

Production and Use of Snake Antivenin

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I. INTRODUCTION

Antivenin was first prepared in 1894, and was the result of several investigations carried out simultaneously in different parts of the world. The successfully scientific immunization of an animal by repeated injections of animal venom was first reported by Fornara in 1877. He successfully protected a dog after several inoculations of small amounts of toad skin secretions. Later in Michigan, Sewall described a similar experiment in 1887. He protected pigeons against the equivalent of six lethal doses (LD) of rattlesnake venom after treating them with gradually increasing doses of venom. Subsequently, in France in 1892, Kaufmann reproduced these experiments on a dog inoculated with *Vipera*

aspis venom. It was demonstrated that animals can be protected against a toxic substance after inoculation of several sublethal doses of the same substance. The development of diphtheria and tetanus antitoxins by Behring and Kitasato in early 1890s was based upon a similar principle. But they showed that protection could be transmitted from one animal to another that had never before received the substance. The way to antitoxin or antivenin therapy was opened.

Discovery of antivenin was claimed the same day (February 10, 1894) by the team of Phisalix and that of Calmette (Brygoo, 1985; Calmette, 1884; Phisalix and Bertrand, 1884). Calmette in Paris, Fraser in Edinburgh, Brazil in São Paulo, and McFarland in Philadelphia began preparation of antivenin against various species of venomous snakes. The main difficulties in the preparation and use of antivenin appeared soon after the beginning of serotherapy. Some are still not completely resolved:

- Inactivation of venom before inoculation of the animal
- Purification of antivenin
- Evaluation of antivenin potency
- Adverse reactions to antivenin

Today, in addition to the epidemiological, biochemical, and immunological considerations, one must add the commercial and economic points of view. Serotherapy is currently the only specific treatment of snake envenomation; however these accidents often occur in regions where antivenin, if available, is difficult to administer and expensive in relation to the way of life.

II. ANTIVENIN PRODUCTION

Since the discovery of antivenin by Calmette in 1894, the method for preparation has changed little. The basic principle is to immunize an animal with venom for production of specific antibodies that will be able to neutralize this venom. Time is leading antivenin producers to modernize the techniques.

A. Sources of Venom

Venom is milked from the snake by mechanical pressure on the venom gland or by electrical stimulation of striated muscles surrounding the gland. A direct electric current of 6-12 V is applied to the mucous membrane of the mouth. The venom is collected and then dried or lyophilized. The quantity of venom obtained by both methods is similar, and only the handling or care of the snakes leads to the choice of the latter, which is safer for both human and snake. At first the snake was decapitated before milking; however, this procedure is no longer used, and snakes are kept alive for several years.

The yield of venom from an individual snake decreases after the first milkings if they are too frequent. We observed that a four-week interval between milking was enough to maintain the average yield of venom from individual snakes. Until recently, the only problem was to supply enough venom for antivenin production; however, within the past decade, the quality of the venom has become a real question. Numerous venom samples appear to be out of

quality or erroneously labeled. Geographic variation in venom has been recently suggested and has now been demonstrated. Therefore, the specificity and efficacy of antivenin can be discussed for a wide range of venomous snakes, the venom of which may differ greatly from place to place. Other causes of venom variations, such as age, season, and sex, are questionable and are probably not as important as genetic diversity (Chippaux et al., 1982). It has been suggested that the venom used for antivenin production should be milked from a large pool of snakes of different origins (Boche et al., 1981). This important question led the World Health Organization (WHO) to create an International Reference Venom repository, which constitutes major reference reagents. Actually, eight snake venoms of major medical importance were chosen as International Reference Venoms (Theakston, 1986).

B. Immunization

1. Animals Used

The horse is the animal of choice. The amount of serum obtained in one bleeding is larger than that from any other easy-to-breed and to handle animal. Donkeys, mules, cows, or oxen, can be used, for similar reasons, but oddly, very few producers use them. Goats, sheep, or rabbits are seldom used, but this is not usual. The reasons for choosing animals other than horses can include difficulties of breeding in tropical areas in which cattle trypanosomes occur, or the expense of breeding when production is restricted, but the most important reason is for provision of a valuable antivenin for persons who are allergic to horse serum.

2. Inoculation

Although inoculation of unmodified venom elicits a better immunization, the use of pure venom, which produces severe clinical reactions in the animal, is not recommended. Since 1894, numerous methods of venom preparations have been proposed that reduce toxicity and preserve antigenic properties. Detoxification has been obtained with various processes: heat-treatment, hypochlorite, soap, mixtures with lipoids, hydrogen peroxide, bile, and others. However, none of these were efficient or of any value. Complexation of venom with formalin, which constitutes the anavenin (Ramon, 1924), is the most interesting method and is still in use today, as are other aldehydes such as glutaraldehyde. Tannin has also been used (Okonogi et al., 1979). Formalin or tannin can be removed from anavenin by dialysis. It is possible to detoxify venom by action of ionizing radiation.

Adjuvant can be mixed into toxoid before inoculation of the animal. This is particularly interesting for booster doses. Christensen (1955) proposed a mixture of venom added with a 2% solution of bentonite, which adsorbs the venom. The effect of adjuvant is to hold venom in tissues and to slowly liberate antigens. Thus, it decreases the acute toxicity of the venom and enhances the immunogenicity of its antigens.

The immunization protocol depends on the

Toxicity and immunogenicity of the venom

Animal species used for immunization

Health of the animal and its immunological response

Ten to 50 injections during 3-15 months can be necessary to obtain a valid immunization of the animal. The titer of immunized animal serum is tested, and if a correct value is reached, the animal is bled into an anticoagulant (usually sodium citrate) before treatment for purification, preservation, and commercialization.

New et al. (1984) proposed the use of liposomes instead of adjuvant. Venom is incorporated into a sphingomyelin and cholesterol emulsion in water, then treated with osmium tetroxide, which interacts with proteins and stabilizes liposomal membranes (New et al., 1985). These authors report an immunological response by animals after a single oral administration of liposomes.

Monovalent antivenin is prepared with venom from an unique species, or pooled venoms from very close species belonging to the same genus. Polyvalent antivenin is produced with venom from various species, genera, or families. Species to be pooled can be chosen for geographic reasons or for immunological purposes. Antigenic properties of close venoms can enhance the immunization of animals. This booster effect of mixed, related antigens immunizes the same animal with several distinct venoms for production of a polyvalent antivenin. This method gives better results, rather than mixing several monovalent antivenins.

C. Purification and Standardization

1. Purification

The purpose of purification is to concentrate and purify the antibody activity of the immunized animal blood. First, cells must be removed from the plasma by centrifugation. Then nonimmunological proteins are removed from the plasma (especially albumin). Several methods are used. Ammonium sulfate fractionation uses the ability of ammonium sulfate to precipitate globulins, rather than albumin. Pepsin digestion splits γ -globulins in two fragments, one is heat-stable and carries the antibody activity (Fab); the other is heat-labile and has no direct immunological action (Fc). Although this sophisticated method does not lead to a better antibody concentration in the final product, the hazards of adverse reaction to antivenin are reduced. Hence, development of such methods is encouraged by WHO. In practice, it consists of adding pepsin to a solution of plasma adjusted to pH 3.25 at 20°C for 30 min. The precipitation is completed by addition of 15% ammonium sulfate. Unwanted proteins can be coagulated by heat (55°C for 10 min or more). Then the plasma is quickly cooled to 40°C, and the denatured proteins are removed by centrifugation or filtration. The supernatant is treated with ammonium sulfate to precipitate the γ -globulins until the desired concentration is reached. Ammonium sulfate is completely removed from the precipitate by dialysis. Finally, lipids are eliminated by aluminum hydroxide gel agglutination. Modified methods may extend the digestion incubation time or shorten the heat treatment or they may change the pH or the temperature of mixtures. The final Fab concentration obtained is about three times more than the initial concentration.

Enzyme purification can be accomplished with various enzymes (papaine, trypsin, or plasmin), that split immunoglobulins into similar fragments that are shorter than those achieved with pepsin. Enzymes other than pepsin are used in laboratories for experimental purposes, but they are not employed on

an industrial scale. Filtration procedures have been improved with use of molecular screening on resins or gels such as Sephadex. The bulk is checked for bacteriological sterility by cultivation on a proper medium, for safety by inoculation of laboratory animals, for immunological specificity by immunoelectrophoresis or immunodiffusion, and for potency, before final conditioning. National and international regulations are explicit about sterility and safety control tests. But procedures for potency verification have not yet been determined.

2. Immunological Control

The immunological composition of an antivenin can be checked by immunoelectrophoresis against the venom used for immunization or against another venom. Venom is submitted to an electrophoresis that separates its components, which then diffused against the antivenom through a gel. Each component is recognized by its specific antibody, the combination precipitates into the gel, and is seen as a line proportionate to the amount of each corresponding constituent. In this manner, it is possible to compare several samples of antivenin prepared against the same venom. It is theoretically possible to measure the quantity of antibody contained in the antivenin. If tested against various venoms, it is possible to find cross-reactions between venoms from different sources and even between venoms belonging to different snake species. This technique is interesting for screening purposes or to choose the correct venom for animal immunization; however, the antibody precipitation sites may be separate from those of neutralization (Menez et al., 1984), consequently, immunological control and potency tests could be unreliable.

3. Standardization

Standardization of antivenin is concerned with the potency or the efficacy of the product and the conservation of these qualities. Snake venoms induce various clinical disorders, such as local necrosis, hemolysis, hemorrhage, and paralysis. For each of these problems, the etiology depends on several factors. Antivenin must neutralize all toxic substances identified in the venom used for immunization. Potency assays, as far as they are significant, can be done *in vitro* or *in vivo*. Preservation of the potency depends mainly on the formulation (e.g., liquid, dried, or lyophilized in glass-sealed vials or in rubber-corked vials).

In vitro potency assays measure the decrease of specific activity of a given amount of venom mixed with increased amounts of antivenin. Several tests recently described are listed by Theakston (1986) and Gutierrez et al. (1987). Hemolytic activity of venom is tested on human, sheep, or rabbit erythrocytes, with or without the presence of lipid. The neutralization of hemolytic activity seems to correlate with the neutralization of lethal activity in mice (Gutierrez et al., 1988). Fibrinolytic activity is measured on sheep or bovine fibrin plates obtained after coagulation of fresh plasma. Proteolytic enzyme activity of venom is checked on various substrates (casein, collagen, artificial polypeptides) after *in vitro* incubation at a suitable temperature. A specific activity unit is defined (for example, the minimum quantity of venom producing 70% hemolysis) and the amount of antivenin able to neutralize 50% of the specific activity of 1 unit of venom is generally considered as the minimum potency

dose of the antivenin. The main problem is to define a reproducible method for measurement.

In vivo potency assays are carried out on laboratory animals (rabbits, rats, or mice) and measure the decrease in a biological activity or lethality of venom mixed with antivenin. The minimum dose of venom killing 50% of inoculated animals (LD_{50}) is calculated by injection of increasing amounts of venom in a given number of mice. After 24 hr of observation, the percentage of dead mice is reported on a probit scale, and the corresponding value of venom injected is reported on a logarithmic scale. Generally, a constant amount of antivenin is mixed with increasing quantities of venom, and after a 30-min incubation at 37°C, the mixture is injected into mice for calculation of the new amount of venom that kills 50% of the mice. Potency of antivenin is expressed by the quantity of antivenin able to neutralize a given number of LD_{50} or a given weight of dry venom. Different methods can be used by laboratories. For example, a constant amount of venom can be challenged by increasing amounts of antivenin. Expression of results can be different too, and the potency is expressed by neutralization of a given number of LD_{90} or LD_{99} . This potency assay is reliable for antivenin prepared against neurotoxins (elapid and scorpion venoms). The assay is questionable for antivenin prepared against enzymes (viper and pit-viper venoms). First, an enzyme can be nontoxic, but it can convert a substrate into a toxic component that is not antigenic. Second, a toxic component for a human can be different from the toxic component for a mouse; hence potency assays test the efficacy for the mouse but not for the human. Finally, incubation of venom with antivenin before injection leads to precipitation of antigen-antibody complexes, which cannot be inoculated into the mouse. In a natural condition, antivenin is inoculated later and separately from venom. Thus, toxic manifestations have begun before treatment. Antibody must reach its target and, then, neutralize it. Boulain and Menez (1982) showed that antivenin was able to remove elapid neurotoxin from acetylcholine receptors (the specific target of neurotoxin). For enzymes, this has not been demonstrated, and it is probably not effective. The thrombinlike enzyme of most viperid or pit-viper snakes converts fibrinogen into fibrin, which is not attacked by antivenin. If the delay before treatment is too long, fibrinogen decreases dramatically, and hemorrhages occur. The clinical and therapeutic aspects of this problem will be discussed next.

Development of LD_{50} s, for which increasing amounts of venom challenge increasing amounts of antivenin, leads to an estimation of the efficacy of antivenin. Three theoretical neutralization curves can be observed (Fig. 1). For each amount of venom, the neutralization curve gives the correct amount of antivenin that is able to neutralize the venom effects. The curve is obtained by calculating the LD_{50} of each dose of venom in the presence of different amounts of antivenin. The first curve (A) represents a good proportional neutralization of venom by antivenin. Here, it is assumed that only one toxic antigen produces clinical effects or, if other toxic components exist in venom, the neutralization by the antivenin is better. The second (B) and the third (C) curves show usual responses if several toxic antigens are mixed in the venom. Reasons for the presence of inflexion in curves B and C are low amounts of a very toxic component that provokes poor antibody production or bad antigenicity of one of the toxic components. Neutralization potency

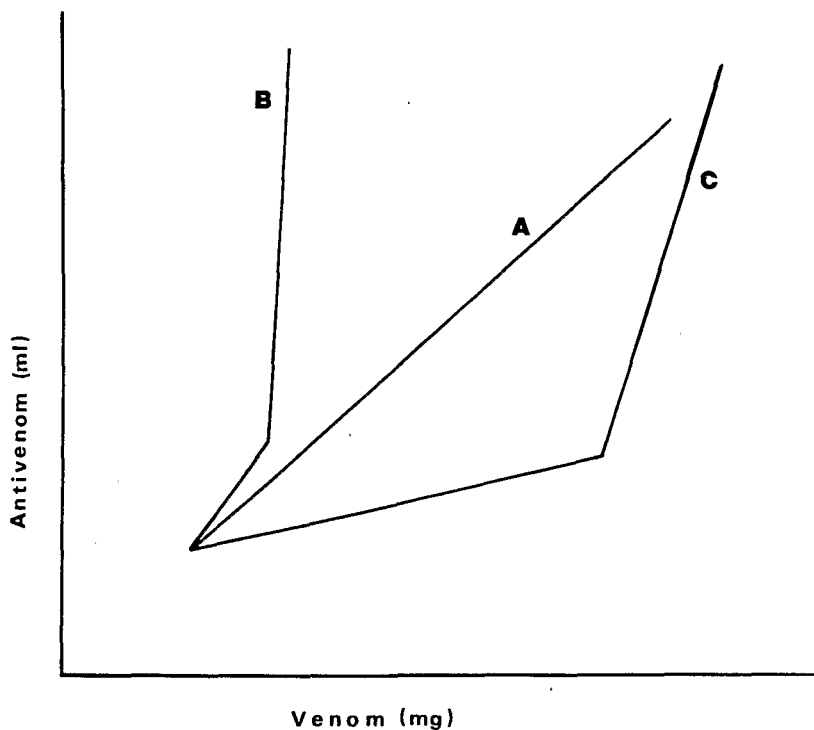


FIGURE 1 Theoretical neutralization curves. Fictitious data: for details see text.

shown by curve B is slight because the toxicity of a nonneutralized component is high. In curve C, neutralization is fairly good as long as the amount or toxicity of minor substances are masked by the principal toxin. However, the neutralization curves assess the potency of antivenin for laboratory animals, which may not be representative of potency of antivenin for humans.

It has been proposed that the main toxic substances should be isolated and each challenged against antivenin (WHO, 1981); but this is also questionable. Viper and pit-viper venom enzymes act synergistically on substrates, and overall toxicity is not the resultant or the summation of proper toxicity of all separate substances, the potential toxicity of which can be drastically decreased. The solution appears to be a combination of *in vivo* and *in vitro* tests depending on the specific properties and toxicities of each venom. Standard antivenin samples would be provided as a reference for further comparisons.

Randomized or controlled clinical field trials of antivenin are not usual for ethical and practical reasons. Protocols should be provided for applying antivenin treatment and for studying the effects on envenomated victims. Supervision of the pharmacologic action of antivenin should be made, with further biological tests, such as coagulation tests; titration of cellular enzymes, in cytotoxicity; and particularly antigens, antibodies, and immune-complex titra-

tion by use of enzyme-linked immunosorbent assay (ELISA). Such surveys are now proposed by producers and researchers (Daudu and Theakston, 1988). Trials can use different antivenins, if available, prepared for local snakes and compare their respective efficacy. Pugh and Theakston (1987) and Daudu and Theakston (1988) showed that antivenin prepared by different producers against venoms belonging to the same species have different clinical and biological efficacies. This is probably because of intraspecies variations of venoms used in antivenin preparation.

Preservation of antivenin is an important problem. Drastic climatic conditions prevail where antivenin is needed. The liquid form of antivenin must be kept at 4°C, which is difficult in tropical areas. The decrease in preservation depends on the holding temperature of the stock, from at least 5 years in a cold area (between 2°C and 8°C) to fewer than a few weeks in a hot area (35-40°C). Dried or lyophilized antivenin lasts longer, but rubber stoppers can become porous and allow moisture or bacterial development within the vials. Thus, most regulations limit the preservation of liquid antivenin to 3 years and that of lyophilized antivenin to 5 years.

III. ANTIVENIN THERAPY

The principle for the use of antivenin as snake envenomation therapy is unquestionable. The proper manner of use should be discussed. Many problems exist, such as the choice of antivenin if several are available, the delay after which antivenin therapy is unsuitable, the administration route, or how to prevent adverse reactions.

A. Indications

The decision for use of antivenin therapy should consider the circumstances of bite, delay time after the bite, identification of the snake, the clinical features, and the medical environment (e.g., availability of a care unit). It has been pointed out that illegitimate bites (i.e., those incurred by persons who own snakes) are more severe than accidental or life-threatening bites, probably because an illegitimate bite occurs when handling the snake. The location of the bite and greater aggressiveness of a snake can explain this observation (Chippaux, 1982a). For illegitimate bite identification of the snake is obvious. So antivenin therapy should be systematic.

For life-threatening bites, the problem is different in developed countries from that in underdeveloped countries (Chippaux, 1982b). Although antivenin is used to avoid the victim's death, in developed countries one of the aims of antivenin treatment is to reduce local complications (Garfin et al., 1985; Homma and Tu, 1970) and the duration of hospitalization (Stahel, 1985). In underdeveloped countries, antivenin therapy is requested to counteract the lethal effect of venom. Other purposes are of less value. Here, delay after the bite can reach several days (at least 5 hr, with an average of 18 hr is not rare), hence, antivenin therapy could be inefficacious for most local complications.

In tropical countries, Chippaux and Bressy (1980) have shown that about 50% of accidental bites are trivial or mild. If after a 3-hr delay, clinical manifestations are only slight, antivenin therapy should not be indicated. Otherwise, clinical complications direct the choice of antivenin, which must be used as soon as possible. Even if a long delay reduces the antivenin efficacy, we again stress the necessity for its use. For instance, in West Africa we observed that hemorrhages after *Echis ocellatus* (formerly *carinatus*) are slow to appear, and death can occur several days after a bite. Defibrination is due to a thrombinlike enzyme, which acts slowly. Antivenin therapy could stop the evolution of fibrin formation. In some other cases, antivenin does not inhibit, *in vitro*, the enzymatic activity, even if the antibody recognizes and precipitates the enzyme. One of the most probable reasons for this observation is that the antibody-binding site is distinct from the enzyme-active site, and the immunological binding does not prevent the enzymatic activity. But this antibody can be efficient *in vivo*, even if precipitation occurs after the appearance of the enzyme's clinical effects. Precipitation of the antigen-antibody complex leads to quick elimination of the complex through the kidneys.

The limits of serotherapy have been recently emphasized in the literature. Although antivenin prevents death, the neutralization of swelling, necrosis, or lethal effect is not always possible. Delay before treatment is of considerable importance for the efficacy of antivenin. Thus, it has been mentioned that antivenin was inefficient for severe local complications. However, it is difficult to distinguish a low efficacy of antivenin from an improper use of it. The choice of antivenin is directed by available stocks and clinical manifestations of envenomation. Monovalent antivenin is better when the snake has been identified and if the species corresponds to one of those venoms used for preparation of the antivenin. This is not always practicable. Polyvalent antivenin should be used if the snake is unknown or if no respective monovalent antivenin is available. Polyvalent antivenin has generally had a better cross-neutralization capacity than a monovalent antivenin. Therefore, it could be employed for a bite by a distinct, but related, species to those against which the antivenin was prepared.

B. Administration

Two major problems are reported that concern the administration route and the quantity of antivenin delivery. Most authors admit that the intravenous route (IV) is the best. Practitioners prefer to mix antivenin with fluid (e.g., saline, dextrose, or glucose solution) to the final dilution of 5-10% antivenin; 500 ml of the mixture should be delivered within the first 30 min. This method is relevant because risk of adverse reactions is reduced and symptomatic or systemic drugs can be added to the mixture. Few physicians slowly inject pure antivenin into the vein. This method should be possible in an extreme emergency, and has to be considered as a type of last-chance treatment. The intravenous route is preferable because it is more efficient (about 75% of injected dose is available) and the effect is quick (Doucet, 1975). The intramuscular route can be recommended if the intravenous route is unfeasible (IV injection set not available, no experienced people for doing IV injection, or in cardiovascular collapse). Chippaux (1982) stressed that local subcutaneous injection is painful and poorly efficient. Diffusion of venom in the body is

very fast, and even if antigens are fixed all around the bite, it is preferable to washing them away. Antivenin injected in situ leads to antigen precipitation and some of these could be still toxic. This could enhance the risk of local damage caused by proteolytic enzymes.

The quantity of antivenin delivered depends on the clinical features and delay time after the bite. It also depends on the known or documented efficacy of available antivenin. For large venomous species, at least four vials (e.g., 40 ml) are recommended by most specialists (Russell, 1980). This recommendation is for highly venomous species and can be doubled for those that inject a large amount of venom. The principle of serotherapy is a target strategy. It means that each molecule of antigen must be captured by antibodies. This is possible only if the quantity of antivenin is larger than the venom quantity in the victim's tissues. Injection of antivenin should be repeated until clinical amelioration or until recovery to the medical staff's satisfaction. For efficacy of antivenin on systemic or local complications, a very large amount of antivenin could be required. Ganthavorn (1971) injected 1150 ml of polyvalent antivenin after an *Ophiophagus hannah* bite (king cobra).

An ELISA is able to detect and to determine the dosage of antigen in the victim's tissues. Thus, efficacy of antivenin treatment is measurable, and the necessity of further antivenin injections can be evaluated. This technique will change the course of snake envenomation medical treatment (Khin-Ohn-Lwin et al., 1984; Theakston and Reid, 1979).

C. Adverse Reactions

One of the main problems of serotherapy is adverse reactions to horse serum in previously sensitized persons. This allergic reaction can be immediate (early reaction). In this case, it can be very severe with urticaria, edema, asthma, or shock. In other cases, the reaction is delayed urticaria, edema, asthma, or shock. In still other cases, the reaction is delayed (late reaction) and occurs 1-15 days later. This type of reaction provokes local or generalized urticaria, fever, and arthralgia. Immediate reactions seem to be rarer than delayed types. A skin test has been proposed for detection of sensitized persons. It consists of injection of a small amount of venom under the skin (or deposit a drop of antivenin on conjunctiva). The test is a legitimate procedure, but it does not preclude caution if negative, because numerous, primarily negative, responses are followed by an unexpected reaction. Otherwise, for severe envenomation, a primarily positive response to the skin test does not contraindicate antivenin treatment. It is just a warning, and further precautions should be taken before or during antivenin administration.

The severity and incidence of allergic reactions vary from place to place. The frequency of serotherapy in the population explains the incidence variations, and the diagnostic criteria can change following people's habits and medical facilities. Therefore, in tropical underdeveloped countries, the incidence of serum sickness could be minimized. It is assumed that fewer than 5% of antivenin-treated persons will have an allergic reaction to equine serum proteins. Severe early reactions are reported in about 0.6% of treated persons. If correctly diagnosed and treated under good medical conditions, the prognosis of allergic reactions is quite fair, and very few deaths have been reported.

Nonallergic reactions beginning soon after the start of antivenin injection could be mistaken for anaphylactic reactions. Pyrogenic shock is caused by poorly refined antivenin, which has not been cleared of nonspecific pyrogenic substances. Complement depletion is provoked by immune complex formation with an excess of IgG Fc-fragment (Day et al., 1984). This fragment does not carry the antibody activity; therefore, it can be considered as an impurity. A better purification and concentration of antivenin should prevent the appearance of nonallergic reactions.

IV. SNAKE ANTIVENIN PRODUCERS

In a recent world survey we reported 50 snake antivenin producers throughout the world (Chippaux and Goyffon, 1983). This number seems to have decreased since 1982. However, it is assumed that antivenin is not a lucrative trade. In underdeveloped countries, where more than 95% of bites—and deaths—occur, most severe envenomations receive no or insufficient antivenin treatment. We observed in French-speaking tropical Africa that no rural state dispensary or medical care unit was supplied with antivenin. An actual list of producers and antivenins (presented on pp. 540-551) is the result of investigation made in 1988 for this chapter. About 60% replied to our last questionnaire.

All antivenins are equine serum and purified (pepsin digestion and ammonium sulfate precipitation), except those indicated with an asterisk, which are not refined. Most antivenins are in liquid form (vials of 10 ml), but those in dried or lyophilized form are indicated with a (D) sign. Potency is expressed per 1 ml of antivenin that neutralizes the indicated minimum number of mouse LD₅₀s or the minimum amount of dried venom, except where indicated differently.

Producer's address	Specific activities (venoms used)	Paraspecific activity	1-ml serum potency
A. Africa			
1. Institut Pasteur d'Algérie Rue Docteur Laveran Alger, Algérie	Antivipéрин <i>Cerastes cerastes</i> <i>Vipera lebetina</i>	<i>C. vipera</i>	40 LD ₅₀ 20 LD ₅₀
2. Institut Pasteur Place Charles-Nicolle Casablanca, Morocco	Polyvalent <i>Cerastes cerastes</i> <i>Vipera lebetina</i>	<i>C. vipera</i>	10 LD ₅₀ 10 LD ₅₀
3. Institut Pasteur 13 Place Pasteur Tunis, Tunisia	Polyvalent <i>Cerastes cerastes</i> <i>Vipera lebetina</i>	<i>C. vipera</i>	20 LD ₅₀ 20 LD ₅₀
4. Al Algousa Share Alvezara Cairo, Egypt	a. <i>Cerastes</i> <i>Cerastes cerastes</i> <i>C. vipera</i> b. Polyvalent <i>Naja haja</i> <i>Cerastes</i> sp.		
5. South African Institute for Medical Research P.O. Box 1038 Johannesburg 2000 South Africa	a. Polyvalent <i>Bitis</i> sp. <i>Dendroaspis</i> sp. <i>Hemachatus</i> sp. <i>Naja</i> sp. v. Monovalent <i>Echis carinatus</i>	<i>Causus</i> sp. <i>D. viridis</i>	40 LD ₉₉ to 80 LD ₉₉
	c. Monovalent <i>Dispholidus typus</i>	West African <i>Echis</i> <i>E. coloratus</i> <i>Cerastes</i> sp.	

<p>6. FitzSimmon's Snake Park P.O. Box 1 Snell Parade Durban, South Africa</p>	<p>a. Dendroaspis <i>D. angusticeps</i> <i>D. jamesoni</i> <i>D. polylepis</i> b. Polyvalent <i>Hemachatus haemachatus</i> <i>Naja nivea</i> <i>Bitis arietans</i> <i>B. gabonica</i></p>	<p><i>D. viridis</i></p>	
<p>B. America</p>			
<p>7. Wyeth International Ltd. P.O. Box 8616 Philadelphia, PA 19101 U.S.A.</p>	<p>a. Crotalidae (D) <i>Crotalus atrox</i> <i>C. adamanteus</i> <i>C. durissus</i> <i>Bothrops atrox</i></p>	<p><i>Sistrurus</i> sp. <i>Agkistrodon</i> sp. <i>Deinagkistrodon</i> sp. <i>Calloselasma</i> sp. <i>Crotalus</i> sp. <i>Lachesis muta</i></p>	
	<p>b. Monovalent (D) <i>Micrurus fulvius</i></p>		
<p>8. Laboratorios MYN S. A. Av. Coyoacan 1707 Mexico 12 D. F. Mexico</p>	<p>Snake Antivenin (D) <i>Bothrops asper</i> <i>B. numifera</i> <i>Crotalus atrox</i> <i>C. nigrescens</i> <i>C. tigris</i> <i>C. durissus</i> <i>Agkistrodon bilineatus</i></p>	<p><i>Crotalus</i> sp.</p>	
<p>9. Gerencia General de Biologicos y Reactivos SSA Mariano Escobedo 10 Col. Popotla, Mexico D.F. CP 11400 Mexico</p>	<p>Antiviperin (D) <i>Bothrops asper</i> <i>C. basiliscus</i></p>	<p><i>Crotalus atrox</i> <i>C. durissus</i> <i>C. mollosus</i> <i>Agkistrodon bilineatus</i></p>	<p>79 DL50 78 DL50</p>

Producer's address	Specific activities (venoms used)	Paraspecific activity	1-ml serum potency
10. Grupo Pharma S. A. de C. V. Lucerna nº7, Col. Juarez C.P. 06600, Mexico D. F.	Polyvalent (D) <i>Bothrops asper</i> <i>Crotalus durissus</i>	20-ml vials	
11. Universidad de Costa Rica Ciudad Universitari Rodrigo facio San José, Costa Rica	a. Anticoral (D) <i>M. nigrocinctus</i> b. Antimipartitus (D) <i>M. mipartitus</i> c. Polyvalent serum liquid or (D) <i>Crotalus durissus</i> <i>Lachesis muta</i> <i>Bothrops asper</i>	<i>M. fulvius</i> <i>M. carinicaudus</i>	0.5 mg 0.35 mg
12. Laboratorios Veterinarios Instituto Nacional de Higiene Casilla Postal 5584 Guayaquil, Ecuador	Anti Bothrops <i>Bothrops atrox</i> <i>B. asper</i> <i>B. xanthogrammus</i>	<i>B. nasuta</i> <i>B. castelnaudi</i> <i>B. schlegelli</i> <i>B. bilineatus</i>	3 mg 3 mg 3 mg
13. Instituto Nacional de Salud Av. Eldorado con Carrera Zona G, Bogota D. E. Colombia	Polyvalent <i>Crotalus durissus</i> <i>Bothrops asper</i>		
14. Laboratoria Behrens Av. Principal de Chapellin Apartado 62 Caracas 101 Venezuela	a. Monovalent <i>Crotalus durissus</i> b. Anti Bothrops <i>Bothrops atrox</i> <i>B. venezuelae</i> c. Polyvalent (a+b)		

<p>15. Instituto Butantan Caixa Postal 65 São Paulo, SP. Brazil</p>	<p>a. Antibotropico <i>Bothrops</i> sp. (most of Brazilian species) b. Antilaquético <i>Lachesis muta</i> c. Anticrotalico <i>Crotalus durissus</i> d. Antielapidico <i>Micrurus</i> sp. (most of Brazilian species) e. Antibrotropica-laquético (a+b) f. Polivalente (a+c)</p>	<p>1 mg 2.5 mg</p>
<p>16. Instituto Vital Brazil Caixa Postal 28 Niteroi, Rio de Janeiro Brazil</p>	<p>a. Anticrotalico <i>Crotalus durissus</i> b. Antibotropico <i>Bothrops</i> sp. (most of Brazilian species) c. Polivalente (a+b)</p>	<p>1 mg 2.5 mg</p>
<p>17. Instituto Nacional de Salud Dept. de Animales venenosos Calle Capac Yupanqui 1400 Apartade 451, Lima Peru</p>	<p>a. Anti-lachesico <i>Lachesis muta</i> b. Anti-crotalico <i>Crotalus durissus</i> c. Anti-botropico <i>Bothrops atrox</i> <i>B. bilineatus</i> <i>B. castelnaudi</i> <i>B. pictus</i> <i>B. brazili</i></p>	<p>0.4 mg 1 mg 2.5 mg 1.5 mg</p>
<p>18. Instituto Nacional de Microbiologia Carlos G. Malbran Av. Velez Sarsfield 563 Buenos Aires Republica Argentina</p>	<p>a. Anticrotalus <i>Crotalus durissus</i> b. Antimicrurus <i>Micrurus frontalis</i> c. <i>Bothrops</i> bivalente <i>Bothrops alternatus</i> <i>B. neuwiedi</i></p> <p>5-ml vials <i>B. ammodytoides</i></p>	<p>0.4 mg 1 mg 2.5 mg 1.5 mg</p>

Producer's address	Specific activities (venoms used)	Paraspecific activity	1-ml serum potency
(All antivenoms distributed only in State Health Sanitary Wards)	d. Bothrops tetravalent		
	<i>B. alternatus</i>	<i>B. ammodytoides</i>	2.5 mg
	<i>B. neuwiedi</i>		1.5 mg
	<i>B. jararaca</i>		1.8 mg
19. Ejercito Argentino Campo de Mayo Batallon 601 Pcia de Buenos Aires Republica Argentina	<i>B. jararacussu</i>		1.5 mg
	a. Antimicrurus (D)		
	<i>Micrurus frontalis</i>	<i>M. corallinus</i>	
	b. Antibothrops (D)		
20. Centro de Zoologia Aplicada Universidad de Cordoba Pcia de Cordoba Republica Argentina	<i>Bothrops alternatus</i>	<i>B. ammodytoides</i>	
	<i>B. neuwiedi</i>		
	a. Anticrotalus		
	<i>Crotalus durissus</i>		
C. Asia	b. Antibothrops		
	<i>Bothrops alternatus</i>	<i>B. ammodytoides</i>	
	<i>B. neuwiedi</i>		
21. Ministry of Health Department of Laboratories P.O. Box 6115 Jerusalem 91060, Israel	a. Anti Echis		
	<i>Echis coloratus</i>		
	b. Anti Vipera		
22. Institut d'Etat des Sérums et Vaccins Razi P.O. Box 11365 1558 Tehéran Iran	<i>Vipera palestinae</i>		
	a. Cobra antivenom		
	<i>Naja naja oxiana</i>	<i>Naja naja</i> sp.	1 mg
	b. Lebetina antivenom		
	<i>Vipera lebetina</i>		2.2 mg
c. Echis antivenom			
<i>Echis carinatus</i>	<i>E. multisquamatus</i>	1.8 mg	
c. Persica antivenom			
<i>Pseudocerastes persicus</i>		1.4 mg	

	e. Agkistrodon antivenom		
	<i>Agkistrodon halys</i>		0.9 mg
	f. Latifi antivenom		
	<i>Vipera latifi</i>		1.2 mg
	g. Poly-specific		
	<i>Naja naja oxiana</i>	<i>Bungarus fasciatus</i>	0.3 mg
	<i>Vipera lebetina</i>	<i>V. russelli</i>	1.3 mg
	<i>V. xanthina (=raddei?)</i>	<i>Cerastes cerastes</i>	0.8 mg
	<i>Echis carinatus</i>	<i>Echis sp.</i>	2 mg
	<i>Pseudocerastes persicus</i>		1.1 mg
		<i>Vipera latifi</i>	0.9 mg
23. Central Research Institute	a. Anti-Cobra liquid or (D)		
Kasauli	<i>Naja naja</i>	<i>Ophiophagus hannah</i>	10 LD ₅₀
173 205 (HP) India	b. Anti-Krait liquid or (D)		
	<i>Bungarus caeruleus</i>	<i>B. fasciatus</i>	10 LD ₅₀
	c. Anti Russell's viper liquid or (D)		
	<i>Vipera russelli</i>	<i>Echis carinatus</i>	10 LD ₅₀
	d. Anti saw-scaled viper liquid or (D)		
	<i>Echis carinatus</i>	<i>Vipera russelli</i>	10 LD ₅₀
	e. Polyvalent liquid or (D)		
	(a+b+c+d)		0.6 mg
24. Haffkine Biopharmaceutical	Polyvalent (D)		
Corp. Ltd.	<i>Naja naja</i>		
Acharya Donde Marg.	<i>Bungarus caeruleus</i>		
Parel, Bombay	<i>Vipera russelli</i>		
400012 India	<i>Echis carinatus</i>		
25. Serum Institute of India Ltd.	a. SII Polyvalent (D)	Complementary information	
212/2, Hadapsar		is given in Chap. 19	
Pune - 411028 India	b. SII Bivalent (D)		
26. National Institute of Health	a. Anti <i>Vipera</i> liquid or (D)		
Biological Productions Div.	<i>Vipera russelli</i>		
Islamabad	b. Anti <i>Echis</i> liquid or (D)		
Pakistan	<i>Echis carinatus</i>		

Producer's address	Specific activities (venoms used)	Paraspecific activity	1-ml serum potency
	c. Polyvalent <i>Naja naja</i> <i>Bungarus caeruleus</i> <i>Vipera russelli</i> <i>Echis carinatus</i>	Effective against all terrestrial venomous snakes of Pakistan	
27. Industrie & Pharmaceutical Corporation Rangoon Burma	a. Siamese cobra (D) <i>Naja kaouthia</i> b. Russell's viper (D) <i>Vipera russelli</i> c. Bivalent (D) (a+b)	<i>Naja naja</i>	
28. Queen Saovabha Memorial Institute The Thai Red Cross Soc. Rama IV Rd. Bangkok Thailand	a. Cobra (D) <i>Naja kaouthia</i> b. King cobra (D)* <i>Ophiophagus hannah</i> c. Banded krait (D)* <i>Bungarus fasciatus</i> d. Russell's viper (D)* <i>Vipera russelli</i> e. Malayan pit viper (D)* <i>Calloselasma rhodostoma</i> f. Green pit viper (D)* <i>Trimeresurus albolabris</i>	<i>Naja naja</i> <i>B. caeruleus</i> <i>Trimeresurus sp.</i>	0.6 mg 0.4 mg 0.4 mg 0.32 mg 1.4 mg 0.6 mg
29. Perusahaan Umum Bio Farma Jl. Pasteur 28 P.O. Bos 47 Bandung, Indonesia	Anti Bisa Ular <i>Calloselasma rhodostoma</i> <i>Bungarus fasciatus</i> <i>Naja sputatrix</i>	<i>Naja naja</i>	10 LD ₅₀ 25 LD ₅₀ 25 LD ₅₀
30. Serum & Vaccine Laboratories Alabang Multinlupa Rizal, Philippines	Cobra <i>Naja philippinensis</i>	<i>Naja naja</i>	

31. National Institute of Preventive Medicine 161 Kun Yang Street Nan-Kang, Taipei Taiwan	a. Monovalent (D) <i>Deinagkistrodon acutus</i> b. Bivalent Trimeresurus (D) <i>Tr. stejnegeri</i> <i>Tr. mucrosquamatus</i> c. Bivalent neurotoxic (D) <i>Naja naja atra</i> <i>Bungarus multicinctus</i>	<i>Tr. gramineus</i>	100 LD ₅₀ 100 LD ₅₀ 100 LD ₅₀ 100 LD ₅₀
32. Shanghai Vaccine & Serum Institute 1262 Yang An Road (W) Shanghai China	a. Mamushi <i>Agkistrodon halys</i> b. Hundred pace <i>Deinagkistrodon acutus</i> c. Krait <i>Bungarus multicinctus</i> d. Cobra <i>Naja naja</i> e. Russell's viper <i>Vipera russelli</i> f. Banded krait <i>Bungarus fasciatus</i>		
(sold on request only)			
(sold on request only)			
33. The Chemo-Sero-Therapeutic Research Institute 668 Okubo Shimizu Kumamoto 860, Japan	a. Habu antivenom (D) <i>Trimeresurus flavoviridis</i> b. Mamushi antivenom <i>Agkistrodon halys</i>		
34. Takeda Chemical Industries Osaka, Japan	Mamushi antivenom (D) <i>Agkistrodon halys</i>		
35. Research Institute for Microbial Diseases Ohsaka University, Kita-ku Osaka, Japan	Mamushi antivenom <i>Agkistrodon halys</i>		

Producer's address	Specific activities (venoms used)	Paraspecific activity	1-ml serum potency
36. Kitasato Institute Minato-ku Tokyo, Japan	Mamushi antivenom <i>Agkistrodon halys</i>		
37. Chiba Serum Institute 2-6-1 Konodai, Ichikawa Chiba 272, Japan	Mamushi antivenom (D) <i>Agkistrodon halys</i>		
38. Serum Laboratory Public Health Dept. Naja, Okinawa Prefecture Japan	Habu antivenom <i>Trimeresurus flavoviridis</i>	No data available	
D. Europe			
39. Pasteur Vaccin 3 Avenue Pasteur 92430 Marnes-La-Coquette France	a. Ipser Europe <i>Vipera aspis</i> <i>V. berus</i> <i>V. ammodytes</i> b. B.E.N. <i>Bitis gabonica</i> <i>B. arietans</i> <i>Echis carinatus</i> <i>Naja haje</i> <i>N. nigricollis</i> <i>N. melanoleuca</i>	<i>V. ursinii</i> 5-ml ready to use syringe <i>B. nasicornis</i> <i>Echis coloratus</i> African <i>Echis</i> sp. <i>N. mossambica</i> <i>N. katiensis</i>	100 LD ₅₀ 50 LD ₅₀ 100 LD ₅₀ 25 LD ₅₀ 25 LD ₅₀ 25 LD ₅₀ 25 LD ₅₀ 20 LD ₅₀ 20 LD ₅₀
(Also available under-20-ml ready to use syringe)	c. Ipser Afrique B.E.N. + <i>Dendroaspis viridis</i> <i>D. jamesoni</i>		20 LD ₅₀ 20 LD ₅₀

(Also available under 20 ml)

40. Institut Ronchèse
Z. I. de la Ballastière
B. P. 126
33501 Libourne, France

41. Behringwerke AG
Postfach 11 40
3550 Marburg
Federal Republic of Germany

d. Antirept Pasteur

Bitis arietans
Echis carinatus
Cerastes cerastes
Vipera lebetina
Naja haje
N. nigricollis

Serum antivenimeux
Vipera aspis
V. berus
V. ammodytes

a. Europe
Vipera aspis
V. ammodytes
V. berus
V. lebetina
V. xanthina

b. Central Affrica
Bitis gabonica
B. arietans
B. nasicornis
Dendroaspis viridis
D. polylepis
Hemachatus haemachatus
Naja haje
N. Melanoleuca
N. nigricollis
c. North and West Africa
B. gabonica
B. arietans
Cerastes cerastes
C. vipera
Vipera lebetina

Echis coloratus 25 LD50
E. pyramidum 25 LD50
C. vipera 20 LD50
V. xanthina 20 LD50
Pseudocerastes persicus 25 LD50
20 LD50

2.4 mg
5 ml ready to use syringe 1 mg
2 mg

30 LD99
500 LD99
60 LD99
30 LD99
60 LD99
30 LD99
30 LD99
10 LD99
10 LD99
10 LD99
15 LD99
20 LD99
10 LD99
10 LD99
30 LD99
30 LD99
10 LD99
10 LD50
10 LD99

Producer's address	Specific activities (venoms used)	Paraspecific activity	1-ml serum potency
	<i>Echis carinatus</i>		10 LD ₉₉
	<i>Naja haje</i>		20 LD ₉₉
	<i>N. melanoleuca</i>		10 LD ₉₉
	<i>N. nigricollis</i>		10 LD ₉₉
	d. Near and Middle East		
	<i>Cerastes cerastes</i>		10 LD ₉₉
	<i>Vipera lebetina</i>		10 LD ₉₉
	<i>Echis carinatus</i>		10 LD ₉₉
	<i>Vipera ammodytes</i>		20 LD ₉₉
	<i>V. xanthina</i>		10 LD ₉₉
	<i>Naja haje</i>		20 LD ₉₉
42. Istituto Sieroterapico Vaccinogeno Toscano "Sclavo" Via Fiorentina 1 53100 Siena, Italy	Antiviperin <i>Vipera aspis</i> <i>V. ammodytes</i> <i>V. berus</i> <i>V. ursinii</i>		
43. Istituto Sieroterapico Via Darwin 20 Milano, Italy	Monovalent <i>Vipera ammodytes</i>		
44. Imunoloski Zavod Rockefellerova 2 Zagreb, Yugoslavia	Antiviperinum <i>V. ammodytes</i>	<i>V. aspis</i> <i>V. berus</i>	
45. Institute of Immunology & Microbiologia Sofia, Bulgaria	Monovalent <i>Vipera ammodytes</i>		
46. Institut sérothérapique et vaccinal Suisse Case Postale 2707 3001 Berne, Suisse	Berna <i>Vipera aspis</i> <i>V. ammodytes</i> <i>V. berus</i>		

47. Chemapol Foreign Trade Co.
Kodanska 46, 10010 Praha 10
Czechoslovakia

Anti vipera
Vipera ammodytes
V. berus

48. Ministry of Public Health
101 431, GSP 4
Moscow K-51
U.S.S.R.

a. Anti vipera
Vipera lebetina
b. Anti naja
Naja naja oxiana
c. Polyvalent
Echis multisquamatus
Vipera lebetina
Naja naja oxiana

C. Oceania

49. Commonwealth Serum
45 Poplar Road
Parkville
Victoria 3052
Australia

a. Death adder
Acanthophis antarcticus
b. Taipan
Oxyuranus scutellatus
c. Eastern brown snake
Pseudonaja textilis

A. pyrrhus

Pn. affinis
Pn. nuchalis

d. Brown snake
Pseudechis australis
e. Tiger-sea snake
Notechis scutatus
Enhydrina schistosa

Ps. porphyriacus

Austrelaps superba
Tropidechis carinatus
Pseudechis sp.

f. Polyvalent
Acant. antarcticus
Oxyur. scutellatus
Pseudonaja textilis
Pseudechis australis
Notechis scutatus

Pseudonaja affinis
Pn. nuchalis
Parademansia microlepidota
Ps. porphyriacus
Ps. papuanus
Austrelaps superba

V. CONCLUSION

Since 1894 little progress has been made in antivenin manufacture. The use of enzymatic digestion and ammonium sulfate precipitation are not sufficient. Efficacy of antivenin is poorly controlled for specific venom activity. In severe envenomation, large amounts of antivenin must be injected and adverse reaction risks are increased. Preservation of antivenin in tropical countries remains problematic. The cost of antivenin limits the supply where such a therapy is strongly useful. One solution could be to improve antivenin manufacturing to better concentrate the specific antibody activity and remove any unsuitable proteins. It would be necessary to identify the toxic components in venoms and to discard venom antigens that are not involved in the envenomation. The difficulties encountered include screening of all venoms and studying venom variations. From a technical view point, it is possible to immunize an animal with selected antigens.

Another solution is to immunize humans against autochthonous snake species. Technically, this is rather easy: safe toxoid could be prepared and injected into humans. Liposomes should provide safe and efficient immunization, even by the oral route. Attempts have been made with various results (WHO, 1981), but none could answer the fundamental question on the speed of antibody response after the penetration of antigen nor the logistical question about administration of vaccine. In vaccination against living organisms or against the toxin-produced, an individual antibody titer is enhanced after penetration of antigen during the antigen development or production within the host. Envenomation usually would not be delayed enough to enable the enhancement of antibodies. The determination of the population at risk and then the immunization of this population is a long shot in underdeveloped countries, where vaccination programs are so difficult to implement.

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