

RELEASE OF HISTAMINE FROM SKELETAL MUSCLE BY SNAKE VENOMS

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It has long been recognized that the physiological effects of an intravenous injection of snake venom bear much resemblance to the acute effects of histamine or anaphylaxis. The work of Gautretet, Halpern and Corteggiani (1934, 1936) has demonstrated the similarity of the action of the venom of *Vipera aspis* and Indian cobra to that of histamine on the peripheral vascular system. Chopra and Chowhan (1934) and Chopra, Chowhan and De (1935) showed that *V. russellii* and *Echis carinata* caused death from acute circulatory failure. The viper venom produced hypotension and paralysis of capillaries resembling the effects of histamine or protein shock. The symptoms of vascular failure in *Echis* poisoning also resembled histamine shock. Feldberg and Kellaway (1937a) explained some of the effects of intravenous injections of cobra venom in cats and dogs in terms of histamine release, and demonstrated (1937b) that after injection of the venom into the pulmonary artery of the perfused guinea-pig's lungs the perfusate contained histamine. These authors (Feldberg and Kellaway, 1938) further showed that the addition of the venom to the fluid used for perfusing the liver or lung of the dog caused histamine to be liberated from these organs.

Several substances such as strychnine (Schild and Gregory, 1947), adrenaline (Eichler and Barfuss, 1940), curare alkaloids (Alam, Anrep, Barsoum, Talaat and Wieninger, 1939), and ammonia (Schild, 1949) are known to set free histamine from skeletal muscle. Since snake venoms liberate histamine from certain tissues, it was considered possible that they might have a similar action in skeletal muscle. The object of this work is to find out whether histamine is released from the rat diaphragm by snake poison and, if so, to compare quantitatively the rate of release of histamine by the venom of three well-known deadly-poisonous Indian snakes—cobra (*Naja naja*), krait (*Bungarus coeruleus*) and saw-scaled viper (*Echis carinata*). An account is also given of the effect of antihistamine drugs on the toxicity of snake venoms. A preliminary account

of the release of histamine by cobra venom has already appeared (Dutta and Narayanan, 1952).

METHODS

The rat diaphragm preparation of Rocha e Silva and Schild (1949) was used. The diaphragm of the albino rat bred at this Institute, and weighing between 200 and 300 g., was dissected out from the surrounding structures. The two lateral lobes of the diaphragm, freed from the middle lobe, were used. One was placed in 5 ml. of oxygenated Tyrode solution at 37° C. and the other in Tyrode solution containing snake venom. These solutions were withdrawn and replaced at regular intervals by fresh solutions of the same composition. The active substances released by venoms were assayed biologically on isolated guinea-pig's ileum suspended in a small bath of Tyrode solution. The total volume of the solution in the bath was always adjusted to 4.5 ml. The active substances were compared with histamine acid phosphate. The activities are given in terms of histamine base.

At the end of the experiment, the histamine remaining in the muscle was first extracted by grinding the muscle with sand and acidified saline. The solution was boiled for a minute and centrifuged. The supernatant liquid was collected and the precipitate was washed with saline and centrifuged again. The process was repeated twice. The final volume of the supernatant fluid after neutralization was so adjusted that 10 ml. corresponded to 0.1 g. of the wet tissue.

RESULTS

Identification of the Substance Released by Venoms

Venoms of cobra, krait and saw-scaled viper released from rat diaphragm substances which produced rapid contraction of the guinea-pig's isolated ileum. When the bath was washed out, the gut relaxed as readily as after a dose of histamine. The contractions produced by the substances released from rat diaphragm, and by equiactive doses of histamine, were reduced to an equal degree by antazoline and tripeleminamine, but were unaffected by atropine. This is illustrated in Fig. 1.

The substance released by cobra venom from rat diaphragm causes a depressor effect in the cat similar to that of histamine; this is unaffected by atropine.

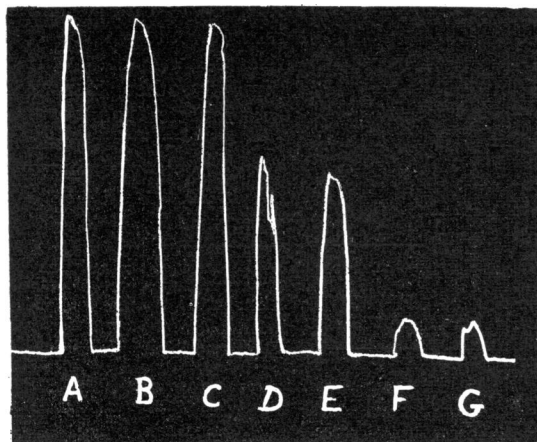


FIG. 1.—Contractions of guinea-pig's ileum in Tyrode solution: A, 0.03 μg . histamine; B, 0.1 ml. test solution containing the substance released by cobra venom (100 $\mu\text{g}/\text{ml}$.) from rat diaphragm; C, 0.1 ml. test solution in presence of 0.1 μg atropine; D, 0.1 ml. test solution in presence of 0.1 μg . antazoline; E, 0.03 μg . histamine in presence of 0.1 μg . antazoline; F, 0.1 ml. test solution in presence of 0.5 μg . antazoline; and G, 0.03 μg . histamine in presence of 0.5 μg . antazoline.

Since the test solution itself contained venom which is known to produce a fall of blood pressure in the cat, it was necessary to remove or destroy the venom before the true hypotensive action of the liberated substance could be demonstrated. This was achieved by using Code's (1937) method for the extraction of histamine. In a control experiment a known quantity of histamine was added to cobra venom solution; when this was treated by the above procedure, the depressor effect of the venom was completely removed whereas the added histamine was quantitatively recovered. Fig. 2 illustrates a typical experiment, and shows that the hypotensive property of the venom is removed by Code's method without the depressor response of the liberated agent being affected. Parallel quantitative assays of the

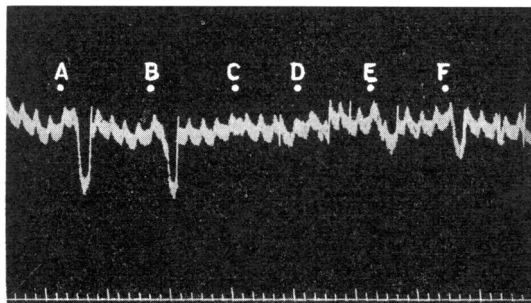


FIG. 2.—Cat, chloralose, atropinized. Blood pressure. A, histamine 0.17 μg .; B, 0.7 ml. test solution containing released substance and cobra venom, treated by Code's method; C, 0.7 ml. Tyrode solution having the same concentration of venom as in B but without the released agent, treated by Code's method; D, 1 mg. antazoline; E, 0.17 μg . histamine; and F, 0.7 ml. test solution. The recording was stopped between injections.

histamine content of test solution on guinea-pig's ileum and on cat's blood pressure showed satisfactory agreement, within the error of the two methods. In a typical experiment, the histamine liberated from four rat diaphragms by cobra venom was pooled and concentrated. Direct assay of this solution on guinea-pig's ileum showed a histamine content equivalent to 21.1 $\mu\text{g}/\text{g}$. of tissue, whereas the same solution when assayed after Code's treatment gave a value of 20.3 $\mu\text{g}/\text{g}$. on guinea-pig's ileum and 22.3 $\mu\text{g}/\text{g}$. on cat's blood pressure. Further experiments showed that the active agent liberated from rat diaphragm by the venoms of saw-scaled viper and of krait was also indistinguishable from histamine.

The Amount of Histamine Released by Venoms

Before the released histamine could be assayed on guinea-pig's ileum, the gut had to be desensitized to the venom, because all three venoms, in doses of 50 to 100 μg ., produce contractions after a latent period of 6–10 sec. This is more pronounced with cobra venom than with the other two. On subsequent addition of similar doses of venom the contractions become less, and finally disappear. To desensitize the tissue against cobra venom about 100 to 200 μg . is required. Guinea-pig's ileum (Schild, 1939) is stated to contain appreciable amounts of histamine and these contractions may result from the liberation of tissue histamine by the venoms.

Fig. 3 shows the progressive decrease of the histamine content of the muscle when bathed repeatedly in Tyrode solution containing 100 $\mu\text{g}/\text{ml}$. of venom. The results for each venom are the average of seven experiments. Cobra venom seems the most active in liberating histamine and saw-scaled viper the least active.

Cobra venom set free 50–60% of tissue histamine in 10 min. and at the end of 45 min. the diaphragm was almost depleted of histamine. It will be observed that with other venoms the release of histamine was more gradual, so that at the end of 45 min. about one-fifth and one-third of the total histamine were retained in the muscle with the krait and the saw-scaled viper venoms respectively. Cobra venom in a concentration of 100 $\mu\text{g}/\text{ml}$. usually released as much as 40% of the total histamine in five min. as against 20% with the other two venoms.

There was a difference in the rate of release of histamine with larger and smaller doses (Table I). Lower concentrations of the venoms could release histamine only up to a certain limit, no matter how long the experiments were continued. For instance, 10 $\mu\text{g}/\text{ml}$. of cobra venom released only 57% of the total histamine from the muscle during the entire

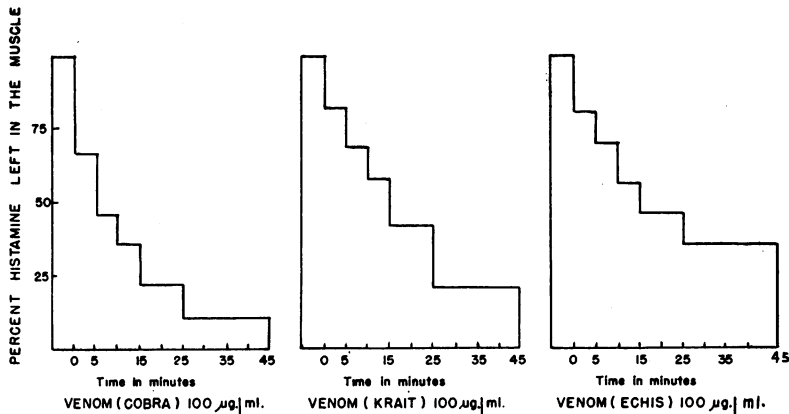


FIG. 3.—Comparison of histamine output from isolated rat diaphragm, in oxygenated Tyrode solution at 37° C., by the three venoms. Average of seven experiments with each venom.

TABLE I
RELEASE OF HISTAMINE FROM RAT DIAPHRAGM, IN OXYGENATED TYRODE SOLUTION AT 37° C., BY SNAKE VENOMS
(Histamine values are expressed as base)

Expt.	Release of Histamine (µg./g.)		Residual Histamine Content of Diaphragm (µg./g.)	Total Histamine Content of Diaphragm (µg./g.)
	During First 5 Min.	During 45 Min.		
<i>Cobra Venom, 10 µg./ml.</i>				
1	2:80	11:25	6:30	17:55
2	2:16	9:10	8:31	17:41
3	3:50	11:95	5:45	17:40
4	2:60	5:76	8:90	14:66
5	3:20	7:33	7:09	14:22
6	2:42	13:41	8:32	21:73
7	1:69	5:96	3:85	9:81
Mean	2:62	9:25	6:90	16:15
<i>Cobra Venom, 50 µg./ml.</i>				
1	4:90	14:25	3:80	18:05
2	4:97	11:47	4:30	15:77
3	3:90	13:28	3:20	16:48
4	6:70	20:54	2:10	22:65
5	2:90	7:97	6:20	14:17
6	5:32	14:32	1:80	15:12
7	4:14	12:14	2:72	14:86
Mean	4:69	13:42	3:49	16:91
<i>Echis Venom, 50 µg./ml.</i>				
1	1:50	6:70	7:50	14:20
2	2:50	11:40	7:90	19:25
3	2:52	7:45	7:52	14:97
4	3:20	9:40	5:60	15:00
5	3:25	11:85	8:60	20:45
6	1:95	8:34	6:32	14:66
7	3:10	10:12	7:72	17:84
Mean	2:57	9:32	7:31	16:63
<i>Krait Venom, 50 µg./ml.</i>				
1	1:90	11:75	5:50	17:25
2	2:50	17:60	7:80	25:4
3	1:75	6:58	3:80	10:38
4	3:25	13:60	7:0	20:6
5	1:28	8:53	3:25	11:78
6	2:63	10:51	6:18	16:69
7	1:62	12:47	4:76	17:23
Mean	2:13	11:57	5:47	17:04

experiment, but in the last five min. the histamine output was reduced to only 2.5 µg./g. of muscle. Thus, had the experiments been continued for more than 45 min. it is unlikely that any appreciable

further quantities of histamine would have been released by the venom. Similarly, other experiments (not reported here) showed that higher concentrations (200 µg./ml.) of the venoms of krait and saw-scaled viper could, like cobra venom, deplete almost all the tissue histamine in 45 min.

Effects of Antihistamines on the Survival Period of Envenomed Animals

It has already been stated that clinical symptoms following snake bites often resemble the acute effects of histamine. Since snake poisons release histamine it seemed likely that at least some of these symptoms would be due to histamine release. The question arose whether antihistamines could prevent, or at least reduce, the effect of released histamine and thus afford some protection against the venom.

The results reported in Table II are based on a series of experiments in which the survival periods of envenomed animals treated with different antihistaminic drugs were compared with those of controls which had received venom but not antihistamines. For each experiment a group of 9 or 10 animals was selected, out of which 3 were used as controls. The groups consisted mostly of litter mates. All the animals in a group were injected on the same day and under identical conditions, the solutions of venom and antihistamine drugs being prepared fresh on the day of the experiment. The experiments were carried out on adult white rats (fasting weight 60–170 g.) and guinea-pigs (320–520 g.) bred and reared at this Institute. Cobra venom (0.5 mg./kg.) was injected intraperitoneally in the rat and intramuscularly in the guinea-pig. The treated animals received in addition two doses of an antihistamine drug intraperitoneally, the first dose being given 15 min. before, and the second 25 min. after, the administration of the venom.

Table II shows that the rats injected intraperitoneally with 0.5 mg./kg. cobra venom survived for a period of 65–175 min., and the guinea-pigs given the same amount of venom for 79–220 min. There is thus no difference between the survival periods of the two species of animals, although the routes of the injection of the venom were different. When the survival periods of envenomed animals

TABLE II
SURVIVAL TIMES OF ENVENOMED ANIMALS TREATED WITH ANTIHISTAMINES
(The antihistamines were given intraperitoneally 15 min. before and 25 min. after 0.5 mg./kg. cobra venom injected intraperitoneally to rats and intramuscularly to guinea-pigs.)

Expt. No.	No. of Animals	Mean Wt. (g.)	Antihistamine and Dose (mg./kg.)	Survival Time (Min.) Minimum and Maximum	Mean Survival Time Min.	Difference of Means \pm S.E. Min.	Significance
<i>Rats</i>							
1	3	160.0	Control	90-112	104.0	8.50 \pm 8.46	t=1.005; p=0.175. Not significant
	6	157.7	Antazoline, 2.5	90-123	112.50		
2	3	140.0	Control	107-149	130.67	-26.67 \pm 13.70	t=1.947; p=0.047. Significant
	6	123.0	Antazoline, 5	82-131	104.00		
3	3	140.0	Control	65-105	88.33	18.10 \pm 13.14	t=1.377; p=0.105. Not significant
	7	125.0	Tripelennamine, 2.5	85-135	106.43		
4	3	138.6	Control	100-175	146.67	-10.84 \pm 18.88	t=0.574; p=0.290. Not significant
	6	150.6	Promethazine, 2.5	115-150	135.83		
<i>Guinea-pigs</i>							
5	3	433.3	Control	90-220	136.00	18.00 \pm 33.03	t=0.545; p=0.300. Not significant
	6	387.5	Antazoline, 2.5	125-208	154.00		
6	3	506.6	Control	79-148	116.33	1.90 \pm 15.32	t=0.124; p=0.460. Not significant
	7	480.7	Tripelennamine, 2.5	99-140	118.23		
7	3	400.0	Control	112-163	134.67	21.33 \pm 24.92	t=0.856; p=0.240. Not significant
	6	378.0	Promethazine, 2.5	107-193	156.00		

treated with an antihistamine were compared with those of untreated animals, some prolongation of life of the treated animals was observed in a few experiments. For example, the control guinea-pigs in Expt. 5 (Table II) lived for an average period of 136 min. whereas the animals treated with 5 mg./kg. antazoline survived for 154 min. When the test was applied this difference was found to be statistically not significant ($P=0.3$). Likewise promethazine did not prolong significantly the life of guinea-pigs (Expt. 7) though the mean survival period of the treated animals was longer by 21.3 min. than that of the controls. In Expt. 2, on the other hand, with a larger dose of antazoline (10 mg./kg.) the mean survival period of the treated rats was 104 min., whereas that of the controls was 130 min. Here, the value of t is 1.95 ($p=0.047$), which is significant at the 5% level. The shorter span of life in the treated animals might possibly have been due to the toxicity of antazoline itself being added to the poisonous effect of the venom. Further experiments (Nos. 1, 3, 4 and 6) with tripelennamine and promethazine on rats and guinea-pigs did not give any indication that these drugs could significantly alter the survival

periods when the animals were poisoned with cobra venom.

DISCUSSION

A "slow-reacting substance" which has some resemblance to the action of histamine is occasionally associated with substances capable of releasing histamine from animal tissues. Feldberg, Holden, and Kellaway (1938) observed it in lung perfusates following perfusion with snake venom. The existence in wasp venom of a potent unidentified substance which produces a delayed, slow contraction of guinea-pig ileum in the presence of atropine and mepyramine has recently been demonstrated by Jaques and Schachter (1954b). Other workers have observed the appearance of a slow-acting substance in the plasma of the cat when injected with 48/80 (Paton, 1951) or anemone extract (Jaques and Schachter, 1954a). In our experiments there was no evidence of the release of a similar slow-reacting substance from rat diaphragm, since the liberated agent was pharmacologically indistinguishable from histamine. The released substance, unlike the slow-reacting substance, produced a quick contraction of the guinea-pig ileum in the presence of

atropine and antihistamine; subsequent relaxation of the gut after washing was as rapid as after a dose of histamine. The histamine equivalent of a given sample was the same before and after treatment by Code's method.

Attempts to counteract the poisonous effect of cobra venom by antihistamines did not meet with success. There are several possible explanations for this failure:

(1) The antihistamine may not reach the site of action of intracellularly-released histamine. It has been pointed out by Feldberg and Paton (1951) that snake and bee venoms release histamine by enzymatic destruction of animal tissue. It is probable that under these conditions histamine is in fact liberated within the cell.

(2) Histamine may kill the animal by some action which is not counteracted by antihistamines. It is known that antihistamines are not very effective in neutralizing the depressor responses of histamine in the cat and dog (Dews and Graham, 1946; Marsh and Davis, 1947). It is also well known that the secretagogue action of histamine on the gastric mucosa is only partially antagonized by antihistamine drugs.

(3) Histamine release may not be the immediate cause of death by snake poison. In clinical cases histamine release probably accounts for the cold extremities, blanched pale skin, low blood pressure, rapid, thready pulse, and extreme prostration. However, the gross damage to the cellular tissues which snake venom produces may be the deciding factor of the fatal outcome, and the release of histamine may be incidental.

SUMMARY

1. The venoms of cobra (*Naja naja*), krait (*Bungarus coeruleus*) and saw-scaled viper (*Echis carinata*) liberate histamine from the isolated rat diaphragm, cobra venom being the most active and saw-scaled viper venom the least active in this respect.

2. The release of histamine by cobra venom is explosive in character, as 50 to 60% of the histamine

content of the muscle is set free during the first ten minutes.

3. Antihistamine drugs did not reduce the toxic effects of cobra venom on rats and guinea-pigs, the survival period being not significantly altered when envenomed animals were treated with antazoline, tripeleminamine or promethazine.

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