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WHO COORDINATION MEETING ON VENOMS AND ANTIVENOMS

A WHO informal meeting took place at the Chemika Zurich from 24 to 27 September 1979. The purpose of the meeting was to coordinate the work in progress throughout the world on the use and standardization of venoms and antivenoms. The list of the participants is shown in Annex I and the agenda in Annex II.

For many years WHO has had an interest in the treatment of bites and stings from poisonous creatures and although there have been informal meetings from time to time none has specifically attempted to collect the data of the clinical effects of snake and scorpion bites and stings and the experiences in their treatment. Furthermore it was recognized that there is an urgent need to correlate such experiences 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 the availability of venoms that had been fully characterized and the establishment of international standard antivenoms.

WHO has taken the first step in designating the Liverpool School of Tropical Diseases as the WHO Collaborative Centre for the Control of Antivenoms. As Director of this Centre, Dr H. Alistair Reid agreed to be Chairman of the meeting.

The meeting agreed that in English 'venom' and 'antivenom' were the preferred names rather than venin/antivenin or venene/antivenene.

A. 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 due mainly to the lack of reliable statistical data.

In the United States of America, approximately 8000 bites by venomous snakes are reported each year. There are about 12 deaths which occur in the untreated, under-treated, 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 sp) 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). 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%.

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In North Africa, scorpion stings are medically more important than snake bites. For example, in parts of southern Libya during 1979 there were 874 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 savannah 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 1936-1960, and an incidence of 163 proven bites during the summer of 1961. In Europe bites by imported venomous snakes are sometimes fatal.

In South East Asia, over 10,000 deaths due to snake bite are reported annually (see Annex III). The mortality is high in India, Sri Lanka, Burma, Thailand and the Philippines; 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 to 14 patients used to die each year but recently the mortality rate has fallen due to better treatment.

The participants agreed that the use of immunodiagnostic methods for assessing venom antigen and antibody, and collaboration with anthropologists and traditional healers should greatly improve the epidemiological data on venomous bites and stings. It was recommended that the data should be reported using the classification recently adopted by WHO.

B. MEDICALLY IMPORTANT SPECIES

Medically important snakes are listed in Annex IV. 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 Americas), Tityus (South America), Androctonus, Buthus, and Leiurus (North Africa, to South-East Asia). The spider Latrodectus sp. occurs in warm areas throughout the world. Loxosceles sp. can cause severe necrosis (mainly in the Americas). Spiders of Phoneutria sp. and Atrax sp. are medically important in South America and Australia respectively.

C. CLINICALLY IMPORTANT FEATURES OF ENVENOMING

C.1 Systemic envenoming

Snake bite envenoming produces changes which, 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.

C.2 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, and emphasises 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 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 usually 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.

C.3 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 which 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 immediate reaction which appears 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.

D. THE CHARACTERIZATION OF VENOMS AND STANDARDIZATION OF ANTIVENOMS

D.1 The provision of venoms

The Group discussed in detail the availability of venoms and their pharmacology. It was clear that 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.

It was agreed that although the provision of venoms from every snake species would be impracticable, it should be possible to obtain venoms from those species causing major health problems.

Differences in pharmacological activities of the snake venoms recognised 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 it was agreed to establish international reference reagents of venoms making a start with those from seven important species: *Naja naja*; *Notechis scutatus*; *Echis carinatus* (West Africa and Iran); *Vipera russelli*; *Crotalus adamanteus*; *Bothrops atrox asper* (Atlantic); and *Trimeresurus flavoviridis* (see Annex V).

It was considered important also 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 it was requested that some of each venom be set aside for the preparation of antivenom.

D.2 The characterisation of venoms

Several laboratories agreed to take part in a collaborative study to measure the lethal, defibrinating, haemorrhagic and necrotising activities. It is important that WHO should encourage these studies. It was recognised that although these tests would not include other activities such as the neurological activity, they would provide useful data for the partial characterisation of the venoms. The details of the collection and the requirements of the suitability of the venoms to be recognised as international reference reagents are shown in Annex V. It was agreed also that there was a need to adopt a common method of testing lethal and other biological activities of the venoms (see Annex V). Each dried venom will be characterised by an international collaborative study according to an agreed protocol. The report of the study will be submitted to the Chief, Biologicals, WHO, Geneva, for presentation to the WHO Expert Committee on Biological Standardisation for adoption as an international reference reagent of venom from a specified species of venomous creature.

D.3 The provision of antivenom

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

In order to develop and establish international standard antivenoms the Group agreed 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 it has been agreed that the international standard antivenoms will be monospecific and raised using the appropriate international reference venom, the antivenoms would not be available commercially and must be prepared specially. Furthermore, it was emphasized repeatedly that the ultimate test of efficacy is in clinical trial. It is important, therefore, that WHO encourage clinical trials of these monospecific antivenoms and attempts to correlate their activity with laboratory tests. The suggested laboratories for the manufacture of the antivenoms are listed in Annex VII.

D.4 Immunization schedules

The schedules used for the immunization of horses depend on so many factors concerning the particular venom and the horse being immunized that no single schedule for all venoms could be established. With some mainly neurotoxic venoms (such as coral snake venoms) the schedule could be relatively short and the total amount of venom of 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 to 1000 mg.

It was considered advisable to include some adjuvant to decrease the time required and to increase the immunogenicity of the venom used. It was recommended also to sterilize the venom, by membrane filtration before the inoculation, in order to minimize abscess formation at the site of the injection. It has been observed that modified venoms (venoids) - unless it has been proven that they retain all the important immunologic components - should not be used to start the immunization schedule and in order to obtain a satisfactory response crude venom should be used. For those venoms not giving rise to necrosis there has been no difficulty in using crude venom. When there are marked necrotising effects, however, venoiding with glutaraldehyde or formaldehyde has been shown to be successful. This method of venoiding (tiger snake venom) which has given venoids protecting small animals against massive challenges with venom is worthy of further investigation with other venoms.

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

There were no satisfactory answers to the inquiries (i) was sufficient attention being paid to avoid the IgM peak of production when spacing the doses of immunization; (ii) would the use of capsules slowly releasing antigen be useful; and (iii) what is the explanation for "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 was important to use the venom obtained by electrical stimulation rather than by maceration of the telson.

D.5 The refining of antivenoms

The Group agreed that the proposed international standards for antivenoms should be refined and produced 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. The technique suggested for purification and concentration of antivenom to be used for human therapy originated with Pope (see Annex VIII).

It was 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 immunoglobulins to diffuse in tissues. This was 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 satisfied the WHO Requirements for Immune Sera of Animal Origin (WHO Technical Report Series, No. 413, 1969).

D.6 The potency assay of antivenoms

Many laboratories throughout the world are neutralizing venoms with antivenoms and calculating the 50% end-point of neutralization by the Reed and Muench method. One of the fundamental principles, 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, however, errors occur in the calculation. A much more acceptable method of calculating the median points is preferably by probit analysis or by the method of Spearman-Kärber.

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 end-point of neutralization a pharmacological property different from that known to be important by clinical observation in man. Thus mice inoculated with Echis carinatus venom die rapidly with massive intravascular coagulation whereas human victims die after one to two 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 were proposed but it was generally agreed that when once an international unit of activity had been assigned to an antivenom with respect to its ability to neutralize a given quantity of venom then standardization of other similar antivenoms could be made 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 D.7.

In order to effect this standardization, it is important to reach an agreement on the methods used and the details of such tests should be established as soon as possible.

D.7 Standardisation of antivenoms

The standardisation 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 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, WHO should 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 but collected from different areas, the snakes from which the venom was collected must be identified geographically. The availability of characterised venoms and standardised antivenoms would greatly assist collaboration among laboratories in different countries. The basic principles of standardisation of antivenoms are summarised in Annex VIII.

Although the mouse protection test is not always reliable in predicting the clinical effectiveness of an antivenom, it is the most widely used single 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 VII.

E. THE DESCRIPTION, STORAGE AND DISTRIBUTION OF ANTIVENOMS

E.1 Labelling of antivenoms

WHO Technical Report Series 1971, No. 463, lays down some simple requirements for labelling antivenoms including the potency of the antivenom, nature of the preparation, method of reconstitution, restrictions if any for 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 should be clear and simple and in print 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 be 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 was recommended 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.

It was agreed that training clinicians through instruction pamphlets was not satisfactory and that education should be supported for both the lay public and medical students. Although much had 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 to actively promote such education.

E.2 Distribution

The discussion identified a number of problems concerning distribution of antivenoms in the developing world. It was clear that in most tropical countries the supply system was deficient and in many areas it was impossible to obtain antivenoms.

On the other hand in the developed countries the distribution is easy and rapid. In 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 two hours under conditions of difficult transportation. In Nigeria paramedical staff 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 was usually in the hands of governments. The antivenoms were distributed to hospitals and medical stations free of charge. It was important, however, for those responsible for the purchase of antivenoms to ask advice concerning the suitability of the products available.

It is important to transport antivenoms in the liquid state to countries with high ambient temperatures with appropriate refrigeration and insulation. Appropriate technology for the transportation and storage is a prerequisite for maintaining high quality antivenoms. The recommendation was made that WHO should develop practical principles for efficient snake and scorpion bite control programmes as part of national primary health care systems.

E.3 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 antivenoms maintained at 37°C retain their potency for at least six months and possibly one year but this does not obviate the necessity for the provision of a satisfactory cold chain (see Annex IX).

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 being available in the liquid form since the former are more stable at high ambient temperatures.

E.4 Expiry date

The period throughout which an antivenom may be expected to retain its activity when stored in the liquid state is two years after the date of issue (some control authorities allow three 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 IX. The optimal range of temperature to store antivenom is between 2° to 10°C. If the antivenom was in the liquid form and kept at 5°C, the observation showed no change in potency within six years, but after eight and ten years a 10 to 20% loss of neutralizing ability may occur against some (though not all) venoms.

The expiry date for a freeze-dried preparation, when sealed by the fusion of glass, should be many years, but for those preparations in vials with a rubber stopper and metal crimped caps the declared expiry date should not be more than five 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 protein content and pH.

F. THE CLINICAL EFFICACY OF ANTIVENOMS

F.1 Clinical efficacy

There is considerable difference in the efficacy 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, except late in the shock state, 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. Seasnake antivenom has proved highly successful even when given as late as two days after the bite. Similar findings of reversing systemic symptoms or signs in other snake venom poisoning cases can be cited as examples of the effectiveness of antivenom.

The effectiveness of antivenoms in preventing or minimising the local effects of venoms, specially the development of local necrosis is uncertain and it was agreed that this problem urgently requires clinical investigation.

F.2 Dosage, time-factor, monitoring problems

The initial dose of antivenom is often calculated from mouse protection tests by scaling the antivenom dose to take account of average venom yields. These calculations ignore the wide variability 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 and at times long delay between the bite and the patient's presentation for treatment introduces yet another difficulty into the clinical study of antivenom. Experimentally, delay in administering antivenom results in a steep increase in the median effective neutralising 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 satisfactory for *in vitro* neutralisation must be given to arrest progress (although first-aid with pressure dressings and splint can reduce the antivenom requirement).

However, in man seasnake antivenom, as already mentioned, has successfully combated life-threatening poisoning even when not given until two 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 four 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, by the wrong route, or even the wrong antivenom.

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 which 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 neutralised. 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 was argued that for bites by the Australian snake species, the initial dose of antivenom could be judged adequately from the symptoms and signs of the patient. For example, the presence of paralytic features or coagulation defects were indications for doubling the initial dose of antivenom. For North American crotalids it was felt that with some exceptions, the dose of antivenom could 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 neutralising dose of antivenom had been given. Clinical judgement remains paramount in this area. It was agreed, however, that there are immunodiagnostic tests which can identify the venom and could, thereby, indicate treatment by monospecific rather than polyspecific antivenom.

F.3 Paraspecific activity of antivenoms

Immunodiffusion tests have shown widespread sharing of venom antigens amongst snakes. Whilst 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 asper, 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 neutralises venoms of five other rattlesnakes but not that from C.durissus terrificus. North American pit viper polyspecific antivenom shows some neutralisation of venoms of Vipera x.palaestinae, V.ammodytes, Bitis gabonica and Cerastes, and not those of V.russelli or Echis. It neutralises 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 neutralises venom from V.berus and is used clinically for this purpose. Considerable cross-neutralisation is seen amongst cobra venoms from both Asia and Africa although venoms of some species, notably Naja nigricollis, are not well-neutralised.

Antivenoms against Notechis, Acanthophis and Oxyuranus neutralise nine Naja venoms from Asian and African sources as well as venoms of six other species of elapids in the mouse protection tests. However, only Notechis antivenom neutralises Micrurus fulvius venom. Dendroaspis venoms are not significantly neutralised by these antivenoms. On the other hand Notechis antivenom neutralised venoms of eight of nine seasnakes better than the seasnake (Enhydrina) antivenom.

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

F. Preferred routes of administration

It was agreed that the intravenous route is the most effective route and that the 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 injected site in patients with incoagulable blood. The topical application of antivenom to the eye in injuries caused by spitting snakes needs to be evaluated but for this purpose the ethics of inducing pain in an experimental animal needs 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. It was considered that such a method of administration of antivenom linked the administrator with the patient through the most critical time of giving the antivenom. This was particularly important under tropical conditions.

F.5. Clinical trials

Antivenoms are some of 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 randomised 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 antivenom 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 is 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 could salvage a proportion of these "lost" cases.

WHO through its regional offices should actively encourage the planning and execution of clinical trials of antivenom. Hospitals in Tamale (Northern Ghana), Bambur (Nigeria), Kufra (Libya), and Bangkok have already collaborated with the WHO Collaborative Centre for the Control of Antivenoms and are suitable for such trials but financial support would be needed from WHO.

In Mexico, a number of 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 Libya, 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.

G. REACTIONS TO ANTIVENOMS

G.1 Early reactions

Antivenom reactions are often classified as "immediate" and "delayed" but these terms have very specific meanings in immunology and the meeting therefore agreed 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 nil to over 40% of all people treated with antivenoms (see Annex X) depending on various factors such as the type of antivenom, dose, route and method of administration, nature of the populations, previous exposure to sensitising 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 sensitising 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 Malaya support this contention (see Annex X). 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 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 have been 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 one immediate reaction occurred and seven 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 problem of pyrogen reactions was also discussed and advice suggested to producers of antivenoms concerning possible ways of combating these reactions.

G.2 Later antivenom reactions

The common clinical features of these reactions are that between five 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 three 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 is possible, however, including complement activation and venom antivenom immune complex formation.

G.3 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 which 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 indigenous 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.

G.4 Prediction and prevention of reactions

True anaphylactic (IgE mediated) hypersensitivity can be detected in some cases by skin tests and this procedure is extensively used in the United States of America. Detection of IgE specific for horse protein by the RAST procedure 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 minimise 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 nonsedating 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.

G.5 Treatment of reactions

Since the predictive value of recommended tests for hypersensitivity such as skin tests and conjunctival tests is questionable, it is recommended that all patients that are to be given antivenom 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 adrenaline and antihistamines before antivenom has been advocated but the side-effects of adrenaline need to be considered.

When the established immediate reactions occur the administration of the antivenom should be stopped and adrenaline 1:1000 (0.5 to 1 ml) should be promptly injected subcutaneously or, if 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 adrenaline. Supportive therapy, including maintenance of the airway and plasma expanders. In many cases it has been possible to continue antivenom administration after recovery from a reaction.

The delayed reactions usually require treatment with steroids.

H. ACTIVE IMMUNIZATION

(a) 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 those in which there is delay or inadequate medical treatment but on the quantity of venom which was introduced into the victims. In such cases of heavy envenoming, the development of the lesion was so rapid that the antivenom alone was insufficient to prevent such a large amount of venom injected. In such cases however, if the victims were immune against the venom even to a small degree, they would be able to delay the onset of symptoms and were therefore treated more successfully.

Separation of the haemorrhagic factors (HR1 and HR2) were carried out to increase the antigenicity of the venoid; 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 M/5 $AlCl_3.6H_2O$ and M/5 $Na_3PO_4.12H_2O$ in the presence of the venoid. Standardization of the venoid was also investigated. Since 1970, field trials of vaccination of the Habu venoids have been carried out on the Amami Island.

Immunization schedules: Human volunteers received three or more injections of either 0.5 ml or 0.1 ml of the venoids at an interval of four weeks and about six months (see Table 1, Annex XI).

Reactions: The main reactions were pain and swelling that appeared at the point of injection. It was found also that the reactions which occurred in the persons who received 0.1 ml of the venoid were less severe than in those having received 0.5 ml.

Levels of circulating antivenom: Antivenoms in sera from immunized persons, anti-HR1 and anti-HR2 were titrated. It was found that most persons who received three injections of 0.5 ml of both venoids attained anti-HR1 titres of 1 unit or higher after the third injection. Since it is known 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 anti-venom titre of about 1.3 u/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 µ/ml.

Hypersensitivity acquired after injection of the venoids: Seven persons who received DHTA venoid for one to five times, and five months to two 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 six of the persons. The reaction in one person who received the venoid before and two persons who had not received the venoid was negative. The positive reaction was apparent within 24 to 48 hours and disappeared within a few days.

Effectiveness of vaccination: Between 1970 and 1978, 1507 bites occurred on Amami Island, among which 168 cases were immunized and 1407 were not immunized; the effectiveness of the vaccination is still under investigation.

(b) Vaccines against scorpion stings

In this meeting a Mexican report was presented about the modification of a purified toxic fraction from the venom of the scorpion *Centruroides noxius* Hoffmann, by treatment with glutaraldehyde. The detoxified fraction was shown to be immunogenic in rabbits.

CONCLUSIONS AND RECOMMENDATIONS

1. Countries should be encouraged to collect more reliable data on the morbidity and mortality due to venomous bites and stings. The WHO classification of injuries due to bites and stings should be used for reporting the cases.

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

3. The terminology of venom and antivenom should be adopted in all future reports.

4. There is a need for a detailed report on the clinical indications for the use of antivenom as well as for the treatment of snake bites and scorpion stings (see recommendation 19).

5. Further work should be done to determine whether the snake or scorpion had injected any venom and if so to quantify the envenoming.

6. Greater attention should be paid to the species of snake or scorpion causing the injury. This is particularly important in relation to the envenoming pattern caused by specific species. WHO should support the wider application of ELISA in this problem.

7. Greater emphasis must be placed on clinical trials on antivenoms rather than relying on efficacy shown only by animal models. WHO should encourage and support such trials in man.

8. There is a need for international reference reagents of venoms for both research purposes and for the preparation of international standards for antivenoms. WHO is requested to take the necessary steps to obtain such venoms and arrange the collaborative studies for their characterisation.

9. There is a need for international standards for antivenoms. WHO is requested to take the necessary steps to obtain such antivenoms and arrange the collaborative studies for their callibration.

10. The assay methods used in the measurement of antivenom potency should be standardised. These include the ability to neutralise the lethal, defibrinating, haemorrhagic and necrotising activities. Where neurotoxicity and neuromuscular activity are important, tests to measure the ability of the antivenom to neutralise these properties also should be developed. The potency of any antivenon should be determined relative to that of a standard antivenon. The availability of an international standard antivenon would greatly assist collaboration among laboratories in different countries.

11. WHO should alert governments to the correct storage of antivenoms and ensure that potent antivenom is more readily available especially in the rural areas.

12. The timing of giving the antivenom may be critical and there should be many more data collected to confirm this. WHO should support such studies.

13. Not enough information is available on the usefulness of immunodiagnostic tests to assist in the diagnosis and treatment of a bitten or stung person. It would be helpful to have more work on this not only for the identification of the venom injected but also for studying the absorption and persistence of venom and quantity of antivenom required.

14. There should be more information on the paraspecific effects of antivenoms particularly where no monospecific antivenom is available.

15. Education of physicians, medical students, paramedicals and lay public regarding snake bite and other venomous bites and stings should be encouraged by WHO.

16. The administration of antivenom should wherever feasible be given by the intravenous infusion of the antivenom. The physician should remain with the patient throughout the early stages of administration of the antivenom to observe for serum reactions.

17. There is a need for more accurate information on both the early and late reactions to envenoming. Governments should be encouraged to collect the data according to an agreed classification using an agreed terminology. WHO should take steps to obtain such data.

18. 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 RAST test should be investigated. It is important that health personnel anticipate that reactions that may occur in any patient given antivenom.

19. There is a need for a detailed and comprehensive manual on the treatment of bites and stings from poisonous creatures. WHO should take steps to provide such a manual.

ANNEX I

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ANNEX II

AGENDA

A. EPIDEMIOLOGY: Incidence, mortality of snake bite, scorpion stings and spider bites on a broad geographical basis

B. MEDICALLY IMPORTANT SPECIES: The commonest snake (etc.) species of medical importance on a broad geographical basis

C. CLINICALLY IMPORTANT FEATURES RELEVANT TO ANTIVENOM

- C.1 Systemic envenoming, especially distinctive diagnostic patterns
- C.2 Local envenoming, especially necrosis (including bacteriological factors)
- C.3 Autopharmacological and other factors

D. AVAILABILITY AND SUITABILITY OF ANTIVENOMS on a broad geographical basis

E. ANTIVENOM PRODUCTION

- E.1 Suitability, variability, availability of venoms and venom fractions
- E.2 Animal immunization schedules
- E.3 Potency assay methods
- E.4 Refining and purification methods
- E.5 Standardization, including standard fractions
- E.6 Labelling, information for clinicians
- E.7 Distribution and costs in the tropics
- E.8 Storage, especially in the tropics
- E.9 Expiry dates

F. ANTIVENOM EFFICACY

- F.1 Clinical efficacy for systemic effects
- F.2 Clinical efficacy for local effects, especially necrosis
- F.3 Dosage, time factor, monitoring problems
- F.4 Paraspecific activity
- F.5 F.1-4, in relation to potency assays (E.3)
- F.6 Preferred routes of administration
- F.7 Clinical trials

G. ANTIVENOM REACTIONS

- G.1 Immediate serum reactions, incidence, severity, relation to antivenom refinement
- G.2 Delayed ditto
- G.3 Complement activation
- G.4 Prediction and prevention of reactions
- G.5 Treatment of reactions

H. ACTIVE IMMUNIZATION: practicabilities

I. OTHER MATTERS

J. SUMMARY AND CONCLUSIONS

ANNEX III

SNAKE BITE MORTALITY IN SOUTH EAST ASIA

Country	Average no. of bites per year	Average no. of deaths per year	Yearly mortality per 100,000 population
Japan	610	5.6	0.570
Hong Kong	203	1.3	0.090
Thailand	3989	302	0.860
Burma	8508	759	2.700
Malaysia	2480	16	0.180
Taiwan		36	0.270
Sri Lanka		104	0.820
India (Maharashtra State)		1093	2.100
Philippines		294	0.770

ANNEX IV

MEDICALLY IMPORTANT SNAKE SPECIES

1. Definition

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

(i) commonly cause death or serious disability;

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

(iii) commonly cause bites but serious effects very uncommon.

2. Geographical areas

	i	ii	iii
North America	Agkistrodon piscivorus Crotalus adamanteus Cr. atrox Cr. viridis	Crotalus scutulatus Micrurus fulvius	Agkistrodon contortrix Crotalus horridus Sistrurus miliarius
Mexico and Central America	Bothrops asper/atrox Crotalus atrox Cr. basiliscus Cr. durissus	Agkistrodon bilineatus Crotalus molossus Cr. triseriatus Cr. polystictus Cr. scutulatus Lachesis muta Micrurus nigrocinctus	Bothrops schlegeli B. lateralis
South America	Bothrops atrox B. jararaca B. neuwiedi Crotalus durissus Cr. durissus terrificus	Bothrops alternatus B. jararacussu Lachesis muta Micrurus corallinus M. lemniscatus M. mipartitus	Bothrops bilineatus B. schlegeli
North Africa	Bitis arietans Echis carinatus Naja nigricollis	Atractaspis sp. Naja haje	Cerastes sp.
Mid-Africa	Bitis arietans Echis carinatus Naja mossambica N. nigricollis	Atractaspis sp. Bitis gabonica Dendroaspis sp. (mainly D. polylepis) Dispholidus typus Naja haje Thelethornis kirtlandi	Causus sp.

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	i	ii	iii
Southern Africa	Bitis arietans Naja nigricollis	Atractaspis sp. Dendroaspis sp. Dispholidus typus Naja haje N. nivea Theletornis kirtlandi	Causus sp.
Europe		Vipera lebetina	Vipera ammodytes V. aspis V. berus
Near and Middle East	Bitis arietans Echis carinatus Naja naja Vipera lebetina V. xanthina	Atractaspis sp. Echis coloratus Naja haje Vipera lebetina	Agkistrodon halys Cerastes sp. Vipera ammodytes
South-East Asia (Pakistan to Celebes)	Agkistrodon rhodostoma Echis carinatus Enhydrina schistosa Naja naja Vipera russelli	Bungarus caeruleus Hydrophis cyanocinctus Lapemis hardwicki Ophiophagus hannah Trimeresurus purpureomaculatus	Trimeresurus albolabris T. wagleri
Far East	Naja naja Trimeresurus flavoviridis (Ryukyu) T. mucrosquamatus	Agkistrodon acutus Bungarus multicinctus (Taiwan) Hydrophis cyanocinctus Lapemis hardwicki Ophiophagus hannah	Agkistrodon blomhoffi A. caliginosus A. halys Trimeresurus albolabris T. stejnegeri (Taiwan)
Australia and Pacific Islands	Acanthophis antarcticus Notechis scutatus Pseudonaja textilis	Austrelaps superba Oxyuranus scutellatus Pseudechis australis Pseudechis papuanus Tropidechis carinatus	Pseudechis porphyriacus

ANNEX V

THE PROVISION, PROCESSING AND CHARACTERIZATION OF VENOMS

1. The provision of venoms

The Group suggested that the most appropriate sources of venoms would be the following:

Snake species	Country of origin	Responsible investigator
Naja Naja	Thailand	D. A. Warrell
Notechis scutatus	Australia	S. Sutherland
Echis carinatus	West Africa	C. Arnett
Echis carinatus	Iran	M. Latifi
Vipera russelli	Thailand	D. A. Warrell
Crotalus adamanteus	United States of America	W. Haast
Bothrops atrox-asper (Atlantic)	Costa Rica	R. Bolanos
Trimerésurus flavoviridis	Japan	A. Ohsaka

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 shall 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.

It was agreed that the appropriate dry weight of the venom in the ampoules would be

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

2 mg for Vipera russelli
Echis carinatus

1 mg for Naja naja
Notechis scutatus

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

2. The characterization of venoms

Determinations of the LD_{50} and other pharmacological properties will be used to select the venom for the international reference reagent.

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

Annex V

(1) centrifugation;

(2) filtration through millipore membranes;

(3) tested for sterility;

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

(5) freeze-dried after freezing in liquid nitrogen

(6) sealed by the fusion of glass;

(7) tested for stability by accelerated degradation tests;

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

The activity of the venoms will be determined in several laboratories. The one test that will be done by each laboratory will be 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*
Route of injection	:	Intravenous
Volume of injection	:	0.5 ml
Rate of injection	:	the 0.5 ml given in 15 seconds
Expression of results	:	the lethal dose 50% 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 three dilutions fall on the steep part of the dose response curve
Number of animals	:	at least five animals are inoculated <u>with each</u> dilution of venom
Period of observation	:	the mice will be observed for 48 hours
Controls	:	five mice injected with saline as controls must survive the injection;

The method of calculating the LD_{50} will 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 (Finney, 1964), which is valid provided:

(i) d, the log dose interval is constant;

(ii) the full response range from 0% to 100% is covered; and

(iii) the response distribution is nearly symmetrical.

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*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., Bull. Wld Hlth Org., 1958, 19, 341).

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

m = log LD_{50};

x_{100} = log dose giving 100% deaths and having 100% deaths for all higher doses;

n = number of mice used at each dose level;

r = number of mice dying at each dose level; and

Σ = denotes summation over all doses between and including x_{100} and x_0 (x_0 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}

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

where Σ denotes summation over the same range as in the previous paragraph.

The 95% fiducial limits to m are taken to be approximately $m \pm t_{0.05}\sqrt{V(m)}$ where $t_{0.05}$ has the value appropriate for Σ (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, d = log 1.25 = 0.097

x_{100} = log 0.86 = -0.065

x_o = log 0.28

n = 4

$t_{0.05}$ = 2.20 for 4+4+4-1 = 11 degrees of freedom.

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

$= 0.065 \pm 0.243$

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

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

$= 0.00196.$

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 V

Other tests

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

Defibrinating activity : 1. Rats are injected intravenously with 1.0 ml of venom solution. After three hours blood is collected by decapitation, citrated, centrifuged and fibrinogen assayed in the plasma according to the method of Blombäck & Blombäck (Ark. Kemi., 10, 415, 1956). The defibrinating unit is defined as the amount of venom necessary to decrease the fibrinogen level to 10% of the normal value.

2. A more simple and cheaper method is to inject mice intravenously with 0.1 ml of venom solution. After one hour venous blood is taken from the tail. The Minimum Defribrinating Dose is the least amount of venom producing non-clotting blood (Reid, H.A. in Animal Toxins, ed. F.E. Russell and P.R. Saunders, p.323. Pergamon Press, Oxford 1967). This method is less sensitive, however, than the first one. It is a good screening test, but less suitable for unit assessment.

Haemorrhagic activity : Haemorrhagic activity is assayed in rabbits, according to Kondo et al..(Jap. J. Med. Sci. Biol., 13, 43, 1960)

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

It is important to reach agreement 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 agreed to coordinate the activities for the different tests.

Test	Coordinator
LD_{50}	Latifi
Defibrinating	Kornalik
Necrotizing	Kornalik
Haemorrhagic	Ohsaka

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 four to six months.

(3) Preparation of the international reference reagents by a WHO laboratory.

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

ANNEX VI

TABLE OF POISONOUS ANIMALS AND AVAILABLE ANTIVENOMS

1. 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, U.S.A.	Crotalus durissus terrificus Bothrops atrox asper Crotalus adamanteus Crotalus atrox asper	Antivenin (Crotalidae) Polyvalent	South American rattlesnake Barba Amarilla Eastern diamondback rattlesnake Western diamondback rattlesnake	Crotalus sp. Sistrurus sp. Agkistrodon sp. (Old & New World) Bothrops sp. Lachesis sp. Trimeresurus sp.	Precipitated with ammonium sulphate, 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 sulphate, and lyophilized.
	Crotalus atrox asper Crotalus d. terrificus Crotalus tigris	Polyvalent Crotalus	Western diamondback rattlesnake South American rattlesnake Tiger rattlesnake	All Mexican crotalids	
	Bothrops atrox Crotalus d. terrificus Crotalus tigris Crotalus atrox	Polyvalent Mexico	Barba Amarilla South American rattlesnake Tiger rattlesnake Western diamondback rattlesnake	All Mexican crotalids	
Instituto Nacional de Higiene, Csda. M. Escobedo No. 20, Mexico City, D.F., Mexico	Bothrops atrox asper	Anti-Bothrops	Barba Amarilla		Pepsin digestion, and ammonium sulphate precipitation. (No recent confirmation).
	Crotalus b. basiliscus Crotalus d. terrificus	Anti-Crotalus	Mexican rattlesnake South American rattlesnake		
	Bothrops atrox Crotalus b. basiliscus Crotalus d. terrificus	Polyvalent	Barba Amarilla Mexican rattlesnake South American rattlesnake		
CENTRAL AND SOUTH AMERICA					
University de Costa Rica Ciudad Universitaria Rodrigo Facio San José, Costa Rica	Lachesis muta stenophrys	Anti-Laquesico	Bushmaster	Lachesis muta muta Lachesis muta noctivaga	Precipitated with ammonium sulfate. Freeze-dried or liquid.
	Bothrops atrox asper Crotalus durissus durissus Lachesis mutus stenophrys	Polyvalent	Terciopelo Central American rattlesnake Bushmaster	Lachesis muta muta Lachesis muta noctivaga Agkistrodon bilineatus Bothrops nummifer Bothrops picadoi Bothrops nasutus Bothrops ophryomegas Bothrops godmani Bothrops lateralis Bothrops schlegeli Bothrops nigroviridis	
	Micrurus nigrocinctus nigrocinctus Micrurus nigrocinctus mosquitensis	Anti-Coral (Central America)		Micrurus carinicaudus dumerili Micrurus fulvius fulvius	
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 Bothrops, Brazilian sp. Lachesis mutus	Bothrops polyvalent	Barba Amarilla		Purified and lyophilized

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	Micrurus nigrocinctus Micrurus mipartitus Micrurus frontalis	Anti-Coral Polyvalent	Giant Coral snake (Cobra coral snake)	Micrurus fulvius fulvius Micrurus alleni Micrurus carinicaudus Micrurus spixi Micrurus lemniscatus Micrurus corallinus	
Instituto Nacional de Salud Ave. Eldorado con Carrera, Zona G, Bogota, D.E., Colombia	Bothrops atrox asper Crotalus d. terrificus	Antiophidico Polivalente	Barba amarilla South American rattlesnake	Bothrops species Crotalus species	Globulin precipitated with ammonium sulphate
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 Bothrops venezuelae		Barba Amarilla Tigra-mariposa	Bothrops colombiensis	
	Bothrops atrox asper Bothrops venezuelae Crotalus d. terrificus		Barba Amarilla Tigra-mariposa South American rattlesnake or cascabel	Bothrops colombiensis Bothrops bilineata Bothrops lansbergi Bothrops lichenosus Bothrops medusa Bothrops neglectus Bothrops schlegeli Crotalus vegrandis	
Instituto Nacional de Microbiología Avdo. Velez Sarsfield 563, Buenos Aires, Argentina	Crotalus d. terrificus		South American rattlesnake or cascabel		Purified by enzamptic and differential thermocoagulation techniques. (No recent confirmation).
	Bothrops alternatus Bothrops neuwiedi	Bothrops Bi-Valent	Yarara or de la Cruz Wied's lance-head, Yarara Chica or painted jararaca		
	Bothrops alternatus Bothrops jararaca Bothrops jararacussu Bothrops neuwiedi Crotalus d. terrificus	Tropical Polyvalent	Yarara or de la Cruz Jararaca Yarara Wied's lance-head South American rattlesnake or cascabel		
	Bothrops alternatus Bothrops neuwiedi Crotalus d. terrificus	Tropical Tri-Valent	Yarara or de la Cruz Wied's lance-head South American rattlesnake or cascabel		
Instituto Butantan Caixa Postal 65, São Paulo, Brazil	Crotalus d. terrificus	Anticrotalic	South American rattlesnake or cascabel	It can be expected that the antivenins of this Institute neutralize other crotalid venoms, even though the producers note in a personal letter that the scarcity of data preclude any specific claims	Pepsin digested and ammonium sulfate precipitation.
	Lachesis mutus	Antilaquetico	Bushmaster or Surucucu		

	Bothrops jararaca	Antibothropico	Jararaca		
	Bothrops moojeni		Moojen's pit viper		
	Bothrops cotiara		Cotiara		
	Bothrops alternatus		Urutu		
	Bothrops jararacussu		Jararacussu		
	Bothrops neuwiedi		Wied's lance-head or painted jararaca		
	Crotalus d. terrificus	Antiophidico Polyvalent	South American rattlesnake		
	Bothrops jararaca		Jararaca		
	Bothrops moojeni		Moojen's pit-viper		
	Bothrops cotiara		Cotiara		
	Bothrops alternatus		Urutu		
	Bothrops jararacussu		Jararacussu		
	Bothrops neuwiedi		Wied's lance-head or painted jararaca		
	Lachesis muta	Antibothropico-lachetico	Bushmaster		
	Bothrops alternatus		Urutu		
	Bothrops jararacussu		Jararacussu		
	Bothrops jararaca		Jararaca		
	Bothrops moojeni		Moojen's pit viper		
	Bothrops cotiara		Cotiara		
	Bothrops neuwiedi		Wied's lance-head or painted jararaca		
	Micrurus frontalis	Antielapidico	Giant coral snake or		
	Micrurus corallinus		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 sulphate precipitation Final solution contains 18% protein.
	Bothrops alternatus		Urutu		
	Bothrops atrox asper		Barba Amarilla		
	Bothrops jararaca		Jararaca		
	Bothrops jararacussu		Jararacussu		
	Bothrops cotiara		Cotiara		
EUROPE					
Institut Pasteur Annexe de Garches 92 (Hauts-de-Seine), Paris, France	Vipera aspis	Ipser V	Jura viper	*Paraspecific	Concentrated and purified to 12 to 13% protein.
	Vipera berus		European viper		
	Vipera ammodytes	Ipser Europe	Long-nosed viper		
	Vipera aspis		Jura viper		
	Vipera berus		European viper		
	Bitis arietans	Bitis-Echis-Naja	Puff adder		
	Bitis gabonica		Gaboon viper		
	Bitis nasicornis*		Rhinoceros viper		
	Echis carinatus		Saw-scaled viper		
	Hemachatus haemachatus*		Ringhals		
	Naja haje		Egyptian cobra		
	Naja melanoleuca		Forest cobra		
	Naja nigricollis		Spitting cobra		
	Naja nivea*		Cape cobra		

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	Vipera ammodytes Vipera lebetina obtusa Vipera xanthina palestinae Cerastes cornutus Cerastes vipera Echis carinatus Naja naja Naja haje	Near and Middle East	Long-nosed viper Levantine viper Palestine viper Horned viper Avicenna's viper Saw-scaled viper Indian cobra Egyptian cobra		
	Naja naja kaouthia	Cobra	Yellow cobra		
	Dendroaspis angusticeps* Dendroaspis jamesoni Dendroaspis polylepis* Dendroaspis viridis	Dendroaspis	Eastern green mamba Jameson's mamba Black mamba Western green mamba		
Behringwerke AG D 3550 Marburg/Lahn Federal Republic of Germany	Vipera ammodytes Vipera berus	Europe		Vipera ammodytes Vipera aspis Vipera berus Vipera libetina Vipera xanthina	Prepared by pepsin digestion, and ammonium sulphate precipitation. Final solution contains 16% protein.
	Bitis gabonica Echis carinatus Naja haje Vipera lebetina	North Africa		Cerastes cerastes Cerastes vipera Bitis arietans Bitis gabonica Echis carinatus Naja haje Naja melanoleuca Naja nigricollis Vipera lebetina	
	Bitis gabonica Dendroaspis polylepis Naja haje	Central Africa		Bitis arietans Bitis gabonica Bitis nasicornis Dendroaspis polylepis Dendroaspis viridis Hemachatus haemachatus Naja haje Naja melanoleuca Naja nigricollis	
	Echis carinatus Naja haje Vipera ammodytes Vipera lebetina	Near and Middle East		Cerastes cerastes Echis carinatus Naja haje Vipera ammodytes Vipera lebetina Vipera xanthina Cerastes cornutus	
Istituto Sieroterapico e Vaccinogeno Toscano "Sclavo", Via Fiorentina 1, Siena, Italy	Vipera ammodytes Vipera aspis Vipera berus Vipera ursinii	Antiviperin	Long-nosed viper Jura viper European viper Ursini's viper	All European vipers	Enzyme refined and supplied in liquid form.
Insitute for Sera and Vaccines W. Pieck Str., Prague, CSSR	Vipera ammodytes Vipera berus	Venise	European viper Long-nosed viper		Digested with pepsin, precipitated with ammonium sulfate. Supplied in liquid form.

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Institute of Immunology Rockefellerova 2 Zagreb, Yugoslavia	Vipera ammodytes	Antiviperinum	Long-nosed viper	Vipera berus Vipera aspis	Solution digested with pepsin, and precipitated with ammonium sulphate.
Institute of Epidemiology and Microbiology Sofia, Bulgaria	Vipera ammodytes		Long-nosed viper	Vipera berus Vipera aspis	Ammonium sulphate precipitation.
Research Institute of Vaccine and Serum Ministry of Public Health Ul. Kafanova 93 Tashkent, U.S.S.R	Echis carinatus	Monovalent Echis carinatus	Saw-scaled viper		No confirmation or recent letter indicating product or processing
	Naja naja	Monovalent Naja naja	Indian cobra		
	Vipera lebetina	Monovalent Vipera lebetina	Levantine viper		
	Echis carinatus Naja naja	Polyvalent Naja and Echis	Saw-scaled viper Indian cobra		
	Naja naja Vipera lebetina	Polyvalent vipera and Naja	Indian cobra Levantine viper		
AFRICA					
Institut Pasteur d'Algerie	Cerastes cerastes Vipera lebetina	Antiviperin	Horned viper Levantine viper		Solution digested with pepsin and precipitated with ammonium sulphate.
The South African Institute for Medical Research P.O. Box 1038 Johannesburg 2000, Republic of South Africa	Hemachatus haemachatus Naja nivea Naja haje Naja melanoleuca Naja nigricollis Dendroaspis angusticeps Dendroaspis jamesoni Dendroaspis polylepis Bitis arietans Bitis gabonica	Polyvalent	Ringhals Cape cobra Egyptian cobra Forest cobra Spitting cobra Eastern green mamba Jameson's mamba Black mamba Puff adder Gaboon viper	Naja naja Ophiophagus hannah Pseudohaje goldi Walterinnesia egyptia Dendroaspis viridis	Digested with pepsin and precipitated with ammonium sulphate
	Echis carinatus	Echis	Saw-scaled viper	Echis coloratus Cerastes cerastes Cerastes vipera	Pepsin refined globulin preparation in liquid form.
	Dispholidus typus	Boomslang antivenom	Boomslang		Pepsin refined globulin preparation is freeze-dried form.

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ASIA					
Central Research Institute Kasauli, (Simla Hills), (H.P.) India	*Bungarus caeruleus* *Naja naja* *Vipera russelli* *Echis carinatus*	Polyvalent	Indian krait Indian cobra Russell's viper Saw-scaled viper	*Bungarus fasciatus* *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 russelli	Vipera	Russell's viper	*Naja naja oxiane*	
	Echis carinatus	Echis	Saw-scaled viper	*Ophiophagus hannah*	
	Bungarus caeruleus *Naja naja* *Echis carinatus* *Vipera russelli*	Polyvalent	Indian krait Indian cobra Saw-scaled viper Russell's viper	*Trimeresurus gramineus* *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		
Institut d'Etat des Serum et Vaccins Razi P.O. Box 656 Teheran, Iran	*Naja naja oxiana*		Oxus cobra		Prepared by pepsin digestion, and ammonium sulphate 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 *Vipera lebetina* *Vipera xanthina* *Echis carinatus* *Pseudocerastes persicus* *Agkistrodon halys*	Polyvalent	Oxus cobra Levantine viper Near East viper Saw-scaled viper Persian horned viper Mamushi	*Cerastes cerastes* *Eristicophis macmahoni* *Vipera aspis* *Vipera cerastes* *Vipera latasti* *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		

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The Chemo-Sero-Therapeutic Research Institute, Kumamoto 860, Kyushu, Japan	Trimeresurus flavoviridis Agkistrodon halys	Habu antivenine Mamushi antivenine	Habu Mamushi	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		
Serum and Vaccine Laboratories, Alabang, Mutinlupa, Rizal, Philippines	Naja naja philippinensis	Cobra	Philippine cobra		Concentrated and purified.
National Institute of Preventive Medicine 161 Kun-Yang St., Nan-Kang, Taipei, Taiwan	Agkistrodon acutus	Agkistrodon	Long-nosed pit viper	Trimeresurus mucrosquamatus	Immunized with formalin-toxoid venom. Venom ammonium sulphate precipitated, and supplied in liquid or lyophilized form.
	Bungarus multicinctus	Bungarus	Many banded krait		
	Naja naja atra	Naja	Chinese cobra		
	Trimeresurus stejnegeri Trimeresurus mucrosquamatus }	Trimeresurus	Bamboo viper Chinese habu	Agkistrodon acutus	
	Bungarus multicinctus Naja naja atra }	Naja-Bungarus	Many-banded krait Chinese cobra		
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 cobra		
	Vipera russelli	Russell's viper	Russell's viper		
	Agkistrodon rhodostoma	Malayan pit viper	Malayan pit viper		
	Timeresurus albolabris }	Green tree viper			
	Timeresurus erythrurus }	Green tree viper			
Industrial and Pharmaceutical Corporation, Rangoon, Burma	Naja naja kaouthia	Siamese cobra			Precipitated with ammonium sulfate and lyophilized.
	Vipera russelli siamensis	Russell's viper			
	Naja naja kaouthia Vipera russelli 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		

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AUSTRALIA					
Commonwealth Serum Laboratories** 45, Poplar Road, Parkville, Victoria 3052 Australia	Acanthophis antarcticus	Death adder	Common death adder	Acanthophis pyrrhus	Prepared by pepsin digestion, and ammonium sulphate precipitation. The products are dialyzed and ultra-filtered to a final concentration of 17% protein.
	Notechis scutatus Enhydrina schistosoa	Tiger-sea snake	Mainland tiger snake Beaked sea snake	Austrelaps superba Pseudechis porphyriacus Tropidechis carinatus Laboratory experiments indicate that antivenin neutralizes at least 12 different sea snake anti-venins.	
	Oxyuranus scutellatus	Taipan	Taipan	Parademansia microlepidota	
	Pseudonaja textilis	Eastern brown snake	Eastern brown snake	Pseudonaja affinis Pseudonaja nuchalis	
	Pseudechis australis	Brown snake	King brown or Mulga snake	Pseudechis australis Pseudechis porphyriacus	
	Oxyuranus scutellatus Acanthophis antarcticus Notechis scutatus Pseudechis australis Pseudonaja textilis	Polyvalent (Australia-New Guinea)	Taipan Death adder Tiger snake King brown snake Eastern brown snake	Austrelaps superba Pseudechis porphyriacus Pseudonaja affinis Pseudonaja nuchalis Pseudechis papuanus Parademansia microlepidota	

* Additional venoms which 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.

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 United States of America	Latrodectus mactans	Black widow	Black widow (spider)		
Instituto Nacional de Higiene Asda. M. Escobedo No. 20 Mexico City, D.F., Mexico	Centruroides noxius	Antialacrás polyvalent			
Laboratorio Zapata Mexico City, Mexico	Centruroides suffusus Centruroides noxius	Antialacrás polyvalent			
Laboratorios "MYN" Av. Coyoacan 1707 Mexico City, 12, D.F., Mexico	Centruroides suffusus Centruroides noxius or C. limidus	Antialacrás polyvalent			
Institutio Nacional de Higiene Lima, Peru	Loxosceles sp.	Anti-Loxoscelico			Ammonium sulfate precipitation. Supplied as liquid.
Instituto Butantan CP 65 05504 Sao Paolo, Brazil	Phoneutria Loxosceles Lycosa	Antiarachnidico polivalente			
Institute of Immunology Rockerfellerova, 2 Zagreb, Yugoslavia	Scorpaena porcus	Scorpion fish antivenom	Scorpion fish		
Institut d'Etat des serum et Vaccins RAZI P.O. Box 656 Teheran, Iran	Androctonus crassicauda Buthotus saulcyi Hemiscorpius lepturus Mesobuthus eupeus Odontobuthus doriae Scorpio maurus	Polyvalent scorpion serum	Scorpions		Ammonium sulfate precipitation. Supplied in liquid form.
Commonwealth Serum Laboratories, Parkville Victoria, Australia	Latrodectes 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		
Institut Pasteur d'Algérie rue Docteur Laveran Algiers	Androctonus australis	Scorpion antivenom			
Lister Institute of Preventive Medicine Elstree, Herts WD6 3AX United Kingdom	Androctonus australis Buthus occitanus Leiurus quinquestriatus	Scorpion			
South African Institute for Medical Research Hospital Street Johannesburg South Africa	Latrodectus (blackwidow) Parabuthus	Scorpion			

ANNEX VII

PROCESSING AND STANDARDIZATION OF ANTIVENOMS

It was recommended that the proposed international standard antivenoms should be produced using the proposed international reference venoms starting with horses that had not been used for any other antibody production.

The following laboratories tentatively agreed to produce the antivenoms:

Laboratory	Antivenom
Commonwealth Serum Laboratories	Naja naja Notechis scutatus
Inst. Clodomiro Picado	Bothrops atrox/asper (Atlantic)
Inst. of Hygiene, Mexico	Crotalus adamanteus
Razi Institute	Echis carinatus (Nigerian and Iranian) Vipera russelli
The Chemo-Sero-Therapeutic Research Institute, Kumamoto	Trimeresurus flavoviridis

It was agreed that the antivenom should be an almost pure $F(ab)_2$ plasma fraction. The volume of fill will be 5 ml of a 10% protein solution in a 20 ml vial. The 5 ml will be isotonic and contain 2% glycine at pH 6.4 to 6.8.

Tests on final freeze-dried product

The freeze-dried antivenoms will 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 ultra-centrifugation.

(6) stability by accelerated degradation studies.

The details of these tests should be agreed between the laboratories producing the antivenoms. WHO will 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 VII

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 V).

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 VIII

PURIFICATION AND CONCENTRATION OF ANTIVENOMS

Monospecific plasma

Neutralization with specific venom to be recorded to have a check on concentration achieved

Digestion

Diluted 1/3 with tap water,
NaCl 0.8%; Temperature 30°C;
pH: 3.2 with HCl: 10%;
Pepsin 0.5%; Time 30 minutes;
pH: 4.2 with normal NaOH;
Toluene 0.1%; $SO_4(NH_4)_2$ 14%

Thermocoagulation

Temperature 56°C; heated 1 hour

↓

Filtration

↓ → Precipitate discarded

Supernatant

pH: 7.2 with normal NaOH;
$(NH_4)_2 SO_4$ 20% T to 34%;

↓ → Supernatant discarded

Precipitate

Dialyzed for 48 hours; Phenol 0.5%;
gel treatment 40% v/v;
temperature 50°C; heated 1 hour;

↓

Filtration

↓ → Precipitate discarded

Supernatant

Saturated $(NH_4)_2 SO_4$ 55%

↓

Filtration

↓ → Supernatant discarded

Precipitate

Pressed; dialyzed; isotonized and
phenol 0.35% added.

The final product will be 10% protein solution and contain 2 % glycine at pH 6.4 to 6.8.

Annex VIII

The plasmas purified and concentrated by this method have the following properties:

SOME PROPERTIES OF THE ANTIVENINS

Tests	Mean ± S.D.
pH	6.4 ± 0.3 (20)
Phenol (g/litre)	2.4 ± 0.6 (20)
Protein N (mg/ml)	18.3 ± 2.9 (20)
Total protein (g%)	11.4 ± 1.7 (20)
Total solid (g%)	15.8 ± 1.8 (20)

Mean and standard deviation (S.D.) are given.
Number of experiments indicated in parentheses.

The Group agreed that for the purposes of the proposed international monospecific antivenom standards this procedure would be adopted or a similar one, ending up with a comparable high activity product.

ANNEX IX

STORAGE, ESPECIALLY IN THE TROPICS

Although no direct data have been available 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 Jerne & Perry, 1956) and the results are summarized in the following Table 1. From these data we may conclude that the potency of an antivenom held in the liquid state will be maintained for at least one 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.

We can conclude that it would be sensible for antivenoms that are to be used in the tropics, to be lyophilized rather than being distributed in the liquid state.

Reference

Jerne, N. K. & Perry, W. L. M. (1956) The stability of biological standards, Bull. Wld Hlth Org., 14, 167-182

TABLE 1. THE 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 (Unpublished data from National Institute of Health, Japan)

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

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

Annex IX

TABLE 2. BATCH-TO-BATCH VARIATION OF POLYSPECIFIC ANTIVENOMS
(Data from Dr M. Latifi)

Antivenoms	pH	Phenol g%	Protein N mg/ml	T. protein g%	T. solid g%
Batch No. 94	6.8	2.7	10.7	6.7	10.3
Batch No. 79	6.4	2.7	13.5	8.4	12.0
Batch No. 66	6.3	2.4	22.8	14.2	16.2
Batch No. 56	6.6	2.5	19.1	11.9	16.0
Batch No. 46	6.8	2.6	21.6	13.5	16.6

For stability see Table 3.

Annex IX

TABLE 3. POTENCIES OF POLYSPECIFIC ANTIVENOMS RELATED TO EXPIRY DATE

Venoms	Neutralized mg/ml									
	Batch No. 94 Exp. Aug. 79		Batch No. 79 Exp. Jun. 77		Batch No. 66 Exp. Apr. 75		Batch No. 56 Exp. Mar. 73		Batch No. 46 Exp. Oct. 71	
	Initial titre	2 Y.	Initial titre	4 Y.	Initial titre	6 Y.	Initial titre	8 Y.	Initial titre	10 Y.
Naja naja oxiana LD_{50} = 8.3 ug	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 ug	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 ug	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 ug	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 ug	0.4	0.4	0.6	0.6	1.0	0.8	1.0	0.8	0.8	0.6

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

ANNEX X

ANTIVENOM REACTIONS IN NORTH-WEST MALAYA

Antivenom[1]			No. of patients	Reactions		
Type	Dose (ml)	Route[2]		Anaphy-lactoid	Early mild	Delayed[3]
Haffkine 1954-5 polyvalent	10-20	IVI	16	5	1	1
Haffkine 1958-9 polyvalent	10	IMI	17	-	1	1 (+1)
	20	IMI	11	-	-	1
	50	IMI	51	-	1	4
Bangkok 1958-9 *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	-

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

[2] 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

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

ANNEX XI

ACTIVE IMMUNIZATION

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

Years	Name of venoid	Number of inoculants	Investigators
1971-1972	APF[a]	1 993	Sawai et al.[c]
1970-1978	Mixed[b]	55 845	Murata et al.[c]

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

[b] Mixed is the HR1 and HR2 haemorrhagic factors toxoided and mixed.

[c] Committee for Research on Habu Toxoid (Chairman: Dr R. Murata).

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

Years	Mixed venoid	APF venoid[a]
1970	93 (59, 54)	
1971	1 349 (1 134, 572)	97 (76, 41)
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 second, third, fourth, fifth and sixth injection are indicated in parentheses.

[a] See note under Table 1.

Annex XI

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
P +	8		2	2		1	16	2	9	3		1
P ++	1						7	2	1			
P +++	2						2					
S (-)	43	37	18	50	42	29	33	25	15	45	28	11
S +	3	3	3	1	3		11	10	4	4	3	2
S ++	2						4	2	2			
S +++			1									
S ++++		1						1				

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

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