

## FACTORS UNDERLYING THE NATURAL RESISTANCE OF ANIMALS AGAINST SNAKE VENOMS

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*The existence of mammals and reptilia with a natural resistance to snake venoms is known since a long time. This fact has been subjected to the study by several research workers. Our experiments showed us that in the marsupial Didelphis marsupialis, a mammal highly resistant to the venom of Bothrops jararaca, and other Bothrops venoms, has a genetically origin protein, a alpha-1, acid glycoprotein, now highly purified, with protective action in mice against the jararaca snake venom.*

Key words: natural immunity – snake venom – protein fraction

It has been observed, since a long time, that there are some reptilia and mammals with a natural resistance against snake venoms. Calmette (1895) makes reference to observations of Fontana, Phisalix, Bertrand, etc., and Calmette himself carried out experiments on the problem, as many other research-workers did, all looking to explain this natural resistance of venomous, or non venomous snakes, as well as the resistance of some mammals to snake venoms.

Noguchi (1904) showed that the snake *O. vernalis* has a natural immunity to crotalic venom, surviving to the administration of 5 mg of the venom. Kegan & Andrews (1942) injected several snake species with the venom of *Crotalus horridus*, *Agkistrodon moccasin* and *Sistrurus catenatus* and observed that some of the injected snakes showed a natural resistance to the venoms up to 449 µg/g. We must take into account that 100% of mice may die after injection of 0.4 µg/g or 5 µg/g of venoms from *Crotalus d. terrificus* or *B. jararaca* respectively. Resistance of snakes to snake venoms has been observed by many other research workers in 1946, 1974, 1975, 1977, 1982.

A short survey on the resistance of other species of vertebrates as birds, mammals or even

reptilia, excepting snakes, showed the scarce opportunity to find animals with the exceedingly resistance found in the snakes. Calmette has reported that the "mangoust", a mammal, Viverridae (*Herpestes mungo*), found in Asia and Africa, is resistant to the cobra venom, however its serum does not protect rabbits against the same venom. It is, perhaps, the oldest observation in mammals.

The pionering observations on birds and mammals have been made by Vellard (1949) showing that the opossum, a marsupial belonging to the genera *Didelphis*, with several species found in all Americas, possesses a high resistance to the venoms of several snakes of the Crotalidae family. The animals survived several days after the injection of doses up to 400 mg/kg, a dosis 40 to 80 times the letal dosis for mice or men. Vellard injected *Didelphis azarae* 100 mg/kg of *Bothrops alternata* venom by muscular route and the animal survived without showing toxic symptoms. We injected a *Didelphis marsupialis* with 400 mg/kg of *Bothrops jararaca* venom by peritoneal route; the animal survived several days after the injection and, at the autopsy, no hemorrhagic signs were observed in the abdominal cavity (unpublished observations).

The opossum serum showed protective action on mice against the venoms of the following snakes: *B. jararaca*, *B. cotiara*, *B. alternata*, *B. jararacussu*, *B. neuwidii* and *Crotalus adamanteus* but not to the venom of *Crotalus d. terrificus*.

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Fig. 1: rabbit hindlegs injected with *Bothrops jararaca* venom and opossum serum. A. Up: Rabbit hindleg injected with *B. jararaca* venom by muscular route (1 mg/0.2 ml saline). B. Down: Rabbit hindleg injected by muscular route with *B. jararaca* venom (1 mg/1 ml opossum serum).

The presence of a serum factor responsible for the resistance of snakes to their own venoms has been showed by several authors. Rosenberg & Glass (1946) showed that both the serum of a rattle snake and a non venomous snake, *Lampropeltis getulus getulus*, inhibited the hemorrhagic effect observed in rats when injected with *Crotalus adamanteus*, *Akgistrodom piscivorum* and *Bothrops atrox* venoms. Similar results have been obtained by Philpot & Smith (1950) showing that serum of *L. getulus* protects against the toxicity of *C. adamanteus* and *A. piscivorum*.

Clark & Wortis (1969) found an albumin in the *Crotalus atrox* serum with protective properties against this venom. Snyder (1976), working with *C. atrox* serum, found by polyacrilamide electrophoresis, a band just behind the albumin fraction, giving carbohydrate reaction and protecting against the venom.

Since hemorrhage is one of the most frequent signs of the effect of snake bite, serum fraction with antihemorrhagic action has been studied

by some researchers. Omori-Sato (1972) obtained from the serum of the venomous snake *Trimerosurus flavoviridis* a serum factor inhibiting both the toxic and the hemorrhagic effect of the venom. Ovadia et al. (1972) isolated from the serum of *Vipera palestina* an antineurotoxic fraction, possessing also anti-hemorrhagic action. Neurotoxicity and hemorrhage are signs of *Vipera palestina* biting. Analysing the neutralising action on the venoms by the factors isolated from Viperidae and Elapidae, led the authors to the concluding remarks that, together with the humoral mechanisms, there are also no humoral mechanisms underlying the resistance to the venomous toxicity.

#### *Isolation and purification of an antiothropic serum fraction from Didelphis marsupialis*

Reference has been made to mammals resistance, specially of marsupialis of the *Didelphis* genera to snake venoms of the Crotalidae family. It was reported for the first time by Vellard (1945, 1949) but without particular

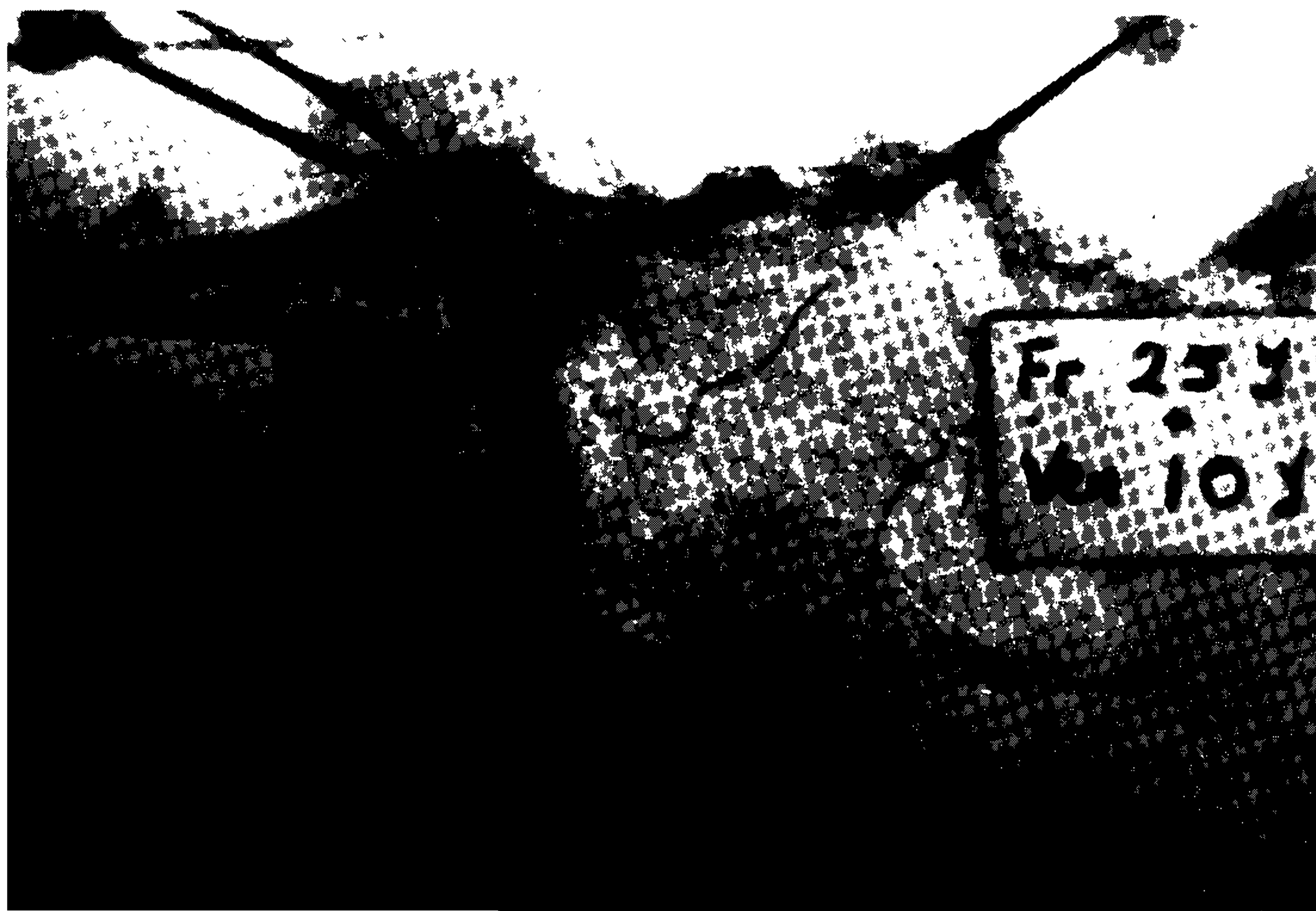


Fig. 2: protection by opossum serum fraction of hemorrhagic action of *Bothrops jararaca* venom. Right side: 10  $\mu\text{g}$  of *B. jararaca* venom (0.25 ml). Left side: 10  $\mu\text{g}$  of *B. jararaca* venom mixed with 25  $\mu\text{g}$  of opossum serum fraction (0.25 ml).

rebound within the research workers in the field. Many years later Werner & Vick (1977) confirmed the results of Vellard and carried out experiments with some other snake venoms of the same family.

Based on the results reported in that paper we started some experiments. In our first results (1977, 1980) we reported that the serum of *Didelphis marsupialis* showed that 5  $\mu\text{g}$  of lyophilised serum added to 0.5 ml of rabbit plasma, increased coagulation time produced by 0.5  $\mu\text{g}$  of *Bothrops jararaca* venom; 1 mg of *B. jararaca* venom injected by subcutaneous route together with 1 ml of *Didelphis* serum in the hindleg of a rabbit inhibited the muscular and skin necrosis produced by the venom (Fig. 1); the opossum serum protected mice against death produced by a 100% mortal dosis of *B. jararaca* venom calculated by log-dose-response; opossum serum injected by venous route (0.1 ml), around 350  $\mu\text{g}/\text{g}$  of protein, on mice (20 g), protected the animals not less than 24 h against the *B. jara-*

*raca* venom (8  $\mu\text{g}/\text{g}$ ) injected by peritoneal route.

By column chromatography on Sephadex G200 five fractions have been isolated from the opossum serum, the third one having protective action on mice against *B. jararaca* venom. Using Shibata's method for acid alpha-1, glycoprotein isolation, a fraction has been obtained with high protective properties against jararaca venom (Table). An assay with human alpha-1, glycoprotein (orosomucoid), injected in mice with 100  $\mu\text{g}/\text{g}$ , showed no protective action for jararaca venom. The opossum isolated fraction has an antihemorrhagic action for the jararaca venom and the hemorrhage produced by a fraction isolated from this venom (Fig. 2).

At present we are working for a higher purification of the opossum serum fraction, trying to discard the contaminating bands showed by the electrophoresis analysis. Column chromatography by ion exchange on DEAE-



Sephacel of opossum serum has given four protein peaks, two of them (the third and fourth) with protective action on mice. Rechromatography of fraction four showed a single bifurcated protein peak, fractions FIV-a and FIV-b, both with action against jararaca venom. Polyacrilamide electrophoresis of both fractions showed heterogeneity with, at least, three common protein bands with protective action on mice. These results led us to admit that the heterogeneity of the fraction IV represents a complex, constituted by electrostatically joined monomers.

TABLE

Protective action of an *Opossum* serum fraction against *Bothrops jararaca* venom

No. of mice	<i>B. jararaca</i> venom µg/g	Protein fraction µg/g	Dead/Total
6	5.1	—	6/6
8	5.1	10	3/8
6	5.1	20	0/6
6	5.1	40	0/6

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