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The Action of the Venom of Sepedon haemachates of South Africa

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VII. *The Action of the Venom of* Sepedon hæmachates *of South Africa.*

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Sepedon hæmachates has received other names, such as *Naja hæmachates*, *Naja Capensis*, and *Aspidelaps hæmachates.** It is found at the Cape of Good Hope, Namaqualand, Clanwilliam, and elsewhere in South Africa. It is known to the

* Boulenger's 'Catalogue of the Snakes in the British Museum,' vol. 3, 1896, p. 389.

Dutch as "Rhinghals" and "Spuwslang," and in Cape Colony also as the "Brown Snake." It measures about 2 feet in length, and has the reputation of being one of the most lethal of the South African snakes. Being a Colubrine of the sub-family of the Elapinæ, it is therefore closely related, in zoological characters, to the members of the Naja genus which includes the deadly Cobras and Hamadryad. So far as we have been able to ascertain, the action of this venom has not before been examined.

The Sepedon venom used in this investigation was extracted from eight dried glands: two of which were sent to one of us, in 1898, by Mr. J. W. VAN PUTTEN, of van Putten's Vlei, Clanwilliam, and the other six, in 1901, by Dr. ROBERTSON, of the Agricultural Department of Cape Colony. To these gentlemen we take this opportunity of expressing our great indebtedness. The glands had been removed in South Africa from recently killed serpents, cleared of adhering fat and other extraneous matter, and then thoroughly and quickly dried by being hung up in a warm and airy place. Along with the dried glands, there were sent the heads of several of the serpents from which these glands had been removed. The latter were submitted to Mr. BOULENGER, F.R.S., of the British Museum, and he confirmed the identification of their being heads of *Sepedon hæmachates*.

The dry venom glands were hard, perfectly dry, and roughly pyramidal in shape. They varied in size from 19×8 to $8 \times 3{\cdot}2$ mm., the majority measuring about 15×7 mm. To ensure perfect dryness, the glands on their reception were placed *in vacuo* over sulphuric acid for several days.

In order to extract the venom from them, they were beaten in a mortar, cut into small pieces, and then triturated; by which means there was obtained a granular powder consisting of dry venom, mixed, however, with many larger particles of gland tissue. The latter were, as far as possible, removed from the powder; and this powder after digestion with thymol water, centrifuging and several filtrations, yielded a clear, golden yellow and viscous solution. When the solution was dried *in vacuo* over sulphuric acid, a solid residue was obtained, weighing 0·623 gramme, brownish yellow in colour, translucent, brittle, and easily soluble in water, the watery solution having a neutral reaction. By this process a venom was obtained that unavoidably contained a small, but unknown, quantity of substances other than true venom, which had been dissolved by the thymol water from the few unremoved fragments of gland structures. To this extent its activity would be somewhat less than that of the venom ejected during the bite of the living serpent. The nature of its action, however, would remain unchanged.*

In a few of the following experiments, we are indebted for assistance to Mr. W. J. MALONEY, at the time Houldsworth Scholar in Pharmacology.

* Although there are "Poison Doctors" in South Africa, who are regarded as experts in the subject of snakes and in the treatment of snake-poisoning, there does not appear to be any class of natives altogether representing the snake-charmers of India, who collect pure venom from living animals by "milking" them as it is there termed.

A. Lethality of Sepedon Venom.

The lethality of the venom was determined for frogs, rabbits, rats, and cats with the following results :—

(a) *In Cold-blooded Animals.*

Table I.—Minimum-lethal Dose by Subcutaneous Injection for Frogs.

No. of experiment.	Weight of frog in grammes.	Dose per kilogramme in grammes.	Actual dose in grammes.	Result.
1	24	0·0007	0·0000168	Recovery—very slight effects.
2	28	0·001	0·000028	Recovery—marked effects.
3	30	0·001	0·00003	Death in 10 days.
4	24	0·002	0·000048	,, 9 ,,
5	20	0·005	0·0001	,, 3 ,,
6	20	0·012	0·00024	,, 3 ,,

In these experiments, male frogs, *R. temporaria*, were used throughout. Injections were made into the dorsal lymph sac.

In the following experiments in warm-blooded animals, a constant time-interval was adopted between the reception of food by the animal and the administration of the venom, so as to lessen errors of body weight due to differences in the quantity of food in the alimentary canal of individual animals.

(b) *In Warm-blooded Animals.*

Table II.—Minimum-lethal Dose by Subcutaneous Injection for Rabbits.

No. of experiment.	Weight of rabbit in grammes.	Dose per kilogramme in grammes.	Actual dose in grammes.	Result.
7	2230	0·0007	0·00156	Recovery.
8	1935	0·00075	0·00143	Death in 29 hours.
9	1662	0·0009	0·0015	Recovery—very ill for 4 days.
10	2900	0·001	0·0029	Death in 24–35 hours.
11	1507	0·0025	0·00377	,, 1 hour 32 minutes.

The injections were made under the skin of the right flank.

Table III.—Minimum-lethal Dose by Intravenous Injection for Rabbits.

No. of experiment.	Weight of rabbit in grammes.	Dose per kilogramme in grammes.	Actual dose in grammes.	Result.
12	1940	0·00051	0·001	Recovery.
13	2470	0·00055	0·0014	Death in 2 hours.
14	1335	0·00075	0·001	,, 1 hour 40 minutes.

The injections were made into the marginal vein of the right ear.

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Table IV.—Minimum-lethal Dose by Subcutaneous Injection for Rats.

No. of experiment.	Weight of rat in grammes.	Dose per kilogramme in grammes.	Actual dose in grammes.	Result.
15	120	0·0015	0 00018	Recovery.
16	200	0·0016	0·00032	Death in 6 hours 20 minutes.
17	147	0·0025	0·000367	,, 2 ,, 30 ,,

The injections were made under the skin of the right flank.

Table V.—Minimum-lethal Dose by Subcutaneous Injection for Cats.

No. of experiment.	Weight of cat in grammes.	Dose per kilogramme in grammes.	Actual dose in grammes.	Result.
18	1920	0·01	0·0192	Recovery.
19	3150	0·015	0·0472	Death in 14 hours.

The injections were made under the skin of the right flank.

Table VI.—Minimum-lethal Dose by Subcutaneous Injection for Pigeons.

No. of experiment.	Weight of pigeon in grammes.	Dose per kilogramme in grammes.	Actual dose in grammes.	Result.
20	230	0·003	0·00069	Recovery.
21	265	0·0033	0·001	Death in 19 hours.
22	275	0·01	0·00275	,, $2\frac{3}{4}$,,

The injections were made under the skin of the right thigh.

Minimum-lethal Dose for Frogs.—Recovery followed from doses of 0·0009 gramme per kilogramme and under, but doses of 0·0012 gramme per kilogramme and over proved fatal. The minimum-lethal dose is, therefore, about 0·0012 gramme per kilogramme.

Minimum-lethal Dose for Rabbits: Subcutaneous.—Doses of 0·0007 and under were followed by recovery, doses of 0·001 and over proved fatal, while between these doses the result was doubtful. The minimum-lethal dose may therefore be assumed to be 0·001 gramme per kilogramme.

Intravenous.—Doses of 0·0005 gramme per kilogramme and under were followed by recovery; doses of 0·00055 and above proved fatal. The minimum-lethal dose by intravenous injection is, therefore, 0·00055 gramme per kilogramme.

Accordingly, the minimum-lethal dose by intravenous injection bears to the minimum-lethal dose by subcutaneous injection a ratio of very nearly 1 to 2.

Minimum-lethal Dose for Rats.—Doses of 0·0015 gramme per kilogramme and under failed to kill; doses of 0·0016 and over proved fatal. The minimum-lethal dose for rats is, therefore, 0·0016 gramme per kilogramme. The dividing line between lethal and non-lethal doses is much sharper in the case of rats than in the case of rabbits.

Minimum-lethal Dose for Cats.—0·015 gramme per kilogramme proved fatal; 0·01 was followed by recovery. It was not deemed advisable to determine the minimum-lethal dose more accurately, owing to the large amount of venom that a single experiment required. Seeing that, with a dose of 0·015 gramme, a cat died after 14 hours, while 0·01 gramme did not produce very serious results, the minimum-lethal dose may be taken with reasonable accuracy as 0·015 gramme per kilogramme.

Minimum-lethal Dose for Pigeons.—Doses of 0·003 gramme per kilogramme and under failed to kill; doses of 0·0033 and above proved fatal. The minimum-lethal dose is, therefore, 0·0033 gramme per kilogramme.

Table VII.—Comparison of Lethality for different Animals.

Animals used.	Frog.	Rabbit.	Rat.	Cat.	Pigeon.
Minimum-lethal dose in grammes per kilogramme	0·0012	0·001	0·0016	0·015	0·0033

The minimum-lethal dose for the rabbit being taken as unit, the proportion may be expressed thus: frog, 1·2; rabbit, 1; rat, 1·6; cat, 15; pigeon, 3·3.

In regard especially to the relatively greater resistance of the cat, this ratio of lethality in the case of *Sepedon hæmachates* venom shows a general resemblance to the following ratio, which has been found by one of us* for Indian cobra venom, viz.: frog, 0·8; rabbit, 1; rat, 1; cat, 20.

The latter venom, however, possesses a higher absolute toxicity, the minimum-lethal dose for the rabbit being 0·0003 gramme per kilogramme.

B. Symptoms produced by Sepedon Venom.

(a) *In Cold-blooded Animals—Frogs.*

The following experiment will serve to illustrate the general symptoms of poisoning by the venom.

Experiment 23.—*Rana temporaria*, male, weight 22 grammes. November 14, 1905.—At 10 A.M. the respirations were 18 in 10 seconds, the cardiac impacts 9 in 10 seconds, the lymph hearts were beating 8 in 10 seconds, and the animal appeared

* Fraser, 'Roy. Soc. Edin. Proc.,' 1896, vol. 20, p. 454.

healthy and vigorous. At 12 noon, 0·33 c.c. of a solution of *Sepedon hæmachates* venom (0·001 gramme dissolved in 10 c.c. saline) was injected into the dorsal lymph sac. This dose represents 0·000033 gramme of venom, equivalent to 0·0015 gramme per kilogramme of frog-weight and, therefore, slightly over the minimum-lethal dose. At 6 P.M. the respirations were 8 in 10 seconds, and the cardiac impacts 9 in 10 seconds. The frog sat with the limbs fully drawn up. No spontaneous movements were made, but when pinched the frog jumped well, and rapidly recovered the usual posture when laid on the back. The lower eyelid was raised so as to cover half the eye. The conjunctival reflex was active.

November 15, at 10.40 A.M., apart from an occasional movement of the nostrils, the respiratory movements had completely ceased. The condition was otherwise unchanged. At 3.30 P.M. the conjunctival reflex was less active, and the frog now weighed 25 grammes. No movement of the lymph hearts could be detected.

November 16, 10 A.M.—No respiratory movement of any kind was observed; the cardiac impacts were 7 in 10 seconds, and very faint. The lower eyelid covered the eye, and no reaction resulted from touching the conjunctiva. When laid on the back, the frog made vigorous tilting movements, but these were ineffectual to restore the usual posture. When pinched, the frog jumped well but alighted somewhat helplessly, and sometimes fell on the back. The weight was now 27 grammes.

November 17, 11 A.M.—There were no respiratory movements, and the cardiac impacts were 6 in 10 seconds, very faint and difficult to count. The frog lay prone with the limbs close to the body, and the throat and abdomen, both much swollen, rested on the slate. Strong pinching elicited a feeble jump. When laid on the back, feeble, ill-sustained, and ineffectual tilting movements were made. The croaking reflex, which had hitherto been present, was inelicitable. The weight was now 32 grammes.

November 18, 1 P.M.—There were no respiratory movements. The cardiac impacts were invisible, but examination under the microscope of the web of the foot showed that the circulation was still going on, but only sluggishly. When the frog was placed on his back, no movements were made. Strong faradic stimulation of the skin of the leg elicited contractions only of the underlying muscles, but no remote movements. The weight was now 35 grammes.

November 19, 12.15 P.M.—No movements were elicitable by mechanical stimulation, and none by electrical stimulation apart from contractions of the underlying muscles when the skin was stimulated by strong faradic excitation. Examination of the web of the foot under the microscope showed that the circulation had ceased. The thorax was opened to expose the heart. A large amount of clear fluid poured out, so that the frog thereafter weighed 30 grammes. The ventricle was found to be dilated and dark red, and arrested in diastole. The auricles were beating very feebly at the rate of 4 in 10 seconds. Mechanical stimulation failed to provoke contraction of the ventricle, and electrical stimulation produced only pallor of the

ventricle at the site of application of the electrodes. The auricles ceased beating at 12.50. The reaction of the ventricular muscle was neutral.

On opening the skin of the thigh to expose the sciatic nerve, serous fluid was found beneath the skin and between the muscles, in considerable quantity. Faradic stimulation of the sciatic nerve failed to affect the muscles with the secondary coil even at zero. Direct stimulation induced contraction of the muscles with the secondary coil at 50 mm. The reaction of the gastrocnemius muscle was neutral.

The symptoms of poisoning with doses up to 10 times the minimum-lethal dose differ from those described only in their earlier onset.

(b) *In Warm-blooded Animals.*

1. *Rabbits: Experiment* 8, May 11, 1906. — White buck rabbit, weight 1935 grammes. At 10.30 A.M., the cardiac impacts were 37 in 10 seconds; and the respirations, when the animal was sitting quietly, were 7 in 10 seconds. The rectal temperature was 101°·6 F.

At 11.5, 0·715 c.c. of venom solution (0·002 gramme in 1 c.c. thymol saline) was injected under the skin of the right flank. This gave an actual dose of 0·00143 gramme, equivalent to 0·00075 gramme per kilogramme.

At 4 P.M., the cardiac impacts were 36, and the respirations 8 in 10 seconds. The rectal temperature was 103° F. Up to this time, no symptoms of poisoning had been shown apart from a gradual rise in temperature.

At 5 P.M., the temperature was 104° F., the maximum rise in this experiment. Food and water were readily taken.

At 6.30 P.M., the cardiac impacts were 37, and the respirations 6 in 10 seconds. Temperature 103°·5. The rabbit was very drowsy.

At 9.30 P.M., the cardiac impacts were 39, the respirations 9 in 10 seconds, and the temperature 102°·8. The rabbit was lying with the head resting on the floor of the tray with the ears folded back, and seemed very drowsy and inert, and refused food.

May 12, 1906.—At 9.50 A.M., the cardiac impacts had fallen to 10, and the respirations to 4, in 10 seconds. Temperature 95°. The eyes were half closed, the pupils were contracted, and the corneal reflex sluggish. The animal was lying with the nose on the floor of the tray, with the head leaning to one side. Breathing was laboured, the mouth being opened, and the nostrils moved strongly with each inspiration. Saliva flowed from the mouth. From 9.50 to 11.20 A.M., the animal remained in much the same condition. The temperature was still falling, the conjunctiva was very insensitive, the ears were livid, and there were frequent convulsive struggles, during which the heart-beats were irregular.

At 10.55 A.M., the cardiac impacts were 9 per 10 seconds. The respirations,

which were now gasping in character, were at the rate of 3 in 10 seconds. The animal lay on the side and had occasional convulsive seizures.

The conditions remained the same up to 2 P.M. The cardiac impacts were as before, 9 in 10 seconds; but the respirations had fallen to 1·5 in 10 seconds, and were still gasping in nature. The conjunctival reflex was very sluggish. The temperature had fallen below 90° F., which was the lowest limit to which the thermometer recorded.

At 2.45 P.M., the pupils, which had hitherto been contracted, began to dilate. At 3.35 the respiratory movements were becoming much shallower, the mouth opening widely at each inspiration but almost no abdominal movement taking place. There were now no struggles, the animal lying quietly on its side. The pupils were dilated, and the conjunctival reflex completely gone. At 3.55, the cardiac impacts were 8 per 10 seconds, and respiration was represented by one gasping inspiration each half minute, but no appreciable movement of the abdomen accompanied it. The pupils were widely dilated. At 4 P.M., all respiratory movement had ceased.

The thorax was opened, and at 4.5 the heart was found to be beating at the rate of 4 per 10 seconds, auricles and ventricles contracting synchronously. At 4.20, the heart beats were 2 per 10 seconds, and at 4.35 they ceased. Both ventricles and auricles were flaccid, the right heart being dilated and the left ventricle was practically empty of blood. No clots were found in the heart, pulmonary arteries or portal system. There was considerable congestion and œdema of the lungs and congestion of the bronchi. There were no hæmorrhages in the serous cavities or elsewhere. Blood taken from the right ventricle clotted in about 5 minutes, and the serum, which separated later, was free from hæmoglobin.

2. *Cats: Experiment* 19.—May 25, 1906. Black and grey cat. Male. Weight 3150 grammes. At 10 A.M., the cardiac impacts were 30 and the respirations 5 per 10 seconds.

At 10.5, 0·94 c.c. of venom solution (0·05 gramme in 1 c.c. thymol saline) was injected under the skin of the right flank. This gave an actual dose of 0·047 gramme, equivalent to 0·015 gramme per kilogramme.

For half an hour after the injection, the cat moved about uneasily, and seemed unable to settle down.

At 10.45, drowsiness appeared, and for the next three hours the cat lay usually with the eyes shut, but occasionally got up and moved about.

At 2.30 p.m., the cat was sleeping and when roused refused food. The conjunctival reflex was less acute; when placed on the floor, the cat walked unsteadily; breathing was somewhat noisy, but was still at the rate of 5 in 10 seconds; and the cardiac impacts were 29 in 10 seconds.

At 3.50 there were frequent jerky hiccough-like movements of the diaphragm. The animal walked with a staggering gait, with the head hanging down and saliva trickling from the mouth. On jumping from a chair on to the ground, the fore limbs collapsed under the body.

At 6 the cat was lying on the side, sleeping. The rate of the heart beats had fallen to 20, the respirations to 4, in 10 seconds. When roused and placed on the ground, the animal made no attempts to walk, but the limbs slowly collapsed until the head touched the ground. The skin felt very cold.

At 10 P.M., the cardiac impacts were 15, and the respirations 3, in 10 seconds. The cat lay stretched out on the side. The mouth opened widely at each inspiration, and the abdominal respiratory movement was more ample than before. The skin felt very cold. The eyes were open, the pupils semidilated, and the conjunctival reflex almost gone. Convulsive seizures began at 10.15 P.M., the respirations being then 3, and the cardiac impacts 16, in 10 seconds. These convulsions became more frequent and severe up to 11.25, at which time they were so persistent that the rate of the heart and respirations could not be counted. The pupils were now widely dilated.

At 11.30, convulsions ceased. The cardiac impacts were 14 per 10 seconds and so forcible that they could be counted by merely observing the movement of the thoracic wall. The respiratory movements were shallow and only at the rate of about one in 30 seconds.

Up to 11.50 P.M. the cat remained in much the same condition, respiration consisting of an occasional deep inspiration, sometimes abrupt, sometimes slow and heaving, with rapid forcible expiration. At 11.57 P.M. the respirations ceased altogether, without any preceding convulsions. The heart was then beating 9 in 10 seconds, and it ceased to contract at 12.1 A.M.

3. *Pigeons: Experiment* 22.—June 22, 1908. Weight of pigeon 275 grammes.

At 9.50 A.M. the respirations were 8 in 10 seconds.

At 10, 0·00275 gramme of Sepedon venom dissolved in 0·6 c.c. of thymol saline was injected under the skin of the right thigh. This was equivalent to a dose of 0·01 gramme per kilogramme.

At 11.30 the respirations were still 8 in 10 seconds. The pigeon supported itself on the left leg alone, the right leg, into which the venom had been injected, being kept in a position of full extension.

At 11, the respirations were 8 in 10 seconds, and the pigeon kept the same posture but was now affected with tremors. The lower eyelid covered half the eye.

At 11.30 the bird could no longer stand or fly and respirations were now more laboured but still at the same rate.

At 12 the rate of the respirations had fallen to 5 in 10 seconds, inspiration being slow and heaving and accompanied by gaping of the mouth. The bird lay on the floor with the wings partly extended and a few feeble convulsions took place.

From this time onwards respiration and motor power became gradually more impaired, until at 12.50 all respiratory movements ceased without any preceding convulsions.

(c) *Summary of Symptoms.*

It is apparent that in the frog the selective action of the venom is on the respiration. Respiratory paralysis is one of the earliest symptoms and occurs at a time when the heart beats are only slightly slowed and when the circulation in the web of the foot is seen to be efficient. In the progress of poisoning, reflex excitability becomes gradually impaired, and complete motor paralysis occurs some time before arrest of the heart. The heart stops in diastole. There is a progressive increase in weight due to accumulation of fluid under the skin and in the serous cavities, from absorption of water on the dish on which the frog was kept during the experiment; there is no increase in weight when the dish is kept dry. Elevation of the lower eyelids is an invariable symptom of poisoning in the frog with this venom as it is with cobra venom.

In the case of warm-blooded animals, the two quoted experiments with mammals, in each of which the dose administered was about the minimum-lethal dose for the particular animal, show that the symptoms of poisoning by *Sepedon hæmachates* venom in the rabbit and cat are qualitatively so similar that they may be conveniently discussed together, though no doubt the relative prominence of certain symptoms varies somewhat according to the animal.

The most obvious and invariable symptom of poisoning is progressive impairment of the respiration, and arrest of respiration is the actual cause of death in all cases. The heart, so far as one can ascertain by palpation, is not markedly impaired in strength or rate until there is pronounced embarrassment of the respiration. The heart is then slowed, reflex inhibition due to asphyxia probably being sufficient to account for this slowing. The heart always continues to beat for several minutes after all respiratory movements have ceased, and it stops in a condition of diastole.

The onset of asphyxia is attended by convulsions, and these convulsions appear to be most violent in the cat, and least so in the rabbit. In the rabbit, especially, increased venosity of the blood is clearly manifested by the colour of the ears and retina, and in these animals it was noted that the convulsions succeeded the appearance of lividity of the ears and retina. There is, therefore, no reason to suppose that the convulsions are due to a direct action of the venom; rather are they secondary to asphyxia. The absence of convulsions in frogs supports this view.

The venom appears to have a direct enfeebling action on the central nervous system. That it exerts a distinct hypnotic action is indicated by the onset, often early in poisoning, of marked drowsiness. There is definite impairment of the reflexes, and marked loss of motor power. In the cat, especially, ataxia is pronounced. The pupil is contracted until shortly before death, dilatation of the pupil being an indication of the near approach of a fatal issue.

The symptoms of poisoning in pigeons resemble in their main features those in

mammals. Convulsions are not violent or long continued, and death, which is preceded by motor paralysis, is due to arrest of the respiration.

With this preliminary generalisation, the action of the venom on different systems and structures may now be considered in somewhat greater detail.

C. Effects on the Cerebro-spinal Nervous System.

(a) *Brain and Spinal Cord.*

The occurrence in mammals of drowsiness, ataxia, and impairment of the reflexes indicates that the venom lowers the functional activity of the brain and spinal cord.

In the frog, apart from experiments specially performed for the purpose of locating the site of the paralysis, the symptoms observed in the course of experiments made to determine the minimum-lethal dose give some indications of the nature of the paralysis. Thus, with doses near the minimum-lethal dose, generally on the second or third day after administration, while respiration is paralysed, the conjunctival reflex inactive or absent, and motor power greatly weakened, vigorous and sustained kicking movements still follow strong mechanical or electrical stimulation of the skin of the leg. The probability, therefore, is that in the earlier stages of poisoning with these doses, the impairment of motility is due to a central paresis rather than to a paresis of the motor nerve-ends. That there is, however, later in the poisoning a paralysing action on the motor nerves is clear from the fact that, during the paretic stage and a day or more before failure of the circulation, contraction of the gastrocnemius muscle cannot be elicited by faradic stimulation of its sciatic nerve, whereas the muscle when stimulated directly reacts to a strength of stimulus which is not much above the minimal for an unpoisoned muscle.

That this venom has a paralysing action on the central nervous system is rendered more evident in the case of large doses, as is seen in the following experiment.

Experiment 24.—A male frog (*R. temp.*), weighing 20 grammes, received by subcutaneous injection 0·0004 gramme of venom, equivalent to 0·02 gramme per kilogramme. Five hours after injection the respirations were completely arrested, the conjunctival reflex was inelicitable, and no reflex movements ensued on strong mechanical stimulation; but the circulation in the web of the foot was found to be active. The cerebrum was pithed and the left sciatic nerve exposed. Faradic stimulation of the left sciatic nerve with the coil at 220 mm. produced contraction of the left gastrocnemius muscle, and this muscle when directly stimulated reacted at 100 mm. Even when the nerve was stimulated with the coil at zero no movements other than of the left gastrocnemius muscle were produced, showing that although there might possibly have been already a slight impairment of excitability of the motor nerve-ends, this impairment was quite inadequate to explain the complete absence of reflex excitability.

A number of experiments were made by which the comparative actions of the

venom on the spinal cord and motor nerves were defined by preventing access of the venom to the nerve-ends and muscles of one limb by ligature of its vessels, and then testing the reflex excitability of both limbs by TURCK's method. The following experiment will serve to illustrate the results obtained. Owing to the protracted nature of the poisoning with merely minimum-lethal doses, it was necessary to use considerably larger doses.

Experiment 25.—November 22, 1905. *R. temp.* Male. Weight 22 grammes.

The brain of the frog was pithed at 9.20 A.M., and at 9.37 the vessels of the right thigh were ligatured, without hæmorrhage.

At 11, the webs of the feet were examined under the microscope, and the circulation was found to be arrested in the right foot, but quite active in the left foot.

The strength of sulphuric acid used was 1 in 1000. Care was taken that the immersion of the foot in the sulphuric acid solution was to the same point each time, and that the foot was, immediately after immersion, carefully washed with water to remove all trace of acid. The results are shown in the following table, intermediate superfluous readings being omitted.

Table VIII.

Time.	Left (unprotected) foot immersed in 1/1000 H_2SO_4.	Right (protected) foot immersed in 1/1000 H_2SO_4.	Notes.
11.30 A.M.	Withdrawn in 3 seconds	Withdrawn in 4 seconds.	0·02 gramme per kilogramme injected into left flank.
11.45 „	„ 3 „	„ 4 „	
12.0	— —	— —	
12.45 P.M.	Withdrawn in 8 seconds	Withdrawn in 8 seconds.	
2.30 „	„ 13 „	„ 12 „	
3.30 „	„ 20 „	„ 15 „	
4.0 „	„ 55 „	„ 45 „	
5.0 „	Not withdrawn in 60 seconds	Not withdrawn in 60 seconds.	

In five hours, therefore, there was a marked prolongation of the time required for withdrawal of the foot after immersion in acid (the reflex activity of a control frog as tested in the same way is not materially weakened in 24 hours). Since this prolongation occurred in the case of the right (protected) foot almost *pari passu* with that of the left, it was mainly due to an action on the spinal cord. It was not due to a paralysis of the sensory or motor nerves, for the time required for withdrawing the right foot, of which the nerve terminals were protected, was lengthened equally with the time required for withdrawing the left foot; nor was it an indirect effect on the cord due to arrest of the circulation, for the circulation in the web of the left foot was still quite active five hours after the injection.

The fact, however, that in four hours the right foot was withdrawn somewhat more

rapidly than the left, indicated that there was probably also a minor effect on the poisoned motor nerve.

To determine this, the sciatic nerves were exposed at 5.30 P.M., when it was found that faradisation of the left sciatic with the secondary coil at 200 mm. induced contraction of its gastrocnemius muscle, but that the right (protected) gastrocnemius reacted to similar stimulation of its sciatic nerve with the coil at 260 mm. No crossed reflex was obtained by stimulation of either sciatic nerve, even with the coil at zero. At 8.20 P.M., the left sciatic reacted at 100 mm., the right sciatic at 230 mm., and the muscles directly stimulated reacted at 90 mm. in each limb. Therefore, in eight hours, there was marked paresis of the nerve-ends in the poisoned limb.

This experiment confirms what was deduced from observation of intact poisoned frogs, namely, that there is produced a primary weakening of the reflex excitability of the spinal cord, and later a curare-like action on the motor nerve-ends.

(b) *Nerves.*

1. *Motor Nerves.*—The action of the venom on the neuro-muscular mechanism was also investigated by CLAUDE BERNARD'S method, and it was found that when a frog's gastrocnemius was immersed in a solution of 1 in 5000 of venom, stimulation of its sciatic nerve ceased to affect the muscle in 90 minutes, while the muscle ceased to react to direct stimulation in 130 minutes. Since this strength of solution applied to a sciatic nerve trunk did not at all affect its excitability in over three hours, and only very slightly in six and a-quarter hours, one may infer that the venom has a selective paralysing action on the nerve-ends, and that it also, in this concentration, afterwards abolishes the excitability of muscle.

Several of our previously described experiments had shown that this action on the motor nerve-ends in frogs is an important factor in poisoning by *Sepedon hæmachates* venom, whereas the action on muscles is relatively unimportant, seeing that the excitability of muscle is only slightly impaired, even at the time of death from many times the minimum-lethal dose.

In the case of mammals, on the other hand, Sepedon venom has little or no action on the motor nerve-ends. In many experiments on rabbits and rats the excitability of the sciatic nerves was determined just after death, and it was invariably found that they responded to weak faradic stimulation; thus in Experiment 16, a rat died about six and a-half hours after injection, and faradic stimulation of the right sciatic nerve induced contraction of the gastrocnemius muscle with the secondary coil at 270 mm., five minutes after death.

2. *Sensory Nerves.*—A solution of venom of 1 in 1000, when injected under the skin of a frog, almost completely abolishes the excitability of the sensory nerves, to which the solution has access, in three hours. Sepedon venom therefore exerts a paralysing action on sensory nerves as well as on motor nerves, but this action is

evidently too feeble to explain the absence of reflex excitability seen after subcutaneous injection, a conclusion to which Experiment 25 had already led.

D. Effects on Skeletal Muscle.

A number of experiments were made in which an isolated muscle was immersed in a solution of venom in 0·65-per-cent. saline, and graphic records were taken on a rapidly revolving drum of single contractions evoked by direct application of break shocks. It was found that no alteration in the character of the muscle contraction was produced beyond what occurs in a dying muscle. No fibrillary twitches occurred.

E. Effects on the Circulation.

(a) *Heart.*

The description given of the symptoms occurring in the frog after subcutaneous administration of slightly over the minimum-lethal dose (*vide supra*, Experiment 23) shows that while the respiratory movements were arrested on the day after injection and motor paralysis was practically complete on the third day, the heart continued to beat for five days after the injection. It was also seen that the rate of the heart beats diminished very gradually, that the amplitude of its contractions was afterwards enfeebled, so far as this could be judged from observation of the cardiac impacts, and that it was arrested in diastole. This would appear to be the effect on the heart with doses even so large as 10 times the minimum-lethal dose, but, as will afterwards be pointed out, with 30 times the minimum-lethal dose, the heart is arrested in systole.

Further experiments were made to determine the action of the venom on the isolated heart, Schäfer's Heart Plethysmograph being used. The bulb of the plethysmograph which contained the ventricle was filled with Ringer's solution. The metal cylinder in which the piston travelled was filled with liquid paraffin. The reservoirs were filled with a mixture of defibrinated ox blood (1 part) and Ringer's solution (2 parts). This mixture was thoroughly aërated, and part of it used as the solvent for the venom. The ventricle was perfused with normal solution for at least 20 minutes before the venom solution was turned on.

Experiment 26 (figs. 1, 2, and 3).—Strength of solution 1 in 5000. The effects produced by this solution are stated in the following table.

The extent of relaxation of the ventricle was diminished almost instantaneously on admission of this solution, so much so that in 1 minute the extent of excursus was diminished from 14 mm. to 1 mm.; coincidentally, the rate of ventricular beat was almost doubled. Four minutes after the venom solution had been turned on, the ventricle began to relax slightly farther, but still its diastole was very incomplete. In 10 minutes, its rate slowed from 29 to 11 per 60 seconds, and its excursus increased from 2 to 4 mm. One minute later, a pause lasting for 40 seconds occurred in this incomplete diastole. Thereafter the rate of beat increased gradually, while the

Table IX.—Experiment 26.

Time.	Amplitude of excursus.	Rate per 60 seconds.	Notes.
	mm.		
12.0	14	14	
12.10	14	15	
12.11	—	—	S.H. venom (1 in 5000) turned on (fig. 1).
12.12	1	29	
12.15	2	29	
12.18	2	29	
12.21	4	11	Succeeded by pause in semidiastole of 40 seconds (fig. 2).
12.26	2	11	
12.29	1	13	
12.35	0·5	17	(Fig. 3.)
12.40	0	0	

ventricle went into a condition of systole and was finally arrested in that state 30 minutes after the venom solution had been turned on.

Table X.—Experiment 27. Strength of Solution 1 in 50,000.

Time.	Amplitude of excursus.	Rate per 60 seconds.	Notes.
	mm.		
11.59	17	8	
12.9	17	8	
12.11	—	—	Solution of venom (1 in 50,000) turned on.
12.16	13	13	Diastolic expansion lessening.
12.20	11	13	,, ,,
12.28	15	14	Systolic contraction lessening.
12.39	13	13	,, ,,
1.0	10	13	,, ,,
1.30	7	13	,, ,,
2.0	6	13	,, ,,
2.30	4	14	,, ,,
3.0	1·0	?	Mere undulatory movements.
3.10	0·5	?	,, ,,
3.12	0	0	Arrest in semidiastole.

For the first six minutes this solution produced effects similar to those produced by stronger solutions, namely, an increase in the rate of beat and a decrease in the amplitude of expansion. Thereafter, however, the ventricle again began to dilate farther during diastole, while the systolic contraction became less complete, and no further increase in the rate occurred. The excursus became gradually less and less until in three hours the ventricle was arrested in a position of incomplete diastole.

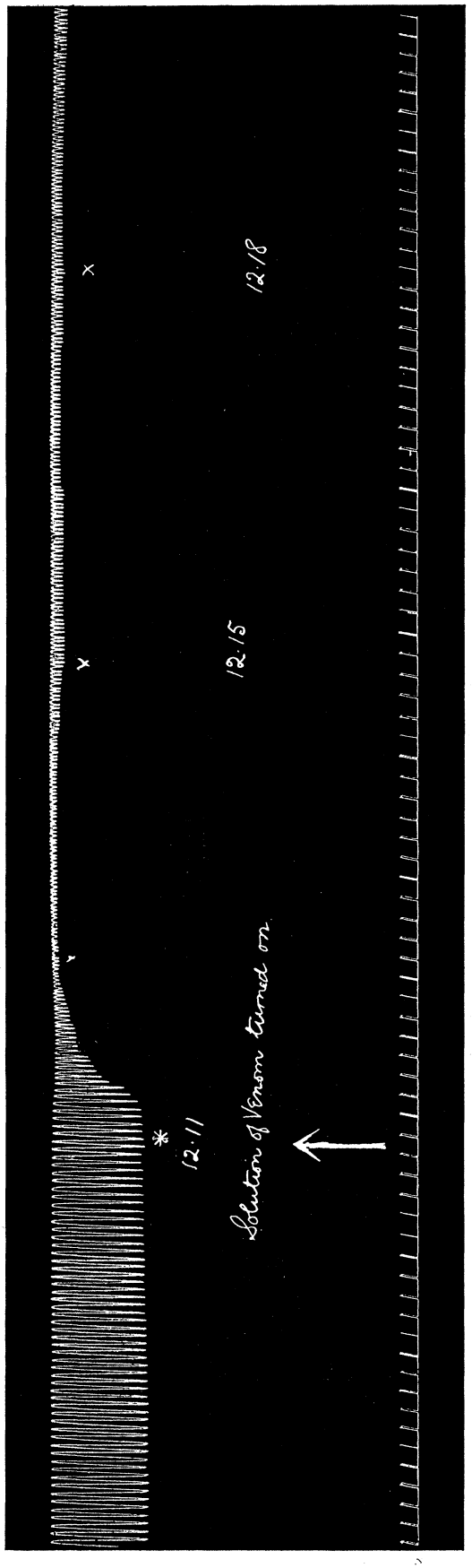

FIG. 1.

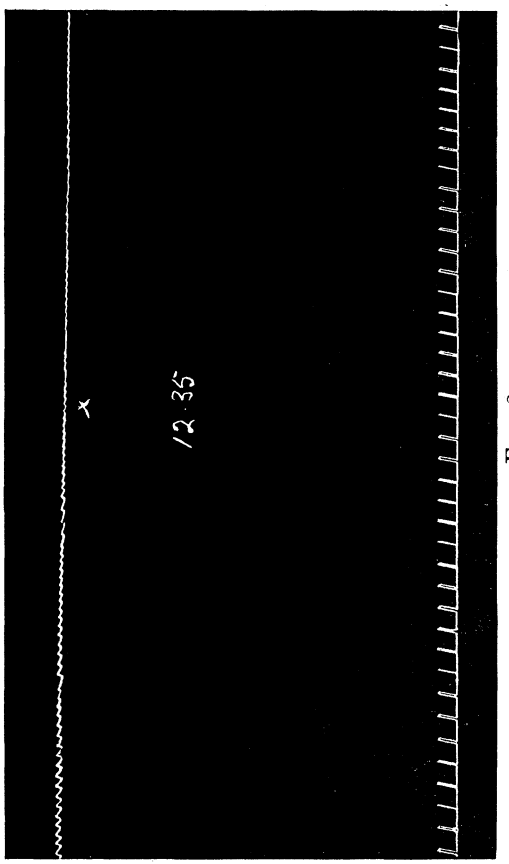

FIG. 3.

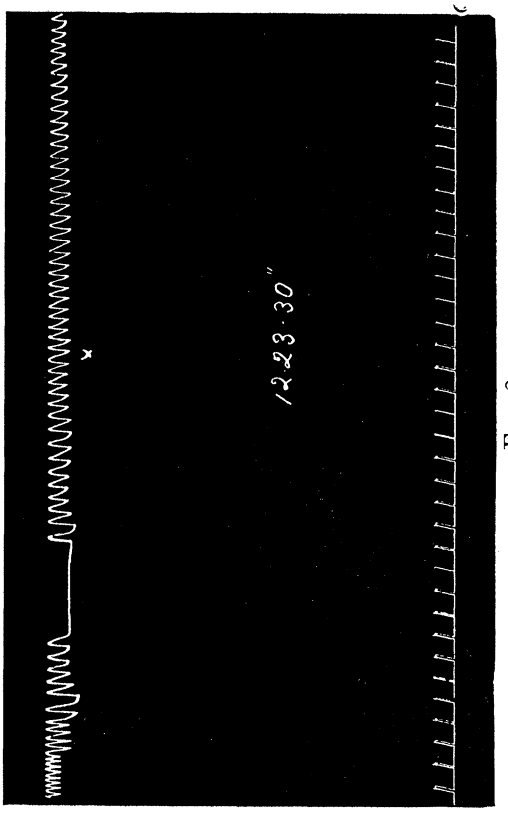

FIG. 2.

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Table XI.—Experiment 28. (Figs. 4, 5, and 6.) Strength of Solution 1 in 100,000.

Time.	Excursus.	Rate per 60 seconds.	Notes.
	mm.		
3.25	10	21	
3.29	10	16	
3.30	—	—	Solution of S.H. venom 1 in 100,000 turned on (fig. 4).
3.36	8	16	Systolic contraction lessening.
3.47	7	17	,, ,,
4.4	6	15	,, ,,
4.34	3	16	,, ,,
5.30	2	14	,, ,,
6.0	1	13	,, ,, Experiment discontinued (fig. 6).

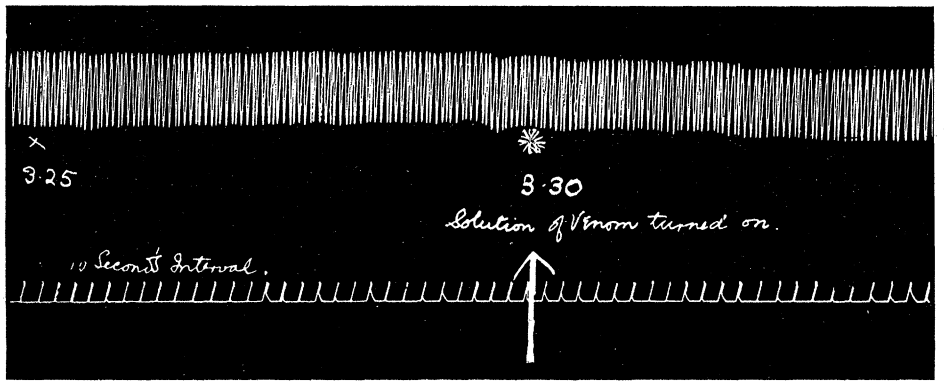

FIG. 4.

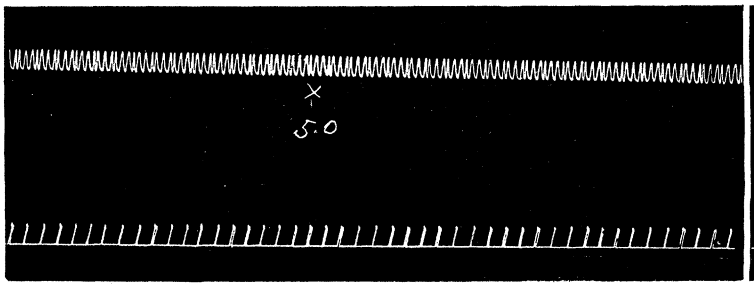

FIG. 5.

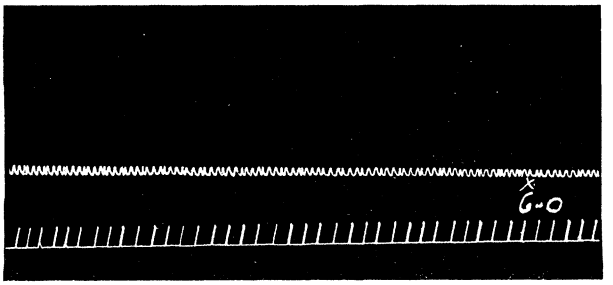

FIG. 6.

The systolic contraction was rendered less complete from the beginning of perfusion with this strength of solution of venom. In two and a-half hours the rate of beat was slightly slowed, and the ventricle was in a state of complete diastolic relaxation, interrupted by extremely feeble systolic contractions.

The results of frog-heart perfusions have been described at some length, partly because the effects vary qualitatively with different strengths of solution and partly

because they are interesting when compared with those produced by cobra venom. Solutions of 1 in 5000 (see Experiment 26), 1 in 10,000, and 1 in 20,000 quicken the rate of beat of the isolated ventricle and bring about its arrest in systole. A solution of 1 in 100,000 slightly slows the heart and brings about arrest in diastole. A solution of 1 in 50,000 produces an intermediate effect.

ELLIOT* found that a solution of cobra venom similarly perfused through the frog's ventricle increases the rate of beat and kills in systole even in so great dilution as 1 in 500,000 or in 1 in 1,000,000. Evidently the action of *Sepedon hæmachates* venom in solutions of up to 1 in 20,000 resembles this action of cobra venom.

ELLIOT also found that with cobra venom in more dilute solutions "there is a gradual tendency for the heart to beat faster at first and slower later on. At the same time its excursus slowly and steadily diminishes, and the organ drifts towards a position of diastole." This action of cobra venom also resembles that of more dilute solutions of Sepedon venom. (Experiments 27 and 28.)

While the minimum-lethal dose of the cobra venom used by ELLIOT was probably about one-half that of the Sepedon venom used in our experiments, the action of the former on the isolated frog's ventricle is at least 25 times as great. RAGOTZI† found that, injected subcutaneously, large doses of cobra venom stop the frog's heart in systole, small doses in diastole. We have stated that with doses of Sepedon venom up to ten times the minimum-lethal the heart is arrested in diastole. We found, however, that, with thirty times the minimum-lethal dose of Sepedon venom, the frog's heart is arrested in systole. The action of Sepedon venom on the frog's heart is therefore qualitatively precisely similar to that of cobra venom as found by RAGOTZI and ELLIOT.

(b) *Blood-vessels.*

The brain and spinal cord of a frog were thoroughly pithed at least half an hour before the beginning of each experiment. A cannula, attached by glass tubes to a series of Mariotte's flasks, the fluids in which were maintained at the same level, was inserted into the left aorta, the right aorta being ligatured. A continuous record was taken of the amount of fluid escaping from the cut *venæ cavæ*. The normal calibre of the vessels was ascertained by causing 0·65-per-cent. sodium chloride solution to flow through them for from 20 to 30 minutes until the flow was uniform. The venom was dissolved in the same strength of saline solution.

It was found that a solution of Sepedon venom of 1 in 20,000 reduced the flow through the vessels from 2·5 c.c. per minute to 0·6 c.c. per minute in 40 minutes; a solution of 1 in 40,000 reduced the flow from 1·9 c.c. to 0·8 c.c. in one hour; a solution of 1 in 50,000 reduced the flow from 1·6 c.c. to 1 c.c. in one hour; while solutions of 1 in 60,000, 1 in 70,000, and 1 in 140,000 produced no effect.

* Elliot, 'Phil. Trans.,' 1905, vol. 197, p. 369.

† Ragotzi, 'Virchow's Archiv,' vol. 122, p. 201.

Elliot* found that a solution of cobra venom of 1 in 10,000,000 produced a distinct constriction of the frog's vessels, the flow being halved in 30 minutes, thus corresponding somewhat in activity to a solution of Sepedon venom of 1 in 40,000. Stronger solutions of cobra venom produced a more powerful constriction effect. It is therefore apparent that, as in the case of the heart, the action of Sepedon venom on the blood-vessels of the frog is qualitatively similar to that of cobra venom, but very much weaker, being at least 250 times less powerful.

(c) *Heart and Blood-vessels.* (Blood-pressure.)

Kymographic experiments were made with a view to determining the effects of the venom on the blood-pressure and respirations.

Experiment 29, Table XII (figs. 7 to 13 inclusive).—June 5, 1907. A rabbit, weighing 1850 grammes, was anæsthetised with chloroform. The trachea was then opened and a cannula tied into it through which the animal respired. Ether was

Table XII.

Time.	Average blood-pressure in mm. Hg.	Pulse rate per 10 seconds.	Respiration rate per 10 seconds.	Respiration excursus in mm.	Notes.
3.24	80	39	10	2·5	Pulse waves about 1 mm.
3.26	84	37	10	2·5	
3.26.30	—	—	—	—	0·0005 gramme per kilogramme injected intravenously (fig. 7).
3.28	80	39	10	3·0	
3.29.30	74	37	10	3·0	
3.30	84	38	10	3·0	
3.30.30	—	—	—	—	0·001 gramme per kilogramme injected intravenously.
3.32	94	38	10	3·0	
3.35	95	36	10	3·0	
3.40	99	35	10	2·0	
3.45	107	34	9	1·0	(Fig. 8.)
3.54	110	33	9	0·5	
4.0	110	29	7	0·5	Pulse waves 3 mm. (fig. 9).
4.3	116	24	7	0·5	
4.5	122	18	6	Mere undulations	„ 5 to 7 mm. (fig. 10).
4.7	112	13	5		
4.8	—	—	—	—	Asphyxial convulsions (fig. 11).
4.9	120	15	4	2·0	Pulse waves (10 mm.).
4.10.20	110	12	1	—	Terminal respiratory gasps. Blood-pressure began to fall (fig. 12).
4.11	76	9	0	—	Pulse waves 12 mm.
4.13	44	8	0	—	„ 7 „ (fig. 13).
4.15	14	7	0	—	„ 0·5 „
4.16	0	0	0		

* Elliot, 'Phil. Trans.,' 1905, vol. 197, p. 365.

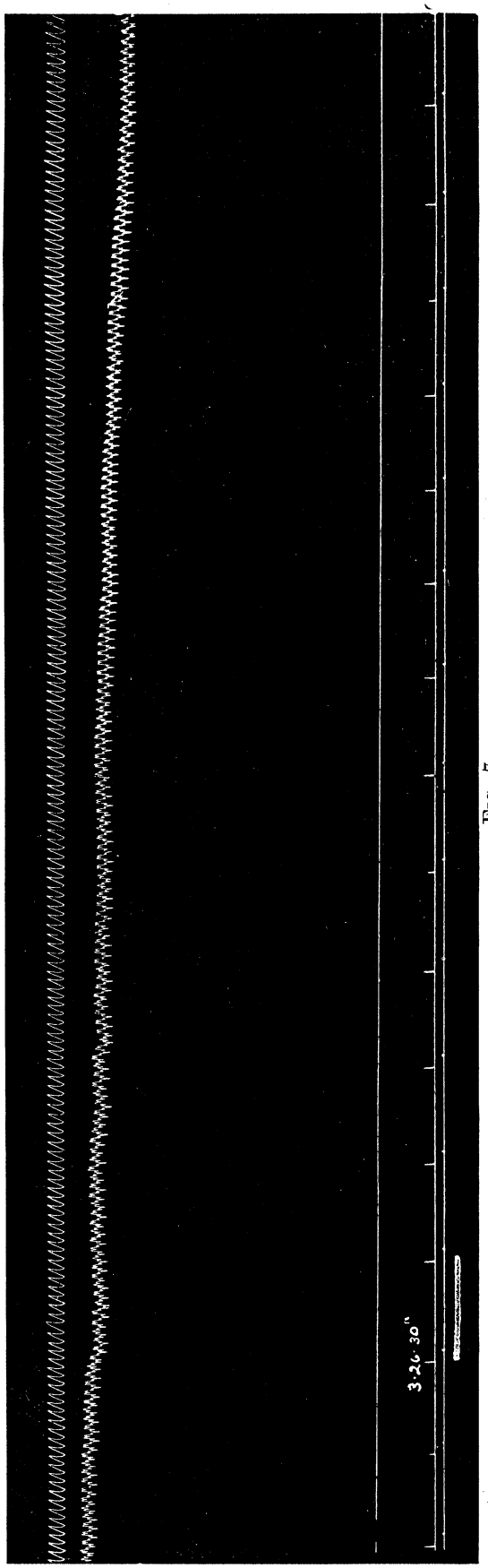

FIG. 7.

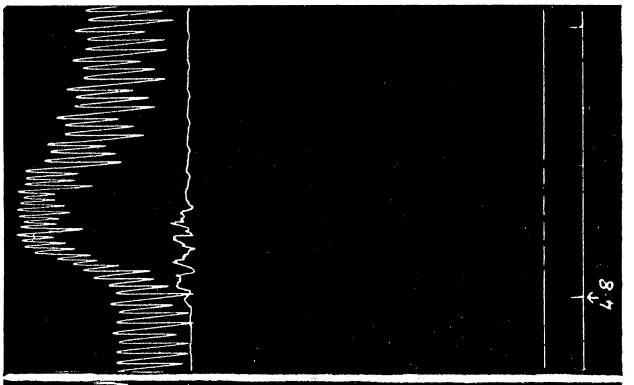

FIG. 11.

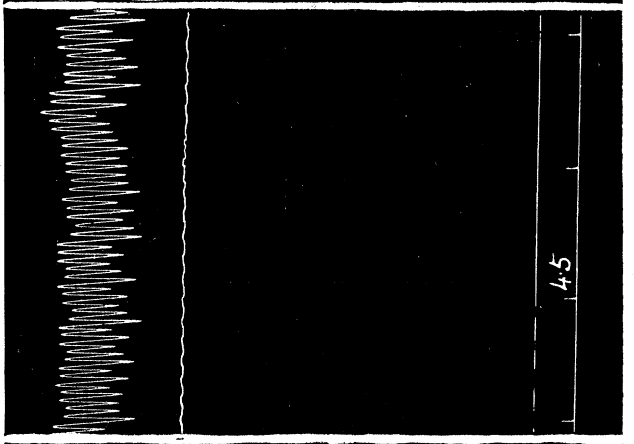

FIG. 10.

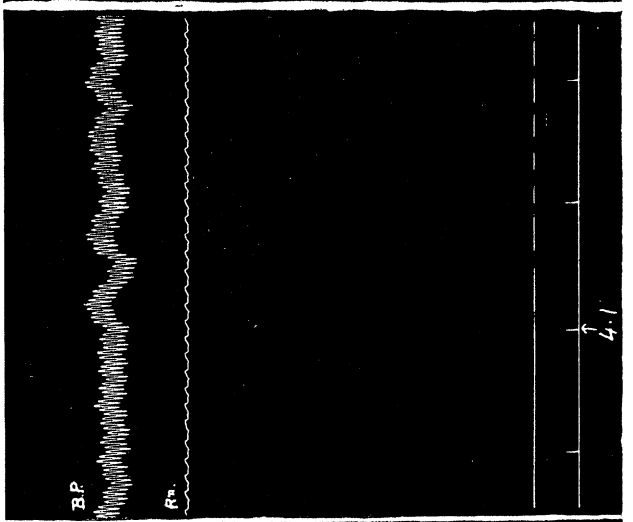

FIG. 9.

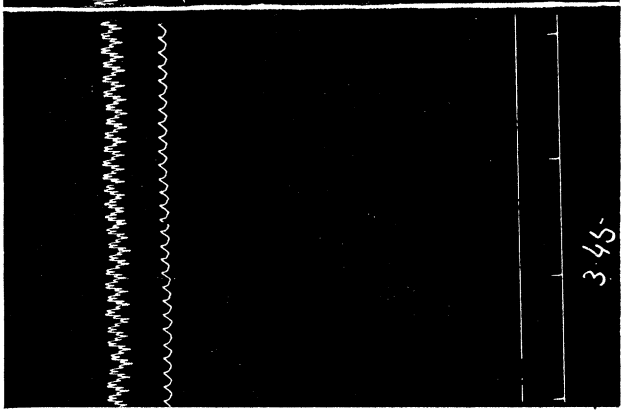

FIG. 8.

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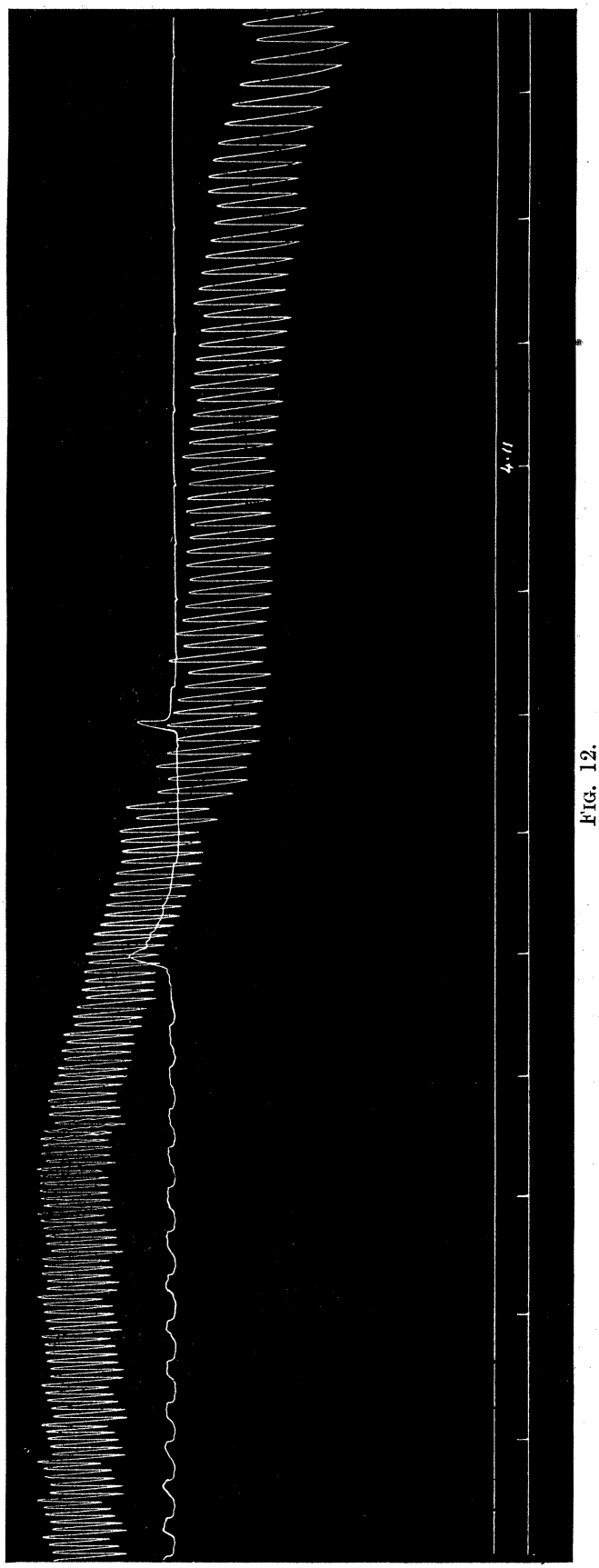

Fig. 12.

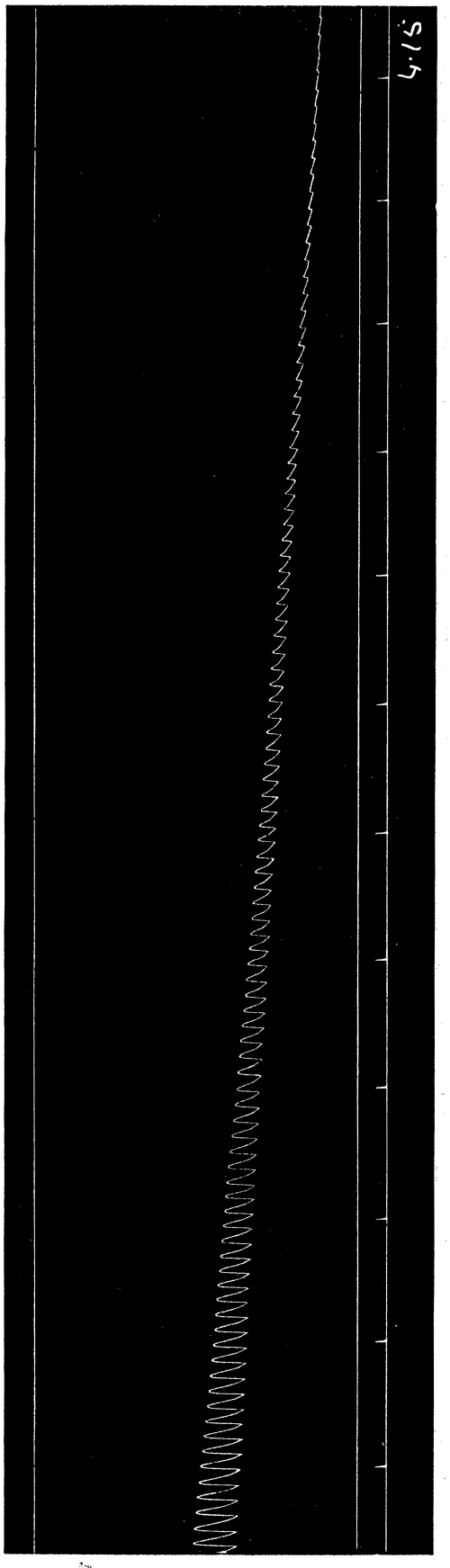

Fig. 13.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

thereafter inhaled through this cannula and chloroform discontinued. The blood-pressure was taken in the carotid, the respirations were recorded by means of a double stethograph, and the venom was administered by a cannula inserted into the external jugular vein.

The venom solution used was 0·004 gramme of Sepedon venom dissolved in 2 c.c. Ringer's solution; and 0·46 c.c. of this solution, representing 0·00092 gramme of venom, or 0·0005 gramme per kilogramme, was injected at 3.26.30. A second injection of 0·92 c.c., or 0·001 gramme per kilogramme, was made at 3.30.

The effects produced by these injections on the blood-pressure and respirations are recorded in Table XII (p. 259).

In this experiment, the first injection of 0·0005 gramme per kilogramme, *i.e.*, just less than the intravenous minimum-lethal dose, caused a slight fall of blood-pressure. The blood-pressure soon began to rise again. When it reached its previous level (four minutes after the first injection), a second injection of 0·001 gramme per kilogramme, *i.e.*, about twice the minimum-lethal dose, was given. The blood-pressure continued to rise in spite of the second injection. Ten minutes later, the amplitude of the respiratory movements began to diminish markedly, and the number of respirations to a less extent. The blood-pressure meantime rose to a maximum of about 40 mm. above the normal. This was accompanied by a pronounced slowing of the heart. Respiration finally ceased 44 minutes after the first injection, the heart stopping about 4 minutes after arrest of the respiration.

Experiment 30, Table XIII (figs. 14 to 18 inclusive), April 1, 1905.—Rabbit, weight 1800 grammes. The method of experiment was the same as in the previous experiment. The venom solution used was 0·006 gramme dissolved in 1 c.c. saline solution; and 0·9 c.c. of this solution, representing 0·0054 gramme of venom, or 0·004 gramme per kilogramme, was injected in one dose into the jugular vein. When natural respiration failed, the animal was kept alive by artificial respiration.

The effects on the blood-pressure and respirations are noted in the following table.

In this experiment, a dose equal to nearly six times the intravenous minimum-lethal dose was given in a single injection. It caused a fall of blood-pressure, transient but much more profound than was produced by merely the minimum-lethal dose as in the preceding experiment. Fifteen minutes after injection, the amplitude of the respiratory movements began to diminish markedly. This was accompanied by a rise of blood-pressure to considerably above the normal, with a pronounced slowing of the pulse rate. Seven minutes later respiration had almost ceased, and the blood-pressure was beginning to fall. Artificial respiration was now begun, and this soon lowered the blood-pressure and increased the rate of the heart.

Artificial respiration was kept up for fully three hours, and in that time there was a slight rise of blood-pressure and some slowing of the heart. When artificial respiration was then stopped, the blood-pressure rose slightly at first and then fell,

Table XIII.

Time.	Average blood-pressure in mm. Hg.	Pulse rate per 10 seconds.	Respiration rate per 10 seconds.	Respiration excursus in mm.	Notes.
1.46	98	45	11	8·5	Pulse waves about 1 mm.
1.47	98	45	11	3·5	
1.47	—	—	—	—	0·003 gramme per kilogramme injected intravenously (fig. 14).
1.47.30	82	43	10	3·5	
1.49	76	42	10	3·5	
1.52	90	43	9	3·5	
2.0	95	41	9	2·0	
2.3	113	33	9	1·5	
2.5	120	21	8	1·0	(Fig. 15.)
2.9	105	13	7	Mere undulations	Pulse waves about 3 mm. Artificial respiration begun (fig. 16).
2.13	90	32			
2.25	74	38			
2.40	76	31			
3.8	79	29			
4.11	91	28			
5.17	92	23			
5.17.20	—	—	—	—	Artificial respiration stopped.
5.17.30	96	16	0	0	
5.17.40	92	12	0	0	
5.18	—	—	—	—	Artificial respiration restarted.
5.19	94	16			
5.20	—	—	—	—	,, ,, stopped (fig. 18).
5.20.30	90	7	0	0	
5.22	70	6	0	0	Artificial respiration restarted.
5.22.10	90	16			

and the heart slowed markedly. No attempt was made at natural respiration, although the blood-pressure and heart beats were well maintained. Succeeding application and discontinuance of artificial respiration produced a repetition of the same phenomena.

In other experiments similar results were obtained.

Summary of Effects on Blood-pressure.

There is a very close resemblance in regard to their effects on blood-pressure between *Sepedon hæmachates* venom and cobra venom. An initial transient fall, followed by a rise (often above normal), with coincident slowing of the heart, is also characteristic of the latter venom. In view of the similarity of their actions on the heart and blood-vessels of the frog, the probability is that the effects produced on blood-pressure are largely due to similar causes in the case of both venoms.

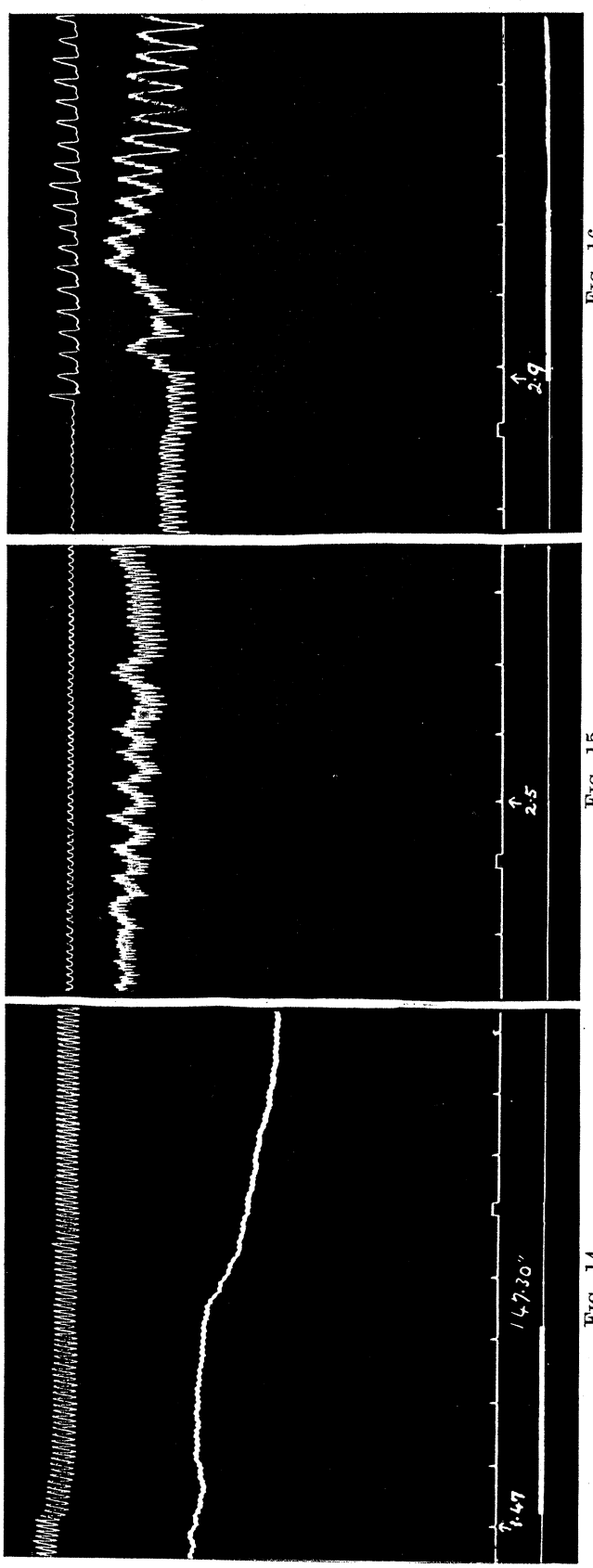

FIG. 14.

FIG. 15.

FIG. 16.

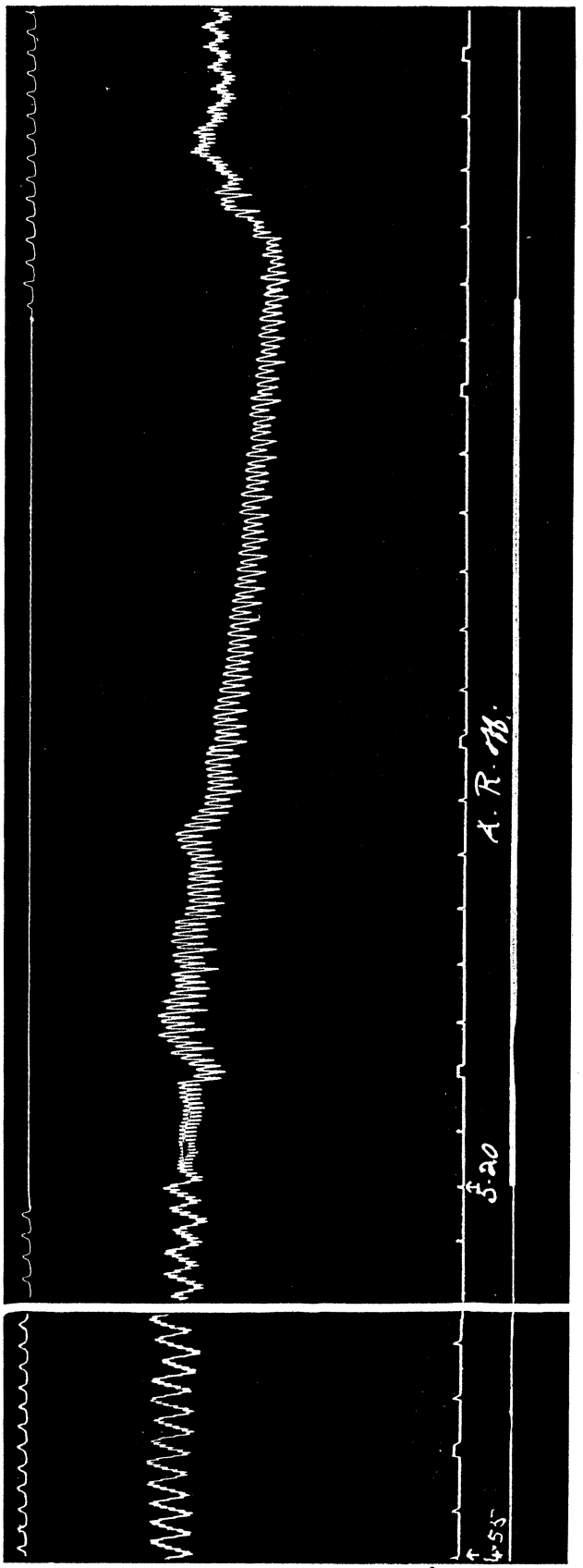

FIG. 17.

FIG. 18.

In regard to cobra venom, ELLIOT states that a point of general interest was that it was impossible to trace any sufficient relationship between the early falls of blood-pressure and simultaneous changes in the rate of the heart-beats.

This also holds good for Sepedon venom, for example in Experiment 55, where the blood-pressure fell from 98 to 76 mm., the heart rate varied only from 45 to 42 per 10 seconds. This fall must therefore be due either to a temporary diminution in the force of the heart's beats or to a transient dilatation of the vessels. As the venom has a constricting action on the vessels themselves, any fall of blood-pressure not due to the heart must be due to a weakness of the vasomotor centre. There is, however, no evidence that Sepedon venom has any paralysing action on the vaso-motor centre, since this centre always remains sensitive to the stimulus of venous blood. It is probable, therefore, that this early fall is due partly to slowing, and partly to diminution in the force of contraction of the heart, caused by the sudden influx of a relatively large quantity of venom. In any case, it is of slight importance, as the fall is neither pronounced nor lasting, even with six times the minimum-lethal dose administered intravenously.

As in the case of cobra poisoning by other than very large doses, the blood-pressure under the influence of Sepedon venom, after a primary fall, rises and remains high until near the end of life. As the blood is at this time insufficiently aërated, because of rapidly increasing inadequacy of respiration, obviously this high blood-pressure occurring with slowing of the heart can at least partly be explained by stimulation of the vasomotor centre by venous blood, and consequent reflex inhibition of the heart. That this is true is clear from Experiment 30, where the blood-pressure was reduced from 105 to 90 mm. and the heart rate increased from 13 to 32 per 10 seconds, by artificial respiration. But since artificial respiration failed to restore the heart quite to its former rate, the venom must also exert an independent action on the heart, tending to slow it. This is probably analogous to the slowing of the heart of the frog, seen after subcutaneous injection and also in perfusion with dilute solutions.

That the slowing of the heart is due mainly to stimulation of the vagus and only slightly to a direct action on the heart was confirmed by blood-pressure experiments in which the venom was injected after the vagal ends had been paralysed by atropine. In such an experiment, after the vagal ends had been paralysed by atropine the pulse rate was 38 in 10 seconds. A dose of Sepedon venom similar to that given in Experiment 29 (*i.e.* 0·0015 gramme per kilogramme) was then injected intravenously. This paralysed the respiration in 34 minutes. Immediately before arrest of the respiration, the pulse rate was still 30 in 10 seconds, as contrasted with 13 in 10 seconds at a corresponding time in Experiment 29, the pulse rate before injection of venom having been the same in both experiments.

The excitability of the vagus nerve-ends is not diminished by Sepedon venom. Direct constriction of the vessels may play some part in maintaining the high level of

blood-pressure, though we have shown that this venom is capable of exerting only a much feebler action in this direction than cobra venom.

The relative unimportance, from a toxic point of view, of the action of the venom on the circulation as compared with its action on the respiration is seen from Experiment 30. In this experiment the animal received six times the minimum-lethal dose. This dose would have arrested the respiration within half an hour. When, however, the animal was kept alive by artificial respiration, the blood-pressure remained at practically its normal level for at least three and a-half hours.

(d) *Lymph Hearts.*

The contractions of the lymph hearts in frogs cease in the course of poisoning by Sepedon venom. The time at which this occurs varies somewhat according to the dose of venom injected; with the minimum-lethal dose the lymph hearts cease beating usually on the day after injection, and therefore much sooner than the blood heart.

(e) *Blood.*

Sepedon hæmachates venom corresponds with Colubrine venoms generally in having little action on the blood. In nearly all the experiments on rabbits, blood was drawn from the ear into capillary tubes at various times after injection, and the serum which separated was in all cases found to be unstained with hæmoglobin. Also, in many experiments blood was collected from the heart after death, and in these there was also a complete absence of hæmolysis. The urine of poisoned rabbits contained no blood corpuscles or hæmoglobin.

Experiments with blood *in vitro* have, however, shown that the venom has a distinct hæmolytic action on washed corpuscles. These experiments were made with human and rabbit's corpuscles, and the results were the same in both cases. The saline solution used throughout was 0·85 per cent. of sodium chloride in distilled water.

Human red blood corpuscles, after the serum has been removed by thrice washing with saline, when suspended in a solution of Sepedon venom, 1 in 2000 or 1 in 4000, are almost completely hæmolysed in 20 hours. There is slight hæmolysis with 1 in 8000 or 1 in 16,000, but no hæmolysis with 1 in 32,000, in 20 hours. Corresponding with the absence of hæmolysis *in corpore*, it is evident that Sepedon venom exercises *in vitro* only a feeble and tardy hæmolytic action.

Post-mortem examinations in rabbits failed to reveal any intravascular clotting. No distinct evidence could be obtained of diminution in the coagulability of the blood. There was also an absence of hæmorrhages in the serous membranes and elsewhere.

F. Effects on Respiration.

Blood-pressure experiments confirm the conclusions drawn from experiments primarily made to determine the minimum-lethal dose, namely, that in mammals

respiratory paralysis is the cause of death in *Sepedon hæmachates* poisoning. Paralysis of the respiratory movements is also early manifested in frogs, but is not fatal to them, as they also breathe through the skin.

In several experiments, the activity of the phrenic nerves was determined immediately after death, and in all cases they responded to weak faradic stimulation, showing that paralysis of the respiratory centre is at any rate the chief cause of respiratory failure. Even with small lethal doses of Sepedon venom, in which case death was postponed for many hours, the excitability of the phrenic nerves was practically unimpaired; *e.g.*, in Experiment 16, where, in a rat which survived for six and one-third hours, stimulation of the phrenic nerves four minutes after death caused contraction of the diaphragm with the secondary coil at 350 mm.; and in an experiment where a rabbit survived for about four hours, the phrenic nerves reacted to stimulation with the secondary coil at 470 mm. seven minutes after arrest of the respiration.

With non-lethal and small lethal doses there was sometimes observed, both in rats and rabbits, a slight primary increase in the rate of respiration, as has been observed with many substances that paralyse the respiratory nerve centre.

In the course of kymographic experiments, an attempt was made to determine whether artificial respiration could restore an animal to life after the respiratory movements had been paralysed by a small lethal dose of Sepedon venom. Thus, a rabbit received intravenously 0·0008 gramme per kilogramme (about one and a-half times the minimum lethal dose). Natural respiration ceased in 1 hour 45 minutes. The rabbit was kept alive by artificial respiration for one and a-half hours thereafter. During that time artificial respiration was interrupted at intervals, and it was found that the animal made no spontaneous effort to respire. It is apparent, therefore, that in rabbits and in the conditions in which these experiments were made, the employment of artificial respiration for one and a-half hours is ineffectual in bringing about recovery even from only slightly more than the minimum-lethal dose.

G. Effects on Temperature.

The effects of the venom on the body temperature were investigated especially in rabbits. The results were not sufficiently invariable to admit of a general statement, but as they are of some interest, it seems advisable to discuss them. The following table shows the main temperature changes produced by subcutaneous injections in rabbits, in the experiments in which special attention was paid to the temperature. The temperature was in all cases taken *per rectum*, and on the Fahrenheit scale.

Doses of 0·001 gramme per kilogramme or over caused either a fall of temperature from the beginning or a transient rise of not more than 0°·4 F., followed by a gradual fall. Smaller doses caused a marked rise of temperature. In the case of the smallest dose, 0·0005 gramme, the temperature returned to normal on the day after injection. The second, third, and fourth experiments on the table are most interesting. In the

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case of 0·00075 and 0·0008 gramme per kilogramme the maximum temperature was attained within six hours. Afterwards it fell steadily, and reached a very low point several hours before the animal died. With 0·0009 gramme—an experiment in which the animal survived an exceptionally large dose—the temperature remained high for a week, and on the fifth day was so high as 105° F.

Table XIV.

No. of experiment.	Dose per kilogramme.	Result.	Temperature.
31	0·0005	Recovery.	Rose from 101° to 103° in 6 hours. 101° next day.
8	0·00075	Death in 29 hours.	Rose from 101°·6 to 104° in 6 hours, then fell until below 95° in 24 hours.
32	0·0008	„ 28½ „	Rose from 100° to 103° in 3½ hours, then fell to 89° in 24 hours.
9	0·0009	Recovery.	Rose from 101°·4 to 104° in 3½ hours. Remained over 103° for a week. 105° on 5th day.
10	0·001	Death in 24–35 hours	Rose from 101°·6 to 102° in 1½ hours, then fell slowly.
33	0·0012	„ „ „	Fell from beginning.
34	0·0015	„ 3⅝ „	Rose from 100°·4 to 100°·8 in 20 seconds, then fell.
11	0·0025	„ 1½ „	Fell from beginning.

The fall of temperature, which occurs in the case of lethal doses, is probably due to a paralysis of the heat regulating mechanism, similar to that produced by many substances that weaken or paralyse the central nervous system. In regard to the rise of temperature, we do not consider any local reaction at the site of injection sufficient to explain it. It is probably a general effect due to the venom, similar to that which occurs in the case of the toxins of specific fevers. In the case of minimum-lethal doses, where death is postponed for over 24 hours, the rise of temperature is marked for the first few hours, and the fall comes on only when there are other conspicuous symptoms of paralysis of the central nervous system.

General Summary.

A. The venom used was an extract from the dried venom glands of the *Sepedon hæmachates*. Its minimum-lethal dose by subcutaneous injection per kilogramme was found to be: for the frog, 0·0012 gramme; for the rabbit, 0·001 gramme; for the rat, 0·0016 gramme; for the cat, 0·015 gramme; for the pigeon, 0·0033 gramme; and, by intravenous injection, for the rabbit, 0·00055 gramme.

B. In the case of all these animals, the venom primarily and with greatest intensity affects the respiration. Respiratory paralysis is the cause of death in mammals and in birds; in frogs the respiratory movements are early paralysed, but

death occurs after several days from gradual failure of the circulation. Other conspicuous effects produced by lethal doses in mammals are drowsiness, ataxia, impairment of reflexes, and fall of temperature. In frogs, the venom produces diminution of reflex excitability, motor paralysis, and progressive increase in weight due to œdema.

C. In warm-blooded animals the venom has a marked enfeebling action on the brain and spinal cord, which is only slightly, if at all, produced on the motor nerve-ends. In frogs, however, motor paralysis is due to a paralysing action both on the central nervous system and on the motor nerve-ends, the former action being characteristic especially of large doses, the latter being more pronounced in the late stages of poisoning with smaller doses.

D. The venom has, comparatively with its action on nerve structures, a very slight action on skeletal muscle.

E. From the point of view of lethality, the effects of the venom on the circulation are of minor importance compared with those on the respiration.

Perfused through the frog's heart, strong solutions of Sepedon venom bring about an increase of the rate followed by arrest of the heart in systole; and weaker solutions slow the heart and arrest it in diastole. The latter effect is the only one manifested after injection of even 10 times the minimum-lethal dose.

Strong solutions slightly constrict the frog's blood-vessels when perfused through them.

In rabbits, the venom injected intravenously causes a slight fall of blood-pressure. This is soon recovered from, and thereafter the blood-pressure rises and remains high till the end of life. The transient fall of blood-pressure is probably mainly due to a weakening of the heart's contractions. When pronounced embarrassment of the respiration comes on, the blood-pressure rises above the normal level. This is mainly due to stimulation of the vasomotor centre by the venous condition of the blood, the heart being at the same time slowed through stimulation of the vagus. The venom also slightly slows the heart by a direct action on it, and the direct but slight constriction of the vessels may be a contributing factor in maintaining the level of the blood-pressure.

In the course of poisoning in frogs, the lymph hearts are paralysed, tardily, but long before the blood heart.

Sepedon venom has little action on the blood. It does not definitely affect the coagulability, and neither hæmorrhages nor intravascular clotting are found *post mortem*. Hæmolysis is not found *in vivo*.

F. Respiratory failure in mammals is due to paralysis of the respiratory centre, the excitability of the phrenic nerve-ends being practically unimpaired.

G. Non-lethal doses of Sepedon venom cause a rise of temperature; lethal doses cause a fall of temperature with sometimes an initial rise.