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MISCELLANEOUS OBSERVATIONS ON SNAKE VENOMS AND ANTIVENINS

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In the course of an extensive study of the immunological properties of the principal *Bothrops* venoms of Brazil and their corresponding antivenins, the results of which have been published elsewhere (30), numerous observations and some supplementary experiments were made, which could not be incorporated in that paper because they do not directly pertain to its subject. These findings and their significance are reported here apart as they may not only be of interest to other workers in this field but, partly, also have a bearing on further venom research now in progress in this Institute. For full details of the experimental data, on which part of the present study is based, the reader is referred to the above mentioned publication (30).

REACTION OF MICE TO PARENTERALLY APPLIED VENOM.

Immediately after intravenous injection of higher doses of venom, the animals showed signs of great irritation, jumped wildly around and had violent convulsions, during which part of them succumbed within a few minutes. Abundant hemorrhage from the lungs was rather common in such cases. Other animals entered in a state of deep coma, in which they died after some minutes to several hours. Still other mice recovered from the coma and, after hours of apparent well-being, died later on, or survived ultimately.

No immediate symptoms of intoxication, except a certain restlessness, were generally observed in animals in which the venom had been injected subcutaneously. Then, progressive collapse according to the severity of the intoxication

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became manifest, from which the animals rarely recovered. At the same time, development of subcutaneous hemorrhage with subsequent gangrene and necrosis at the site of injection set on. However, this symptom alone, if not accompanied by general collapse, is no measure by which the fate of the animals can be predicted, as mice with very extensive local damage survived rather often.

After both types of venom inoculation, behavior and symptoms of the animals were alike independent from the species of venom administered, with the only exception of the venom sample *jararacussu* L17 on subcutaneous injection. Here, about 10% of the animals, which received this venom alone or in combination with antivenin and survived at least one day, developed unilateral exophthalmus, which attained enormous proportions. This lesion was generally distinguishable 24 hours after venom injection and reached its maximum on the second day. A few days later, the affected eye-ball necrotized (Fig. 1). In the most advanced stages, dissection revealed caseous necrosis of the eye-ball and surrounding tissues. Histological examination of another severe case showed a hemorrhagic zone under the epithelium of the palpebral conjunctiva, an extensive hemorrhagic focus in the anterior chamber, under the cornea and around the lens, and pronounced hyperemia of the optic nerve. In a further case, there was present hemorrhage in the entire anterior and posterior chambers and in the periocular orbital tissues, extending to the adipose tissue and the striped muscles of the orbit; a high degree of vasodilation, and beginning diapedesis of leucocytes in some vessels. In light cases, only congestion and smaller perivascular hemorrhages could be distinguished. The phenomenon, which was not related to death or survival and for which no reasonable explanation can be offered at present, was not observed with two other samples of the same species of venom.

The intensity of the local reactions elicited by subcutaneous injection of the venoms alone and of the venom-antivenin mixtures is recorded in Table I. It must be understood that the division of the continuous scale of local damage in five arbitrary degrees is based on the personal impressions of the author only. As intermediate subdegrees were liable to be under- or overrated in order to be placed within the scheme, only differences of ++ or more may be considered significant.

Inspection of this table reveals that the degree of local damage provoked by the seven venom samples under investigation does not depend on the toxicity of a venom but on the absolute amount of toxin administered. Thus, the LD_{50} of the *alternata* venom (15.80mg/kg) is situated in the zone of light to medium local reactions, whereas that of the *atrox* venom (80.99 mg/kg) is deep in the region of severest local tissue destruction. The hemorrhagic factor of the venoms in neutralized to a certain degree by homologous as well as heterologous (but genus-specific) antivenins. However, there is no congruence

between the neutralization of the local activities of these venoms and that of their total toxicity, as demonstrated by the case of the *cotiara* venom and its specific antiserum.

DISCUSSION

The resemblance of the clinical features of snake poisoning to the symptoms which accompany various forms of shock has been commented on by Essex & Markowitz (5), Moon (16) and others and is confirmed by the findings of Witham et al. (37). Crushed muscle tissue in a quantity of less than 0.5% of the bodyweight and introduced in the peritoneal cavity has been shown by Moon & Kennedy (17) to be sufficient to produce fatal shock in dogs. These and similar observations by other authors made the establishment of a causal connection between the extensive local damage wrought by numerous snake venoms (23 — 27) and the appearance of a shock-like collapse of the blood circulation very suggestive. Thus, Schaumann (22) distinguished two toxic principles in locally active venoms inoculated subcutaneously, one being the properly toxic factor, to which the animals succumb in 24 hours or less, and the other the hemorrhagic component, which causes "late deaths", i. e. after more than 24 hours, by the severe damage of tissues at the site of injection or, in other words, by traumatic shock. This separation of the two mechanisms is, of course, rather arbitrary, for it is a well-known fact that death from local trauma, such as muscle contusion, burns, etc., may ensue in from a few hours to several days, but the survival time before death on intoxication with snake venoms devoid of local activity falls in the same range in laboratory animals (24, 27) as well as in man (1). The disconcerting observations of Schöttler (24), namely that animals with seemingly severe local trauma often did not appear very much affected, cast some doubt on the importance of the damage induced by venom at the site of injection. It is well possible that the local destruction of tissue and the formation of sanguineous edema, which may hardly exceed one third of the body surface of small animals, is greatly overestimated by the observer. On the other hand, it has been frequently reported that fatal shock may result from relatively small injuries, as also evident from the experiments of Moon & Kennedy (17).

The mechanism of shock in general is still a highly disputed matter, and that of shock by locally active venoms given subcutaneously, by the way the most frequent route of venom introduction in snake bite accidents is further complicated by the indirect effects the toxin may elicit through local reactions. Thus the factors which may be involved in the local trauma produced by poisoning with such venoms, are:

a. *Production of local hemorrhagic edema resulting in a decrease of fluid in the circulatory system.* Schwiegk & Schöttler (32) have demonstrated that the loss of plasma into the tissues caused by local trauma may be voluminous enough to constitute by itself the causa mortis. However, it is not necessary to assume such large edemas in the case of venoms, as much smaller losses of plasma are capable of starting the circulus vitiosus (Moon (16) and Schwiegk (31)) of secondary collapse induced by generalized internal loss of plasma or blood. On the other hand, the intensity of a local trauma does not warrant a corresponding degree of systemic reaction. It is possible that the destructive action of venom on the endothelial tissues is so strong that, after initial increase of capillary permeability with consequent leakage of blood fluid, the circulation is entirely interrupted in the affected area due to necrosis of the blood vessels. This assumption would supply an acceptable explanation of the incongruity between the apparent severity of local reactions and the respective rates of mortality. Though practically nothing is known about the mechanism of local venom action, except that it is probably associated with the proteolytic activity, the experimental evidence, especially the neutralization of the hemorrhagic factor by antivenin without significant alteration of the lethal dose, tends to indicate that the local trauma per se does not greatly contribute to death by snake poisoning, unless the peritoneum is perforated and the guts are exposed (24).

b. *Liberation of pharmacodynamically active substances, which are normally cell-bound, and formation of toxic split-products from inoffensive material by the various enzymic actions of venoms* (38). Feldberg & Kellaway (6, 7), Rocha e Silva et al (21), Werle et al. (36) and other workers have shown the release, or formation respectively, of histamine and other depressor substances by venoms. Nothing, however, seems to be known of the proportions at which these substances appear and in which amounts they might affect the organism. In the present investigation on mice, the possible influence of histamine should be irrelevant because, according to Dekanski (3), the intravenous injection of histamine doses ten times larger than exist in the whole mouse is tolerated by this notably histamine-resistant animal species with scarcely any discomfort. The insignificant rôle of histamine in snake poisoning is also evident from the therapeutic ineffectiveness of antihistamine medication in such experiments (29). The production of local hemorrhage is not necessarily coupled to the mechanism which brings about the liberation, or formation, of toxic compounds, as evident from the fact that the latter also occurs with venoms which do not provoke macroscopically perceptible symptoms at the site of inoculation. Furthermore, such an activity need not be limited to the immediate surroundings of the venom deposit but may spread throughout the whole body along with the absorption of the venom, although with decreasing intensity of reaction. If and

to what degree the physiologic principle treated in this paragraph is neutralized by antivenin has not yet been assayed.

It is almost certain that the combined local actions of venoms are associated with their proteolytic enzymes. Therefore it is interesting to investigate the proteolytic capacity of lethal doses of the venoms under consideration. Unfortunately, pertinent data were only available for the venom of *B. jararaca*. Here, Martirani & Azevedo (15) found that this venom digests gelatin in 60 minutes at pH 8.0 in a proportion of from 1:83 to 1:1,122. The lethal effect of the same species of venom, relative to solid material under the assumption that three fourths of the mouse body are water, occurs at a ratio of from 1:29,500 to 1:581,400. If the digestibility of the body proteins were equal to that of gelatin, this would mean that a lethal dose of venom should be capable of splitting 0.01 to 3.8% of the body substance. Recalling that the dissolution of less than 0.5% may suffice to provoke death, it is evident that the importance of the proteolytic action of venoms in quite a number of cases cannot be denied. The supposition of a common factor at least for part of the mechanism contributing to shock in snake poisoning on one side and in numerous forms of more or less localized trauma on the other is further supported by the similarity of the histo-pathologic findings in both conditions. The possibility that such wide-spread alterations may be due to the direct effect of the extremely minute lethal doses of venom appears rather remote, although the latter might and probably will directly interfere with selected organs of higher susceptibility as, for instance, the nervous system.

SURVIVAL TIME AFTER INJECTION OF LETHAL VENOM DOSES

A survey of the survival time after fatal intoxication is given in Table II, in the compilation of which mortalities at dose levels above the certainly lethal one were not included. No statistically significant difference between the two routes of venom application can be deduced from these figures, which have been obtained by recording the mortality rate at 24 hour intervals. A more detailed investigation of the times of death within the first 24 hour period after venom inoculation, which was technically impossible, might have revealed that death ensues more rapidly after intravenous injection.

The death times of the animals in the antivenin assays are recorded in Table III. A comparison of these figures with those obtained with venom alone (Table II) shows that there is no difference in the intravenous tests and that the difference of the means in the subcutaneous experiments, though not significant, tends to indicate that antivenin retards death in fatal cases. If the observations are arranged after the various types of antivenin (Table IV), it

is interesting to note that the periods of survival become more regular in the subcutaneous tests as evident from the considerably smaller standard deviation (σ). Furthermore, after this arrangement death ensues definitely faster on intravenous than on subcutaneous injection of lethal venom-antivenin mixtures, which is obvious from the figures of the first two 24 hour recording intervals.

ANTIVENIN TITRATION BY FLOCCULATION AND BIOASSAY

After incubation of venom-antivenin mixtures for one hour at 37° C, flocculation occurred only with the pepsin digested antibothropic serum B-114, whereas the other antivenins, which were investigated in the present study and which had been treated by other purificatory procedures or, perhaps, had not been processed at all, did not show the slightest trace of turbidity. It is possible that incubation was too short for the formation of a precipitate of the venoms and the latter sera, as Hansen (10, 11) and Petermann & Pappenheimer (18) have observed that peptic digestion greatly decreased the flocculation time of antitoxin.

In none of the experiments here referred to, maximum flocculation coincided with the neutralization of the toxic properties of the venoms (Table V), which confirms previous observations on this subject (28). Such findings are not surprising as, in the light of the research on purified diphtheria toxin and antitoxin by Pope et al. (19, 20), congruence between in vivo and in vitro assays of so highly complex antigens as snake venoms and their corresponding antibodies cannot even be expected in theory. As a matter of fact, Christensen (2) even observed that floccules obtained at optimal venom; antivenin ratios contained no toxin at all in certain cases. A plausible explanation of this phenomenon would be supplied by the venom toxoids of Githens & Butz (8) or group II of the "Giftdrüsensekret" of Schöttler (24).

ORDER OF TOXICITY AND SUBCUTANEOUS:INTRAVENOUS INDEX

The order of toxicity within a series of venoms is obtained by calculating the ratio between the mean lethal dose of each venom and that of the most active sample in the series. The respective figures for the venoms used in the present investigation ar given in Table VI. The inspection of this table demonstrates that the more active a venom is on intravenous injection the less toxic it seems to be if administered subcutaneously, with the exception of *neuwiedii* L-10, which is weak in either case. This reversed order of toxicity depending on the route of injection is admittedly not established beyond doubt, as the

ranges of the indices overlap in nearly every instance. However, if the mean lethal doses are directly compared, e. g. *atrox* L-11 is superior to *alternata* L-8 on intravenous and inferior on subcutaneous injection, 32 pairs of combination are possible, leaving out, of course, the comparison between different samples of the same species of venom. This direct comparison shows that in 9 cases the relation between two venoms is definitely reversed in the two types of assay and that there is not one statistically tenable case to supply evidence of the contrary. The greater part of the remaining cases, though dubious in a statistical sense, is favorable for the indicated observation. The same is true with regard to the subcutaneous: intravenous index, which is the quotient between the subcutaneous and the intravenous mean lethal doses of a venom. In spite of the very wide ranges of this index, the differences between venoms characterized by it are statistically significant in at least 13 cases out of the 32 possibilities of comparison.

Magnitude and differences of the subcutaneous: intravenous indices of the venoms combined with antivenin (Table VII) are smaller than those of the venoms alone (Table VI). Though the differences between the indices of the venom-antivenin mixtures are not significant, it is interesting that the venoms with the highest subcutaneous: intravenous index in the toxicity test — *atrox* L-11 and *jararacussu* L-7 — have also the highest index in combination with antivenin and that, in the intravenous antivenin assay, considerably more lethal doses are neutralized of these two venoms than of the others (30). It is further noteworthy that the combination with the anti-*cotiara* serum resulted in the highest subcutaneous: intravenous index in five of the venom samples and in second and third highest once each.

DISCUSSION

A possible explanation of these findings might be supplied by the aforementioned observation that on intravenous venom inoculation the mice frequently enterd in immediate shock, from which they soon recovered but, after hours of apparent well-being, died later on. By the complex nature of venoms it would not be unlikely that two toxic principles are involved in intravenous poisoning, one which acts directly on its effector organs, in immediate contact with which it is brought by the blood stream, and another which either has to leave the circulation in order to encounter its receptors or/and which needs time to liberate, or form, toxic substances within the animal body. On subcutaneous injection, the former factor would be barred from its points of attack by masses of tissue so that its entrance in the circulation is delayed and gradual, thus rendering it relatively harmless. The latter may be concluded from the

experiments of Schaumann (22) and Vellard & Huidobro (34), who observed that the immediate fall of blood pressure caused by intravenous injection of locally active venoms is less severe or even absent on replication of the same dose, which indicates a desensitization of the effector organs to this fraction of the venom. Hence, a factor of great importance in intravenous poisoning has less or no bearing on the outcome of subcutaneous venom application, and variations in the quantitative distribution of this factor in the venoms might well account for the reversed order of toxicity of the venom samples in the two routes of inoculation.

Another explanation was offered by Martirani (14), who suggested that differences in the hyaluronidase activity of the venoms might determine the degree of the latter's toxicity on subcutaneous injection but might be of negligible influence in intravenous inoculations. The comparison of the amounts of hyaluronidase present in lethal doses of the venom samples under investigation, as assayed by the method of Tolksdorf et al. (33) (Table VI), shows considerable variation in the hyaluronidase activity of the venom doses that provoke death on intravenous injection, whereas the quantities of this enzyme contained in the rather diverging subcutaneous lethal doses are remarkably similar. Therefore a closer examination of this matter is justified.

INFLUENCE OF HYALURONIDASE ACTIVITY OF VENOMS ON THEIR SUBCUTANEOUS TOXICITY

The possible rôle of hyaluronidase in the subcutaneous intoxication by venoms should become evident if its titer is artificially raised, or if the hyaluronidase of the venom is used up by its proper substrate prior to the injection of the venom. In these experiments, a commercial brand of bovine testicular hyaluronidase *, or hyaluronic acid, was added to the venom solutions, and the mixtures were incubated at 37°C for one hour before subcutaneous injection in female white nice. Control tests with the same venom solutions plus saline were run at the same time. Results are reported in Table VIII. The hyaluronidase increase in experiments No. 55, 56, 58 over the controls No. 34 or 35, respectively, was 25, 18 and 72% in the mean lethal doses. The amounts of hyaluronic acid added to the venom solutions in experiments No. 57, 59, 60 were sufficient to absorb 10,720, 580 and 850% of the hyaluronidase activity of the controls relative to the LD_{50}s. The results of these assays are rather confusing. Experiments No. 55, 56, 57 show that the reduction of hyaluronidase activity by large amounts of hyaluronic acid significantly decreases the toxicity of the venom

* Supplied under the trade-name "Hyalozima" by Opoterapica NESPA Ltda., São Paulo, S. P., through the courtesey of Dr. I. Martirani.

in comparison with that of the solutions to which hyaluronidase had been added. Just the opposite occurred in experiments No. 58, 59, 60, where hyaluronic acid enhanced the toxicity relative to the hyaluronidase enriched venom solution, in one case (experiment No. 58/60) even significantly from a statistical viewpoint. However, the extremely large error of the toxicity estimations in the control tests with venom alone makes it very probable that the conflicting results are merely due to chance. Thus, no evidence of the suggested influence of the venom hyaluronidase on the subcutaneous toxicity of venom is conceivable from the results of these experiments, which, however, are open to the criticism that, according to Haas (9) and Hechter (12), more factors than were controlled here are involved in the mechanism of spreading in vivo. Hence, the conclusion that hyaluronidase does definitely not enhance the toxicity of venoms would be premature.

DISCUSSION

In the vast literature on the spreading factor, or factors, of venoms, which is supposed to be hyaluronidase or a hyaluronidase-like substance, it has generally been accepted bona fide that a mechanism which promotes invasion of an organism is favorable for the action of a toxic or otherwise physiologically important substance. This view holds true for certain conditions such as the observations of Krech (13), who found that, in subcutaneous experiments, markedly less antitoxin is necessary to neutralize a previously injected dose of diphtheria toxin if hyaluronidase is added to the serum. In this case, the hyaluronidase will hasten the distribution of the antitoxin throughout the infected organism at a rate which without the aid of the enzyme might only be attained by higher doses of serum. The time element, however, is of no importance in snake poisoning under natural circumstances, because it is entirely irrelevant whether a serpent kills its prey in 5 or in 30 minutes. Moreover, in the case of venoms with pronounced local activity, there is still another aspect of the problem of spreading, namely that of the threshold concentration of venom required to overcome the natural resistance of the tissues to its action. It is obvious that the higher the hyaluronidase content of a given amount of venom, the faster the latter will spread and, thus, become diluted towards the critical level, below which no local damage occurs. This way, the final effect of venom hyaluronidase might be just the opposite of what is generally assumed.

A discussion of these disconcerting possibilities is rather useless before much more is known about the essential features of the physiologic mechanism by which venoms provoke death. In spite of the lack of evidence on the importance of the spreading factor in *Bothrops* poisoning supplied by the exper-

iments of this paper, a closer investigation of this matter also in other types of venom might yield interesting results, which possibly would be of help for a better understanding of these substances. For the benefit of snake bite therapy such research should also be extended to the interactions between antivenin and hyaluronidase. This will not be an easy task because by the proteic nature of sera their influence on hyaluronidase can hardly be determined turbidimetrically (33). On the other hand, the interpretation of viscosimetric assays (9) will be complicated by the concomitant reactions between distinct factors, which influence the viscosity of the system in different and independent ways: viscosity of hyaluronic acid, viscosity of antivenin, depolymerization of hyaluronic acid by venom, unspecific inhibition (9) and specific neutralization (4) of venom hyaluronidase by antivenin, proteolytic and thus viscosity reducing action of venom on serum, alteration of surface tension by venom (35), if the factor responsible for the latter phenomenon is different from the afore-mentioned ones.

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SUMMARY

This paper presents a collection of supplementary observations and experiments made in connection with an extensive study on *Bothrops* venoms and their antivenins published elsewhere (30).

A unilateral eye lesion provoked by subcutaneous injection of a certain sample of *Bothrops jararacussu* venom in mice is described.

The severity of the local reactions elicited by subcutaneous venom administration depends on the dose and not on the general toxicity of a venom. They are neutralized to a certain degree by species- and genus-specific antivenins. Local activity and total toxicity are neutralized at different rates. The significance of local damage in snake poisoning is amply discussed.

Survival times of mice after intravenous and subcutaneous inoculations of lethal venom doses with and without addition of antivenin are reported.

No congruence between in vivo titration and flocculation of antivenin was observed.

The order of toxicity of the venoms established by intravenous assay is generally reversed in subcutaneous determinations, which results in different

subcutaneous: intravenous indices for the various venom species of the same snake genus. As an explanation of this phenomenon, the possibility of a dual mechanism of death by venom is discussed.

Under the conditions of the experiment, no influence of hyaluronidase on the subcutaneous toxicity of venom was detected.

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FIG. I — Necrosis of the eye-ball provoked by subcutaneus injection of a certain sample of *Bothrops jararacussu* Venom.

Table I

SEVERITY OF LOCAL REACTIONS (O — ++++) ON SUBCUTANEOUS INJECTION OF VENOM AND VENOM-ANTIVENIN MIXTURES IN FEMALE WHITE MICE.

alternata L—8

LOG-DOSE OF VENOM	saline control	anti-alternata	anti-atrox	anti-cotiara	anti-jararaca	anti-jararacussu	anti-neuwiedii
0.1	—	—	—	—	—	—	—
0.2	—	—	—	—	—	—	—
0.3	—	—	—	—	—	—	—
0.4	0*	—	—	—	—	0*	—
0.5	0*	—	—	—	—	—	—
0.6	0*	—	—	—	—	0*	—
0.7	0	—	—	—	—	—	—
0.8	+	—	0*	0*	—	0	—
0.9	+	—	—	—	—	—	—
1.0	++	—	0*	0*	0*	0	0*
1.1	++	—	—	—	—	—	—
1.2	++	0*	+	0	0*	++	0*
1.3	+++	—	—	—	—	—	—
1.4	++++	++*	++	+	+	+++	++
1.5	**	—	—	—	—	—	—
1.6	**	+++	**	++	++	++++	**
1.7	**	—	—	—	—	—	—
1.8	**	+++	**	+++	+++	**	+++
1.9	—	—	—	—	—	—	—
2.0	—	**	**	**	**	**	**
2.1	—	—	—	—	—	—	—
2.2	—	—	—	—	—	—	—
2.3	—	—	—	—	—	—	—
2.4	—	—	—	—	—	—	—
2.5	—	—	—	—	—	—	—
2.6	—	—	—	—	—	—	—
2.7	—	—	—	—	—	—	—
2.8	—	—	—	—	—	—	—

atrox L—11

LOG-DOSE OF VENOM	saline control	anti-alternata	anti-atrox	anti-cotiara	anti-jararaca	anti-jararacussu	anti-neuwiedii
0.1	—	—	—	—	—	—	—
0.2	—	—	—	—	—	—	—
0.3	—	—	—	—	—	—	—
0.4	—	—	—	—	—	—	—
0.5	—	—	—	—	—	—	—
0.6	—	—	—	—	—	—	—
0.7	—	—	—	—	—	—	—
0.8	—	—	—	—	—	—	—
0.9	—	—	—	—	—	—	—
1.0	++*	—	—	—	—	—	—
1.1	—	—	—	—	—	—	—
1.2	++*	—	—	—	(+)*	—	—
1.3	+++*	—	—	—	—	—	—
1.4	+++	—	—	—	+*	—	—
1.5	++++*	—	—	—	—	—	—
1.6	++++	—	—	+++	++*	—	—
1.7	++++	—	—	+++*	—	++*	—
1.8	++++	+++	—	++++*	+++*	+++	—
1.9	++++	—	++++	++++*	—	+++	—
2.0	++++	+++	++++	++++*	+++	++++	+++
2.1	**	—	++++	++++	+++	++++	++++*
2.2	++++	++++	++++	++++	++++	++++	++++
2.3	**	—	++++	++++	++++	++++	++++
2.4	**	**	**	++++	++++	++++	++++
2.5	—	—	**	—	—	**	**
2.6	—	**	**	—	—	—	**
2.7	—	—	—	—	—	—	—
2.8	—	**	—	—	—	—	—

cotiara L—6

LOG-DOSE OF VENOM	saline control	anti-alternata	anti-atrox	anti-cotiara	anti-jararaca	anti-jararacussu	anti-neuwiedii
0.1	—	—	—	—	—	—	—
0.2	—	—	—	—	—	—	—
0.3	—	—	—	—	—	—	—
0.4	—	—	—	—	—	—	—
0.5	—	—	—	—	—	—	—
0.6	—	—	—	—	—	—	—
0.7	0*	—	—	—	—	—	—
0.8	—	0*	0*	0*	—	0*	—
0.9	+	—	—	—	—	0*	—
1.0	?	0*	0*	0*	0*	0	0*
1.1	++	—	—	—	0*	+	—
1.2	?*	0*	0*	0*	0*	+	0*
1.3	+++	—	—	—	0*	++	—
1.4	?	+	0	0*	0	+++	0
1.5	+++	—	—	—	++	**	—
1.6	?	++	**	+	+	**	0
1.7	**	—	—	—	+++	**	—
1.8	?	**	**	++	**	**	**
1.9	**	—	—	—	**	—	—
2.0	?	+++	**	+++	—	—	—
2.1	**	—	—	—	—	—	—
2.2	**	**	—	**	—	—	—
2.3	—	—	—	—	—	—	—
2.4	**	**	—	—	—	—	—
2.5	—	—	—	—	—	—	—
2.6	—	**	—	—	—	—	—
2.7	—	—	—	—	—	—	—
2.8	—	—	—	—	—	—	—

jararaca L—5

LOG-DOSE OF VENOM	saline control	anti-alternata	anti-atrox	anti-cotiara	anti-jararaca	anti-jararacussu	anti-neuwiedii
0.1	—	—	—	—	—	—	—
0.2	—	—	—	—	—	—	—
0.3	—	—	—	—	—	—	—
0.4	—	—	—	—	—	—	—
0.5	—	—	—	—	—	—	—
0.6	+	—	—	—	—	—	—
0.7	+	—	—	—	—	0	—
0.8	++	—	—	—	—	—	—
0.9	++	—	—	—	—	0	—
1.0	+++	—	0*	0*	0*	+	—
1.1	+++	—	—	—	—	0	—
1.2	+++	+	0*	0*	0*	+	0*
1.3	+++	(+)*	—	—	—	+	—
1.4	++++	++	0	+	0*	++	+*
1.5	**	+	—	—	—	++	—
1.6	++++	**	++	++	+	**	++
1.7	**	—	—	—	—	**	—
1.8	—	**	**	+++**	+++	**	+++
1.9	—	—	—	—	—	—	—
2.0	—	**	**	**	**	**	**
2.1	—	—	—	—	—	—	—
2.2	—	**	**	—	—	—	**
2.3	—	—	—	—	—	—	—
2.4	—	—	—	—	—	—	—
2.5	—	—	—	—	—	—	—
2.6	—	—	—	—	—	—	—
2.7	—	—	—	—	—	—	—
2.8	—	—	—	—	—	—	—

jararaca L—14

LOG-DOSE OF VENOM	saline control	anti-alternata	anti-atrox	anti-cotiara	anti-jararaca	anti-jararacussu	anti-neuwiedii
0.1	0*	—	—	—	—	—	—
0.2	—	—	—	—	—	—	—
0.3	0*	—	—	—	—	—	—
0.4	0*	—	—	—	—	—	—
0.5	+*	—	—	—	—	—	—
0.6	+	—	—	—	—	—	—
0.7	++	—	—	—	—	—	—
0.8	++	—	—	—	—	—	—
0.9	+++	—	—	—	—	—	—
1.0	+++	0*	—	0	—	0	—
1.1	+++	0*	—	—	—	—	—
1.2	+++	0*	0*	0*	—	0	0
1.3	+++	+	—	—	0*	—	—
1.4	**	**	0	—	0*	++	0*
1.5	**	**	—	+	0*	—	—
1.6	—	++	**	+++	++	**	++
1.7	—	**	—	—	+++*	—	—
1.8	—	**	**	**	+++	**	+++
1.9	—	**	—	—	++++	—	—
2.0	—	**	**	**	**	**	+++
2.1	—	—	—	—	**	—	—
2.2	—	—	**	—	—	—	**
2.3	—	—	—	—	—	—	—
2.4	—	—	—	—	—	—	—
2.5	—	—	—	—	—	—	—
2.6	—	—	—	—	—	—	—
2.7	—	—	—	—	—	—	—
2.8	—	—	—	—	—	—	—

jararacussu L—7

LOG-DOSE OF VENOM	saline control	anti-alternata	anti-atrox	anti-cotiara	anti-jararaca	anti-jararacussu	anti-neuwiedii
0.1	—	—	—	—	—	—	—
0.2	—	—	—	—	—	—	—
0.3	—	—	—	—	—	—	—
0.4	—	—	—	—	—	—	—
0.5	—	—	—	—	—	—	—
0.6	0*	—	—	—	—	—	—
0.7	—	—	—	—	—	—	—
0.8	+*	—	—	—	—	—	—
0.9	—	—	—	—	—	—	—
1.0	++*	—	—	—	—	—	—
1.1	—	—	—	—	—	—	—
1.2	+++*	—	—	—	—	—	—
1.3	+++	—	—	—	—	—	—
1.4	+++	—	—	+	—	—	—
1.5	+++	—	—	—	—	—	—
1.6	++++	—	0	+*	—	—	—
1.7	++++	—	—	—	—	+*	—
1.8	++++	+++*	++	++	++*	—	++*
1.9	++++	—	—	—	—	++	—
2.0	++++	+++	+++	+++	+++*	—	+++
2.1	++++	—	—	—	—	+++	—
2.2	++++	++++	+++	+++	+++	—	+++
2.3	++++	—	—	—	—	++++	—
2.4	++++	++++	—	++++	++++	—	++++
2.5	**	—	—	—	—	**	—
2.6	—	++++	++++	**	++++	—	++++
2.7	—	—	—	—	—	**	—
2.8	—	++++	**	**	**	—	**

neuwiedii L—10

LOG-DOSE OF VENOM	saline control	anti-alternata	anti-atrox	anti-cotiara	anti-jararaca	anti-jararacussu	anti-neuwiedii
0.1	—	—	—	—	—	—	—
0.2	—	—	—	—	—	—	—
0.3	—	—	—	—	—	—	—
0.4	—	—	—	—	—	—	—
0.5	—	—	—	—	—	—	—
0.6	+*	—	—	—	—	—	—
0.7	—	—	—	—	—	—	—
0.8	+*	—	—	—	—	—	—
0.9	—	—	—	—	—	—	—
1.0	++*	—	—	—	—	—	—
1.1	+++*	—	—	—	—	—	—
1.2	+++*	—	—	—	—	—	—
1.3	++++	—	—	—	—	—	—
1.4	+++	—	—	+*	—	—	—
1.5	+++	—	—	—	—	—	—
1.6	++++	—	+++	++	—	—	—
1.7	++++	—	++++	—	—	—	—
1.8	++++	+++	+++	+++	+++	+++	+++*
1.9	**	—	—	—	++++*	+++	—
2.0	++++	++++	++++	++++	+++*	+++	++++
2.1	++++	—	+++	—	++++	+++	—
2.2	**	++++	++++**	++++	++++	**	++++
2.3	—	—	**	—	++++	++++	—
2.4	—	**	++++	**	**	++++	**
2.5	—	—	—	—	**	—	—
2.6	—	**	—	—	—	—	**
2.7	—	—	—	—	—	—	—
2.8	—	**	—	—	—	—	**

Bars indicate the approximate position of the LD_{50}; * (**) indicates that no death (survival) occurred with this dose; log-dose 1.0 = 10.0 mg/kg.

TABLE IV

PERCENTAGE OF MORTALITY AS A FUNCTION OF SURVIVAL TIME AFTER INJECTION OF LETHAL DOSES OF VENOM-ANTIVENIN MIXTURES

Type of antivenin	Days after injection on which death occurred							Number of observations
	1	2	3	4	5	6	8	
A. intravenous injection								
anti-alternata.....	91	7	2	0	0	0	0	120
anti-atrox	93	5	2	0	0	0	0	85
anti-cotiara.....	96	2	1	1	1	0	0	303
anti-jararaca.....	97	3	0	0	0	0	0	274
anti-jararacussu	90	6	1	1	0	0	1	145
anti-neuwiedii.....	90	0	10	0	0	0	0	78
anti-Bothrops	92	6	1	1	1	0	0	131
Weighted average $\pm$ 1.96 σ	94±6	4±4	2±6	0±1	0±1	0±0	0±0	1136
B. subcutaneous injection								
anti-alternata.....	80	14	3	3	0	1	0	147
anti-atrox	81	14	4	1	0	1	0	160
anti-cotiara.....	75	17	6	2	0	1	0	162
anti-jararaca	80	15	4	1	0	1	0	139
anti-jararacussu	73	18	7	1	0	1	0	325
anti-neuwiedii.....	76	17	5	1	0	1	0	150
Weighted average $\pm$ 1.96 σ	77±6	16±3	5±3	1±1	0±0	1±0	0±0	1083

TABLE V

COMPARISON BETWEEN PERCENTAGE OF INTRAVENOUS MORTALITY AND FLOCCULATION IN THE COMBINATION OF THE VENOMS WITH A FIXED DOSE OF ANTIBOTHROPIC SERUM B-114.

Log-dose of venom	Venom samples											
	alternata L-8		atrox L-11		cotiara L-6		jararaca L-5		jararacussu L-7		neuwiedii L-10	
	mortality	flocculate	mortality	flocculate	mortality	flocculate	mortality	flocculate	mortality	flocculate	mortality	flocculate
0.6									0	0		
0.7									0	0		
0.8	0	0	12	0	0	0			0	0		
0.9	0	0	0	0	0	0	0	0	0	0		
1.0	0	19	25	10	0	0	0	0	0	0	0	
1.1	20	31	12	10	0	0	0	trace	0	0	0	
1.2	22	94	29	0	20	27	0	trace	0		0	
1.3	89	98	14	0	40	32	0	85	10		10	
1.4	80	51	0	23	100	35	40	100	30		10	
1.5	100	27	50	31	100	98	90	100	90		0	
1.6	100	18	62	33			100	96	100		70	
1.7	100	0	100	15			100	29	100		100	
1.8							100	20			100	

increasing turbidity

The figures for the flocculation test represent the height of the precipitate as percentage of the entire column of liquid.

TABLE VI

VARIOUS INDICES OF *BOTHROPS* VENOM ACTIVITIES

Venom sample	Order of toxicity (range)		Subcutaneous / intravenous Index (range)	mg of venom containing one unit of hyaluronidase activity	Order of hyaluronidase activity in one LD_{50} (range)	
	intravenous	subcutaneous			intravenous	subcutaneous
alternata L-8..	5.3 (1.4-20.5)	1.4 (0.7-2.6)	8.1 (3.1-26.2)	0.073	26.8 (11.5-42.2)	2.1 (1.3-2.9)
atrox L-11	1.0 (0.4-1.6)	7.0 (2.3-13.3)	221.4 (84.0-755.2)	0.380	1.0 (0.4-1.5)	2.1 (1.3-2.9)
cotiara L-6....	5.7 (1.9-20.3)	2.1 (0.9-4.0)	11.5 (4.5-30.6)	0.058	35.9 (19.1-52.4)	4.0 (2.3-5 7)
jararaca L-5...	4.5 (1.5-15.9)	1.0 (0.7-1.3)	7.1 (3.5-16.8)	0.030	54.3 (29.0-79.7)	3.8 (2.8-4.8)
« L-14..	3.3 (0.4-14.4)	1.4 (0.8-2.4)	13.4 (5.5-83.9)	0.050	24.0 (4.8-43.2)	3.1 (2.3-3.9)
jararacussu L-7	1.1 (0.3-4.1)	6.0 (2.7-11.6)	171.4 (65.2-493.8)	0.390	1.0 (0.5-1.6)	1.7 (1.0-2.5)
« III	1.3 (0.2-5.5)	3.0 (1.1-6.3)	74.8 (18.8-596.9)	0.105	4.0 (0.9-7.9)	3.2 (1.5-5.0)
« IV	1.2 (0.2-6.2)	3.4 (1.2-7.2)	76.3 (19.0-609.9)	0.150	3.5 (0.7-6.2)	2.6 (1.2-4.0)
neuwiedii L-10	6.3 (1.1-26.5)	5.7 (2.7-10.8)	28.3 (9.9-141.2)	0.640	3.6 (1.0-6.2)	1.0 (0.6-1.4)

TABLE VII

MEAN SUBCUTANEOUS:INTRAVENOUS INDICES OF THE LD_{50}S OF VENOM-ANTIVENIN MIXTURES

Antivenins	Venom samples						
	alternata L-8	atrox L-11	cotiara L-6	jararaca L-5	jararaca L-14	jararacussu L-7	neuwiedii L-10
anti-alternata.....	2.99	11.54	2.43	3.29	3.48	46.98	5.86
anti-atrox	3.46	7.26	2.70	2.64	2.31	10.36	5.05
anti-cotiara.....	3.58	19.34	5.72	5.49	2.77	18.83	13.72
anti-jararaca	2.87	9.92	3.63	3.12	3.28	17.05	10.27
anti-jararacussu	3.00	8.14	4.74	1.62	2.55	6.28	9.89
anti-neuwiedii.....	2.81	8.30	2.90	2.39	3.19	10.51	5.28
Average (1.96 σ)	3.12 (0.57)	10.75 (8.00)	3.67 (2.31)	3.09 (2.35)	2.93 (0.82)	18.33 (26.44)	8.34 (6.87)

TABLE VIII

INFLUENCE OF HYALURONIDASE ON THE SUBCUTANEOUS TOXICITY OF VENOM SAMPLE BOTHROPS JARARACA L-5 FOR FEMALE WHITE MICE, AT LOG-DOSE INTERVALS OF 0.2 AND WITH 10 ANIMALS PER DOSE; PROBIT ANALYSIS.

Experiments			Animals				LD_{50}		χ^2 (degrees of freedom)		Hetcroge-neity factor	Regression coefficient		
			Weight in g		Number									
N°.	Date	Substance added to venom	average	range	total	Included in statistical analysis	mg/kg	Fiducial limits at p = 0.05	normal	pooled		b	Fiducial limits at p = 0.05	g criterion
34	3-12-51	saline	18.5	16-21	140	70	8.74	2.42-31.42	$13.2_{(5)}$	$9.6_{(4)}$	2.39	2.91	1.09-4.72	0.78
55	"	hyaluronidase 0.15 γ/g.	18.7	17-21	140	80	10.40	7.80-13.48	$11.5_{(6)}$			3.35	2.08-4.63	0.14
56	"	" 0.30 γ/g.	18.5	16-21	140	60	9.16	7.40-11.53	$3.1_{(4)}$			4.92	2.87-6.97	0.75
57	"	hyaluronic acid 0.6 mg/g	18.5	17-20	140	70	16.95	13.11-21.74	$2.3_{(5)}$			3.79	2.35-5.24	0.15
35	11-1-52	saline	19.0	17-21	80	70	14.68	6.78-34.89	$11.4_{(5)}$	$9.4_{(3)}$	2.27	3.15	1.35-4.96	0.56
58	"	hyaluronidase 10:3	20.2	18-23	90	80	11.85	9.01-15.63	$5.0_{(6)}$			3.45	2.20-4.69	0.13
59	"	hyaluronic acid 1:2.5 ...	18.7	17-20	110	60	9.94	7.53-13.11	$4.6_{(4)}$			3.65	2.16-5.14	0.17
60	"	" " 1:5.0 ...	18·7	17-20	110	60	7.78	6.10-10.00	$3.7_{(4)}$			4.56	2.64-6.49	0.18

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ESCORPIÕES E ESCORPIONISMO NO BRASIL

IV. Considerações em torno de substâncias escorpionicidas e outras medidas de combate aos escorpiões.

WOLFGANG BÜCHERL

(*Laboratório de Zoologia Médica, Instituto Butantan*)

INTRODUÇÃO

Desde 1949 temos realizado experiências para verificar até que ponto se poderiam empregar *substâncias insecticidas* e outras medidas quaisquer no combate às duas espécies de escorpiões mais comuns e perigosas, em nosso meio, para o homem, o *Tityus serrulatus* e o *T. bahiensis*.

De 1949 para cá foram feitos ensaios isolados.

Octavio de Magalhães (1) experimentou o D. D. T. Etiénne Sergent (2) demonstrou as qualidades escorpicionicidas da mesma substância em realção a alguns escorpiões africanos. Lordello (3) usou o Rhodiatox e o B. H. C.. Lopes da Silva (4) obteve resultados promissores no tocante ao D. D. T. e principalmente o B. H. C., quando da campanha anti-escorpiônica de Ribeirão Preto. O Serviço de Profilaxia da Malaria veio confirmar estes resultados na recente luta profilática de Belo Horizonte.

Não era interessante, evidentemente, repetir simplesmente o que já tinha sido feito. Uma análise acurada, porém, dos resultados globais, obtidos com êstes insecticidas na luta contra os escorpiões, deixava entrever certas falhas, a exigir maior número de experiências, em ambiente de trabalho mais favorável, com testes de crítica mais precisos e, antes de tudo, com maior número de escorpiões por experiência, fazendo-se acompanhar a cada ensaio com o mesmo número de testemunhas, mantidos, o mais possível, nas mesmas condições de ambiente. No cômputo do cálculo final dos sucumbidos pela substância escorpionicida, podem, então, ser deduzidos os de *morte natural*.

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