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34. VENOM AND ANTIVENIN SPECIFICITY: MODERN CONCEPT

AFRÂNIO DO AMARAL

(*Brazil*)

An expression of the changes our knowledge of biological phenomena constantly undergoes may be found in the gradual evolution of the notion of specificity of venoms and antivenins since the beginning of the present century.

At the implantation stage of Serumtherapy, when Calmette (1894), based on tests and injections made with the first batches of antivenin he had prepared to counteract the effects of the envenomation caused by "Cobra" (*Naja naja*), stated the anti-neurotoxin was devoid of specificity, a serious generalization of the concept thus expressed by a such a prominent pioneer of Science, commenced rapidly spreading out. Fortunately, Calmette's radical theory did not last long. As a matter of fact, since 1897 and, more precisely, 1901, following Vital Brazil's original demonstration in favour of the opposite view, a succession of experimental studies was published by specialists from all over the world (McFarland, 1901; Rogers and Lamb, 1906; Ishizaka, 1907; Noguchi, 1909; Arthus, 1911; Gomes, 1920; Houssay and Negrete, 1923; Amaral, 1921-27, besides other investigators at a more recent period) showing specificity to be a normal phenomenon between venoms and antivenins. The adoption and generalization of this conception, however, did not occur before successive technical advancements were made in Bio-Chemistry and Physico-Chemistry so as to dissipate many doubts and remove serious obstacles researchers had encountered in their way.

The existence of specificity in this field is no longer a matter of controversy. Any restriction still to be heard in this respect is rather a reaction to prove the notion of specificity to be broader than we were at first led to suppose.

It is true we admit that this property, normally confined to the morphological range of any species of veneniferous animal, is apt eventually, but partly anyhow, to reach closely allied forms through the intervention of factors leading to speciation.

Be as it may, such an extensiveness would in no case cross the boundaries of the genus wherein those forms happen to have been placed. In thesis, such a limitation does exist in view of the fact that the specificity principle implies the solidary and uniform intervention of the entire succession of activities on the part of the numerous constituents of the molecules forming every venom, most of which have a protein nature.

The behaviour of such substances in regard to heat, diffusion, dialysis, chemical affinities and bio-immunologic reactions is so consistent as to have led researchers to include them in the group of the "antitoxinogens" (Zinsser, 1923).

In the course of numerous investigations it was possible to connect venom nocuousness towards the organism of other animals with the presence of certain toxins and especially of a large series of enzymes.

The number of enzymes found in venoms has been steadily increasing as a direct result of many improvements introduced in laboratory technic, which are rendering their identification possible.

The very number of toxins that have been recognized in a few venoms, such as "crotoxin" connected with the South American rattlesnake (Slotta *et al.*, 1938) has increased through further researches (Gonçalves, 1950; Neumann and Habermann, 1955), "crotamin" (1) and "crotactin" deserving special mention as new principles.

As a matter of fact, variation in the composition of that crotalic venom was foreseen some forty years ago (Amaral, 1925-6) (2), when the presence of yellow pigment was consistently noticed in that excretion so as to characterize the rattlesnake population found in North-Eastern Brazil.

Further knowledge of the chemical constitution of that venom was enhanced by the application of improved and more sensitive technical processes such as electrophoresis (Slotta, 1938; Gonçalves, 1950), chromatography and double microdiffusion (Schenberg, 1959-63). Under the stimulus received from such findings, the characterization of other venoms was also attempted with a view to identifying the nature of their chemical constituents, the constancy of which, connected with genetic factors, is used as a means for telling apart specimens representing even sub-racial forms.

As regards the presence of immunologic variants already traced in some venoms (that of *B. neuwiedii*, for instance), their possible connexion with racial differences is a question under investigation (Schenberg, 1963).

In view of the existence of variations in the content of the venom from different specimens of the same morphologic species, even the composition of mixtures or batches (prepared either at different periods or at only one occasion) of several samples of a definite venom even though it be secured from numerous specimens all from the known species range may not always be the same (Schoettler, 1951). This variability is likely to assume a clinal or ecologic character related to the "niche" where those specimens have been captured (Amaral, 1956). In the light of these observations it is easy to admit the existence of "biological races" in such species.

A good example of that occurrence we pointed out (Amaral, 1956, WHO/B5/373) while comparatively examining two populations of *B. jararaca*, one from Cruz Machado (Paraná), the other from Timbó (Santa Catarina), separated from each other by the Iguaçu river. Morphologically, they were undistinguishable. Pharmacologically, although the venom from specimens really from C. Machado showed a toxicity (MLD) comparable to that from specimens really from Timbó following intra-venous inoculation (rapid blood-clotting and toxinic effects), the latter venom was about 50% more deadly than the former when given hypodermically (slow, general enzymic effects). This is one of the reasons for us not to advise the use, either in titration of venoms or in standardization of antivenins, of intravenous injections, since this is apt to provoke the primary intervention of substances involved in the blood clotting mechanism.

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(1) Acting like "apamin", the bee-venom basic polypeptid.

(2) Rev. Mus. Paulista, **15**:91; Bull. Antivenin Inst. of America, 1928, **3**:6.

Moreover, the neurotropic venoms (typically toxiniferous), when kept under ordinary, uncontrolled, conditions, are likely to keep their activity for a long time, whilst the cytotropic ones (typically enzymophorous) gradually lose activity. This inactivation as measured through toxicologic tests may reach 60 to 80% of the original figure.

In the light of these facts the conception of homogeneity and stability of the general composition of venoms (at least those under scrutiny) is no longer tenable.

Specificity reflects a combination at definite proportions of a series of principles in any venom; its derangement will follow any handling or treatment that may be partly or totally destructive to such biochemical principles by enhancing splitting or cleavage of their basic constituents and by altering their balance and normal ratio.

Although most of such principles are known to act as "antitoxinogens" a few of them (including mucin, an impurity mixing with them through the much used and abused process of oversqueezing the snake glands) appear to be non-antigenic. This might explain why, in certain cases, not all of the active principles are neutralized even by an otherwise potent uni-specific antivenin. Moreover, even uni-specific antivenins, when given late in the course of ophiotoxicoses, are likely not to neutralize those new noxious substances resulting from the interaction of preformed, normal constituents of the venom with the victim's tissue and blood proteins.

In their present general connotation, zootoxicoses represent chain-reactions initiated by proteinases and intensified by the intervention of other enzymic substances (besides specific toxins) successively acting on different tissues as well as on the very products issuing from cell lysis (Amaral, 1959) (3).

Due to the variable composition of the venom from specimens representing a definite morphologic species but proceeding from different clines or "niches" the preparation of an uni-specific antivenin for exclusive use in the corresponding area appears to deserve consideration. However, this ideal solution, no matter how justifiable it may be from a scientific standpoint, really is economically unsound. Indeed, it would involve the necessity of multiplying beyond acceptable limits the collecting and preserving in a separate container every individual venom (this without mentioning the influence of possible seasonal variations in venom composition) to be employed in immunizing any group of animals for serum production.

In view of the economic contra-indication to the routine use of such a solution, one might resort to the following expediency:

a) securing, preferably through electric stimulation, the venom from specimens proceeding from the greatest number of localities lying within the recognized range of the corresponding species;

b) preparing antivenins for the greatest number of species (races and subraces) within a certain genus so as to possibly cover all the types and subtypes of toxic and antigenic representative principles of that group (multi-specific but uni-generic antivenins);

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(3) Ciência e Cultura, 11:176.

c) titrating, biometrically, every batch of antivenin against either the venoms employed in immunizing the animals or at least that in the molecule of which the greatest number of active principles common to that group may be found.

Due to the complexity of their composition, venoms seem to stimulate, at variable degrees, the production of neutralizing substances: anti-neurotoxic, anti-cytotoxic or anti-enzymic. In certain venoms, especially in the highly enzymophorous group, the rate of inert, non-antigenic, substances appears to be higher than in the toxiniferous group. This may explain why it happens quite often for the antivenins produced even through an advanced immunization procedure not to be so potent as the usual bacterio-antitoxins. In South America, the anti-cytotoxic potency of antivenins happens to be ponderally higher than the anti-neurotoxic potency. As a matter of fact, among venoms used in immunization the neurotoxic ones have a greater activity, as expressed in MLDs, than the cytotoxic.

For titrating antivenins the best process — although not yet the ideal nor equally efficient for every case — among those thus far devised and developped implies the biometric calculation of the results secured on white mice, all homozygotic young males of the same weight, serially injected subcutaneously with decreasing dilutions of antivenin mixed with a fixed dose of the corresponding venom (× MLDs), this antigen being lyophilized and preserved away from light and humidity (Amaral and Schoettler, 1956, WHO/BS/364).

Brazilian contribution — The Brazilian contribution to the advancement of this branch of Science has a rather long and uneven evolution. At first, it coincided with the very growth of this Institute, which happened to become one of the world centres devoted to the study of zootoxicoses thanks to V. Brazil's pioneering activity. This fact notwithstanding, the present stage of our knowledge has not resulted either from a rapid progress or from unforseen discoveries. On the contrary, it has come about through several, successive and discontinuous attempts at abating the surrounding gloom until a few rays of light would appear that started clarifying this highly tangled field of investigation.

We are just beginning to unhide a few of the curious mechanisms, which, by intervening with the development of the most complex phenomena connected with the physiopathologic activities of venoms, have for so long a time kept among the *Naturae arcana* (the *Physeos mysteria* of Aristotelianism) the real meaning of those toxic secretions in their multiple effects on the human and animal tissues.

Our modest role and meager share in those developments had incipience, firstly while we had the luck to be one of V. Brazil's collaborators at Butantan (as a matter of fact, we represent the only survivor of that group still to be interested in zootoxicoses); secondly, as the result of a mere accident, after we were promoted to head the Butantan Laboratory of Medical Zoology, previous to our being commissioned in this Institute's directorship as one of V. Brazil's first successors. In that double capacity have we been both an instrument and a witness in the amazing, gradual unveiling of the secrets surrounding the phenomena of venoms.

Although quite long, the history of our "prise de contact" with problems related to venom and toxin titration, is not sufficiently known. For this reason we feel that, at the last quarter of our life of scientific researcher, we ought at least to touch upon the series of the most impressive experiences we had while dealing with such questions. And so, it might be pertinent for us to do it at

the very moment so large a group of exponential figures in the field of zoo-toxicoses is attending this International Symposium. This will also give us an opportunity to explain to all of you, particularly those who have shown interest in learning the scientific evolution of Butantan particularly for the last 40 years or so (which were complicated by the world's greatest economic depression and political instability leading to the recent international conflict), the changes that have taken place here, to wit:

By the middle of 1919, when V. Brazil decided to retire from the position he so much elevated in excellence at this Institute, we were engaged in an investigation, on the mosquitoes of São Paulo, at the Parasitological Laboratory under J. Florêncio Gomes. Much to our misfortune, Gomes was then caught by the epidemic influenza called "espanhola" and died from it. We were thus compelled, all of a sudden, to assume the heavy duties of chief of Medical Zoology, this being Butantan's main Section.

1 — As though this responsability were not sufficient to challenge our youth's energy, one more, out of the six laboratories existing at Butantan at that remote period, that devoted to Tetanus Serumtherapy had also be assigned to us, as one more contribution to avoid Butantan's work from collapsing.

There, while using the process of Anderson and Rosenau for testing tetanus toxin on guinea-pigs reared at the Institute Breeding Station, we came across an extraordinary case of individual variation as disclosed by their capacity to react against the established MLD of that antigen.

2 — In the 1919-21 period we found the *B. jararaca* venom, as extracted from several specimens, purified by centrifugation and intravenously injected (according to the technic used here) into adult pigeons (350 g) (of the race *Columba livia domestica*) not tc show consistent toxicity, its MLD varying sometimes between 0.016 and 0.025 mg.

3 — In that period we also verified that the venom of *B. insularis*, a Crotalid we described from Queimada Grande Island, where it lives on trees and feeds on small birds, was much more toxic to the pigeon than the venom of the homologous "jararaca" of the mainland, living on the ground and feeding generally on rodents (4).

4 — In 1921, we showed that the experimental intoxication caused by the Texas rattler (*C. atrox*) venom, although yielding much more markedly to the injection of the specific antivenin (that we had just prepared by using a batch of venom sent by our good collaborator, R. Ditmars, of the Bronx Zoo), also favourably reacted to the injection of the antivenin specific for the S. A. rattler still called *terrificus*. The cross-tests as performed intravenously into pigeons showed the following striking differences in toxicity:

A)	Venoms: *atrox* MLD	0.200 mg (in weight)	
	terrificus MLD .	0.001 mg (in weight)	
B)	Sera: anti-*atrox* titre ...	15 MLD (3,000 mg in weight)	of *atrox* venom
		23 MLD (0.023 mg in weight)	of *terrificus* venom
	anti-*terrificus* titre	1.000 MLD (1,000 mg in weight)	of *terrificus* venom
		6 MLD (1,200 mg in weight)	of *atrox* venom (5)

(4) Col. Trab. Inst. Butantan, 1920, 2:53; Anex. Mem. Inst. Butantan, Ofiologia, 1921, I: 44, 88.

(5) Col. Trab. Inst. Butantan, 1921, 2:171.

These observations, which date from about 45 years ago, were all borne in our mind and, if not published, registered in our note-books for further investigations whenever we could find the means for correctly performing them and subjecting their results to scientific analysis. No matter how faithfully we had accomplished our student's duties at College and the Medical School, we could not satisfactorily explain the amazing facts we have just tried briefly (not to tire this audience) to point out, in the light of the knowledge of biological phenomena prevailing at least in our midst at that really remote period of our activity.

Faced with the impossibility of finding the reason for such differences and variations, and led by the desire to penetrate the secrets underlying those intricacies, we decided to take advantage of the traveling prize we had received from the Medical School in order that we could take post-graduate courses at the outstanding University centres and research laboratories both in North America and Europe, our longer stay abroad being facilitated by a special fellowship we received from the International Health Board. We had thus a chance carefully to study different subjects connected with this Institute's work line and especially to enlighten our mind concerning bio-tests and titrations.

Through the personal contact we succeeded in establishing with a few of the great scientists of that time, not only (and particularly) at Harvard University but at other American and European institutions, we learned the ways and technic of investigation as followed by such prominent men as Th. Barbour (Comp. Zoology) — with whom we enjoyed the privilege of collaborating for several years — and G. Parker (Comp. Physiology) — in Cambridge; W. Cannon, C. Drinker, W. Porter and A. Redfield (Exp. Physiology), W. T. Richards and E. Bovie (Physico-Chemistry), E. J. Cohn (Physiol. Chemistry), H. L. Henderson (Blood Phys.-Chemistry), E. W. Wilson (Bio-Mathematics), R. Pearl (Bio-Statistics), W. Castle (Genetics), H. Zinsser (Immunology), M. Rosenau (Serology), E. E. Tyzzer (Comp. Pathology), R. Strong (Trop. Medicine) — all in Boston; J. Macleod and F. Banting (Bio-Titration:insulin) — in Toronto; E. V. McCollum (Nutritional Chemistry) — in Baltimore; Wm. Park (Serumtherapy) — in New York; J. Kolmer (Lab. Testing) — Philadelphia and E. C. Kendall (Hormonal Biochemistry) — in Rochester, our experience in Europe having covered the Lister Institute (London), Inst. Pasteur (Paris), St. Serum Inst. (Copenhagen), Inst. f. Schiffs-u. Tropenhygiene (Hamburg) and Instituto Sieroterapico (Milano). During our second sojourn abroad and before returning home we talked with some of those teachers and investigators over the main problems — both of technic and personnel — we had to face at Butantan. In Europe, we also secured advise as to the selection of a group of specialists we wanted to invite to come to São Paulo as collaborators in our plan of expanding this Institute so as to transform it into a centre of Experimental Medicine especialized in Human Pathology. This seemed, indeed, to be the logical, the necessary step for us to take, in the development of the ideal that led V. Brazil to found this institution. We thus attempted to catch up with the never so mobile trend scientific investigation was already revealing at that time.

That plan as presented to and approved by our Government following their invitation for us to return and modernize Butantan, called for the organization, to start by 1931, of whole new Departments to deal, respectively, with Experimental Genetics (and Cyto-Embryology), Bio-Chemistry (and Pharmacology), Physico-Chemistry, Immunology (and Serum-Therapy), Virus (and Virus-The-

rapy), Physio-Pathology (with Physiology, Endocrinology and Histo-Pathology), Parasitology (with Entomology) and Medical Botany (with Pharmacognosia), most of which were then a novelty in our "milieu".

Needless to say that the dynamics of that transformation implied the real integration of research through a close cooperation of the numerous scientists involved therein, with the hope that it might set an example in an environment such as ours, so well known for its individualism. A sketch of that plan may be found in Mem. Inst. Butantan, **VI**, 1931 *et* **X**, 1935 in their "Noticiário" section.

Concerning venoms and antivenins: we immediately decided to synchronize the action of the new group of scientific collaborators and to take advantage of the modern laboratory equipment we were installing at Butantan, in order to tackle the fundamental problem of properly analysing the chemical composition of venoms with a view to purifying them in such a way as to make it possible for the exact properties of their active constituents to be pharmacologically determined. Moreover: the dream we started to cherish at Harvard as early as 1924 called for the eventual synthetic production of pure principles to be applied as antitoxinogens instead of whole, crude, venoms, so that the preparation of really specific antivenins could leave the empiric stage in which it lay for so long a time, and follow a really scientific direction, thus also opening the way for the establishment of a rational procedure in venom titration and antivenin standardization.

Besides several promising colleagues such as Lemos Monteiro, J. Travassos and Vallejo-Freire (Virus), Flavio da Fonseca and Paulo Artigas (Parasitology and Entomology), J. R. Valle and R. F. Mello (Physiology and Endocrinology) and others, the following specialists came to work here: G. v. Ubisch (Prof. Heidelberg Univ.), in Genetics; K. H. Slotta (Prof. Breslau Univ.) and his assistants H. F.-Conrat and G. Szyska, besides Kl. Neisser (Berlin Univ., assistant to Nobel Prize Prof. A. Windaus), in Bio-Chemistry; D. v. Klobusitzky (Frankfurt Univ. Prof. W. Pauli's pupil) and P. Koenig (Wien Univ.), in Physico-Chemistry; Prof. Thales Martins (Inst. Oswaldo Cruz, Rio) and M. F. Amorim (São Paulo Med. Faculty), in Physio-Pathology; the great Prof. Pirajá da Silva (Bahia Med. Faculty, retired), in Medical Botany, besides the famous Prof. Ludwig Fraenkel (Breslau Univ.), who joined us as a volunteer at the Endocrinological Laboratory; the dynamic Werner Schoettler (who as a student at Berlin Univ. had already been one of our collaborators through the Antivenin Institute of America); and, at a later date, the cautious Saul Schenberg, who, like, W. Schoettler, became engaged in pharmacological experimentation.

Results — Among the many facts that were brought to light at Butantan at that period, besides many others but unrelated to the object of this Symposium, the following seem to deserve special mention at this time:

a) At the Genetic Dept., v. Ubisch showed the guinea-pigs reared at our Breeding Station not to represent a homozygotic colony but to descend from an extensive and long-standing hybridization between *Cavia porcellus* and *C. rufescens*, hence their variable response to toxin and venom.

b) Our local pigeon is about twice as susceptible to *B. jararaca* stabilized venom as its N. American homologous, in the light of comparative experiments we

made at Butantan Ophiologic Dept., at Harvard Univ. while teaching there and at the Antivenin Institute of America in its organization period.

c) At the Physico-Chemical Dept., "bothropotoxin" as a blood coagulant was prepared from *B. jararaca* venom by v. Klobusitsky and Koenig.

d) At the Bio-Chemical Dept., Slotta and colls., started the isolation of the neurotoxic principle from *terrificus* crotalic venom, the substance still bound to phospholipase A having been called "crotoxin" and "crotactin" (N. *et* H.), when separated from it. This pioneering piece of work has enhanced further investigators in Brazil and abroad (M. Gonçalves, Ribeirão Prêto Med. School, 1950; A. Barrio, Inst. Malbran, 1954; S. Schenberg, 1959, O. V. Brazil *et al.*) to develop the analysis of that substance and recognize "crotamin" also as an active principle in that venom.

e) At the Ophiological Dept., an extensive study of the Brazilian and the Neotropic serpents was made, two general Check-Lists having appeared preparatory to the publication of our "Iconographic Catalog of the Serpents of Brazil" with coloured plates and Portuguese and English texts (in press). In the 1929-1937 period, our official journal "Memórias do Instituto Butantan" was issued quite regularly, numerous studies having been published in its volumes IV to XI (8) as original contributions from some Departments of this Institution. Vol. IV of our "Memórias" covered 2,764 pages, all taken up by the afore-mentioned Check-Lists besides many other articles prepared by the Director of this Institute and Chief of its main Ophiological Department. Previous to that period, a preliminary study as based on the examination we carried out from 1920 to 1924 of the differential characters of over 6,000 specimens (mostly living ones) of the main species of Neotropic pit-vipers of the genus *Bothrops*, which had been misidentified by the great G. A. Boulenger (in Cat. Sn. Brit. Mus. IV, 1896), was published as No. 2 Contribution from the Harvard Institute for Tropical Biology and Medicine, 1925. That revision pointed out many misleading differences particularly connected with the ontogenetic evolution and frequent individual variations in the chromatic characters of those pit-vipers. And as a complement to such a work we published an article (in Amer. J. Trop. Med., **IV**, 5 1924) on the differentiation of *B. atrox*, *B. jararaca* and *B. jararacussu* venoms by their M. L. D., coagulability by heat, proteolytic, hemolytic and hemocoagulant activities, venom-antivenin cross-neutralization and serum-precipitin tests.

f) At the Ophiological and Physio-Pathological Depts., the method of treating human ophiotoxicoses was scrutinized and, following improvement, has of late been introduced into the routine work at the Butantan Infirmary.

In this connexion we may say that our first personal contribution towards rationalizing specific therapeutics of envenomation consisted in the establishment of the following fundamental principle that had thus far been overlooked: the dose of any antivenin to be given to a patient (either human or animal) must be inversely proportional to his body weight, since the lighter the victim proportionately the greater the concentration of the venom in his tissues (Amaral — in *Bull. Antivenin Inst. America*, **1**:77, 80, 1927, et *Mem. Inst. Butantan*, **5**:223, 1930).

g) Through the joint work of the Ophiological and Pharmacological Laboratories, an analysis of the most rational process for testing venoms and titrating antivenins has been under way for many years although not with the desirable continuity.

As explained in a special report we prepared in collaboration with W. Schoettler for publication through the WHO (BS/364/1956) and now brought up to date, in that work a few preliminary precautions must be taken so as to eliminate many causes of error and reduce variants to an acceptable minimum, to wit:

A) *Concerning the snake:*

a) Specimens must proceed from or be secured in definitely identified places;

b) When taken into the laboratory, every specimen must be kept undisturbed and under constant environmental conditions, in a separate cage (with number and full data on tag), to be properly fed and cared for (WHO/BS/373/1956).

B) *Regarding the venom:*

a) Forceful extraction must be avoided to prevent mixing the proper excretion with other gland constituents and mucus;

b) For titration and standardization purposes, it is advisable to secure the venom by means of the electrically induced bite through a special rubber membrane so as to avoid injuring the snakes teeth and exceeding the normal limits of pressure set by the natural contraction of the muscles involved in the bite mechanism;

c) The venom, ejected into a laboratory glass, must be immediately and successfully centrifuged and dehydrated, either from the liquid or from the frozen state, under vacuum at either room or lower temperature, or through Stokes' lyophilizer at 0.1 mm Hg;

d) As to storage, apparently keeping in neutral-glass tubes filled with N at athmospheric pressure and kept in dark at $-10^{o}C$ is a safe way to warrant preserving every active principle in a venom. For obvious reasons, the desirable establishment of any "reference preparation" must be based on a rationally extracted and properly preserved venom.

C) *Respecting venom testing:*

Among the technics thus far devised for this purpose there seems to deserve preference, for reasons both economic and biologic, the probit method through hypodermic injections into standard homozygotic mice, every precaution being taken to warrant a correct statistical computation of results and all tangible causes of error being avoided, inclusive such shortcomings as: age/weight variations; sex differences (pregnant female mice usually being more resistant than the male); greater or lesser local trauma, with loss of

plasma as held up in the oedema area (resulting particularly from injection of enzymophorous venoms), responsible for the dehydration followed by collapse of some of the test animals; environmental physical factors prevailing at the breeding quarters, etc.

D) *As to antivenin titration:*

Preliminary, the following facts must be borne in mind: a) The MLD of a venom type per unit of weight varies from species (perhaps also from race to race) of animal; the relative potencies of antivenins, for reasons until unknown and calling for further investigation, may show variations when assayed on different species of laboratory animal; different animals show different susceptibility to different venom types. b) The absolute and relative resistance of man to venoms is unknown. c) The intimate mechanism of death of man and even laboratory animals in every type of ophiotoxicosis is still a matter of speculation and so is its possible and complete inhibition by antivenin.

REMARKS — In view of so many fundamental and unknown factors being involved in the mechanism of death caused by the numerous types of venom, it seems necessary to extend our comparative scrutiny to larger animals such as dogs and monkeys as an approach to our learning the ways man reacts to venoms and antivenins. We feel that, at the present stage of our knowledge, even the method (which we consider reasonable) based on the determination of the relative potency of an antivenin by assaying various amounts of serum against a fixed dose of venom would be generally accepted only when its efficiency should pass a test performed on larger animals. In this connexion we might mention that, while organizing the Antivenin Institute in the U.S.A. some 40 years ago, we decided to resort to *Rhesus* monkeys on which to test the activity of the first batches of the Nearctic antivenin we had prepared, in order to give satisfaction to the officers of the Hygienic Laboratory, in Washington, as to the therapeutic efficacy of that product. Concerning dogs and monkeys as testing animals, one important aspect that seems to have been overlooked is that, in order for the most tangible variants to be removed, it would be necessary for those animals also to be reared at Breeding Stations so as to warrant the formation and maintenance of homozygotic stocks.

CONCLUDING HINTS — The reorganization of Butantan always as a State institution, having commenced in 1931, was near completion by 1937, having thus passed a most difficult period following the 1930 Revolution and opening the era of successive political crises, social unrest and economic distress Brazil started to experience. Much to our regret and shame, at the very moment we were beginning to get the fruit of our reorganizational plan, there came the well-known "coup d'État" of 1937, responsible for the improvised statization of this country. As happened in Europe all through traditional centers of culture such as Germany, Italy and other countries, in Brazil the new regime made havoc and preferred negative selection as the rule for filling positions of responsability. Instituto Butantan as a State organization was profoundly affected: many of the members of its scientific staff were displaced. Whilst some of them luckly were engaged by local Biological Laboratories and others were made teachers at

Medical Schools, a few had to leave Brazil to continue serving Science. Amongst these we may mention G. v. Ubisch who went to work in Genetics at Leyden University; K. H. Slotta, who, as the head of the Biochemical Dept. of the Miami University Medical School, immediately took up chemical analysis of the ative constituents of blood involved in coagulation; and H. F.-Conrat, who joined, at the University of California, the group under Prof. W. M. Stanley, with whom he soon succeeded in separating and re-synthetizing the active molecule of a virus of vegetable mosaic; not to mention Prof. L. Conrat, who left for Uruguay where he continued cooperating towards the progress of Medical Science.

In more recent years, scientific research was taken up again on Physio-pathology by G. Rosenfeld and his assistants, on Bio-chemistry by S. B. Henriques and his group, as well as on Cyto-genetics, first under the leadership of Prof. G. Schreiber with collaboration from H. Belluomini and others, and lately by W. Beçak and assistants, on Arachnidology by W. Bücherl and on Ophiology by A. R. Hoge.

Fifteen years had not yet elapsed when, in view of the Brazilian reconstitutionalization, we made a new attempt at modernizing Butantan (cf. "Noticiário" in *Mem. Inst. Butantan*, **XXVI**, 1954). In order that it might be freed from strange influences used to causing periodic slowing crises at Butantan, we tried to have it changed into an autarchic organization. Unfortunately, Brazil, still as an underdevelopped country in want of an organized, watchful public opinion apt to uphold any sensible plan of scientific investigation, proved not to be ready yet to take Science seriously or even to comprehend our programme to keep this institution true to the sprit that dictated its foundation, as an homage rendered to its first director, in whose honour this International Symposium is being held. Anyhow, Butantan scientific structure has suffered so deeply, that, following our decision to retire from official duties here, after having honestly and faithfully tried to serve Brazil for 50 years, and confine our attention to the international organizations (International Commission on Zoological Nomenclature, in London, and World Health Organization, in Geneva) wherewith we have long been connected, our successor, Dr. A. Vallejo-Freire, decided to devote his energies to the extreme ("heróico" as it is called in Portuguese) plan of establishing here a foundation whereby research might recover vitality. This is our earnest hope, which we feel is shared by you all, who know the progress of human knowledge to lie on investigation.