Mem. Inst. Butantan
52 (1): 17-23, 1990

CARBON DIOXIDE AS AN AUXILIARY IN THE VENOM EXTRACTION OF *BOTHROPS JARARACA* SNAKES (Viperidae, Crotalinae)

Frederico Fontoura LEINZ*
Thélia R. F. JANEIRO — CINQUINI*
Masaio Mizuno ISHIZUKA**

ABSTRACT: Snakes of the species *Bothrops jararaca*, destined for periodical venom extraction, maintained in captivity during the period from November 1988 to April 1989 in the ''Seção de Venenos'' of the ''Instituto Butantan'', were separated in two lots: Lot I, composed by 24 snakes extracted under carbon dioxide anaesthesia, and Lot II, composed also by 24 snakes, extracted however without the aid of this gas. The death rate, weight of dry venom individually produced in relation to the weight of snake, dry venom rate in relation to sex, dried venom yield in relation to the length of captivity of both snake lots, was analized. The observations proved that carbon dioxide was efficient and practical as an aid in the venom extraction of *Bothrops jararaca* reducing noticeably the risk of accidents without affecting survival of the snakes and venom production. The rate of dry venom obtained from the females was higher than from the males and the yield of dry venom in relation to the liquid was of 22%. There occurred a small drop of the production in the 2nd month of captivity followed by a gradative increase up to the 6th month.
KEYWORDS: Snakes, venom extraction, carbon dioxide, captivity.

INTRODUCTION

The safe handling of venomous snakes is preoccuping researchers along the last years on account of the high risk of accidents that this operation includes. De Biasi[12] estimates that at the ''Instituto Butantan'' one accident occurs every 10.000 venom extractions, a number easily reached, since this process is repeated more or less 7.200 times during the year.

* Seção Venenos
** Faculdade de Medicina Veterinária e Zootecnia-USP
Instituto Butantan - C.P. 65 - 01051 - São Paulo - SP - Brasil
Recebido para publicação em 14.12.1989 e aceito em 13.2.1990

With the aim to reduce the accident rate, is was verified that the most used method for snake contention has been the general anaesthesia, using for this purpose injectables and inhalants substances. The injectable anaesthesics were tested by various authors (Calderwood[9], Brazenor[7], Hinsch and Gandal[17], Karlstrom[21]), the barbiturics being the drug of selection in these cases. When however a great number of venomous snakes is to be handled within a short space of time, this method becomes impracticable, not only by the indispensable handling, but also by the prolonged induction and recuperation. According to Calderwood[9], the induction period due to the low metabolism of these animals, may vary from 5 to 60 min and recuperation from 2 to 48 hs.

The use of inhalants involves less risk for the handler. Depending on the system used for the induction, the animal is introduced into the container with the aid of an appropriate hook, thus restraining the direct handling, practiced only when the snake is anaesthetized.

Ether and chloroform, although already having been employed, are not recommended on account of the delay in induction, prolonged recuperation time, small margin of safety between the anaesthetic and toxic doses, and also by risk of explosion (Brazenor and Kaye[7], Kaplan and Taylor[19], Stecher[26].

Volatile liquids as Halothane and its by products are the substances currently more used as inhalant anaesthesics. Depending on the species, induction and recuperation are prompt and only a few deaths are mentioned in consequence of their use. However, when many animals are to be handled, its use is limited by the necessity to supplement oxygen during the induction and by eventual post-anaesthesic care, that would complicate the process(Kaplan[20], Calderwood[9].

Ishii and Noburu[18] used carbon dioxide as anaesthesic in carrying out surgery in *Trimeresurus flavoviridis*. Based on this methodology, De Biasi[12] observed a lot of 28 snakes *Bothrops moojeni*, extracted with the help of this gas, and concluded that the method besides propiciating a higher protection to the extractor, prevents loss of venom when aggressive animals are handled.

At the moment, the venom extraction of all the snake species maintained at the ''Seção de Venenos'' are done with the aid of carbon dioxide, with the purpose to diminish the risk of accidents.

Since there exists a marked variation in sensibility of the species to anaesthesic drugs (Gans, in Burke and Wall[8]), it is attempted to determine the mortality rate, weight of dry venom individually produced in relation to the weight of the snake, dry venom rate in relation to sex, dried venom yield in relation to the liquid venom, and dried venom weight obtained in relation to the lenght of captivity, when two groups of *Bothrops jararaca* were extracted with and without the use of carbon dioxide as anaesthesic.

MATERIAL AND METHODS

Snakes: A total of 48 snakes *Bothrops jararaca*, were extracted at the ''Seção de Venenos'' of the ''Instituto Butantan'' during the period of November 1988 to April 1989. On receiving them from nature, they were treated against ecto and endo-parasites, maintained in quarantine for 21 days, then placed into rooms destined for maintenance. Veterinary medi-

cal care was available from the admission up to the conclusion of the experiment. The snakes that died were submitted to necropsy.

Maintenance: The snakes were individually maintained in wooden boxes, lined with corrugated cardboard, 50cm in lenght, 40cm in width and 25cm in height, within rooms with the enviromental temperature varying from a minimum of 19°C to a maximum of 30°C. No control of humidity was done, and the enviroment was kept under artificial light of fluorescent type for 8 hs per day. The cleaning of the boxes was done always as soon as the animal defecated, and once a month, all boxes were substituted by clean and desinfected boxes. The substitution of boxes was accompained by a general desinfection of the rooms.

Alimentation: Adult albinic mice *Mus musculus* were given 7 days after venom extraction, with variable acceptance of the food. Water was daily exchanged and maintained at disposal of the animals in earthen jugs, washed and desinfected every second day.

Venom extraction: At first, two lots were set up: Lot I, composed by 24 snakes, i.e., 18 females and 6 males, extracted with the aid of carbon dioxide anaesthesic. The induction followed the process described by De Biasi[12], however individually applied and not in groups. The anaesthetized animal was considered ready for extraction when it showed itself immobilized, thus impeded to make aggressive movements. Lot II, constituted also by 24 snakes, 3 males and 21 females, extracted without the help of carbon dioxide. The interval between the extractions was 28 days for both lots. The manual extraction method described by Belluomini[5] was habitally used in the ''Seção de Venenos''.

Attainment, weighing and drying of the venom: The venom was collected on individual plates weighed on an analytical precision balance, and then vaccum dessicated in a container with calcium chloride for 24 hs, then again wheighed to determine the dry weight.

Statistical analysis: The test of the difference between two ratios with normal approach (Z) and the test ''t-Student'' wete done. The rejection level of the nullity hypothesis was established as 0,05.

RESULTS

During the realization of the experiment, 4 deaths were verified in Lot I, submitted to anaesthesia with carbon dioxide and 2 deaths in Lot II, not submitted to the action of the gas. Based on these results, the test of Two Proportions with normal approximation was applied, obtaining the value of $Z = 0,88$. This value showed that there was no significance in the number of deaths in both lots the adopted 5% level of rejection, during the the 6 months of observation. The results of the necropsies of the snakes that died, are found in Table 1.

Table 1

Bothrops jararaca maintained in captivity, causa mortis and treatment.

Treatment Causa mortis	With gas	Without gas
PNEUMOENTERITIS	1	2
GASTROENTERITIS	2	—
NDN*	1	—

* NDN = nothing to note

For calculation, the dried venom production was standardized on account of the very great variation of the snakes' weight. Thus there was obtained, during the 6 months duration of the experiment, a mean production of 0,3 mg dried venom per weight (g) of the snake that produced the venom. Table 2

Table 2

Mean values of the dry venom rate (*) according to treatment and venom extraction of *Bothrops jararaca* in captivity

Extraction Treatment	1º	2º	3º	4º	5º	6º
WITH GAS	0,330 ± 0,152	0,296 ± 0,081	0,300 ± 0,121	0,311 ± 0,124	0,322 ± 0,111	0,367 ± 0,159
WITHOUT GAS	0,350 ± 0,147	0,283 ± 0,105	0,300 ± 0,110	0,343 ± 0,147	0,357 ± 0,116	0,371 ± 0,115

* Weight of dry venom (mg) per weight of the snake (g)

When the dried venom rate produced in relation to sex was compared, a significant difference was observed between both, the production of females being larger than that of the males ($t = 10,0$) for the two lots.

The mean yield of dry venom in relation to the liquid venom in both groups was 22,8%.

An analysis of successive extractions disclosed a drop in the venom production during the 2nd month of extraction, when compared with the 1st month. Afterwards, there was a gradative increase up to 6th month, when the dry venom production became larger than that of the 1st month of extraction. Table 2

The mean period of induction was 3 min and that of recuperation, 8 min.

DISCUSSION

The observation showed that the carbon dioxide was efficient as anaesthetics when a rapid induction and recuperation is desired. It is a prac-

tical method to eliminate aggressive movements of the snakes, diminishing radically the risk of accidents. Therefore it is well qualified for the necessities of the ''Seção de Venenos'', where many snakes are handled within a short time interval.

In relation to the mortality, althought the death rate occurring in both groups is not significant, an index of 16,7% of deaths was verified in the lot submitted to CO_2, and 8,3% in the lot not submitted to the gas. This index may be considered low, since the animals were monthly submitted to the trauma provoked by venom extraction (Cowan)[11]. Belluomini[4], cites a death rate of 81,7% for *Bothrops jararaca* maintained in captivity at the ''Instituto Butantan'' during 1963. For the 5 months duration of the experiment developed by De Biasi[12], the death rate of *Bothrops moojeni* reached and index of 50%. This difference may be atributed to a higher sensibility of the species, to the changes recently introduced in the management and mainly to the methodology adopted by the author, where the animals were anaesthetized in groups, thus difficulting the observation.

The two method used for the contention of the snakes, led us to analize the individual production of venom in both groups. The mean weight of the obtained dry substance maintained itself constant for both groups, corroborating the observations of several authors (Amaral[1], Klauber[22]) who affirmed that there exists a direct connection between the size of the snake and the production of venom. In *Bothrops jararaca*, the females produced more dry matter, not only because they are naturally larger and heavier, but also because the show proportionally to their weight, a higher rate of dry matter, when compared with the males, corroborating the observation of Deoras[13].

Snakes maintained in captivity, according to Klauber[22] and Belluomini[3], present a drop in the venom production in the succeeding extractions. Other authors (Wolf[28], Kochva[23], do not admit this phenomenon, continuing stable the production. The analysis of the successive extractions of *Bothrops jararaca*, either submitted or not to carbon dioxide, disclosed an abrupt drop in the 2nd extraction, when compared to the 1st, and soon after an increase of the produced dried venom rate. According to Snyder[24], adaptation to an enviroment other than the natural constitues itself in stress, when there may occur fat consumption in order to maintain the inner equilibrium of the animal, to an immediate detriment to the vital functions. The variation in the dry venom weight obtained in this phase, perhaps occurred in consequence of an adaptation period of the animal to captivity.

CONCLUSIONS

1 — The carbon dioxide proved to be practical and efficient for the venom extraction of *Bothrops jararaca* diminishing noticeably the risk of accidents without affecting the survival of the animals and the venom production.

2 — Females produced more dried venom proportionally to their body weight then the males.

3 — During the adaptation period to captivity, a drop in venom production was observed.

LEINZ, F.F.; JANEIRO-CINQUINI, T.R.F.; ISHIZUKA, M.M. Carbon dioxide as an auxiliary in the venom extraction of **Bothrops jararaca** snakes (Viperidae, Crotalinae). **Mem. Inst. Butantan, 52** (1): 17-23, 1990

RESUMO: Serpentes *Bothrops jararaca* destinadas à extração periódica de veneno e mantidas em cativeiro no período de novembro de 1988 a abril de 1989, na Seção de Venenos do Instituto Butantan, foram divididas em dois lotes: Lote I, composto de 24 serpentes extraídas com o auxílio do dióxido de carbono como anestésico, e Lote II, constituído também de 24 serpentes extraídas porém sem o auxílio deste gás. Foram analisados os índices de mortalidade, o pêso de veneno sêco produzido individualmente, a taxa de veneno sêco em relação ao líquido e o pêso do veneno sêco obtido em relação ao tempo de cativeiro das serpentes de ambos os lotes. As observações demonstraram que o dióxido de carbono foi eficiente como auxiliar na extração de veneno de *Bothrops jararaca*, diminuindo sensivelmente o risco de acidentes sem afetar a sobrevida dos animais e a produção. A taxa de veneno sêco obtido das fêmeas foi maior que a dos machos, e o rendimento de matéria seca em relação à líquida foi de 22,8%. Ocorreu uma pequena queda na produção no 2º mês de cativeiro e em seguida houve um gradativo aumento até o 6º mês.

UNITERMOS: Serpentes, extração de veneno, dióxido de carbono, cativeiro.

ACKNOWLEDGMENTS

The authors wish to thank the technicians Zilma L. da Silva e Pedro N.M. Cerveira for the meticulous weighing and extraction of the venom.

REFERENCES

1. AMARAL, A. Improved process of venom extraction. *Bull. antiv. Inst. America, 1*: 100-102, 1928.
2. AMARAL, A. Studies on snake venoms. I-Amounts of venom secreted by Neartic Pit Vipers. *Bull. antiv. Inst. America, 1*: 103-104, 1928.
3. BELLUOMINI, H. Produção de veneno de serpentes em cativeiro. *Arq. Inst. Biol.* São Paulo, *31*(4): 149-154, 1964.
4. BELLUOMINI, H. Venom production of snakes in captivity. Mem. *Inst. Butantan, 32*: 79-88, 1965.
5. BELLUOMINI, H. Extraction and quantities of venom obtained from some Brazilian snakes. In: BUCHERL, W., ed. *Venomous animals and their venoms*. New York, Academic Press, v. 1, p. 9-117, 1968.
6. BETZ, T.W. Surgical anesthesia in reptiles, with special reference to the water snake *Natrix rhombifera. Copeia, 2*: 284-287, 1962.
7. BRAZENOR, C.W. & KAYE, G. Anesthesia for reptiles. *Copeia, 3*: 165-170, 1953.
8. BURKE, T.J. & WALL, B.E. Anesthetic deaths in cobras *Naja naja* and *Ophiophagus hannah* with Methoxyflurane. *JAVMA, 157* (5): 620-621, 1970.
9. CALDERWOOD, H.W. Anesthesia for reptiles. *JAVMA, 159*(11): 1618-1627, 1971.
10. CALOW, P. Ecology, evolution and energetics; a study in metabolic adaptation. In: MCFAYDEN, A., ed. *Adv. Ecological Res. 10*: 1-62, 1977.
11. COWAN, D.E. Adaptation, maladaptation and disease. In: MURPHY, J.B. *Reproductive biology and diseases of captive reptiles*. Lawrence, Society for the Study of Amphibians and Reptiles, 1980. p. 191-6.
12. DE BIASI, P.; BELLUOMINI, H.E.; HOGE, A.R.; PUORTO, G. Uso do gás carbônico na extração de veneno de serpentes. *Mem. Inst. Butantan, 40/41*: 167-172, 1976/77.
13. DEORAS, P.J. Probable significance of venom Yield record studies. *Mem. Inst. Butantan, 33*(3): 767-774, 1966.
14. FRYE, F.L. Husbandry, medicine and surgery in captive reptiles. Kansas, V.M. Publishing, Bonner Springs, 1973. p. 112-126.
15. GANS, C. *Biology of the reptilia*. London, Academic Press, 1976. V.5.

LEINZ, F.F.; JANEIRO-CINQUINI, T.R.F.; ISHIZUKA, M.M. Carbon dioxide as an auxiliary in the venom extraction of **Bothrops jararaca** snakes (Viperidae, Crotalinae). **Mem. Inst. Butantan, 52** (1): 17-23, 1990

16. HACKENBROCK, C.R. & FINSTER, M. Fluothane; a rapid and safe inhalation anestesic for poisonous snakes. *Copeia, 2*: 440-441, 1963.
17. HINSCH, H. & GANDALL, C.P. The effects of Etorphine (M99) Oxymorphone Hydrocloride and Meperidine Hydrocloride in reptiles. *Copeia, 2*: 404-405, 1969.
18. ISHII, A. & NOBURU, Y. Eletrocoagulation of the venom duct of Habu, *Trimeresurus flavoviridis* under carbon dioxide anaesthesia. *The Snake*, 3: 35-38, 1971.
19. KAPLAN, H.M. & TAYLOR, R. Anesthesia in turtles. *Herpetologica, 13*: 43, 1957.
20. KAPLAN, H.M. Anesthesia in amphibians and reptiles. *Fed. Proc., 28*: 1541-1546, 1969.
21. KARLSTROM, E.L. & COOK JR., S.F. Notes on snake anesthesia. *Copeia, 1*: 57-58, 1955.
22. KLAUBER, L.M. Rattlesnakes; their habits, life histories and influence on mankind. Berkeley, Univ. of California, 1956.
23. KOCHVA, E. A Quantitative study of venom secretion by *Vipera palestina*. *Am. J. Trop. Med. Hyg., 9*: 381-390, 1960.
24. SNYDER, R.L. *The biology of population Grwth. St*. New York, Martin Press, 1976.
25. STADELMAN, R.E. Some venom extraction records. *Bull. antiv. Inst. America, 3*(1):29, 1929.
26. STECHER, P.G., ed. *The Merck Index*. 8 ed. New Jersey, Merck Rahway, 1968.
27. VIEIRA, S. *Introdução à bioestatística*. São Paulo, Campus, 1988. 294 p.
28. WOLFF, N.C. & GITHENS, T.S. Yield and toxicity of venom from snakes extracted over a period of two years. *Copeia, 4: 234, 1939.*