. Supplied as a liquid. |
| Instituto Nacional de Higiene, Lima, Peru | Bothrops atrox asper<br>Bothrops, Brazilian sp.<br>Lachesis muta | Bothrops polyvalent | Barba amarilla | | Purified and lyophilized. |
| | Micrurus nigrocinctus<br>Micrurus mipartitus<br>Micrurus frontalis | Anti-coral polyvalent | Giant coral snake (Cobra coral snake) | Micrurus fulvius fulvius<br>Micrurus alleni<br>Micrurus carinicaudus<br>Micrurus spixi<br>Micrurus lemniscatus<br>Micrurus corallinus | |
| Instituto Nacional de Salud, Ave. Eldorado con Carrera, Zona G, Bogotá, Colombia | Bothrops atrox asper<br>Crotalus d. terrificus | Antiophidico polivalente | Barba amarilla<br>South American rattlesnake | Bothrops sp.<br>Crotalus sp. | Globulin precipitated with ammonium sulfate. |
| Laboratorio Behrens, Ave. Principal de Chapellin, Apartado 62, Caracas, 101 Venezuela | Crotalus d. terrificus | | South American rattlesnake or cascabel | Crotalus vegrandis | Foreign protein reduced. |
| | Bothrops atrox asper<br>Bothrops venezuelae | | Barba amarilla<br>Tigra-mariposa | Bothrops colombiensis | |
| | Bothrops atrox asper<br>Bothrops venezuelae<br>Crotalus d. terrificus | | Barba amarilla<br>Tigra-mariposa<br>South American rattlesnake or cascabel | Bothrops colombiensis<br>Bothrops bilineata<br>Bothrops lansbergi<br>Bothrops lichenosus<br>Bothrops medusa<br>Bothrops neglectus<br>Bothrops schlegeli<br>Crotalus vegrandis | |
| Instituto Nacional de Microbiologia, Avdo. Velez Sarsfield 563, Buenos Aires, Argentina | Crotalus d. terrificus | | South American rattlesnake or cascabel | | Purified by enzymatic and differential thermocoagulation techniques. (No recent confirmation.) |
| | Bothrops alternatus<br>Bothrops neuwiedi | Bothrops bivalent | Yarara or de la Cruz<br>Wied's lance-head<br>Yarara chica, or painted jararaca | | |
| | Bothrops alternatus<br>Bothrops jararaca<br>Bothrops jararacussu<br>Bothrops neuwiedi<br>Crotalus d. terrificus | Tropical polyvalent | Yarara, or de la Cruz<br>Jararaca<br>Yarara<br>Wied's lance-head<br>South American rattlesnake or cascabel | | |
| | Bothrops alternatus<br>Bothrops neuwiedi<br>Crotalus d. terrificus | Tropical trivalent | Yarara, or de la Cruz<br>Wied's lance-head<br>South American rattlesnake or cascabel | | |

TABLE OF POISONOUS ANIMALS AND AVAILABLE ANTIVENOMS (continued)

| Producer or distributor | Venoms used in preparation | Trade or common name | Common name of snake | Additional venoms neutralized* | Comments |
|---|---|---|---|---|---|
| CENTRAL AND SOUTH AMERICA (continued) | | | | | |
| Instituto Butantan, Caixa Postal 65, 05504 São Paulo, Brazil | Crotalus d terrificus | Anticrotalic | South American rattlesnake or cascabel | It can be expected that the antivenoms of this Institute neutralize other crotalid venoms, even though the producers note in a personal letter that the scarcity of data precludes any specific claims. | Pepsin digested and ammonium sulfate precipitation. |
| | Lachesis muta | Antilaquetico | Bushmaster, or Surucucu | | |
| | Bothrops jararaca<br>Bothrops moojeni<br>Bothrops cotiara<br>Bothrops alternatus<br>Bothrops jararacussu<br>Bothrops neuwiedi | Antibothropico | Jararaca<br>Moojen's pit viper<br>Cotiara<br>Urutu<br>Jararacussu<br>Wied's lance-head, or painted jararaca | | |
| | Crotalus d. terrificus<br>Bothrops jararaca<br>Bothrops moojeni<br>Bothrops cotiara<br>Bothrops alternatus<br>Bothrops jararacussu<br>Bothrops neuwiedi | Antiophidico polyvalent | South American rattlesnake<br>Jararaca<br>Moojen's pit-viper<br>Cotiara<br>Urutu<br>Jararacussu<br>Wied's lance-head, or painted jararaca | | |
| | Lachesis muta<br>Bothrops alternatus<br>Bothrops jararacussu<br>Bothrops jararaca<br>Bothrops moojeni<br>Bothrops cotiara<br>Bothrops neuwiedi | Antibothropico-lachetico | Bushmaster<br>Urutu<br>Jararacussu<br>Jararaca<br>Moojen's pit viper<br>Cotiara<br>Wied's lance-head, or painted jararaca | | |
| | Micrurus frontalis<br>Micrurus corallinus | Antielapidico | Gaint coral snake, or Veradeira | | |
| Syntex do Brasil S/A, Industria e Comercio, Caixa Postal 951, São Paulo, Brasil | Crotalus d. terrificus | | South American rattlesnake or cascabel | | Pepsin digestion, and ammonium sulfate precipitation. Final solution contains 180 g/litre protein. |
| | Bothrops alternatus<br>Bothrops atrox asper<br>Bothrops jararaca<br>Bothrops jararacussu<br>Bothrops cotiara | | Urutu<br>Barba amarilla<br>Jararaca<br>Jararacussu<br>Cotiara | | |

TABLE OF POISONOUS ANIMALS AND AVAILABLE ANTIVENOMS (continued)

| Producer or distributor | Venoms used in preparation | Trade or common name | Common name of snake | Additional venoms neutralized* | Comments |
|---|---|---|---|---|---|
| EUROPE | | | | | |
| Institut Pasteur, Annexe de Garches, 92 (Hauts-de-Seine), Paris, France | Vipera aspis<br>Vipera berus | Ipser V | Jura viper<br>European viper | Paraspecific* | Concentrated and purified to 120-130 g/litre protein |
| | Vipera ammodytes<br>Vipera aspis<br>Vipera berus | Ipser Europe | Long-nosed viper<br>Jura viper<br>European viper | | |
| | Bitis arietans<br>Bitis gabonica<br>Bitis nasicornis*<br>Echis carinatus<br>Haemachatus haemachatus*<br>Naja haje<br>Naja melanoleuca<br>Naja nigricollis<br>Naja nivea* | Bitis-Echis-Naja | Puff adder<br>Gaboon viper<br>Rhinoceros viper<br>Saw-scaled viper<br>Ringhals<br>Egyptian cobra<br>Forest cobra<br>Spitting cobra<br>Cape cobra | | |
| | Vipera ammodytes<br>Vipera lebetina obtusa<br>Vipera xanthina palestinae<br>Cerastes cornutus<br>Cerastes vipera<br>Echis carinatus<br>Naja naja<br>Naja haje | Near and Middle East | Long-nosed viper<br>Levantine viper<br>Palestine viper<br>Horned viper<br>Avicenna's viper<br>Saw-scaled viper<br>Indian cobra<br>Egyptian cobra | | |
| | Naja naja kaouthia | Cobra | Yellow cobra | | |
| | Dendroaspis angusticeps*<br>Dendroaspis jamesoni<br>Dendroaspis polylepis*<br>Dendroaspis viridis | Dendroaspis | Eastern green mamba<br>Jameson's mamba<br>Black mamba<br>Western green mamba | | |
| Behringwerke AG, D 3550 Marburg/Lahn, Federal Republic of Germany | Vipera ammodytes<br>Vipera berus | Europe | | Vipera ammodytes<br>Vipera aspis<br>Vipera berus<br>Vipera lebetina<br>Vipera xanthina | Prepared by pepsin digestion, and ammonium sulfate precipitation. Final solution contains 160 g/litre protein. |
| | Bitis gabonica<br>Echis carinatus<br>Naja haje<br>Vipera lebetina | North Africa | | Cerastes cerastes<br>Cerastes vipera<br>Bitis arietans<br>Bitis gabonica<br>Echis carinatus<br>Naja haje<br>Naja melanoleuca<br>Naja nigricollis<br>Vipera lebetina | |

Annex 4 (continued)

TABLE OF POISONOUS ANIMALS AND AVAILABLE ANTIVENOMS (continued)

| Producer or distributor | Venoms used in preparation | Trade or common name | Common name of snake | Additional venoms neutralized* | Comments |
|---|---|---|---|---|---|
| EUROPE (continued) | | | | | |
| | Bitis gabonica<br>Dendroaspis polylepis<br>Naja haje | Central Africa | | Bitis arietans<br>Bitis gabonica<br>Bitis nasicornis<br>Dendroaspis polylepis<br>Dendroaspis viridis<br>Haemachatus haemachatus<br>Naja haje<br>Naja melanoleuca<br>Naja nigricollis | |
| | Echis carinatus<br>Naja haje<br>Vipera ammodytes<br>Vipera lebetina | Near and Middle East | | Cerastes cerastes<br>Echis carinatus<br>Naja haje<br>Vipera ammodytes<br>Vipera lebetina<br>Vipera xanthina<br>Cerastes cornutus | |
| Istituto Sieroterapico e Vaccinogeno Toscano "Sclavo", Via Fiorentina 1, Siena, Italy | Vipera ammodytes<br>Vipera aspis<br>Vipera berus<br>Vipera ursinii | Antiviperin | Long-nosed viper<br>Jura viper<br>European viper<br>Ursini's viper | All European vipers | Enzyme-refined and supplied in liquid form. |
| Institute for Sera and Vaccines, W. Pieck Str., Prague, Czechoslovakia | Vipera ammodytes<br>Vipera berus | Venise | European viper<br>Long-nosed viper | | Digested with pepsin, precipitated with ammonium sulfate. Supplied in liquid form. |
| Institute of Immunology, Rockefellerova 2, Zagreb, Yugoslavia | Vipera ammodytes | Antiviperinum | Long-nosed viper | Vipera berus<br>Vipera aspis | Solution digested with pepsin, and precipitated with ammonium sulfate. |
| Institute of Epidemiology and Microbiology, Sofia, Bulgaria | Vipera ammodytes | | Long-nosed viper | Vipera berus<br>Vipera aspis | Ammonium sulfate precipitation. |
| Reseach Institute of Vaccine and Serum, Ministry of Public Health, Ul. Kafanova 93, Tashkent, USSR | Echis carinatus | Monovalent Echis carinatus | Saw-scaled viper | | No details available. |
| | Naja naja | Monovalent Naja naja | Indian cobra | | |
| | Vipera lebetina | Monovalent Vipera lebetina | Levantine viper | | |
| | Echis carinatus<br>Naja naja | Polyvalent Naja and Echis | Saw-scaled viper<br>Indian cobra | | |
| | Naja naja<br>Vipera lebetina | Polyvalent Vipera and Naja | Indian cobra<br>Levantine viper | | |

Annex 4 (continued)

TABLE OF POISONOUS ANIMALS AND AVAILABLE ANTIVENOMS (continued)

| Producer or distributor | Venoms used in preparation | Trade or common name | Common name of snake | Additional venoms neutralized* | Comments |
|---|---|---|---|---|---|
| AFRICA | | | | | |
| Institut Pasteur d'Algérie, rue Docteur Laveran, Algiers, Algeria | Cerastes cerastes<br>Vipera lebetina | Antiviperin | Horned viper<br>Levantine viper | | Solution digested with pepsin and precipitated with ammonium sulfate. |
| South African Institute for Medical Research, P.O. Box 1038, Johannesburg 2000, Republic of South Africa | Haemachatus haemachatus<br>Naja nivea<br>Naja haje<br>Naja melanoleuca<br>Naja nigricollis<br>Dendroaspis angusticeps<br>Dendroaspis jamesoni<br>Dendroaspis polylepis<br>Bitis arietans<br>Bitis gabonica | Polyvalent | Ringhals<br>Cape cobra<br>Egyptian cobra<br>Forest cobra<br>Spitting cobra<br>Eastern green mamba<br>Jameson's mamba<br>Black mamba<br>Puff adder<br>Gaboon viper | Naja naja<br>Ophiophagus hannah<br>Pseudohaje goldi<br>Walterinnesia egyptia<br>Dendroaspis viridis | Digested with pepsin and precipitated with ammonium sulfate. |
| | Echis carinatus | Echis | Saw-scaled viper | Echis coloratus<br>Cerastes cerastes<br>Cerastes vipera | Pepsin-refined globulin preparation in liquid form. |
| | Dispholidus typus | Boomslang antivenom | Boomslang | | Pepsin-refined globulin preparation in freeze-fried form. |
| ASIA | | | | | |
| Central Research Institute, Kasauli, (Simla Hills), (H.P.) India | Bungarus caeruleus<br>Naja naja<br>Vipera russelii<br>Echis carinatus | Polyvalent | Indian krait<br>Indian cobra<br>Russell's viper<br>Saw-scaled viper | Bungarus fasciatus<br>Naja hannah | Enzyme-refined, equine globulin supplied in liquid and lyophilized forms. |
| Haffkine Bio-pharmaceutical Corporation Ltd., Parel, Bombay, India | Bungarus caeruleus | Bungarus | Indian krait | Bungarus fasciatus | Digested with pepsin, concentrated and lyophilized. |
| | Naja naja | Naja | Indian cobra | Naja naja kaouthia | |
| | Vipera russelii | Vipera | Russell's viper | Naja naja oxiane | |
| | Echis carinatus | Echis | Saw-scaled viper | Ophiophagus hannah | |
| | Bungarus caeruleus<br>Naja naja<br>Echis carinatus<br>Vipera russelii | Polyvalent | Indian krait<br>Indian cobra<br>Saw-scaled viper<br>Russell's viper | Trimeresurus gramineus<br>Trimeresurus labialis | |
| Perusahaan Negara BioFarma, 9, Jalan Pasteur, Bandung, Indonesia | Agkistrodon rhodostoma | | Malayan pit viper | | Purified serum supplied in liquid form. |
| | Bungarus fasciatus | | Banded krait | | |
| | Naja sputatrix | | Malayan cobra | | |

TABLE OF POISONOUS ANIMALS AND AVAILABLE ANTIVENOMS (continued)

| Producer or distributor | Venoms used in preparation | Trade or common name | Common name of snake | Additional venoms neutralized* | Comments |
|---|---|---|---|---|---|
| ASIA (continued) | | | | | |
| Institut d'Etat des Serums et Vaccins Razi, P.O. Box 656, Teheran, Iran | Naja naja oxiana | | Oxus cobra | | Prepared by pepsin digestion, and ammonium sulfate precipitation. |
| | Vipera lebetina | | Levantine viper | | |
| | Echis carinatus | | Saw-scaled viper | | |
| | Pseudocerastes persicus | | Persian horned viper | | |
| | Vipera latasti | | Snub-nosed viper | | |
| | Agkistrodon halys | | Mamushi | | |
| | Naja naja oxiana<br>Vipera lebetina<br>Vipera xanthina<br>Echis carinatus<br>Pseudocerastes persicus<br>Agkistrodon halys | Polyvalent | Oxus cobra<br>Levantine viper<br>Near East viper<br>Saw-scaled viper<br>Persian horned viper<br>Mamushi | Cerastes cerastes<br>Eristicophis macmahoni<br>Vipera aspis<br>Vipera cerastes<br>Vipera latasti<br>Vipera x. palaestinae | |
| Rogoff Medical Research Institute, Beilinson Medical Centre, Tel-Aviv, Israel | Echis coloratus | Arabian Echis | Arabian saw-scaled viper | | Whole venom plus resin-bound "neurotoxin" used as antigen. Supplied as globulin fraction of horse serum in liquid form. |
| | Vipera xanthina palaestinae | Palestine viper | Palestine viper | | |
| The Chemo-Sero-Therapeutic Research Institute, Kumamoto 860, Kyushu, Japan | Trimeresurus flavoviridis | Habu antivenine | Habu | Partial neutralization of Agkistrodon halys | Pepsin digestion, ammonium sulfate precipitation. Supplied in lyophilized form. |
| The Takeda Pharmaceutical Company, Osaka, Japan | Agkistrodon halys | Mamushi antivenine | Mamushi | | Pepsin digestion, ammonium sulfate precipitation. Supplied in lyophilized form. |
| Research Institute for Microbial Diseases, Osaka University, Suite 565, Japan | Agkistrodon halys | Mamushi antivenine | Mamushi | | |
| Kitasato Institute, Minato-ku, Tokyo, Japan | Agkistrodon halys | Mamushi antivenine | Mamushi | | |
| Chiba Prefectural Serum Institute, Inchikawa, Japan | Agkistrodon halys | Mamushi antivenine | Mamushi | | |

TABLE OF POISONOUS ANIMALS AND AVAILABLE ANTIVENOMS (continued)

| Producer or distributor | Venoms used in preparation | Trade or common name | Common name of snake | Additional venoms neutralized* | Comments |
|---|---|---|---|---|---|
| ASIA (continued) | | | | | |
| Serum and Vaccine Laboratories, Alabang, Mutinlupa, Rizal, Philippines | Naja naja philippinensis | Cobra | Philippine cobra | | Concentrated and purified. |
| Queen Saovabha Memorial Institute, Rama 4 Road, Bangkok, Thailand | Bungaru fasciatus | Bungarus | Banded krait | | Lyophilized whole serum. |
| | Naja naja | Cobra | Indian cobra | | |
| | Ophiophagus hannah | King cobra | King corba | | |
| | Vipera russelii | Russell's viper | Russell's viper | | |
| | Agkistrodon rhodostoma | Malayan pit viper | Malayan pit viper | | |
| | Timeresurus albolabris<br>Timeresurus erythrurus | Green tree viper | | | |
| Industrial and Pharmaceutical Corporation, Rangoon, Burma | Naja naja kaouthia | Siamese cobra | | | Precipitated with ammonium sulfate and lyophilized. |
| | Vipera russelii siamensis | Russell's viper | | | |
| | Naja naja kaouthia<br>Vipera russelii siamensis | Bivalent | | | |
| Shanghai Vaccine and Serum Institute, 1262 Yang An Road (W), Shanghai, China | Agkistrodon halys | Mamushi, monovalent | Mamushi | | Precipitated with ammonium sulfate and lyophilized. |
| | Agkistrodon acutus | Monovalent | 100-Pace snake | | |
| National Institute of Preventive Medicine, 161 Kun-Yang St., Nan-Kang, Taipei, Taiwan, China | Agkistrodon acutus | Agkistrodon | Long-nosed pit viper | Trimeresurus mucrosquamatus | Immunized with formalin-toxoid venom. Venom ammonium sulfate precipitated, and supplied in liquid or lyophilized form. |
| | Bungarus multicinctus | Bungarus | Many banded krait | | |
| | Naja naja atra | Naja | Chinese cobra | | |
| | Trimeresurus stejnegeri<br>Trimeresurus mucrosquamatus | Trimeresurus | Bamboo viper<br>Chinese habu | Agkistrodon acutus | |
| | Bungarus multicinctus<br>Naja naja atra | Naja-Bungarus | Many-banded krait<br>Chinese cobra | | |
| AUSTRALIA | | | | | |
| Commonwealth Serum Laboratories**<br>45, Poplar Road, Parkville, Victoria 3052, Australia | Acanthophis antarcticus | Death adder | Common death adder | Acanthophis pyrrhus | Prepared by pepsin digestion, and ammonium sulfate precipitation. The products are dialysed and ultrafiltered to a final concentration of 170 g/litre protein. |
| | Notechis scutatus<br>Enhydrina schistosoa | Tiger-sea snake | Mainland tiger snake<br>Beaked sea snake | Austrelaps superba<br>Pseudechis porphyriacus<br>Tropidechis carinatus<br>Laboratory experiments indicate that antivenom neutralizes at least 12 different sea-snake antivenoms. | |

TABLE OF POISONOUS ANIMALS AND AVAILABLE ANTIVENOMS (continued)

| Producer or distributor | Venoms used in preparation | Trade or common name | Common name of snake | Additional venoms neutralized* | Comments |
|---|---|---|---|---|---|
| AUSTRALIA (continued) | | | | | |
| | Oxyuranus scutellatus | Taipan | Taipan | Parademansia microlepidota | |
| | Pseudonaja textilis | Eastern brown snake | Eastern brown snake | Pseudonaja affinis<br>Pseudonaja nuchalis | |
| | Pseudechis australis | Brown snake | King brown or Mulga snake | Pseudechis australis<br>Pseudechis porphyriacus | |
| | Oxyuranus scutellatus<br>Acanthophis antarcticus<br>Notechis scutatus<br>Pseudechis australis<br>Pseudonaja textilis | Polyvalent (Australia-New Guinea) | Taipan<br>Death adder<br>Tiger snake<br>King brown snake<br>Eastern brown snake | Austrelaps superba<br>Pseudechis porphyriacus<br>Pseudonaja affinis<br>Pseudonaja muchalis<br>Pseudechis papuanus<br>Parademansia microlepidota | |

* Additional venoms that the said antivenom may neutralize, according to the producer. It can be expected that the antivenom will afford some protection, even though it might be slight, against the venoms of snakes of closely related species.

** Manufacturer states that no true monospecific commercial antivenoms are available. Horses are first "sensitized" to all major venoms and may then be used to produce a succession of separate antivenoms.

TABLE OF POISONOUS ANIMALS AND AVAILABLE ANTIVENOMS (continued)

II. ARTHROPODS (AND SOME OTHERS)

| Producer or distributor | Venoms used in preparation | Trade or common name | Common name of arthropod | Additional venoms neutralized | Comments |
|---|---|---|---|---|---|
| Merck, Sharp and Dome, Westpoint, Pennsylvania 19486, USA | Latrodectus mactans | Black widow | Black widow (spider) | | |
| Instituto Nacional de Higiene, Av. M. Escobedo No. 20, Mexico City D.F., Mexico | Centruroides noxius | Antialacrás polyvalent | | | |
| Laboratorio Zapata, Mexico City D.F., Mexico | Centruroides suffusus<br>Centruroides noxius | Antialacrás polyvalent | | | |
| Laboratorios "MYN", S.A. Av. Coyoacan 1707, Mexico City, 12, D.F. Mexico | Centruroides suffusus<br>Centruroides noxius or C. limidus | Antialacras polyvalent | | | |
| Institutio Nacional de Higiene, Lima, Peru | Loxosceles sp. | Anti-Loxoscelico | | | Ammonium sulfate precipitation. Supplied as liquid. |
| Instituto Butantan, Caixa Postal 65, 05504 São Paulo, Brazil | Phoneutria<br>Loxosceles<br>Lycosa | Antiarachnidico polivalente | | | |
| Institute of Immunology, Rockefellerova, 2, Zagreb, Yugoslavia | Scorpaena porcus | Scorpion fish antivenom | Scorpion fish | | |
| Institut d'Etat des serums et Vaccins Razi, P.O. Box 656, Teheran, Iran | Androctonus crassicauda<br>Buthotus saulcyi<br>Hemiscorpius lepturus<br>Mesobuthus eupeus<br>Odontobuthus doriae<br>Scorpio maurus | Polyvalent scorpion serum | Scorpions | | Ammonium sulfate precipitation. Supplied in liquid form. |
| Commonwealth Serum Laboratories, 45 Poplar Road, Parkville, Victoria 3052, Australia | Latrodectus mactans hasselti | Red-back spider antivenom | Red-back spider | | Pepsin digestion and ammonium sulfate precipitation. |
| | Chironex fleckeri | Sea-wasp | Sea-wasp | Chiropsalmus quadrigatus | |
| | Synanceja trachynis | Stonefish | Stonefish | | |

TABLE OF POISONOUS ANIMALS AND AVAILABLE ANTIVENOMS (continued)

| Producer or distributor | Venoms used in preparation | Trade or common name | Common name of arthropod | Additional venoms neutralized | Comments |
|---|---|---|---|---|---|
| Institut Pasteur d'Algérie, rue Docteur Laveran, Algiers, Algeria | Androctonus australis | Scorpion antivenom | | | |
| Lister Institute of Preventive Medicine, Elstree, Herts WD6 3AX, England | Androctonus australis<br>Buthus occitanus<br>Leiurus quinquestriatus | Scorpion | | | |
| South African Institute for Medical Research, Hospital Street, Johannesburg, South Africa | Latrodectus<br>Parabuthus | Black widow<br>Scorpion | | | |

Annex 5

PROCESSING AND STANDARDIZATION OF ANTIVENOMS

The proposed international standard antivenoms should be produced using the proposed international reference venoms, starting with horses that have not been used for any other antibody production.

The following laboratories have tentatively agreed to produce antivenoms:

| Laboratory | Antivenom |
|---|---|
| Commonwealth Serum Laboratories, Parkville, Australia | Naja naja<br>Notechis scutatus |
| Instituto Clodomiro Picado, San José, Costa Rica | Bothrops atrox asper (Atlantic) |
| Institute of Hygiene, Mexico D.F., Mexico | Crotalus adamanteus |
| Razi State Vaccine and Serum Institute, Teheran, Iran | Echis carinatus (Nigerian and Iranian)<br>Vipera russelii |
| The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Kyushu, Japan | Trimeresurus flavoviridis |

The antivenom should be an almost pure $F(ab)_2$ plasma fraction. The volume of fill should be 5 ml of a 100 g/litre protein solution in a 20-ml vial. The 5 ml should be isotonic and contain glycine (20 g/litre) at pH 6.4 to 6.8.

Tests on final freeze-dried product

The freeze-dried antivenoms must be subjected to the following tests:

(1) protein nitrogen;

(2) moisture content;

(3) sterility;

(4) determination of ability to neutralize the pharmacological properties of the venom used in its production ($ED_{50}$, antihaemorrhagic, antidefibrinating, antinecrotizing);

(5) determination of purity by immunoelectrophoresis, polyacrylamide gel electrophoresis, and analytical ultracentrifugation;

(6) stability by accelerated degradation studies.

The details of these tests should be agreed between the laboratories producing the antivenoms. In appropriate cases, WHO may assist in having those tests done for laboratories in which the equipment may not be available.

Quantities required

In view of the time-consuming and expensive work involved, it would be advisable to set aside at least 5500 vials of each antivenom as an international standard so that the stocks may last for at least 10 years. This means that a pool of at least 27.5 litres of each antivenom would be required. It would be advisable to aim for 30 litres so that 5500 vials remain after the standardization of the antivenom.

Annex 5 (continued)

Standardization

The details of the tests to be used in the standardization of the antivenoms should be agreed as soon as possible. There are some basic principles that should be followed. These are:

(1) the same animal and route of inoculation as that used to determine the particular pharmacological property of the venom should be used;

(2) the mixtures of venom and antivenom should be incubated under appropriate conditions, for example, for one hour at 37 °C before being inoculated into the animals.

It would be appropriate for the laboratories responsible for a specific pharmacological property of the venom to be responsible also for defining the details of the test to measure the neutralization of that property (see Annex 3).

All laboratories taking part in a collaborative study for the assigning of a unit of activity to an antivenom should become familiar with the study method as soon as possible.

Annex 6

## STORAGE, ESPECIALLY IN THE TROPICS

Although no direct data have been found on the stability of antivenoms, relevant data are available on the stability of bacterial antitoxins exposed to various experimental conditions.

The number of years during which a 10% loss occurred in potency of antitoxin preparations held in the liquid state and stored at different temperatures was calculated according to the method of Jerne & Perry[1] and the results are summarized in Table 1. From these data, it may be concluded that the potency of an antivenom held in the liquid state will be maintained for at least 1 year when stored at 30 °C. It is well known, however, that lyophilized antisera are much more stable at elevated temperatures than are liquid antisera.

It would therefore seem sensible for antivenoms that are to be used in the tropics to be lyophilized rather than being distributed in the liquid state.

TABLE 1. NUMBER OF YEARS REQUIRED TO CAUSE A 10% LOSS IN POTENCY OF ANTITOXIN PREPARATIONS HELD IN THE LIQUID STATE AND STORED AT THE INDICATED TEMPERATURES[a]

| Antitoxin[b] | 5 °C | 10 °C | 25 °C | 30 °C |
|---|---|---|---|---|
| D-antitoxin (Chiba-1) | 44 710 | 5 001 | 10.9 | 1.6 |
| D-antitoxin (Chiba-2) | 30 540 | 3 610 | 9.2 | 1.4 |
| D-antitoxin (Chiba-3) | 19 970 | 2 791 | 11.3 | 2.0 |
| D-antitoxin (Chiba-4) | 26 070 | 3 138 | 8.4 | 1.3 |
| D-antitoxin (Chiba-5) | 226 000 | 20 770 | 26.1 | 3.3 |
| D-antitoxin (Saikin-1) | 374 900 | 38 720 | 67.4 | 9.3 |
| D-antitoxin (Saikin-2) | 45 390 | 5 766 | 17.9 | 3.0 |
| D-antitoxin (Saikin-3) | 1 236 | 215 | 1.6 | 0.4 |
| D-antitoxin (Yoken-1) | 9 372 | 1 267 | 4.7 | 0.8 |
| T-antitoxin (Biken-1) | 15 690 | 2 148 | 8.2 | 1.5 |
| T-antitoxin (Biken-2) | 9 020 | 1 331 | 6.3 | 1.2 |
| W-serum (Yoken-2) | 24 900 000 000 | 810 000 000 | 55 980 | 2 835 |

[a] Unpublished data from National Institute of Health, Japan.

[b] D-antitoxin = diphtheria antitoxin; T-antitoxin = tetanus antitoxin; W-serum = Weil's disease therapeutic serum.

[1] According to Jerne, N. K. & Perry, W. L. M. (1956) The stability of biological standards. Bulletin of the World Health Organization, 14: 167-182.

TABLE 2. BATCH-TO-BATCH VARIATION OF POLYSPECIFIC ANTIVENOMS[a]

| Antivenoms | pH | Phenol (g/litre) | Protein N (g/litre) | Total protein (g/litre) | Total solid (g/litre) |
|---|---|---|---|---|---|
| Batch No. 94 | 6.8 | 27 | 10.7 | 67 | 103 |
| Batch No. 79 | 6.4 | 27 | 13.5 | 84 | 120 |
| Batch No. 66 | 6.3 | 24 | 22.8 | 142 | 162 |
| Batch No. 56 | 6.6 | 25 | 19.1 | 119 | 160 |
| Batch No. 46 | 6.8 | 26 | 21.6 | 135 | 166 |

[a] Data from Dr M. Latifi.

TABLE 3. POTENCIES[a] OF POLYSPECIFIC ANTIVENOMS RELATED TO EXPIRY DATE FOR A STORAGE TEMPERATURE OF 5 °C[b]

| Venoms | Dilution neutralized (g/litre) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Batch No. 94 (expiry Aug. 79) | | Batch No. 79 (expiry June 77) | | Batch No. 66 (expiry April 75) | | Batch No. 56 (expiry March 73) | | Batch No. 46 (expiry Oct. 71) | |
| | Initial titre | 2 years | Initial titre | 4 years | Initial titre | 6 years | Initial titre | 8 years | Initial titre | 10 years |
| Naja naja oxiana $LD_{50}$ = 8.3 µg | 0.3 | 0.3 | 0.3 | 0.2 | 0.3 | 0.3 | 0.4 | 0.2 | 0.4 | 0.3 |
| Echis carinatus $LD_{50}$ = 4.6 µg | 2.2 | 2.2 | 2.0 | 2.0 | 2.8 | 2.6 | 2.6 | 2.6 | 1.8 | 1.8 |
| Vipera lebetina $LD_{50}$ = 7.6 µg | 1.4 | 1.4 | 1.4 | 1.4 | 1.4 | 1.4 | 1.8 | 1.4 | 1.6 | 1.2 |
| Pseudocerastes persicus $LD_{50}$ = 16.2 µg | 1.0 | 1.0 | 1.0 | 1.0 | 1.8 | 1.4 | 2.0 | 1.2 | 1.2 | 1.0 |
| Agkistrodon halys $LD_{50}$ = 13.7 µg | 0.4 | 0.4 | 0.6 | 0.6 | 1.0 | 0.8 | 1.0 | 0.8 | 0.8 | 0.6 |

[a] Potency was determined intravenously in mice (16-18 g).

[b] Data from Dr M. Latifi.

Annex 7

ANTIVENOM REACTIONS IN NORTH-WEST MALAYSIA

| Antivenom[a] | | | No. of patients | Reactions | | |
|---|---|---|---|---|---|---|
| Type | Dose (ml) | Route[b] | | Anaphylactoid | Early mild | Delayed[c] |
| Haffkine 1954-55 polyvalent | 10-20 | IVI | 16 | 5 | 1 | 1 |
| Haffkine 1958-59 polyvalent | 10 | IMI | 17 | - | 1 | 1 (+1) |
| | 20 | IMI | 11 | - | - | 1 |
| | 50 | IMI | 51 | - | 1 | 4 |
| Bangkok 1958-59 Agkistrodon rhodostoma | 10 | IMI | 53 | - | 1 | 4 (+1) |
| | 20 | IMI | 195 | 2 | 13 | 12 (+3) |
| | 50 | IMI | 140 | 5 | 22 | 20 (+3) |
| Bangkok 1960-64 Agkistrodon rhodostoma | 50 | IVD | 42 | 6 | 12 | 2 (+3) |
| Naja naja | 50 | IVD | 4 | 1 | 2 | (+3) |
| Haffkine 1960-64 polyvalent | 100 | IVD | 4 | 1 | - | - |
| Commonwealth Serum Laboratories 1961-64 Agkistrodon rhodostoma | 28-112 | IVD | 4 | - | 1 | - |
| Enhydrina schistosa | 31-72 | IVD | 8 | - | 3 | - |
| | 180 | IVD | 2 | - | 1 | - |

[a] Antivenom refining methods were: Bangkok, nil; Haffkine, ammonium sulfate; CSL, enzyme/ammonium sulfate.

[b] IVI - intravenous injection of undiluted antivenom
IMI - intramuscular injection of antivenom with 1 ml hyaluronidase
IVD - intravenous drip-infusion of antivenom diluted in 300 ml isotonic saline

[c] In parentheses - delayed reactions in patients who have already had either anaphylactoid or early mild reactions.

Annex 8

ACTIVE IMMUNIZATION

TABLE 1. VACCINATIONS AGAINST HABU VENOMS BY THE USE OF DIFFERENT VENOIDS IN JAPAN[a]

| Years | Name of venoid | Number of inoculants |
|---|---|---|
| 1971-72 | APF[b] | 1 993 |
| 1970-78 | Mixed[c] | 55 845 |

[a] Data supplied by the Committee for Research on Habu Toxoid.

[b] APF is venom precipitated with alcohol. This venoid was studied for 2 years and then abandoned because of the relatively severe side-reactions.

[c] Mixed means the HR1 and HR2 haemorrhagic factors toxoided and mixed.

TABLE 2. NUMBER OF PARTICIPANTS GIVEN HABU VENOID FROM 1970 TO 1978

| Years | Mixed venoid | APF venoid[a] |
|---|---|---|
| 1970 | 93 (59, 54) | 97 (76, 41) |
| 1971 | 1 349 (1 134, 572) | |
| 1972 | 160 (123, 90, 31) | 1, 96 (1 545, 1 339) |
| 1973 | 1 126 (900, 675, 339, 495, 24) | |
| 1974 | 928 (784, 176, 136, 175) | |
| 1975 | 1 007 (514, 285, 332) | |
| 1976 | 803 (546, 135) | |
| 1977 | 304 (212, 123) | |
| 1978 | 75 (73, 22) | |

Number of participants receiving second, third, fourth, fifth, and sixth injections are indicated in parentheses.

[a] See note b under Table 1.

Annex 8 (continued)

TABLE 3. REACTION TO THE HABU VENOID

| | | Mixed venoid lot 13 | | | | | | APF venoid lot 1 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.5 ml | | | 0.1 ml | | | 0.5 ml | | | 0.1 ml | | |
| | | 1st | 2nd | 3rd | 1st | 2nd | 3rd | 1st | 2nd | 3rd | 1st | 2nd | 3rd |
| | | 48 | 41 | 22 | 51 | 45 | 29 | 48 | 37 | 21 | 49 | 32 | 13 |
| P | (-) | 37 | 41 | 20 | 49 | 45 | 28 | 26 | 35 | 11 | 46 | 32 | 12 |
| | + | 8 | | 2 | 2 | | 1 | 16 | 2 | 9 | 3 | | 1 |
| | ++ | 1 | | | | | | 7 | 2 | 1 | | | |
| | +++ | 2 | | | | | | 2 | | | | | |
| S | (-) | 43 | 37 | 18 | 50 | 42 | 29 | 33 | 25 | 15 | 45 | 28 | 11 |
| | + | 3 | 3 | 3 | 1 | 3 | | 11 | 10 | 4 | 4 | 3 | 2 |
| | ++ | 2 | | | | | | 4 | 2 | 2 | | | |
| | +++ | | | 1 | | | | | | | | | |
| | ++++ | | 1 | | | | | | 1 | | | | |

1st, 2nd, 3rd = order of injections. Pain (P): No pain (-); pain on pressure (+); spontaneous pain (++); pain on movement (+++). Swelling(s): No swelling (-); less than 5 mm diameter (+); less than 10 mm (++); reaching the elbow (+++); beyond the elbow (++++).

Annex 9

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