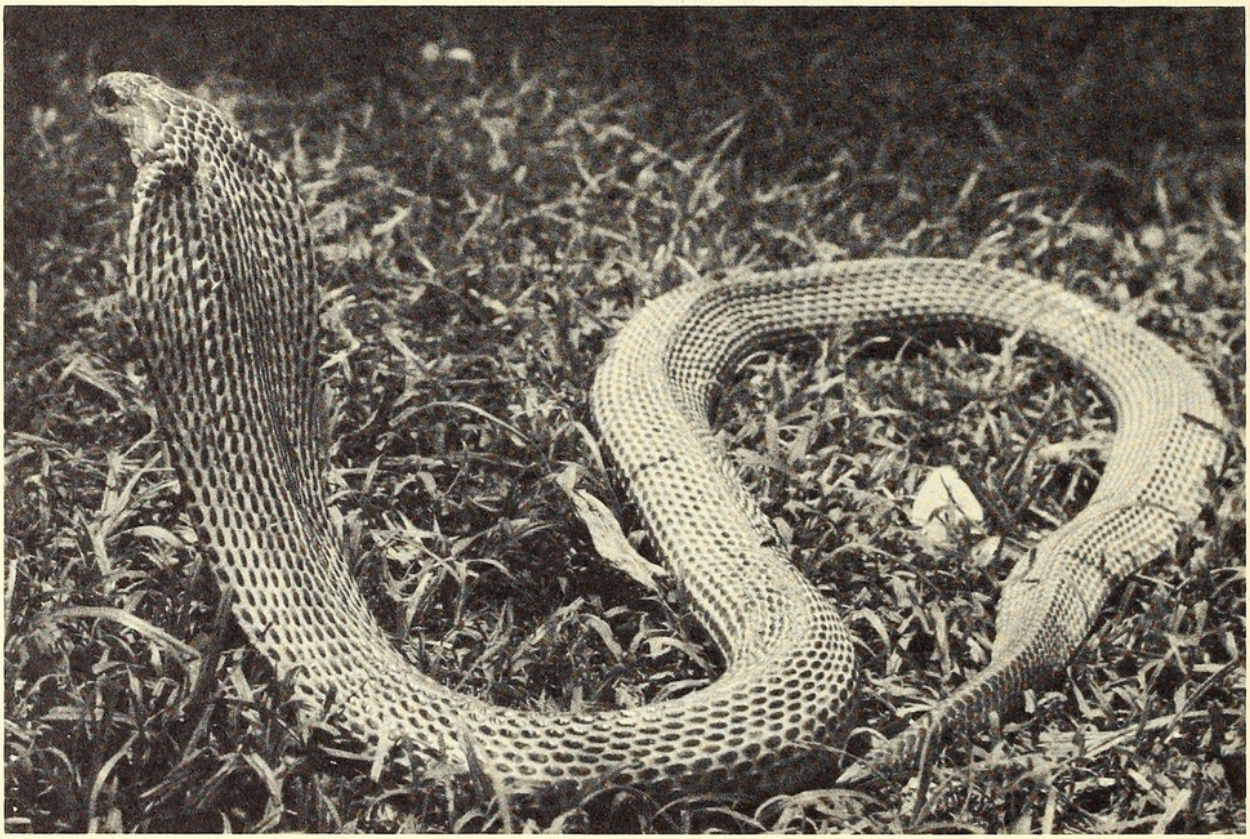

PLATE I. Philippine Cobra, *Naja naja philippinensis*.

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Venom Yields of the Philippine Cobra, *Naja naja philippinensis*

(Plate I; Text-figure 1; Tables I-II)

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Data on venom yields of 150 cobras (*Naja naja philippinensis*) gave an overall average venom yield per cobra per extraction (AVY/C/E) of 0.33 ml or 70.14 mg and an overall average total lifetime venom yield per cobra (ATLVY/C) of 2.23 ml or 527.77 mg for the fresh and corresponding dried venom, respectively.

The ATLVY/C were greater at schedule every 14 days than those at schedule every 7, 21, or 28 days. The all-male groups gave bigger yields than the corresponding all-female groups. The AVY/C/E in the former increased as the intervals between extractions lengthened. In the latter the reverse was observed.

The solid content of the venom does not appear to be affected by either sex, time of the year, or schedule of collection, but the serial records of extractions show a tendency for this to gradually decline with time. Venom extraction at close intervals resulted in a marked decline of the solid content, the rate of reduction becoming more marked in direct proportion to frequency of extraction.

The data on AVY/C/E of several groups indicate a general tendency for this to decline with time.

INTRODUCTION

IN VIEW of the absence of venom suppliers in the Philippines, it is essential that the Serum and Vaccine Laboratories (SVL) undertake the production of the venom to carry on its antivenin preparation program. For this purpose, a serpentarium is maintained for cobras of the species *Naja naja philippinensis*, and sufficient number of snakes are kept to ensure an adequate and uninterrupted supply of good quality venom. The number of snakes is replenished with cobras caught during field collection trips undertaken every three or four months.

To provide a basis for a realistic estimate of the number of cobras needed to supply an adequate amount of the venom to meet the requirements of the laboratory for antivenin production, records of venom yields are necessary. The present study was conducted for this purpose as well as to enable us to work out a schedule of venom collection which will assure maximum yields with the facilities available. Experiments were designed to furnish information on the possible effects of factors like sex and schedule or frequency of extraction on venom yields. The possibility of trends in yields with time was also investigated.

To the clinicians, data on venom yields may serve as aids in making an estimate of the amounts of the specific antivenin which may be required to neutralize the venom injected in a bite.

Oliver (1944) states that "the quantity of venom secreted in the act of biting varies according to the species, the size, age, and the condition of the snake at the time of the bite. In general, the larger the snake, the greater the quantity of venom injected, though there are many exceptions to this generality. The amount of venom injected depends on the time interval since the last bite, the venom glands usually requiring approximately two weeks to regain their maximum capacity of venom. In a normal bite, a snake does not expel its full quantity of venom, but only a small portion and is still capable of inflicting a fatal bite. Evidence indicates that an enraged snake injects a greater quantity of venom than one which has not been angered prior to biting. The amount of venom released during a spontaneous bite is greater than that obtained by investigators through 'milking' or forced expulsion of the venom."

He gives the following figures on the approximate amounts of dry venom obtained at a single

extraction from common poisonous snakes:

	Mg.
A. North American Species	
Copperhead	45– 65
Water moccasin	90–150
Timber rattler	40– 90
Texas rattler	120–300
Florida rattler	240–450
B. South and Central American Species	
Tropical rattlesnake	60–150
Fer-de-lance	80–160
Bushmmaster	300–500
C. Indian Species	
Asiatic cobra	250–350
Russell's viper	200–300
D. African Species	
Mamba	50– 80
Puff adder	70–120
E. Australian Species	
Tiger snake	35– 50
Death adder	60– 80

Christensen (1955), referring to the works of Grasset, Zoutendyk, and Schaafsma, and Grasset and Schaafsma gave the following records of yields of venom of various snakes:

Snake	Average Yield	Limit of Individual Yields	Remarks
N. flava	0.12 g (128 specimens)	Maximum–0.25 g	
D. angusticeps	0.1 g		
N. haje	0.72 g (1 snake)		Length–7'3" An enormous yield
S. haemachates	0.42 ml (0.1 g) (over 150 specimens)	0.1 ml–1.8 ml (0.033 g–0.242 g)	Solid content just under 25%
B. arientans	0.18 g first milking 0.07 g five days in captivity 0.1 g three weeks in captivity	Maximum–0.75 g	
D. typus	0.015 g average yield from pair of glands		41% W/W solid content; (obtained direct from gland)

Other data on venom yields mentioned by Christensen include:

Fifteen full grown puff adders gave an average yield of 0.67 ml (0.186 g).

The following table gives data on venom obtained from different snakes in his laboratory:

Species	No.	Size Cm.	Yields* Min.	Max.	Ave.	Percentage** Solid Min.	Max.	Ave.
S. haemachates	10	90–120	0.11	0.72	0.35	17.5	28.3	23.9
N. flava	1	150			0.11			27.1
N. haje	1	150			0.12			33.5
C. rhombeatus	9	45– 75	0.07	0.75	0.34	19.3	28.1	23.6
B. cornuta	5	32– 35	0.008	0.087	0.045	24.5	36.4	31.1
B. caudalis	1	60			0.085			27.6
B. gabonica	1	113			1.90			26.4

* Venom from B. cornuta and B. caudalis in g, the others in ml.

** W/W for B. cornuta and B. caudalis venom W/V for others.

Christensen states that the quantity, composition, and toxicity of the venom of a given species may vary considerably. He mentions that age, state of health, climate, and even the "mood" of the snake may affect the character and quantity of venom. Conant (1952) states that the snake has some control over the amount of venom injected in a bite. Minton (1957) gives the average amount of venom delivered in a bite by the following venomous species:

	Mg.
A. Elapidae	
North American coral snake (*Micrurus fulvius*)	3– 5
Blue krait (*Bungarus candidus*)	5– 10
Tiger snake (*Notechis scutatus*)	35– 45
Indian cobra (*Naja naja*)	175–250
Mamba (*Dendroaspis angusticeps*)	75–100
B. Viperidae	
African puff adder (*Bitis arientans*)	160–200
Russell's viper (*Vipera russelli*)	150–250
C. Crotalidae	
Fer-de-lance (*Bothrops atrox*)	80–160
Bushmaster (*Lachesis muta*)	300–500
Western diamondback rattlesnake (*Crotalus atrox*)	200–300

Deoras (1966) mentions environment, sex, seasonality, and frequency of milking as possible factors affecting venom yields, and in his studies showed that cobras and kraits produced more venom when kept in a farm under conditions simulating that of their natural habitat than when kept in separate cages in a room. The vipers however, produced more venom when kept in the room.

Materials and Methods

A total of 150 cobras, *Naja naja philippinensis,* 69 males and 81 females, with an overall average length of 47.7 inches collected in the province of Camarines Sur, Luzon Island, were used to provide the data presented in this study. All were apparently healthy and freshly caught at the beginning of each experiment except as indicated. Distribution of specimens into groups for comparison was done in a completely random manner from batches comprised of specimens approximately of same lengths. The care and management of the snakes and the method of venom collection employed, using a beaker with rubber diaphragm, have been previously described (Salafranca, 1967). The freshly collected venom was spread thinly in petri dishes and placed inside a dessicator over calcium chloride. The dessicator was sealed and evacuated using a high vacuum pump. It was kept in the cold room (4°-10°C) until the dried crystalline venom could be easily peeled off the glass. This required one to three or more days depending upon the thickness of the layer or the amount of the venom and the condition of the calcium chloride. The schedule of venom collections for any given experiment was observed until all the snakes had died.

EXPERIMENT I. Venom was collected from a group of 29 cobras, 7 males and 22 females with an average length of 48 inches, every 14 days. The pooled amount was recorded for each collection schedule.

The average venom yield per cobra per extraction (AVY/C/E) was computed by dividing the total amount collected by the number of snakes involved at each extraction.

Following the last collection, the overall AVY/C/E and median were computed.

The average total lifetime venom yields per cobra (ATLVY/C) was computed by dividing the total amount of venom collected from each schedule group by the number of snakes involved in the group.

The percent solid was computed by dividing the weight of the dry venom by the weight of the corresponding "wet" venom.

EXPERIMENT II. Thirty cobras with an average length of 48.9 inches, 15 males and 15 females, were selected from a catch of 37 specimens on the basis of similarities in sizes. Each sex group was randomly divided into three equal groups and each group of males paired at random with a group of females. Each of the three resulting groups was assigned to one of three schedules of venom collection, as follows:

Group	Schedule of Venom Collection
IIa	Every 7 days
IIb	Every 14 days
IIc	Every 28 days

EXPERIMENT III. Thirty-eight cobras with an average length of 48.9 inches, 19 males and 19 females, were distributed at random into four groups and their venom collected as indicated below:

Group	Number and Sex	Scheduel of Venom Collection
IIIa	10 females	Every 14 days
IIIb	10 males	Every 14 days
IIIc	9 females	Every 28 days
IIId	9 males	Every 28 days

EXPERIMENT IV. Fifty cobras of average length, 46.6 inches, 25 males and 25 females,

were distributed into six groups and their venom collected according to the schedule indicated below:

Group	Number and Sex	Scheduel of Venom Collection
IVa	8 males	Every 14 days
IVb	8 females	Every 14 days
IVc	9 males	Every 21 days
IVd	9 females	Every 21 days
IVe	8 males	Every 28 days
IVf	8 females	Every 28 days

EXPERIMENT V. To determine the capacity for venom production of individual cobras, the following experiment was performed:

Three male cobras were selected and subjected to repeated venom extractions at regular intervals during a whole working day according to the following schedule:

Cobra Number	Length (inches)	Schedule of Venom Collection
1	45	Every 2 hours
2	43.5	Every hour
3	39	Every half-hour

Cobra no. 1 and cobra no. 2 were obtained in our regular periodic hunt and had been in the laboratory serpentarium 22 days at the time of this experiment. Cobra no. 3 was a specimen caught within the laboratory premises two days before this experiment.

A 50-ml beaker of known weight was assigned to each snake. The venom was extracted as described previously (Salafranca, 1967). Due to the anticipated difficulty of getting accurate volumetric measurements, the weight of the venom collections were determined instead. The initial amount collected was rated 100% and the subsequent collections as percentages of the initial collection.

The data on the serial amounts of venom collected from certain groups in experiments one to four were plotted in a graph to determine possible trends with time.

Results and Discussion

The results of Experiments I to IV are summarized in Table I. A comparison of the ATLVY/C in three comparable groups of cobras, IIa, IIb, and IIc of Experiment II, subjected to 7, 14, and 28-day schedules, respectively; in four comparable groups, IIIa and IIIb, and IIIc and IIId, of Experiment III, subjected to 14 and 28-day schedule, respectively; and in six comparable groups, IVa and IVb, IVc and IVd, and IVe and IVf of Experiment IV, on 14, 21, and 28-day schedules, respectively, shows that, in each case, the average was greater in the group or groups on the every 14-day schedule of extraction. In Experiment II, using the dry venom data, the ATLVY/C on the 14-day schedule (IIb) was 16% and 26% (or 12% and 11% on the "wet" data) greater than those on 7-day and 28-day schedules (IIa and IIc), respectively. In Experiment III, the all-female and all-male groups on the 14-day schedule (IIIa and IIIb) averaged 35% and 37% (or 55% and 15% on the "wet" data) more venom than the corresponding all-female and all-male groups on the 28-day schedule (IIIc and IIId). The data for the all-male and all-female groups on the 14-day schedule in Experiment IV (IVa and IVb) show 15% and 9% and 18% and 25% (or 26% and 17% and 24% and 45% on the "wet" data) greater ATLVY/C than those in the corresponding all-male and all-female groups on the 21 and 28-day schedules (IVc and IVd and IVe and IVf), respectively.

From the data on the dry venom yields for comparable all-male and all-female groups, it will be observed that the former consistently gave greater ATLVY/C. In Experiment III, the all-male groups on the 14 and 28-day schedules (IIIb and IIId) gave 32% and 73% (or 50% and 102% on the "wet" data) more venom than the corresponding all-female groups (IIIa and IIIc), while in Experiment IV the all-male groups on 14, 21, and 28-day schedules (IVa, IVc and IVe) gave 87%, 93%, and 114% (or 102%, 100%, and 149% on the "wet" data) more than the corresponding all-female groups (IVb, IVd, and IVf).

The AVY/C/E on the other hand, in comparable groups, was observed to increase with the increase in intervals between extraction both in the mixed sex groups as well as in all-male groups. In Experiment II, we have 58.14 mg, 77.6 mg, and 101.73 mg for 7, 14, and 28-day schedules, and in Experiment IV, 72.73 mg, 75.75 mg, and 95.71 mg for the all-male groups on 14, 21, and 28-day schedules, respectively. The increase in AVY/C/E with the increase in intervals between extraction appear logical, since the gland is given correspondingly more time to recover its full capacity. With the all-female groups, however, the order is reversed; that is, the AVY/C/E decreases with increasing intervals. Thus we have for the all-female groups in Experiment III, 63.66 mg and 57.1 mg for the 14 and 28-day schedules, and in Experiment IV, 40.74 mg, 40.46 mg, and 35.66 mg for the 14,

TABLE I. SUMMARY OF RESULTS, EXPERIMENTS I-IV.

Experiment Number	Period of Handling	Schedule of Venom Extraction (interval in days)	No. of Snakes		Venom Yields				Average % Solid
					AVY/C/E*		ATLVY/C*		
			M	F	Wet (ml)	Dry (mg)	Wet (ml)	Dry (mg)	
I	to 8/15/63 3/20/64	14	7	22	a - 0.20 m - 0.18	a - 53.95 m - 58.39	1.94	520.16	a - 26.76 m - 27.65
IIa	to 2/ 4/64 4/27/64	7	5	5	a - 0.21 m - 0.20	a - 58.14 m - 53.37	2.24	633.39	a - 29.54 m - 25.50
IIb	to 2/ 4/64 7/ 7/64	14	5	5	a - 0.29 m - 0.28	a - 77.60 m - 79.09	2.60	709.42	a - 26.30 m - 28.50
IIc	to 2/ 4/64 7/21/64	28	5	5	a - 0.33 m - 0.33	a - 101.73 m - 97.70	2.07	641.28	a - 29.25 m - 29.80
IIIa	to 5/16/64 10/18/64	14	0	10	a - 0.28 m - 0.28	a - 63.66 m - 60.41	2.33	562.23	a - 22.66 m - 21.00
IIIb	to 5/16/64 11/17/64	14	10	0	a - 0.36 m - 0.34	a - 92.86 m - 78.21	3.08	844.41	a - 24.69 m - 23.00
IIIc	to 5/16/64 11/ 5/64	28	0	9	a - 0.29 m - 0.30	a - 57.10 m - 53.80	1.72	363.29	a - 27.72 m - 17.42
IIId	to 5/16/64 11/12/64	28	9	0	a - 0.52 m - 0.55	a - 115.96 m - 109.45	2.97	733.91	a - 21.20 m - 19.00
IVa	to 8/25/64 1/15/65	14	8	0	a - 0.39 m - 0.40	a - 72.73 m - 77.70	2.92	613.71	a - 17.45 m - 19.00
IVb	to 8/25/64 1/15/65	14	0	8	a - 0.24 m - 0.23	a - 40.74 m - 41.32	1.56	303.52	a - 16.86 m - 18.00
IVc	to 8/25/64 1/27/65	21	9	0	a - 0.41 m - 0.41	a - 75.75 m - 67.43	2.53	487.89	a - 17.56 m - 15.75
IVd	to 8/25/64 1/29/65	21	0	9	a - 0.24 m - 0.21	a - 40.46 m - 37.05	1.31	244.13	a - 17.15 m - 16.40
IVe	to 8/25/64 2/16/65	28	8	0	a - 0.54 m - 0.53	a - 95.71 m - 106.35	2.68	521.74	a - 17.85 m - 19.00
IVf	to 8/25/64 2/14/65	28	0	8	a - 0.26 m - 0.21	a - 35.66 m - 29.89	1.25	209.73	a - 15.07 m - 15.00
Totals			66	81					
Overall Averages					0.33	70.17	2.23	527.77	22.14

AVY/C/E - Average venom yield per cobra per extraction
ATLVY/C - Average total lifetime venom yield per cobra

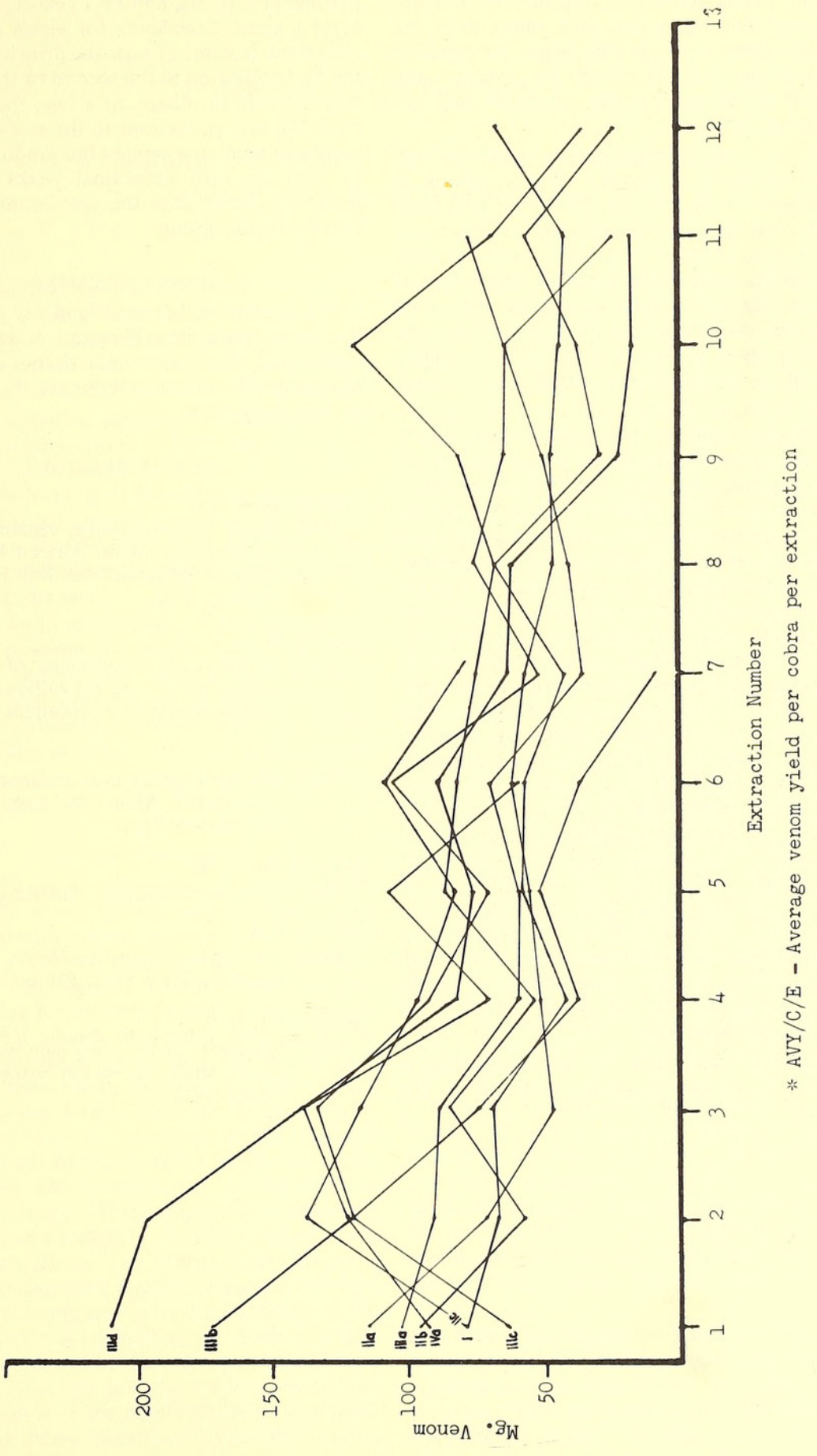
a - Average
m - Median

Cobra I.D.	Schedule and Record of Extractions* (Wet - mg)	Total Amount Collected (Dry - mg)	% Solid **
No. - 1 Sex - M Length - 45"	Every 2 hours 287.25 (100%) 166.84 (58%) 78.38 (27%) 94.68 (33%) 173.32 (60%)	81.4	10%
No. - 2 Sex - M Length - 43.5"	Every hour 200.94 (100%) 102.25 (51%) 129.46 (64%) 144.31 (72%) 86.29 (43%) 71.60 (36%)	53.27	7%
No. - 3 Sex - M Length - 39"	Every half hour 480.72 (100%) 433.02 (90%) 392.47 (82%) 203.24 (42%) 163.67 (34%) 228.29 (47%) 125.47 (26%) 161.25 (34%) 152.06 (32%) 113.03 (24%) 142.14 (20%) 73.63 (15%) 98.15 (20%) 74.70 (16%) 50.25 (10%)	122.13	4%

* Amount of venom given are in the chronological order of extractions. Initial extractions are rated 100% and subsequent extractions as percentages of the initial amount.

** Obtained by determining the relation of total dry weight to total weight of corresponding wet venom.

TABLE II. SUMMARY OF RESULTS, EXPERIMENT V.



TEXT-FIGURE 1. Graphic presentation of AVY/C/E* of indicated groups in Experiments I, II, III, and IV.

21, and 28-day schedules, respectively. The observation that the males give more than the females, however, is as true when we consider the data on ATLVY/C as the data on the AVY/C/E in comparable all-male and all-female groups.

The data on average percent solids of the venom collected from the various groups in Experiments I, II, III, and IV do not give indications that this may be affected by either sex, season, (period of the year when the experiment was undertaken), or the schedule of venom extraction observed. The records of the serial extraction of the majority of individual groups, however, show a tendency for this to decline gradually as the number of extractions increased.

The results of Experiment V are summarized in Table II. It will be observed that the amounts obtained generally decreased in the order of the chronology of extraction. The amounts obtained from cobra no. 3, notwithstanding its smaller size and greater frequency of extraction (every half-hour), are greater. This may be explained by the fact, as pointed to above, that this specimen at the time of the experiment was only two days in captivity and therefore more vigorous than the other two which have been in captivity 22 days at the time of this experiment. The percent solids of the combined extractions from each snake decreased as the frequency of extraction increased, from 10% for the extractions every two hours to 4% for those at every half-hour. The percent solids of 10%, 7%, and 4% obtained for the pooled collections from snakes 1, 2, and 3, respectively, are much lower than the overall average of 22.14 (15.07% to 29.54%) percent solids from the yields in Experiments I to IV (Table I). Apparently, repeated extractions at the close intervals of every two hours, hourly, and every half-hour, results in marked dilution of the venom, the dilution increasing in that order.

Graphical examination of the chronological AVY/C/E of several groups included in Experiments I, II, III, and IV (Text-figure 1) indicates a general tendency for yields to decrease with time. In some groups, the drop in yield from the first collection to the second or third is more marked than in others. In a few, there is a rise from the first extractions to the second or third, and from then on a general but gradual tendency to diminish with occasional peaks which are generally lower than the maximum noted for that particular group.

Acknowledgment

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