

VENOM PRODUCTION OF SNAKES IN CAPTIVITY

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INTRODUCTION

Until some years ago snakes which arrived at the Instituto Butantan were kept in the traditional snakepits of the Institute. Different species from various localities were kept together under the same surroundings and climatic conditions. The yield of venom was sufficient for the Institute's requirements.

However, while the demands for antivenoms for Brazil and South America increased progressively, the number of arriving snakes remained stationary. In order to increase venom production, several modifications were introduced in the snake maintenance conditions and a new experimental bioterium was organized with the purpose of studying the biology of snakes in captivity to prolong their lifetime and increase the number of venom extractions. Diseases occurring in captivity are also investigated.

A snake colony was constructed in an adapted building. The result of the venom production in this colony during the first five months of 1963 was published by Belluomini (1).

During the initial phase of this study an epidemic disease, later diagnosed as visceral gout by Sørensen et al. (2), killed a large number of rattlesnakes (3). An outbreak of another epidemic infectious disease-acute necrotic hemorrhagic lesion in the mouth-caused by a diphteroid was reported (4). The epidemic was controlled by prophylactic vaccination.

MATERIAL AND METHODS

SNAKES: The data reported herein were obtained from observations made from January to December, 1963. During this period the Institute received 12 901 poisonous snakes including dead ones and specimens in poor health, belonging to seven different species. Only healthy strong specimens were used and new born ones rejected. There was no selection as to size or species. The following species

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were studied: *Crotalus durissus terrificus*, *Bothrops jararaca*, *Bothrops alternatus*, *Bothrops atrox*, *Bothrops cotiara*, *Bothrops jararacussu* and *Bothrops neuwiedi*.

SNAKE COLONY

Electrically heated rooms: The heating of the rooms was controlled by electric resistances at the lateral walls at the height of 40 cm. above floor level.

Room A — Daily temperature 28-32°C and overnight 25-28°C; humidity 55-65%. Snakes were kept in wooden cages measuring 58 cm in length by 40 cm in width and 30 cm in height, with a screen in front.

Room B — Continuous temperature from 18°C to 25°C; humidity 60-70%. Snakes kept under the same conditions as in Room A.

Room C — With the continuous use of an electrically heated fan. Daily temperature 28-32°C and at night 25-28°C; humidity 60-70%. Snakes free on the floor. Drinking place with running water instead of the little ceramic dishes as in the cages in Room A and B.

The temperature and humidity were selected following data obtained after previous field observations carried out in the State of São Paulo (Avaré, Barra Grande, Itapetininga, Ribeirão Preto, Barretos and Franca); Minas Gerais (Furnas, Uberaba, Uberlândia); and Goiás (Catalão), and also following studies of climatic maps and geographical distribution of species by Köepen (5); and Setzer (6), and studies on the behaviour of snakes in captivity by Klauber (7) and Backhaus (8).

VENOM EXTRACTIONS: Venom extractions were performed every 15 days by electric shock from a 4 volts current source, with terminals applied to the oral mucosa (9). Manual extractions were made by a soft pressure on the venom glands; two days later, the snakes were fed with baby or adult mice. Individual cages were used for larger specimens of rattlesnakes, the smaller ones being placed two in a cage. In the case of *Bothrops*, four snakes were placed in each cage.

The snake colony was maintained at dim light and lit with artificial light from normal lamps during all work with the serpents. Cages and floors were cleaned every eight days without any disinfectants, soap or detergents, only water under pressure.

In order to allow comparison, the data were compiled as follows:

- 1 — From 1956 to 1962, in open snakepit;
- 2 — In 1963, obtained in the new or heated animal colony;
- 3 — Data about *Crotalus durissus terrificus*:
 - a) In open snakepits and non heated rooms (1957-1960).
 - b) Partially heated rooms, temperature ranging from 22° to 24°C (1961-1962).
 - c) Heated rooms (28-32°C temperature and 55-65% humidity).

- 4 — Data from *Bothrops neuwiedi*, *Bothrops atrox*, *Bothrops alternatus*, obtained under the same conditions as *Crotalus durissus terrificus*, 1963 (item 3, c).
- 5 — Data of *Bothrops jararaca*, *Bothrops jararacussu* and *Bothrops cotiara*, from snakes kept under variable conditions of temperature (ranging between 18-25°C) and relative atmospheric humidity (55-65%), always lower than those measured at the snakes' natural habitat.

RESULTS

Table I shows the data of venom obtained in 1963, distributed by species. The figures of the first column represent the total number of received snakes and the second, the number of snakes selected for investigations in the animal colony. The last three columns of the table contains the number of extractions performed and the respective amount of venom in ml (fluid) or in grams (vacuum — dried).

TABLE I — DATA FROM 1963 IN THE ANIMAL COLONY. NUMBER OF RECEIVED SNAKES BY SPECIES, NUMBER OF SNAKES STUDIED, NUMBER OF EXTRACTIONS AND VENOM OBTAINED IN ml (FLUID) AND mg (DRIED).

Species	Received by I. Butantan	Received by Animal Colony	Number of extractions in Animal Colony	Quantity of Venom	
				ml	mg
<i>C. d. terrificus</i>	4 259	3 312	18 661	2 279,5	532.045,8
<i>B. jararaca</i>	6 788	4 287	18 775	2 263,2	546.856,5
<i>B. cotiara</i>	430	309	1 049	157,1	34.312,2
<i>B. alternatus</i>	483	371	1 198	364,7	88.748,9
<i>B. atrox</i>	404	268	1 557	467,7	118.801,8
<i>B. neuwiedi</i>	410	341	1 612	154,8	32.155,3
<i>B. jararacussu</i>	127	85	216	127,5	30.901,4
Total	12 901	8 973	43 048	5 814,5	1.348.821,9

Table II contains the total number of snakes received by the Instituto Butantan, from 1956 to 1963; number of extractions performed in snakes maintained in open snakepits, the amount of fluid and dry venom obtained and also data from animal colonies in 1963.

As there is no record available on the number of snakes suffering venom extractions, only the total number of received snakes from 1956 to 1962, it was assumed that the same proportion was practically maintained in 1963, since the differences between the number of received snakes and extractions seem to be fairly constant. In tables III to IX similar comparisons were made for each species. As can be seen, the amount of venom yielded by the heated animal colony of *Crotalus durissus terrificus*, *Bothrops neuwiedi* and *Bothrops atrox* is larger than that of *Bothrops jararaca*, *Bothrops alternatus*, *Bothrops cotiara* and *Bothrops jararacussu*.

TABLE II — TOTAL NUMBER OF SNAKES RECEIVED BY INSTITUTO BUTANTAN, NUMBER OF EXTRACTIONS, VENOM PRODUCTION IN ml (FLUID) AND mg (DRIED) FROM 1956 TO 1963 (*).

Years	Snakes received	Snakepit	Animal Colony		Quantity of Venom	
	Number	Number of extractions	Snakes received	Number of extractions	ml (fluid)	mg (dried)
1956	14 058	10 703	—	—	1 082,2	273.720,2
1957	13 480	11 033	—	—	1 209,4	301.779,0
1958	11 966	10 360	—	—	1 338,6	334.104,9
1959	12 208	11 142	—	—	1 671,6	419.818,8
1960	11 310	10 875	—	—	1 513,1	378.663,0
1961	12 868	13 316	—	—	1 838,8	466.691,4
1962	11 076	13 340	—	—	2 026,0	475.601,7
1963	12 901	—	8 973	43 048	5 814,5	1 384.821,9

(*) As the number of snakes actually extracted from 1956 to 1963 was not recorded, it was assumed that the proportion observed in 1963 was the same as in the precedent years (from 1956 to 1962).

TABLE III — *Crotalus d. terrificus* — NUMBER OF SNAKES RECEIVED AND NUMBER OF EXTRACTIONS (1956-1962) COMPARED WITH THOSE OBTAINED IN THE ANIMAL COLONY IN 1963.

Years	Snakes received	Snakepit			Animal Colony			
		No. of extractions	Venom		Snakes		Venom	
	Number		ml (fluid)	g (dried)	Received	No. of extractions	ml (fluid)	g (dried)
1956	5 364	5 459	533	131	—	—	—	—
1957	4 564	4 955	545	129	—	—	—	—
1958	3 553	3 377	425	99	—	—	—	—
1959	3 919	4 125	590	139	—	—	—	—
1960	3 313	3 810	549	132	—	—	—	—
1961	3 188	4 431	796	194	—	—	—	—
1962	3 035	5 039	787	161	—	—	—	—
1963	4 259	—	—	—	3 312	18 661	2 279	532

Table X shows the data of each species for every year. Here the comparison was made using the percentage of an index calculated with the number of received snakes and number of extractions from 1956 to 1963, regardless of the number of snakes actually used.

As the difference between received snakes and the ones really used in the extractions was only reported for the year of 1963, it can be seen that after correction, the index in column 1963, shows a greater yield of venom production.

TABLE IV — *Bothrops neuwiedi* — NUMBER OF SNAKES RECEIVED AND NUMBER OF EXTRACTIONS (1956-1962) COMPARED WITH THOSE OBTAINED IN THE ANIMAL COLONY IN 1963.

Years	Snakes received	Snake pit			Animal Colony			
	Number	No. of extractions	Venom		Snakes		Venom	
			ml (fluid)	g (dried)	Received	No. of extractions	ml (fluid)	g (dried)
1956	555	361	27	6	—	—	—	—
1957	520	407	29	7	—	—	—	—
1958	336	165	11	3	—	—	—	—
1959	370	58	5	1	—	—	—	—
1960	432	404	31	7	—	—	—	—
1961	498	482	37	9	—	—	—	—
1962	476	516	5	12	—	—	—	—
1963	410	—	—	—	341	1 612	155	32

TABLE V — *Bothrops atrox* — NUMBER OF SNAKES RECEIVED AND NUMBER OF EXTRACTIONS (1956-1962) COMPARED WITH THOSE OBTAINED IN THE ANIMAL COLONY IN 1963.

Years	Snakes received	Snake pit			Animal Colony			
	Number	No. of extractions	Venom		Snakes		Venom	
			ml (fluid)	g (dried)	Received	No. of extractions	ml (fluid)	g (dried)
1956	637	388	57	15	—	—	—	—
1957	474	385	66	18	—	—	—	—
1958	380	373	75	20	—	—	—	—
1959	378	560	142	39	—	—	—	—
1960	436	624	153	43	—	—	—	—
1961	371	410	85	24	—	—	—	—
1962	371	540	134	36	—	—	—	—
1963	404	—	—	—	268	1 557	468	119

TABLE VI — *Bothrops alternatus* — NUMBER OF SNAKES RECEIVED AND NUMBER OF EXTRACTIONS (1956-1962) COMPARED WITH THOSE OBTAINED IN THE ANIMAL COLONY IN 1963.

Years	Snakes received	Snake pit			Animal Colony			
	Number	No. of extractions	Venom		Snakes		Venom	
			ml (fluid)	g (dried)	Received	No. of extractions	ml (fluid)	g (dried)
1956	646	315	63	17	—	—	—	—
1957	571	277	54	14	—	—	—	—
1958	485	315	77	21	—	—	—	—
1959	457	342	101	27	—	—	—	—
1960	504	432	89	24	—	—	—	—
1961	564	410	89	24	—	—	—	—
1962	457	402	107	29	—	—	—	—
1963	483	—	—	—	371	1 198	365	89

TABLE VII — *Bothrops jararaca* — NUMBER OF SNAKES RECEIVED AND NUMBER OF EXTRACTIONS (1956-1962) COMPARED WITH THOSE OBTAINED IN THE ANIMAL COLONY IN 1963.

Years	Snakes received	Snakepit			Animal Colony			
	Number	No. of extractions	Venom		Snakes		Venom	
			ml (fluid)	g (dried)	Received	No. of extractions	ml (fluid)	g (dried)
1956	6 361	3 848	343	86	—	—	—	—
1957	6 810	4 572	434	112	—	—	—	—
1958	6 585	5 401	557	138	—	—	—	—
1959	6 493	5 443	638	161	—	—	—	—
1960	6 127	5 163	613	148	—	—	—	—
1961	7 630	7 111	752	190	—	—	—	—
1962	5 915	5 848	744	186	—	—	—	—
1963	6 788	—	—	—	4 287	18 755	2 200	547

TABLE VIII — *Bothrops cotiara* — NUMBER OF SNAKES RECEIVED AND NUMBER OF EXTRACTIONS (1956-1962) COMPARED WITH THOSE OBTAINED IN THE ANIMAL COLONY IN 1963.

Years	Snakes received	Snakepit			Animal Colony			
	Number	No. of extractions	Venom		Snakes		Venom	
			ml (fluid)	g (dried)	Received	No. of extractions	ml (fluid)	g (dried)
1956	387	283	37	8	—	—	—	—
1957	426	373	44	11	—	—	—	—
1958	539	569	72	17	—	—	—	—
1959	474	432	55	13	—	—	—	—
1960	391	383	41	10	—	—	—	—
1961	481	409	43	11	—	—	—	—
1962	432	896	123	28	—	—	—	—
1963	430	—	—	—	309	1 049	157	34

TABLE IX — *Bothrops jararacussu* — NUMBER OF SNAKES RECEIVED AND NUMBER OF EXTRACTIONS (1956-1962) COMPARED WITH THOSE OBTAINED IN THE ANIMAL COLONY IN 1963.

Years	Snakes received	Snakepit			Animal Colony			
	Number	No. of extractions	Venom		Snakes		Venom	
			ml (fluid)	g (dried)	Received	No. of extractions	ml (fluid)	g (dried)
1956	108	49	29	8	—	—	—	—
1957	115	64	36	10	—	—	—	—
1958	88	160	120	36	—	—	—	—
1959	117	182	140	38	—	—	—	—
1960	107	59	37	14	—	—	—	—
1961	136	63	37	14	—	—	—	—
1962	120	99	78	25	—	—	—	—
1963	127	—	—	—	85	216	127	31

TABLE X — PERCENTAGE OF EXTRACTIONS FOR EACH SPECIE. COMPARISON BETWEEN THE DATA OBTAINED FROM 1956 TO 1962 WITH THOSE IN 1963 IN THE ANIMAL COLONY.

Species	S n a k e p i t							Animal Colony	
	Y E A R S								
	1956	1957	1958	1959	1960	1961	1962	1963 a	b(*)
<i>B. atrox</i>	0,6	0,8	1,0	1,5	1,5	1,1	1,5	3,9	5,8
<i>B. neuwiedi</i>	0,7	0,8	0,5	0,2	0,9	1,0	1,1	4,0	4,7
<i>C. d. terrificus</i>	1,0	1,1	1,0	1,1	1,2	1,4	1,5	3,4	4,6
<i>B. jararaca</i>	0,6	0,7	0,8	0,8	0,8	0,9	1,0	2,8	4,4
<i>B. cotiara</i>	0,8	0,9	1,0	0,9	1,0	0,7	1,1	2,5	3,4
<i>B. alternatus</i>	0,5	0,5	0,6	0,7	0,9	0,7	0,9	2,5	3,2
<i>B. jararacussu</i>	0,5	0,6	1,9	1,6	0,6	0,5	0,8	1,7	2,5

(*) a — Percentual data taking as absolute the relationship between the number of snakes received and the number of extractions for comparison with data of the years from 1956 to 1962, period in which the number of snakes used in the extractions was not recorded.

b — Percentual data of extractions and relationship between number of snakes used and number of extractions.

DISCUSSION

The animal colony with controlled temperature improved the life conditions of the snakes: *Crotalus durissus terrificus*, *Bothrops neuwiedi* and *Bothrops atrox*; was less effective with *Bothrops alternatus* and ineffective with *Bothrops jararaca*, *Bothrops cotiara* and *Bothrops jararacussu*.

In the two former groups the prolonged lifetime allowed a larger number of venom extractions and as a result a larger yield of venom "per capita". The volume of venom obtained in each extraction was not improved, on the contrary, a gradual decrease of the amount of venom obtained was observed in successive extractions.

From 1956 to 1962, 86.966 snakes were received belonging to seven species and 80.769 extractions were performed during this period. In 1963, 12.901 specimens were received, with 43.048 venom extractions of 8.973 snakes, which were placed in the heated animal colony; that means that about 70% of the snakes were used for venom extractions. Although the quantity of snakes employed in the service of venom extractions from 1956 to 1962 is not recorded, we can assume that the same average of 70% has been used (Table II). In one year of observation in this laboratory, the number of extractions was a little larger than half of the general total of extractions performed in the seven years used for comparison, and in 1963, more extractions were made than in the last three years together.

From 1956 to 1962 the above mentioned number of snakes supplied 10.680 ml of fluid venom, which yielded, 2.650 g. of vacuum dried venom. In 1963, the animal colony yielded 5.814,5 ml of fluid venom, or 1.385 g. of vacuum dried venom. The yield of venoms in 1963 was half the total volume of venom obtained in the seven previous years. The production of 1963 was greater than that of the last three years (5.378 ml of fluid venom or 1.321 g. of dry venom).

Unfortunately, from 1956 to 1962 only the number of received snakes and the number of extractions are recorded. There was no possibility to compare the number of extractions with the number of snakes really used for this purpose. Therefore, column (a) 1963 of Table X shows data collected at the snake colony for comparison. Column (b) 1963 of Table X contains data that refer to the real utilization of snakes maintained in the animal colony and used for the extractions.

It can be concluded that higher yield of venom is obtained in the animal colony than in the open snakepit.

Table X also gives data on the high mortality of snakes in the service of venom extraction at Instituto Butantan. All snakes undergo extraction of venom regularly twice a month, or 24 times per year. We can assume that as in 1963, in the former years only 70% of the snakes received by the Instituto suffered venom extraction. The correction of the data from 1956 to 1962 of Table X, allows to compare them with the real data of column (b) of the same Table. It is noticeable, that the mortality, in percentage, at the Institute's snakepits is of about 92 to 98% yearly. That means, that each snake was used for extraction 0,6 to 1,5 times, according to the species.

The yearly percentual mortalities in the snake colony in 1963 were the following: *Bothrops atrox* (75,8%), *Bothrops neuwiedi* (79,0%), *Crotalus durissus terrificus* (80,5%), *Bothrops jararaca* (81,7%), *Bothrops cotiara* (85,8%), *Bothrops alternatus* (86,7%) and *Bothrops jararacussu* (89,5%), and consequently each snake was used for extractions about 2,5 to 5,8 times.

Among the snakes observed in captivity at the snake colony, the *Bothrops jararacussu* strikes attention, because it presents the lowest index of productivity, similar to that obtained in the snakepit (Table X) or even lower than the data of 1958 and practically equal to that of 1959. In the snakepits, the yearly utilization of snakes for venom extraction was only 2 to 8% of all species, whereas in the snake colony it increased to from 10,5 to 24,2% varying according to the species.

Former observations registered by Fonseca (10), refer to the number of received snakes, number of extractions, extracted volume in ml, during the years of 1908 to 1945 and also show the high index of mortality in the snakepits, with rare exceptions, similar to those observed from 1956 to 1962.

Under the conditions of the experimental animal colony after one year of observations, there occurred an outbreak of visceral uric gout (3 & 4) only among

rattlesnakes but not among snakes of the genus *Bothrops*, being responsible for a larger mortality of snakes.

An *Corynebacterium* of the group *diphtheroid* later caused mortality in a group of 2.700 snakes, which could be controlled by specific vaccination (6).

RESUMO

Os serpentários tradicionais do Instituto Butantan permitiam um aproveitamento da ordem de 5 a 8% das serpentes recebidas e utilizadas nos serviços de extração de veneno. O Biotério Experimental de Serpentes permitiu, na sua fase de observação piloto, um aproveitamento da ordem de 10,5 a 24%, de acordo com as espécies de serpentes. O alto índice de mortalidade é provocado: a) por doenças, entre as quais já são conhecidas a gota úrica visceral em "cascaveis" e uma epizootia provocada por um *Corynebacterium* do grupo difteróide; além de vários tipos de verminoses; b) as serpentes eram mantidas em cativeiro nos serpentários expostos em desacôrdo com o "habitat". A parte de climatologia, pouco estudada, é importante para a sobrevivência dos ofídios em cativeiro; c) extrações quinzenais são contra-indicadas; o manuseio contínuo das serpentes provoca traumatismos indesejáveis pela técnica de contenção e concorre para o aumento da mortalidade. A extração por choque elétrico é mais indicada.

Apesar do alto índice de mortalidade, conseqüente principalmente à contínua necessidade de manuseio para a obtenção de venenos, o Biotério Experimental apresentou resultados auspiciosos, permitindo apreciável aumento de produção de venenos, mesmo nas precárias condições de trabalho. Biotérios com alto nível de higiene e climatização adequada para observações mais controladas poderão levar a uma sobrevivência de ofídios em cativeiro ou mesmo a permitir a sua multiplicação.

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