13. PROBABLE SIGNIFICANCE OF VENOM YIELD RECORD STUDIES

P. J. DEORAS

Haffkine Institute, Bombay, India

The venom yield records are necessary for sera producing laboratories to know the output and numbers required for immunization. They are useful to the clinician to know the maximum amount of venom given by a snake so that in the case of a snake bite he is prepared for neutralizing that much amount of venom. It is also necessary for a biochemist to know the periodicity during the year and the toxicity thereby, to plan experiments for enzymes.

In the production of venom by snakes some of the factors that are supposed to affect the yield are, environment, sex, seasonality and frequency of milking; provided there is uniformity of feeding and age.

This Institute has been working on these aspects for some years and Deoras (1) gave the description of a snake farm wherein *Naja naja*, *Vipera russelli*, and *Bungarus coeruleus* were kept, after giving them a habitat which was seen for these snakes in nature. The snakes were milked for venom from the snake farm and this yield after lyophilization was compared to similar yield by the same snakes kept in cages in the room. The results of the comparison of these venom yield records have been given in the same paper and in the proceedings of the symposium held at the Pan Pacific Science Congress (2). In these comparative studies specific attention was given to taking different number of samples and sexes separately. The results of these individual studies showed that the output of venom in the farm by a cobra and Krait snakes both for male and female sexes was more than in rooms. This was not the case with the viper as is shown by table below which indicates significance or otherwise

TABLE I — STATISTICAL ANALYSIS OF AVERAGE VENOM PRODUCTION DATA TO SHOW WHETHER THE ENVIRONMENTAL DIFFERENCE IS SIGNIFICANT OR NOT

Year of Production	M/F	Cobra (*Naja naja*)	M/F	R. Viper (*Vipera russelli*)	M/F	Krait (*Bungarus coeruleus*)
1953	Vf-Vr	Significant		Not significant	Vf-Vr	Significant
1954	Vf-Vr	Significant		Not significant		Not significant
1955	Vf-Vr	Significant	Vr-Vf	Significante		Not significant
1956	Vf-Vr	Significant		Not significant	Vf-Vr	Significant

TABLE II — STATISTICAL ANALYSIS OF AVERAGE VENOM PRODUCTION DATA TO SHOW, WHETHER THE SEX DIFFERENCE IS SIGNIICANT OR NOT

Year of Production	M/F	Cobra (*Naja naja*)	M/F	R. Viper (*Vipera russelli*)	M/F	Krait (*Bungarus coeruleus*)
1955	Vm-Vf	Significant		Not significant	Vm-Vf	Significant
1957	Vm-Vf	Significant		Not significant	Vm-Vf	Significant
1958	Vm-Vf	Significant		Not significant	Vm-Vf	Significant

Vf = Venom of farm snake M/F = Vm verses Vf
Vr = Venom of room snake
Vm = Venom of male snake
Vf = Venom of female snake

The actual figures appear at Table No. V and VI of reference No. 2 quoted in this paper.

only; the figures are not given. A periodicity in milking and the production of venom output during different months as well as differential output of venom from different fangs especially by the Russell's viper was established (2). Since then improvements have been done in the keeping of snakes in the rooms and milking has been done in groups of both male and female snakes of different lengths together as will normally be practical for any laboratory. In the routine working of a laboratory, routine work cannot always be done with statistical data. It was therefore necessary to see if the results obtained in previous studies with controlled conditions are available in routine working. Vipers yield better in rooms. The caging conditions were improved as such it was necessary to see if the yield was still maintained. The present studies give an account of these subsequent observations and their probable significance.

Materials and Methods

The snakes in the rooms were kept in uniform size of tin cages. These had on two sides, a 16 mesh wire gauze and a 2 inch tall partition inside the cage, dividing it into two chambers. One that is below the cover, has a drinking water in a brass pot (clamped) and a number of rough stones. The other side has a small platform to allow the snake to rest on a dry surface. The stones are for rubbing the body during casting. The details about the rearing have been mentioned in the proceedings of the symposium on Animal husbandry in International Congress of Laboratory Animals, Dublin 1965. The room 46′ × 26′ contained 300 snake cages on racks. It had two exhaust fans running 12 hours during the night when doors and windows were closed. The maximum and minimum temperatures in room and farm were 31°C, 25.8°C, 31°C, and 24.9°C, respectively. The maximum and minimum relative humidity during the year in the room and the farm was 81%, 64%, 82%, and 67% respectively.

Total number of times that snakes were milked for these studies are 7960.

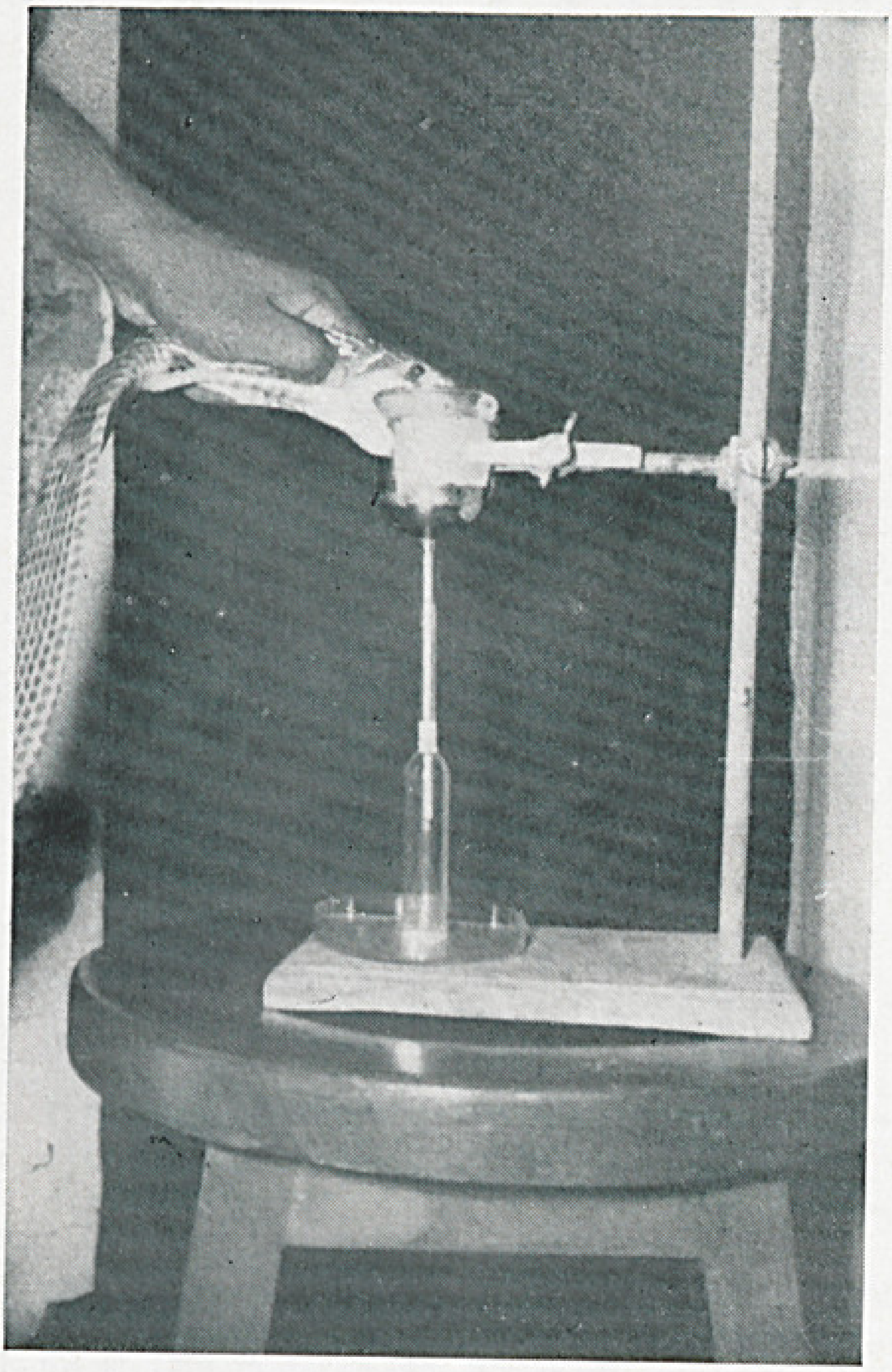

Fig. 1 — *Naja naja* biting a plastic coverstretched over a stainless steel funnel (whose stem leads into an ampoule for collection of venom).

Observations

Having seen significance in the output of venom by taking a group of snakes and even individual snakes of different sexes, we have now seen this in a large mixture group as will normally be available in any laboratory. Table III below, gives the observations from 1961 to 1965 wherein both male and female snakes have been taken together from farm and room. The results again shown as in the past work statistically analysed, that the output of venom given out by cobra and krait snakes is significantly more in the farm than the room. This is not the case with Russell's viper.

The periodicity of milking in the case of these snakes was one month. Table III gives the maximum and mininum output of venom for the different snakes. Table IV gives the entire yearly data showing the periodicity of output of venom in the room and farm in these snakes. The maximum outputs being concentrated during April to September months.

Individual snakes of different length and sexes were taken and milked in farm and room separately. Table V gives the data in detail for Krait and

TABLE III — SHOWING THE AVERAGE OUTPUT OF VENOM IN GMS AFTER LYOPHILIZATION, OF DIFFERENT SNAKES KEPT IN THE ROOM AND THE FARM FOR BOTH THE SEXES, AS WELL AS THE MAXIMUM & MINIMUM YIELDS

Kind of snake	Year	Total No. milked in 12 months.		Average venom output		Maximum venom output		Minimum venom output	
		Room	Farm	Room	Farm	Room	Farm	Room	Farm
Krait (*Bungarus coeruleus*)	1961	238	332	0.0066	0.0307	0.0100	0.0385	0.0028	0.0240
	1962	308	216	0.0078	0.0208	0.0131	0.0337	0.0044	0.0173
	1963	584	444	0.0103	0.0181	0.0153	0.0267	0.0071	0.0120
	1964	752	286	0.0082	0.0138	0.0136	0.0210	0.0058	0.0109
	1965	1.059	381	0.0090	0.0116	0.0115	0.0325	0.0044	0.0033
Russells viper (*Vipera russelli*)	1961	294	98	0.1081	0.1023	0.1700	0.1224	0.0570	0.0790
	1962	266	91	0.1357	0.1164	0.1729	0.1518	0.1189	0.0979
	1963	406	175	0.1514	0.1003	0.2000	0.1500	0.1060	0.0310
	1964	260	97	0.0851	0.0923	0.1290	0.1556	0.0651	0.0622
	1965	563	66	0.0725	0.0925	0.1640	0.1200	0.0490	0.0489
Cobra (*Naja naja*)	1962	39	10	0.1807	0.2660	0.2012	0.3616	0.1692	0.1225
	1964	105	20	0.1758	0.1515	0.2950	0.1866	0.0835	0.1221
	1965	657	223	0.1131	0.1272	0.1805	0.2136	0.0754	0.0275

TABLE IV — TABLE SHOWING THE AVERAGE OUTPUT AND PERIODICITY OF VENOM YIELD IN DIFFERENT SNAKES. (VENOM OF ONLY 13 SNAKES IN ONE AMPOULE).

1954	Cobra (*Naja naja*)		Viper (*Vipera russelli*)		Krait (*Bungarus coeruleus*)	
	Farm	Room	Farm	Room	Farm	Room
January	0.1480	0.0933	0.1110	0.0650	0.0167	0.0414
February	0.1710	0.1240	0.1310	0.0770	0.0121	0.0200
March	0.1810	0.1120	0.1290	0.1040	0.0250	0.0180
April	0.1810	0.1250	0.1310	0.1130	0.0290	0.0290
May	0.1810	0.1180	0.1670	0.1000	0.0280	0.0220
June	0.1700	0.1400	0.1670	0.1050	0.0275	0.0192
July	0.1390	0.1280	0.1250	0.0890	0.0240	0.0180
August	0.1327	0.0926	0.1760	0.0605	0.0354	0.0180
September	0.1589	0.1220	0.1564	0.1346	0.0360	0.0240
October	0.1235	0.0904	0.1237	0.1187	0.0260	0.0207
November	0.1714	0.0966	0.1280	0.1067	0.0210	0.0160
December	0.1193	0.1497	0.1062	0.1343	0.0314	0.0126
Average	0.1564	0.1160	0.1376	0.1015	0,0261	0.0216

average for Russell's viper and cobra indicating that the sexes and length inspite of being different, the results show that the output of venom in farm is better than in room for Krait and cobra and not significant fo the Russell's viper. It may here be noticed that beyond certain length, there is not much difference in the output of venom and that similar sizes may give more venom than bigger ones.

Venom collected from snakes was centrifuged and the supernatant liquid as well as the debris lyophilised. There was no or extremely small debris left in the case of cobra and krait snakes, but Russell's vipers gave a lot of debris. Table VI below shows the amount of venom in volume before centrifuging and the toxicity of the supernatant as well as the debris part. The table indicates that there is debris in vipers and the amount of debris left over may be only a dead bulk which is not toxic. Venom used in horses for immunization recently has shown that the present purified venom where debris has been discarded does not give the sloughing and other side reactions in horses as was available when this had not been removed.

Discussion

Stadelman (3), Fairley and Splatt (4), Amaral (5), and Schoettler (6) have recorded the differences in the output of venom in vipers of different locations. Kochwa (7) has tried to study the yield of venom in *Vipera palestinae*, but he has not made any attempts to keep snakes under natural surroundings. These

TABLE V — SHOWING THE PERIODICITY IN VENOM PRODUCTION DURING DIFFERENT MONTHS. WEIGHT OF VENOM IN GM AFTER LYOPHILIZATION

Krait (*Bungarus coeruleus*)

Months 1955	ROOM				FARM			
	Male length	Wt. of venom	Female length	Wt. of venom	Male length	Wt. of venom	Female length	Wt. of venom
January	4' 10"	0.0368	3' 11"	0.0012	4' 8"	0.0550	3' 4"	0.0050
February	4 1	0.0305	3 1	0.0006	4 11	0.0746	3 3	0.0086
March	4 9	0.0210	3 1	0.0044	4 0	0.0179	3 10	0.0135
April	3 9	0.0113	3 5	0.0067	5 0	0.0697	3 6	0.0097
May	4 5	0.0165	3 2	0.0092	5 0	0.0722	3 8	0.0832
June	4 8	0.0226	3 2	0.0028	4 4	0.0403	3 7	0.0223
July	3 8	0.0168	3 1	0.0176	4 9	0.0324	3 4	0.0166
August	4 2	0.0072	3 0	0.0104	4 1	0.0326	3 3	0.0148
September	4 11	0.0172	2 10	0.0064	4 8	0.0426	3 9	0.0159
October	4 4	0.0332	3 1	0.0112	3 8	0.0765	3 7	0.0218
November	3 1	0.0132	3 1	0.0092	3 11	0.0950	3 10	0.0319
December	3 9	0.0082	3 8	0.0098	3 10	0.0218	3 8	0.0419
Average	4' 3"	0.0195	3' 7"	0.0075	4' 5"	0.0525	3' 7"	0.0238

Russell's viper (*Vipera russelli*)

Average venom yield in gm in 1955.

Average	3' 10"	0.0952	3' 7"	0.0938	3' 9"	0.2072	3' 9"	0.2114

Cobra (*Naja naja*)

Average venom yield in gm in 1955.

Average	3' 10"	0.1145	3' 11"	0.1015	4' 6"	0.3003	4' 9"	0.1920

TABLE VI — SHOWING THE WEIGHT IN GMS OF LYOPHILIZED SUPERNATANT LIQUID AND DEBRIS IN VENOM UNDER PURIFICATION

Snake	No. snakes milked	Volume of Venom	Weight of Venom	Weights after centrifugation and Lyophilizing			
				Wt. of super-natant	Wt. of debris	M. L. D. of super-natant	M. L. D. of debris
Naja naja (Male)	5	3.0 cc.	2.8307	1.0230	Nil	1:120000	No debris
Naja naja (Female)	5	2.0 cc.	1.9291	0.7059	Nil	1:135000	Do.
Vipera russelli (Male)	5	5.5 cc.	5.1790	0.5371	0.0104	1:140000	Not toxic even at 1:10
Vipera russelli (Female)	5	4.7 cc.	5.0679	0.8553	0.0149	1:130000	Not toxic even at 1:10

surroundings were therefore made after studying the natural habitat and the results of experiments published in 1961 and 1963 (1, 2). These observations needed confirmation under routine conditions and data established for taking on over-all view about the keeping of snakes in the laboratory. There are a number of Institutions which are keeping snakes for immunological studies or for venom in connection with biochemical studies. Many have a structure called snake farms, in which the snakes are kept and exhibited. Some expenses are involved in keeping and exhibiting them. Our present studies have shown that the production of venom from cobra and Krait snakes is more from snakes kept in the farm where natural habitat is given than those kept in the room. Both these snakes have neurotoxic poison. The vipers which have haemotoxic venoms give more venom in the rooms. It has also been observed by us that the mortality of vipers kept in the farm is more than those kept in rooms as compared to cobra and krait snakes. It is therefore argued that if a snake farm is to be maintained in an institution for the production of venom, then it should have the natural surroundings of the habitat of that snake. Secondly the snakes like cobra and krait may be reared in such a kind of farm because there is more production of venom. For Russell's viper, the production is more in the room and the conditions there be improved to prevent the infection of viper throats by giving them cages where living and feeding compartments are separate. Laboratories working on immunological aspects should have venom production data for the year to plan immunological programmes for keeping the required number of snakes. It will be worthwhile experimenting whether the period when maximum amount of venom is produced is also the time to get most toxic venom. If so then the maximum collection be done during this time and venom used for antigenicity. The same could be available for biochemical studies. The figures for the maximum and average venom output for snakes give an indication to clinicians as to how much venom to expect in the worst cases when that particular snake bites. Lastly venom used for immunization be better centrifuged and supernatant liquid only used for the purpose.

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