PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Contributions to the Study of the Action of Sea-Snake Venoms. Part I. Venoms of Enhydrina valakadien and Enhydris curtus

Thomas R. Fraser and R. H. Elliot

Phil. Trans. R. Soc. Lond. B 1905 **197**, 249-279
doi: 10.1098/rstb.1905.0009

VIII. *Contributions to the Study of the Action of Sea-snake Venoms.*—Part I. *Venoms of* Enhydrina valakadien *and* Enhydris curtus.

By Sir Thomas R. Fraser, *M.D., F.R.S., Professor of Materia Medica in the University of Edinburgh, and* Major R. H. Elliot, *I.M.S., on Special Duty for Snake Venom Research under the orders of the Secretary of State for India.*

(From the Pharmacology Laboratory of the University of Edinburgh.)

Received May 10,—Read June 9, 1904.

Contents.

Introductory.

The following paper is the first of a series of articles which we hope to publish on the Action of Sea-snake Venoms. The work dealt with herein was carried out mainly in the Materia Medica Laboratory of the University of Edinburgh. The kymographic work, however, was done in the Physiology Laboratory of that University, and we desire to express our sense of indebtedness to Professor E. A. Schäfer for permitting us to use his apparatus.

Previous Literature of the Subject.

This is very scanty. The main contributions have been the recent ones by Captain LEONARD ROGERS, I.M.S., to which we shall have frequent occasion to refer. They appear in the 'Proceedings' of the Royal Society, May 7th, 1903, and June 18th, 1903.

We may observe that the plan of the present work had been laid down by us long before we were aware that Captain ROGERS was engaged on similar lines, and that we have but very little departed from our original scheme since reading his papers. We need not say that we have read these papers with great interest.

Species of Snakes used.

The venom used in this research was obtained for us by Dr. P. P. PINTO, of Madras, who was put on special duty for this and kindred purposes by the Government of Madras, whose kind assistance we desire gratefully to acknowledge. Two species only were used, viz., *Enhydrina valakadien*, and *Enhydris curtus*. The former is said by Dr. PINTO to be the commonest Sea-snake infesting the coast of Madras Presidency. Most of the specimens were small. The venom collected from six snakes, averaging 2 feet 6 inches in length, amounted to between 30 and 40 milligrammes, but owing to an unfortunate accident the exact amount cannot be stated. The *Enhydris curtus* proved a comparatively rare snake. From eight specimens, Dr. PINTO obtained ·022 gramme of poison or ·00275 gramme per snake. The *Hydrophis gracilis* was the only other moderately large Sea-snake brought in by the fishermen, but it was too rare to enable any useful amount of venom to be collected.

We are greatly indebted to Mr. EDGAR THURSTON, Superintendent of the Madras Government Museum, and to Mr. RAMUNI MENON, of the Presidency College, Madras, for the kind assistance they gave Dr. PINTO in the work of identification.

Method of collecting the Venom.

The snakes were brought to Dr. PINTO by the fishermen, who frequently catch them in their nets along with their fish. The glands were removed with aseptic precautions, and if they were sufficiently large the venom was expressed from them into a watch glass and dried in a desiccator over sulphuric acid. The smaller sacs were dried in a similar manner and sent home in sealed bottles. They were here again dried over sulphuric acid in the exhausted receiver of an air pump. After carefully removing all tissues adhering to them, the contained venom was extracted by first opening up and minutely sub-dividing the glands, macerating them in successive small quantities of thymol water, and filtering the solutions, which were

afterwards mixed together and evaporated to dryness, over sulphuric acid, in the exhausted receiver of an air pump. There was thus obtained a yellow, brittle and scaly product, freely soluble in water and having much the same appearance and characters as the venom expressed from the fresh glands. This product, however, necessarily contained other substances than venom; but it may be noted that maceration of the glands in water produced perfectly clear solutions and very little swelling and softening of the particles of undissolved substances, unlike the hazy solutions with swollen and softened undissolved particles, which are obtained when the poison glands of the Cobra and of many other serpents are similarly treated. By this treatment, 60 dry glands of the *Enhydrina valakadien*, weighing 1·09 gramme, yielded ·28 gramme of impure venom, whose lethal power will be dealt with presently.

Appearance of the Venom.

The venom of both species, as we received it from India, consisted of thin scales of a very pale yellow colour. It readily dissolved in RINGER'S fluid, which was the solvent and vehicle we employed.

The M.L.D. of Enhydrina Venom.

ROGERS (*loc. cit. supra*) estimated the potency of the mixed Sea-snake venoms employed by him "by means of numerous experiments carried out with the mixed dried venom of a number of these snakes." He found that the M.L.D. for rats was ·00007 gramme per kilo. He appears to have made only two experiments on rabbits. In one of these, ·00004 gramme per kilo. killed in about four hours, in the other, ·00001 gramme per kilo. produced no symptoms but loss of appetite, but a second dose of ·00002 gramme per kilo., five days later, killed in a few hours.

We estimated the M.L.D. of the two kinds of venom at our disposal quite separately. That of *Enhydrina valakadien*, being in the greater quantity, was tested first. The accompanying tables (I. to VI.) show the results obtained :—

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE I.—Estimation of the M.L.D. of the Venom of *Enhydrina valakadien* for White Rats.

(The effect of the venom on the nerve-ends is also shown. It was tested immediately after the death of the animal.)

Rat's weight in kilos.	Dose per kilo. in grammes.	Results as to life.	Effect on nerve-ends.	Remarks.
·102	·001	Dead in 50 mins.	Not tested.	10 mins. after injection, Ht. 50. R. 22 in 10 secs. 45 ,, ,, Ht. 32. R. 8 ,, *P.M.* ½ hr. after death. Heart tightly contracted and hard.
			millims.	
·097	·0005	,, 63 ,,	Phrenic response at . . 450 Occasional spontaneous movements of diaphragm observed after death.	10 mins. after injection, Ht. 50. R. 26 in 10 secs. 55 ,, ,, ——— R. 14 ,, 60 ,, ,, Ht. 16 in 10 secs. *P.M.* at once. Heart in diastole.
·117	·00025	,, 2 hrs.	Phrenic response at . . 450	10 mins. after injection, Ht. 50. R. 20 in 10 secs. 80 ,, ,, Ht. 36. R. 8 ,, 120 ,, ,, Ht. 12. R. 45 ,,
·195	·0001	,, 3 ,,	Phrenic response at . . 300 *P.M.* was delayed 10 mins.	Heart in diastole; contained some *P.M.* clot. *Rigor mortis* pronounced in 25 mins.
·107	·0009	,, 5 ,, 55 mins.	Phrenic response at . . 360 Sciatic ,, . . 220 *P.M. at once.*	80 mins. after injection, Ht. not felt. R. 30 in 10 secs. 4½ hrs. ,, ,, Ht. 12. R. 15, irregular. 5⅓ ,, ,, ,, Ht. ? R. 7 in 10 secs. 5 ,, 50 mins. ,, R. 2 in 10 secs.
·098	·00008	Recovered. Seemed to be dying a few hours later. Respiration rhythm very irregular. 24 hrs. later was still very ill; rhythm still irregular. Ht. 51. Killed 5 days later.	Phrenic response at . . 500 Sciatic ,, . . 400 Vagal ,, . . 120	—
·134	·00007	Recovered. Ill for 1 day.	—	—
·132	·00006	,, ,, ,,	—	—
·177	·00005	Recovered. Seemed to be dying at first. Apparently well next day.	—	2 hrs. after injection, Ht. 50. R. 16 in 10 secs. 3 ,, ,, Ht. 42. R. 15 ,, 4 ,, ,, Ht. 46. R. 17 ,, 4½ ,, ,, Ht. 36. R. 17 ,, Respiration laboured.
·127	·000035	Recovered. Never really ill.	—	—
·173	·000025	,, ,, ,,	—	—
·143	·000015	,, ,, ,,	—	—

Note.—The nerve responses are stated in millimetres of distance of the secondary from the primary coil. The NEAVE's hammer was used, and short shocks were given by sharply opening and closing a key in the primary circuit. The tongue could appreciate the interrupted current, when the shocks were continued for some time at 215 millims. The phrenic nerves were always tested first, then the vagi, and lastly the sciatics.

TABLE II.—Estimation of the M.L.D. of the Venom of *Enhydrina valakadien* for Rabbits.

(The effect of the venom on the nerve-ends is also shown.)

Rabbit's weight in kilos.	Dose per kilo. in grammes.	Results as to life.	Effect on nerve-ends.	Remarks.
			millims.	
1·980	·0001	Dead in 2 hrs.	Phrenic response at 260 Sciatic ,, 260	*P.M.* at once. Heart distended with blood, and still flickering.
1·990	·000075	,, 2¾ hrs.	Phrenic ,, 220 Sciatic ,, 190	Heart still flickering, and distended with blood.
2·115	·00007	,, 4 hrs.	Phrenic ,, 400 Sciatic ,, 180	Heart in diastole; it stopped soon after the chest was opened.
2·005	·00006	,, 4 hrs.	Phrenic ,, 400 Sciatic ,, 250 Vagal ,, 120	—
1·650	·00005	Dead in 5 hrs. 50 mins.	Phrenic ,, 250 Sciatic ,, 240 Vagal ,, 120	—
1·260	·00005	Ill for one day. Recovered. Killed on 6th day.	Phrenic ,, 450 Sciatic ,, 330 Vagal ,, 180	Lost ·140 kilo. in the 6 days after injection.
2·275	·00004	Very ill for one day. Recovered.	—	—
2·345	·00003	Ill for one day. Recovered.	—	—
1·610	·000025	Recovered. Never very ill.	—	—

Note.—The nerve responses are stated in millimetres of distance of the secondary from the primary coil. The NEAVE'S hammer was used, and short shocks were given by sharply opening and closing a key in the primary circuit. The tongue could appreciate the interrupted current, when the shocks were continued for some time at 215 millims. The phrenic nerves were always tested first, then the vagi, and lastly the sciatics.

TABLE III.—Estimation of the M.L.D. of the Venom of *Enhydrina valakadien* for Cats.

(The effect of the venom on the nerve-ends is also shown.)

Cat's weight in kilos.	Dose per kilo. in grammes.	Results as to life.	Effect on nerve-ends.	Remarks.
			millims.	
1·980	·001	Dead in 50 mins.	Phrenic response at 360 Sciatic ,, 340 Vagal ,, 110	*P.M.* at once. Heart beating. Blood very dark. Heart in diastole.
2·090	·0005	,, 2 hrs.	—	—
2·770	·00025	,, 2½ hrs.	Phrenic ,, 320 Sciatic ,, 300 Vagal ,, 60	—
2·395	·00015	Recovered, but was very ill. It seemed to be moribund 6 or 7 hrs. after the injection, but had pulled round by next morning.	—	—
2·490	·00010	Recovered.	—	—
3·030	·00009	Recovered.	—	—

Note.—The nerve responses are stated in millimetres of distance of the secondary from the primary coil. The NEAVE'S hammer was used, and short shocks were given by sharply opening and closing a key in the primary circuit. The tongue could appreciate the interrupted current, when the shocks were continued for some time at 215 millims. The phrenic nerves were always tested first, then the vagi, and lastly the sciatics.

This series of experiments could not be completed, as the supply of venom had become exhausted. The M.L.D. for the cat is apparently ·0002 gramme per kilo.

White Rats.—It will be seen that a dose of ·00009 gramme per kilo. killed in 5 hrs. 55 mins., all larger doses causing death with proportional celerity, whilst no dose less than ·00009 gramme per kilo. proved fatal, though doses from ·00008 to ·00005 gramme per kilo. produced well-marked effects.

Rabbits.—A dose of ·00005 gramme per kilo. proved fatal on one occasion in 5 hrs. 50 mins., whilst on another occasion it merely made the animal very ill. ·00006 gramme per kilo. and all doses above this proved fatal, whilst ·00004 gramme per kilo. and all lower doses failed to kill.

Cats.—·001 gramme per kilo. killed in 50 mins.; ·0005 gramme per kilo. killed in 2 hrs.; ·00025 gramme per kilo. killed in 2½ hrs.; and ·00015 gramme per kilo. reduced the animal to such a state that for 6 or 7 hours it seemed moribund,

but by the next morning it had practically recovered. Doses of ·0001 and of ·00009 gramme per kilo. failed to kill. As the stock of pure venom at our disposal had now become exhausted, we were unable to continue farther, but it is evident that ·0002 gramme per kilo. might with every probability of accuracy be put down as the M.L.D. for the cat.

It will be observed that our estimations of the M.L.D.'s of *Enhydrina valakadien* venom agree fairly well with those of ROGERS for rats, and with his fragmentary estimates for rabbits. In both cases his dose is lower than ours. Both he and we find the dose for the rabbit lower than that for the rat. As to the latter fact, it is partly explainable by the difficulty of getting the rabbit weighed food-free, and this, obviously, would raise its apparent weight and so lower the apparent M.L.D. This difficulty is not so great with the rat, whose real weight can be got fairly nearly after 24 hours' starvation, especially as this animal always micturates when handled.

ROGERS collected his venom by getting the snakes to bite through indiarubber sheeting; ours was obtained by expression from the dissected glands. Whilst our method facilitates the procuring of a perfectly aseptic product, it appears to yield a venom diluted with mucus, and consequently of a lower toxicity. We have observed a similar difference in specimens of Cobra venom obtained by these two methods.

It is of interest to compare the lethality of Cobra venom with that of the *Enhydrina valakadien*. The venoms were in both cases obtained for us by Dr. PINTO, under the same conditions and by the same methods, and the experiments were also made under exactly parallel conditions. The results are shown in the accompanying table (IV.).

TABLE IV.

Animals used.	M.L.D. of Cobra venom.	M.L.D. of Enhydrina venom.
Rats	·0005 gramme per kilo.	·00009 gramme per kilo.
Rabbits	·0006 „ „	·00006 „ „
Cats	·010 „ „	·0002 „ „

It will be observed that the relative lethality of the two venoms for the various kinds of animal experimented with differs widely. Taking the lowest M.L.D. in each case as the unit, we obtain the following relative values for lethality :—

Cobra venom rats = 1 ; rabbits = 1·2 ; cats = 20.

Enhydrina venom (valakadien) rabbits = 1 ; rats = 1·5 ; cats = 3·3.

These figures, and especially the cat-rodent relationship, suggest most strongly that the two venoms differ materially from each other in their actions.

As our stock of *Enhydrina valakadien* venom, obtained by compression of the glands in the fresh state, had failed, we were obliged to fall back on the dried glands we had received, the venom from which was extracted in the manner already described.

The lethal power of the venom so obtained was ascertained for rats and rabbits. The following table shows the results of our experiments :—

TABLE V.

Animal used.	Weight.	Dose per kilo.	Result.
	kilos.	grammes.	
Rat	·152	·00009	Never very ill.
,, ...	·120	·0001	No effect observed.
,, ...	·145	·00015	Slight symptoms.
,, ...	·140	·0002	Found dead 19 hrs. afterwards.
,, ...	·090	·00025	Dead in 5 hrs. 40 mins.*
Rabbit . . .	1·320	·00005	No symptoms observed.
,, ...	2·030	·000075	Decidedly ill, but recovered.
,, ...	1·217	·0001	Very ill on day of injection. In 30 hrs. the heart rate was 18, and the respiration rate 13 in 10 secs., and the breathing was laboured. The animal then slowly recovered.
,, ...	1·870	·0001	Found dead 19 hrs. afterwards.
,, ...	1·680	·00015	7 hrs. after the injection the animal was seriously ill, but not paralysed. 19 hrs. after the injection found dead and stiff.

The lethal power of this venom-extract of the dried glands is therefore a little less than half that of the purer specimen of venom expressed from fresh glands by Dr. PINTO. It may, however, be noted that a purer and much more active venom than the above could readily be obtained from dry glands, as one of us has shown, if the glands are subjected to a smaller number of successive extractions with thymol water than was adopted in the present case.

The M.L.D. of Enhydris curtus Venom.

A Belgian hare, weighing 2·34 kilos., was given ·00006 gramme per kilo. of *Enhydris curtus* venom, but only slight symptoms were produced.

A cat, weighing 2·265 kilos., received ·0002 gramme per kilo. of the venom, and it also was only slightly affected.

It was therefore obvious that the M.L.D.'s of our specimens of this venom and of that of *Enhydrina valakadien* were not the same. Our stock being limited, we selected white rats as the subjects on which to fully determine the lethality of the venom under consideration. The results were as follows :—

Rats recovered from doses of ·00007 gramme per kilo., ·00008 gramme per kilo., ·00009 gramme per kilo., ·0001 gramme per kilo., ·00015 gramme per kilo., ·00025 gramme per kilo., ·0003 gramme per kilo., ·0004 gramme per kilo., and on two occasions from a dose of ·0005 gramme per kilo.

* The left phrenic nerve responded at 420 millims., the left vagus at 40 millims., and the left sciatic at 290 millims.

A dose of ·0005 gramme per kilo. proved all but fatal to a rat weighing ·275 kilo. The animal lay for several hours apparently moribund. The next day it was much better, and it then steadily recovered.

Higher doses killed. The following are the details :—

TABLE VI.

No.	Weight of rat.	Dose per kilo.	Result.
	kilo.	gramme.	hrs. mins.
1	·135	·0006	Dead in 3 12
2	·125	·0007	,, 1 9
3	·137	·0008	,, 1 0
4	·233	·0009	,, 2 18
5	·280	·001	,, 1 25
6	·259	·002	,, 0 58
7	·270	·003	,, 0 46

The state of the nerve-ends was tested, when possible, by means of the secondary current. The following results were obtained; the distance of the secondary coil from the primary being stated in millims.

TABLE VII.

No. of experiment.	Distance of the secondary coil at which a response was obtained with the electrodes applied to		
	Phrenic nerve.	Sciatic nerve.	Vagus nerve.
	millims.	millims.	millims.
2	370	280	50
5	450	—	Complete inhibition not obtained with maximal stimulation, but slowing at 120.
6	300	—	80
7	400	—	No complete inhibition with the maximal stimulus available.

In all the above experiments, the injections were made subcutaneously at the abdomen, and absorption was hastened by rubbing the part. The venom was dissolved in RINGER's fluid, and the bulk of each injection was about 1 cub. centim.

These results indicate that the specimen of *Enhydris curtus* venom with which we were working was of considerably lower lethality than that possessed by our specimens of *Enhydrina valakadien* venom, a dose of ·00009 gramme per kilo. of the more

powerful of the latter having proved fatal, as against one of ·0006 gramme per kilo. of *Enhydris curtus* venom.

Whilst recording this result, we do not desire to lay any great stress on it, although both venoms obtained from fresh glands were, to the best of our belief, collected under precisely similar conditions. We have found a similar discrepancy between the lethal power of different specimens of Cobra venom expressed for us from fresh glands, under conditions which, apparently, were identical with each other.

Symptoms, &c., of Sea-Snake Poisoning.

Rogers has given an excellent description of the symptoms produced by Sea-snake venom in the lower animals. We have only two comments to make on the subject.

1. Rogers says that it is impossible to distinguish between the symptoms in an animal poisoned with Sea-snake venom, and one poisoned with Cobra venom. We have, however, observed that in the former the urgency of the dyspnœa is much greater than in the latter. The animal (rat, rabbit, or cat) rushes from place to place as if suffocated, instead of remaining nearly motionless as a cobraised animal does. The explanation of this difference becomes a simple one, when it is realised that, as we shall afterwards show, in Sea-snake poisoning, the venom acts on the respiratory apparatus alone, and leaves the heart and blood vessels practically unaffected, in so far as any direct action on them is concerned. One of us has recently pointed out in a communication to the Royal Society that Cobra venom, on the other hand, has a powerful action on the heart: (*a*) directly on its muscle fibres or on the nerve-ends therein; (*b*) through its vago-inhibitory apparatus; and (*c*) through the increased pressure against which the organ has to work, in consequence of the action of the venom on the arterioles. Consequently, though death takes place in cobraism primarily through the respiratory system, there is a steadily progressing depression of the cardiac functions going on *pari passu* with the respiratory embarrassment. It naturally follows that the dyspnœa is masked thereby.

2. One of us had observed that in frogs poisoned with Cobra venom, the lower eyelid invariably rises, and that the extent of this rise is a distinct indication of the intensity of the poisoning. A similar symptom is noticed in cobraised human beings, and was recorded also by one of us as being of constant occurrence in cobraised monkeys. We refer to lid-drop of the upper eyelid. A poisoned monkey will throw back his head to obtain sight through the narrow slit left below by the increasing ptosis, and will thus assume a characteristic position. Dr. F. H. A. Marshall, whom we consulted on the subject, very kindly informed us that we were correct in inferring that the lower lid of the frog is depressed by muscular effort, and raised by elastic rebound on the cessation of such effort. It is of interest to note that no

such symptom is observed when an animal is dying from Daboia poisoning; indeed, it is easy to pick out frogs poisoned by Cobra and Daboia venom from each other by attention to this symptom alone. It is therefore of special interest to find that this lid-rise is of invariable occurrence in frogs poisoned by the venom of the Sea-snakes we have worked with. The point allies Cobra with Sea-snake venom in action, and distinguishes these venoms from the Daboia venom, if not also from the venom of all other Viperine snakes. We may add that the same sign has been noticed in yet another form of Colubrine snake poisoning.

It only remains to add that a frog which received ·002 gramme of *Enhydris curtus* venom per kilo. died in 18 hrs.; one which received ·001 gramme of the venom per kilo. died in 50 hrs.; and a third which received ·0005 gramme per kilo. survived the injection 3 days, and then died. The symptoms of the poisoning were indistinguishable from those met with in cobraised frogs, failure of pulmonary respiration being an early and constant phenomenon.

The Antagonism between Calmette's Serum and Enhydris Venom.

Rogers found Calmette's serum "of no use against the poison of the Hydrophidæ."* He tested it by adding minimal and slightly supra-minimal doses of the poison of Enhydrina to ½ cub. centims. of fresh (Calmette's) serum; white rats being used in the experiments. Whilst accepting the conclusion that the antivenene was, for all practical purposes, powerless against Enhydrina venom, we desired to satisfy ourselves as to the existence or otherwise of even a small measure of antagonism. We accordingly devised two parallel series of experiments. The first was undertaken with Cobra venom in order to standardise the sample of antivenene with which we were working; the second was carried out with the poison of *Enhydris curtus*. M. Calmette most kindly furnished us with a specimen of his antivenene, which was received in August, 1903, and used in February, 1904, *i.e.*, 6 months later. The M.L.D. of the specimen of Cobra venom used had been very carefully determined for white rats and was ·0005 gramme per kilo. The Sea-snake venom used was of an almost equal lethality, since a dose of ·0005 gramme per kilo. reduced a rat to a moribund condition for several hours, while a dose of ·001 gramme per kilo. killed in 1 hr. 25 mins.

The doses of venom and of serum were carefully calculated per kilo. weight of each animal; they were mixed in small conical glasses and allowed to stand for exactly 30 mins.; they were then injected each into the corresponding animal, and the subcutaneous tissue of the abdomen was the site selected for the injections. The tables are self-explanatory.

* 'Roy. Soc. Proc.,' vol. 71, No. 475, March, 1903.

TABLE VIII.—To test the Measure of Antagonism of Antivenene against a definite Sample of Cobra Venom of known Lethal Power.

No.	Weight of animal (white rat).	Dose per kilo. of cobra venom.	Dose per kilo. of antivenene.	Result.
	kilo.		cub. centims.	
1	·183	10 × M.L.D. or ·005 gramme	2	Dead in 6 hrs. 8 mins.
2	·175	Ditto	3	Found dead 18 hrs. after injection.
3	·188	Ditto	4	Ditto.
4	·166	Ditto	5	Recovered. Never really ill.
5	·087	Ditto	Nil (control)	Dead in $1\frac{3}{4}$ hrs.

TABLE IX.—To test the Existence of any Measure of Antagonism of the Antivenene against a Sample of the Venom of *Enhydris curtus*.

No.	Weight of rat.	Dose per kilo. of venom.	Dose per kilo. of antivenene.	Result.
	kilo.	gramme.	cub. centims.	
1	·275	·0005	Nil (control)	Appeared moribund for some hours, but recovered.
2	·155	·005 = 10 × sub-M.L.D.	4	Dead in 47 mins.
3	·150	Ditto	5	Dead in 69 mins.
4	·157	Ditto	10	Dead in 91 mins.

The order in which these animals died suggests that a feeble measure of protection was afforded by the antivenene. There is, however, a great contrast between this action and that shown by the previous table, where Cobra venom was experimented with. One of us has brought forward evidence to show that the anti-dotal effect of antivenene is most probably due to a chemical reaction between it and the venom it is capable of antagonising.* In accordance with this explanation of serum antidotism, the above experiments indicate that there is a chemical difference between the composition of the two venoms under comparison. From this we would not unnaturally expect to find a corresponding difference between their actions. To this point we shall return later on.

* "Immunisation against Serpent's Venom, &c.," by Professor FRASER, 'Journal of the Royal Institution of Great Britain,' 1896, and 'Nature,' April 23, 1896.

Action of Enhydrina valakadien Venom on the Blood.

At our request, the following observations on this subject were made by G. Scott-Carmichael, M.B., Ch.B., Junior Assistant in the Department of Materia Medica, who had previously made many similar observations, and in whose accuracy we have full confidence.

(*a*) *Action* in vivo.—1. Red corpuscles from blood, taken immediately after death from the left side of the hearts of white rats killed by Enhydrina venom, showed in films no evidence of hæmolysis having occurred. The corpuscles were of normal size and contour and of uniform intensity of colour, and rouleaux formation was present as in health.

Serum which had separated spontaneously from this blood placed in capillary tubes showed hæmoglobin coloration to the naked eye. The fluid in all cases was of a dark venous colour.

2. Red corpuscles from the blood of rats which had received less than the minimum lethal dose of this venom likewise showed no evidence of hæmolytic change, and the serum from this blood was unstained with hæmoglobin.

The blood was of a darker colour than that of health. It was examined at different intervals after the injection in each case, one examination having been made on the day of injection and another on the following day.

(*b*) *Action* in vitro.—These observations were made in test-tubes and on microscope slides.

1. When the hæmolytic action on the blood of rabbits and of man was tested, it was found that no effect was produced within 18 hrs. in mixtures of blood and Enhydrina venom containing 1 of venom in 500, 1 : 1000 and 1 : 2500. With 1 : 500, however, a very slight effect was produced in 21 hrs., and with 1 : 250 in 18 hrs.; and in both cases controls with mixtures of blood and saline had not yet been affected.

2. In contrast with these extremely slight effects, when similar observations were made with Daboia venom, it was found that in solutions of 1 : 500, 1 : 1000, and 1 : 2500 this venom has a relatively strong hæmolytic action, both pigment diffusion and agglutination being distinctly produced by all of the solutions within 21 hrs., and by 1 : 500 solutions in less than 18 hrs. No hæmolysis was observable in control experiments within 24 hrs.

Action of Sea-Snake Venom on the Heart.

Rogers applied solutions of Sea-snake venom directly to the heart of pithed frogs, and obtained "no appreciable effect" thereby. He says:—"As a few drops of a 1/1000 solution of Enhydrina poison given *per venam*, and, therefore, further greatly diluted in the circulation, is very rapidly fatal, it is evident that the poison

should produce a very marked action on the heart when directly applied to it if the lethal effect is in any degree due to cardiac paralysis."

Whether these conclusions be right or wrong, we considered that they were founded on insufficient data, since FAYRER and BRUNTON* pointed out about 30 years ago that there was a great difference in the action of a presumably allied poison, viz., that of the Cobra, according as it was applied to the surface of a frog-heart or perfused through its cavity.

In a recent contribution to the pharmacology of Cobra venom,† one of us has shown that a solution so weak as 1/10,000,000 of this poison exerts an appreciable effect on the isolated frog-heart, when perfused through its cavity, and that a solution of 1/100,000 takes but a few minutes to paralyse that organ in a condition of extreme systole. When a 1 : 1000 solution of the same venom was dropped continuously on a suspended frog-heart, only a very slight quickening of the beat and a slight tendency of the organ to pass towards systole was observed. The contrast between these two sets of results is very marked, and confirms FAYRER and BRUNTON'S work. We were, therefore, of opinion that it was not possible to definitely decide that Sea-snake venom had no cardiac action till experiments had been made both with the isolated heart and with the heart exposed *in situ*. It seemed important to make both sets of experiments, since previous work with Cobra venom had shown that the heart is acted on by that poison in two manners ; on the one hand, by the direct systole-increasing action of the venom on the muscle tissue or on its nerve-ends, and, on the other, by the tendency to inhibition caused by the direct action of the venom on the vago-inhibitory centre as well as by its indirect action on the same centre through the medium of failing respiration. Our first object was to ascertain if either or both of these actions was likewise exerted by Sea-snake venom.

Perfusion of the Frog-Heart with Solutions of Enhydrina Venom.

A modification of SCHÄFER'S plethysmograph was employed for this purpose. The details of the experimental method were in all respects the same as those described by one of us† in a similar series, in which Cobra venom was under investigation. The fluid perfused consisted of one part of ox-blood and two parts of RINGER'S fluid.

Experiment 1.—An isolated frog-ventricle was perfused with a 1 : 20,000 solution of Enhydrina venom, with negative results.

Experiment 2.—An isolated frog-ventricle was perfused with a 1 : 5000 solution of Enhydrina venom. The beat quickened slightly, and the heart passed into fatigue in the ordinary course.

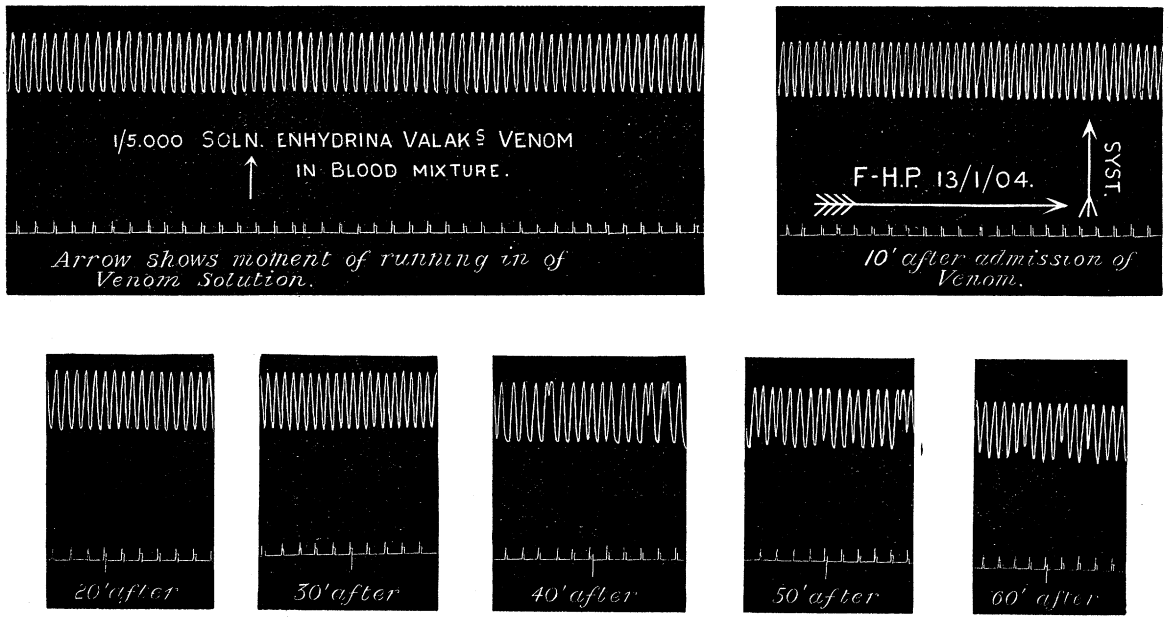
* 'Roy. Soc. Proc.,' vols. 21 and 22.

† 'A Contribution to the Study of the Action of Indian Cobra Poison,' by Captain R. H. ELLIOT, communicated to the Royal Society, January 18, 1904.

Experiment 3.—Was undertaken to make sure that the quickening observed was actually due to the venom. A heart was perfused for 2 hrs., and whilst its beat was very steady a 1 : 5000 solution of Enhydrina was admitted. The following were the changes observed in the heart's rate :—

4	mins.	before	venom	was admitted	there were 21·5 beats per min.
3	,,	,,	,,	21	,,
2	,,	,,	,,	21	,,
1	min.	,,	,,	21·5	,,
1	min.	after	,,	21·5	,,
2	mins.	,,	,,	22·5	,,
3	,,	,,	,,	22·5	,,
4	,,	,,	,,	22·5	,,
5	,,	,,	,,	23	,,
6	,,	,,	,,	24	,,
7	,,	,,	,,	24·5	,,
8	,,	,,	,,	25	,,
9	,,	,,	,,	25·5	,,
10	,,	,,	,,	25	,,
20	,,	,,	,,	23	,,

Parts of the tracing are also shown.



Extracts from Frog-heart Perfusion Tracing.

Rogers* found that the average quantity of dried venom obtained from single bites of thirteen different living specimens of the Enhydrina was just under 1 centigramme. It will be remembered that our own estimates of the available

* 'Roy. Soc. Proc.,' vol. 71.

amounts of venom, in the glands of the species we have had to do with, were much lower than this.

Accepting ROGERS' figure of about a centigramme of venom per bite, the maximum possible concentration in the blood of an average man would be about 1 : 500,000. This figure is arrived at by taking the weight of an average man at 70 kilos. and his blood at about one-fourteenth of this figure. The calculation stands thus: 70,000 grammes $\times$ 1/14 $\times$ 1/100 = 1/500,000.

Since a solution 100 times as strong as this has but very feeble action on the isolated ventricle, whilst the results of perfusion with a solution twenty-five times as strong as that which could be in operation after a bite are negative, we may safely conclude that Enhydrina venom differs markedly from Cobra venom, in that it has no direct action on the heart in any strength of solution ever likely to be present in the bodies of human victims of this species of Sea-snake.

Perfusion of the Vessels of the Frog with Enhydrina Venom.

Two experiments were made with a solution of *Enhydrina valakadien* venom of a strength of 1 : 50,000. The results were entirely negative.

It is of interest to recall, as one of us has shown,* that Cobra venom, even in a solution so dilute as 1 : 10,000,000, produces decided constriction of the vessel walls. The contrast between this powerful action and the absence of direct constriction by Enhydrina venom has a distinct bearing, as might have been expected, on the level of blood-pressure in these two different kinds of snake poisoning. This point will afterwards be returned to.

We are indebted to Mr. JOLLY, who carried out these experiments for us under our supervision.

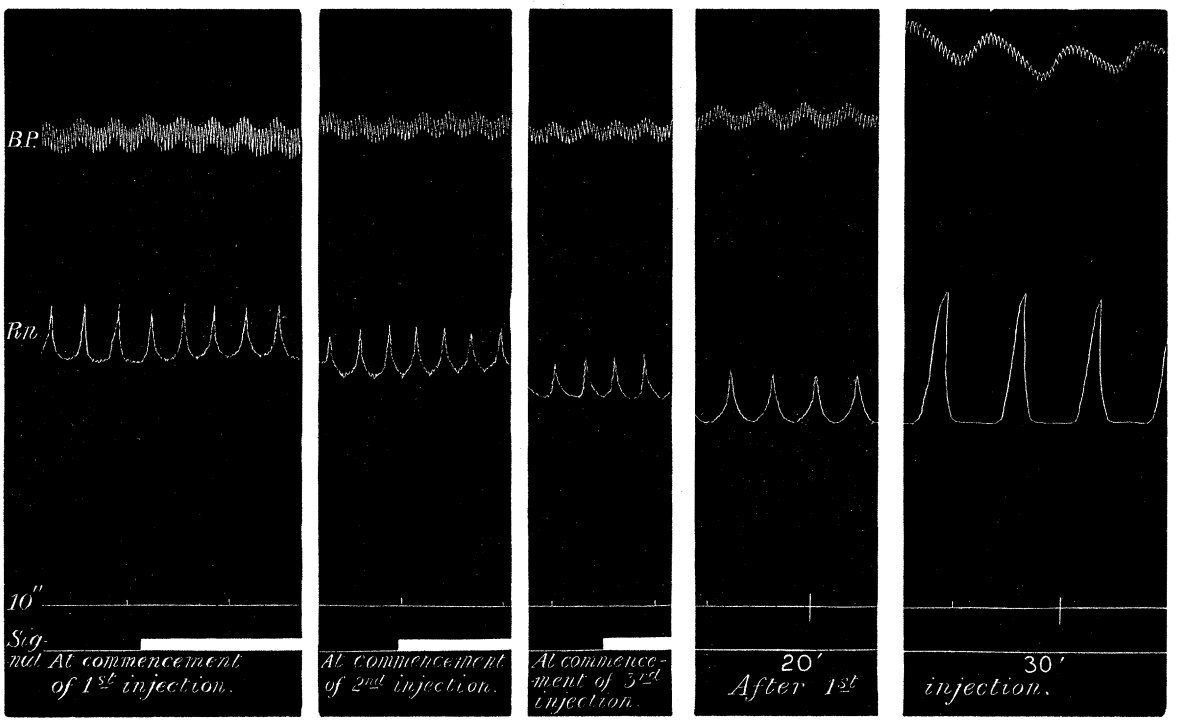
Kymographic Experiments with the Venom of Enhydrina valakadien.

Experiment 1.—A cat, weighing 2·5 kilos., was etherised and a tracheal cannula inserted, through which ether was then given; respirations were recorded by means of a double stethograph; the blood pressure was taken in the carotid; a cannula was tied into the external jugular; and the venom solution was placed in a graduated cylinder connected with the cannula by a short piece of rubber tubing. On this tubing a clip was placed, by the removal of which the solution could be admitted, and the fluid thus found its way slowly into the heart by its own weight, or, if required, it could be introduced faster, by means of a rubber ball arrangement which screwed on to the top of the cylinder.

After a normal had been obtained, one-fifth of the S.M.L.D. (subcutaneous minimum

* Captain R. H. ELLIOT, I.M.S., 'A Contribution to the Study of the Action of Indian Cobra Poison,' communicated to the Royal Society, January 18, 1904.

lethal dose) was introduced into the circulation, dissolved in 10 cub. centims. of RINGER'S fluid. The injection lasted 2½ mins. A distinct rise of blood pressure took place, and continued after the injection had been finished; but the rate and force of respiration did not much alter. 6 mins. after the commencement of the first injection, a second injection was made, which occupied 3 mins. Very little effect was produced, and 12 mins. after the commencement of the first injection a third was given, equivalent to 4 M.L.D.'s, in 20 cub. centims. of RINGER'S fluid, and this took 6 mins. to pass in, and was accompanied first by a slight fall and then by a very large rise of blood pressure. The rise continued after the injection had been completed, and was synchronous with the increasing failure of respiration. A fall occurred only when the heart stopped beating, or at least recording. The heart-rate remained very constant throughout the experiment (Table X.).

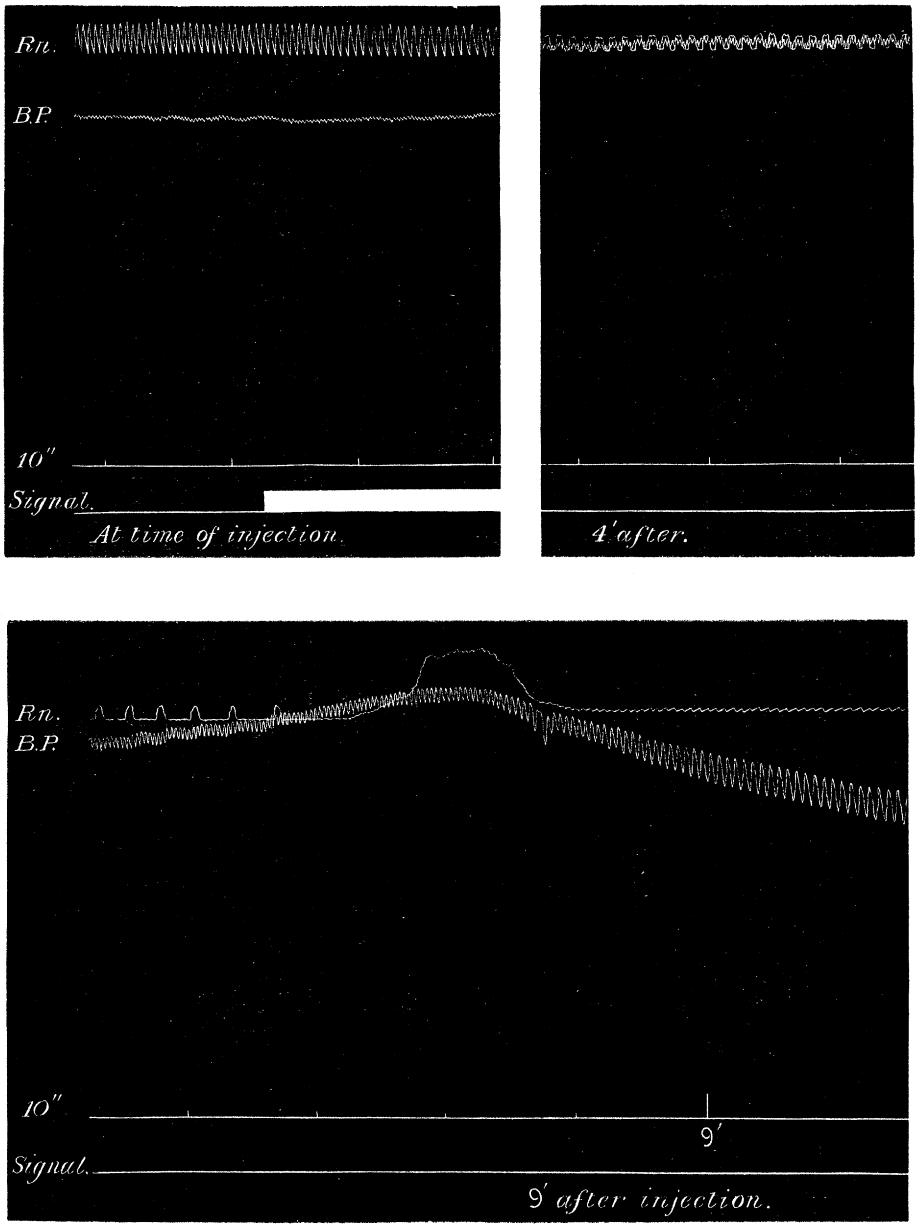


Experiment 1.—Extracts from Blood-pressure Tracing. Time marked = 10 secs.

Experiment 2.—A rabbit, weighing 2·17 kilos. All details of preparation were as in the previous experiment; 2 S.M.L.D.'s were given intravenously in 20 cub. centims. of RINGER'S solution; the injection, which was much faster than had been intended, took 55 secs. The blood pressure rose rapidly, then fell back to its original level, but it rose again as respiration failed, and then fell sharply to death. The first rise was probably mainly due to the large bulk of the injection, and the second was obviously asphyxial. The slowing of the heart, which accompanied the respiratory failure, was also probably asphyxial, and due to an action on the vago-inhibitory centre. Respiration was rapidly paralysed by the venom (Table XI.).

TABLE X.—Experiment No. 1.

Time.	Blood pressure.	Heart beats.	Respirations.	Resp. excursions.
	millims.	per min.	per min.	millims.
1 min. before injection	115	30	3	6 to 9
One-fifth subcutaneous M.L.D. of *Enhy. val.* venom in 10 cub. centims. of RINGER'S fluid intravenously in $2\frac{1}{2}$ mins.				
1 min. after commencement of injection	118	27	2·5	6·5
2 mins. " "	119	27	2·25	3·5
3 " " "	118	26	3	7·5
4 " " "	118	26	3	5 to 8
5 " " "	120	26	3·25	8 to 10
6 " " "	119	27	3	7
One-fifth subcutaneous M.L.D. in 10 cub. centims. of RINGER, given in 3 mins.				
7 mins. after commencement of injection	122	26	3·25	**6·5**
8 " " "	119	27	3	6·5
9 " " "	118	27	3·25	6 to 8
10 " " "	117	27	3·5	6·5
11 " " "	118	27	3·5	9
12 " " "	118	27	3	5
Four subcutaneous M.L.D.'s in 20 cub. centims. of RINGER, given in 6 mins.				
13 mins. after commencement of injection	122	29	3·75	7·5 (struggle)
14 " " "	122	29	4·5	7 to 10
15 " " "	115	27·5	3·5	7·5
16 " " "	112	27	3·25	6
17 " " "	108	26	3·25	5
18 " " "	110	27	2·75	7
19 " " "	115	28	2·75	12
20 " " "	122	27	2·5	5 to 8
21 " " "	122	26	2·2	9
22 " " "	124	26	2·2	9
23 " " "	127	26	2	12
24 " " "	135	25	1·75	15
27 " " "	137	26	1·5	16·5
28 " " "	154	24	1	21
29 " " "	144	25	1·5	22
30 " " "	140	27	1·5	21
31 " " "	140	27	1·25	19·5
32 " " "	146	27	1·25	17·25
33 " " "	148	29·5 (flickering)	1	12
34 " " "	124	0	0	0



Experiment 2.—Extracts from Blood-pressure Tracing.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

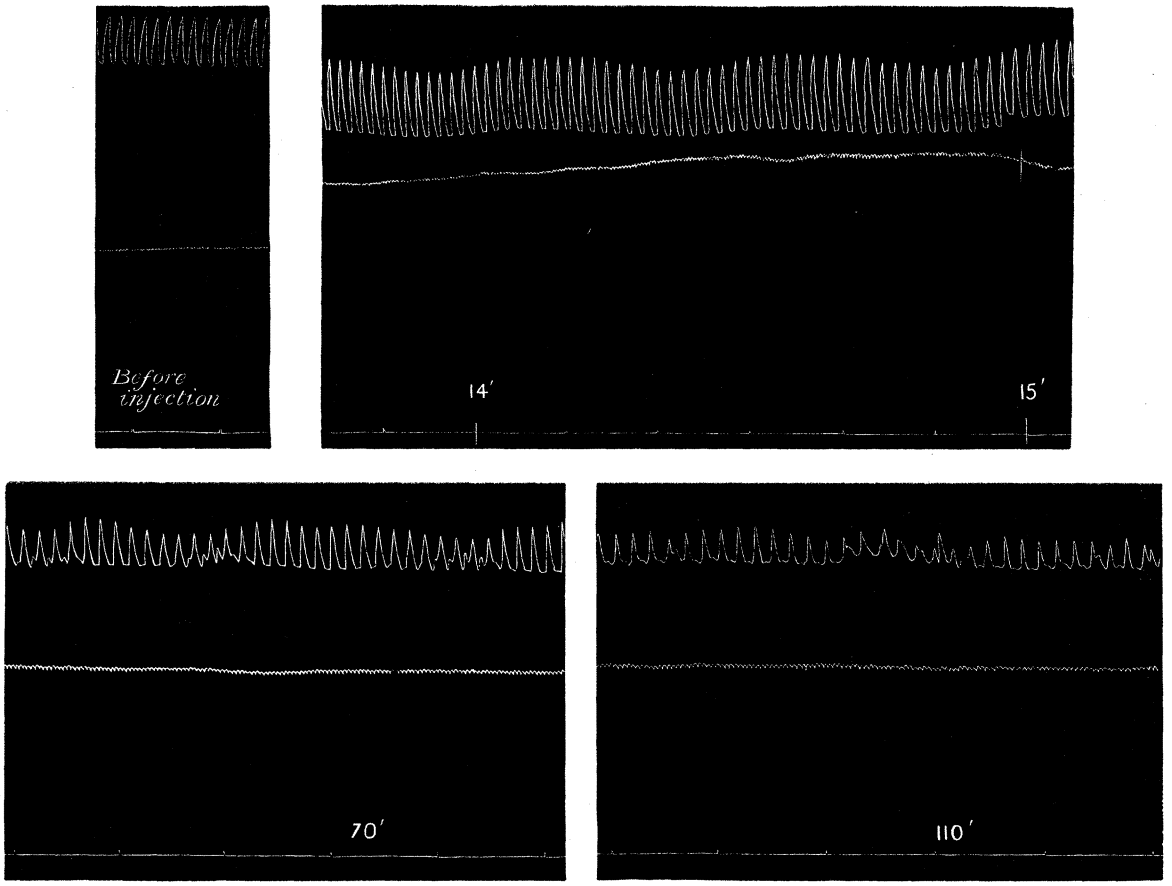
TABLE XI.—Experiment No. 2.

Time.	Blood pressure.	Heart beats.	Respirations.	Resp. excursions.
	millims.	per min.	per min.	per min.
½ min. before injection . .	105	36	23	4·5
One subcutaneous M.L.D. of *Endy. val.* venom given by external jugular in 20 cub. centims. of RINGER'S fluid.				
1 min. after commencement of injection	105	36·5	12	3·25
2 mins. „ „	115	37·5	10·5	3
3 „ „ „	122	37	10	2
4 „ „ „	124	36	9·5	2
5 „ „ „	113	35	9	2·25
6 „ „ „	107	32	7·75	2·5
7 „ „ „	104	31	6·5	2·5
8 „ „ „	111	30	4·75	2
8 „ 40 secs. „	128	25	0	0
9 „ 0 „ „	108	17	0	0
10 „ „ „	73	10·5	0	0
11 „ „ „	65	11·5	0	0
12 „ „ „	45	12	0	0
13 „ „ „	45	0	0	0

Experiment 3.—A rabbit, weighing 2·18 kilos., was etherised, and a tracheal cannula inserted, through which the anæsthetic was continued to be given. Blood pressure was taken in a carotid, and respiratory movement was recorded by means of a double stethograph. Injections were made through an external jugular vein.

After taking a normal, one-fifth of the subcutaneous M.L.D. was injected intravenously, dissolved in 4 cub. centims. of RINGER'S fluid. There was a sharp rise of blood pressure, followed by an equally sharp fall. At the time of the second injection the pressure had fallen till it stood at the same point as that which it had occupied at the beginning of the experiment. At the 85th minute, a second injection, the same in amount, &c., as the last, was given; and at the 105th minute, a third injection, double the dose of either of the two preceding, was given. The rises which followed each of these injections were probably largely due to the bulk of fluid injected. The heart rate remained very nearly constant for a long time and then fell slowly. Respiratory rhythm was early affected, as is shown by the accompanying extracts from the tracings. ROGERS recorded his observation "that Cheyne-Stokes breathing is sometimes simulated" in Enhydrina poisoning, experimentally produced. Our extracts from this tracing (p. 269) are an interesting confirmation of his statement. As death approached the blood pressure tended to fall. This observation is not really in opposition to ROGERS' experience of a marked rise in blood pressure ushering in death, for in all his recorded experiments death occurred much more rapidly than in the

case we are now considering. Indeed, it was our conviction that ROGERS had confined himself too exclusively to large doses, and this led us to administer much smaller doses in addition to large ones. We need hardly insist on the advisability of approximating the doses (so far as experimental requirements admit) as closely as possible to those likely to be met with in actual experience. Details of the tracing will be found in the accompanying table (Table XII.). The main inference would appear to be that the venom has no cardio-inhibitory action such as one meets with in cobraism,



Experiment 3.—Extracts from Blood-pressure Tracing, showing marked changes of respiratory rhythm.

but that the increasing venosity of the blood, which is induced by slow failure of respiration, exerts an indirect inhibitory influence on the heart through the vagal centre in the medulla oblongata. When rapid failure of respiration occurs, with a heart still undamaged, abrupt rises of blood pressure accompany asphyxiation. This is not the case in the slower cases whose course we have just sketched out above. It may be urged that in cobraism, abrupt asphyxial rises of the blood-pressure curve immediately precede death, even though Cobra venom has a marked inhibitory action on the heart, both direct and indirect. There is, however, an element in cobraism, which we have shown to be lacking in Sea-snake poisoning, viz., the action

TABLE XII.—Experiment No. 3.

Time.	Blood pressure.	Heart beats.	Respirations.	Resp. excursions.
	millims.	per min.	per min.	per min.
½ min. before injection	100	30	7·5	7·5
One-fifth subcutaneous M.L.D. intravenously in 4 cub. centims. of RINGER.				
1 min. after commencement of injection	116	28	6·5	9
2 ,, ,, ,,	116	28	5·75	9·5
3 ,, ,, ,,	119	27	5·5	10
4 ,, ,, ,,	123	27	5·5	11
5 ,, ,, ,,	124	27·5	5·75	11
6 ,, ,, ,,	124	26	5·75	11
7 ,, ,, ,,	126	27·5	5·75	12
8 ,, ,, ,,	128	27·5	6	12·25
9 ,, ,, ,,	130	28	6	11·5
10 ,, ,, ,,	129	27·5	6	12
15 ,, ,, ,,	130	29	6·75	12
20 ,, ,, ,,	121	30	9	8
25 ,, ,, ,,	104	29	6·25	4·25
30 ,, ,, ,,	102	28	6·75	7·5
35 ,, ,, ,,	110	29	7	7 to 14
40 ,, ,, ,,	110	28	7	6 to 12
50 ,, ,, ,,	108	28	7	6 to 8·5
55 ,, ,, ,,	107	28	7	6 to 10
60 ,, ,, ,,	105	28	7	7 to 9·5
70 ,, ,, ,,	102	27	7	4·5 to 7·5
80 ,, ,, ,,	101	26	7	6·5
85 ,, ,, ,,	100	26	6·75	4 to 7·5
One-fifth subcutaneous M.L.D. intravenously in 4 cub. centims. of RINGER given in 1 min.				
90 mins. after commencement of first injection	100	26	6·75	4·5 to 6·5
95 ,, ,, ,,	102	25	7	4 to 6·5
105 ,, ,, ,,	104	25	6·25	3 to 5·5
Two-fifths of subcutaneous M.L.D. intravenously in 8 cub. centims. of RINGER given in 3 min.				
110 mins. after commencement of first injection	103	24	6	4 to 5·5
120 ,, ,, ,,	110	23	5	1·5
125 ,, ,, ,,	80	21	—	—

After this the heart slowed steadily, respiration rapidly failed and the animal was dead 23 mins. later. Blood pressure fell steadily to the end. Respiratory failure was presumably the cause of the cardiac slowing.

of the venom on the blood-vessels, which leads to powerful constriction of their walls and so maintains a high pressure, despite other opposing influences. As we have

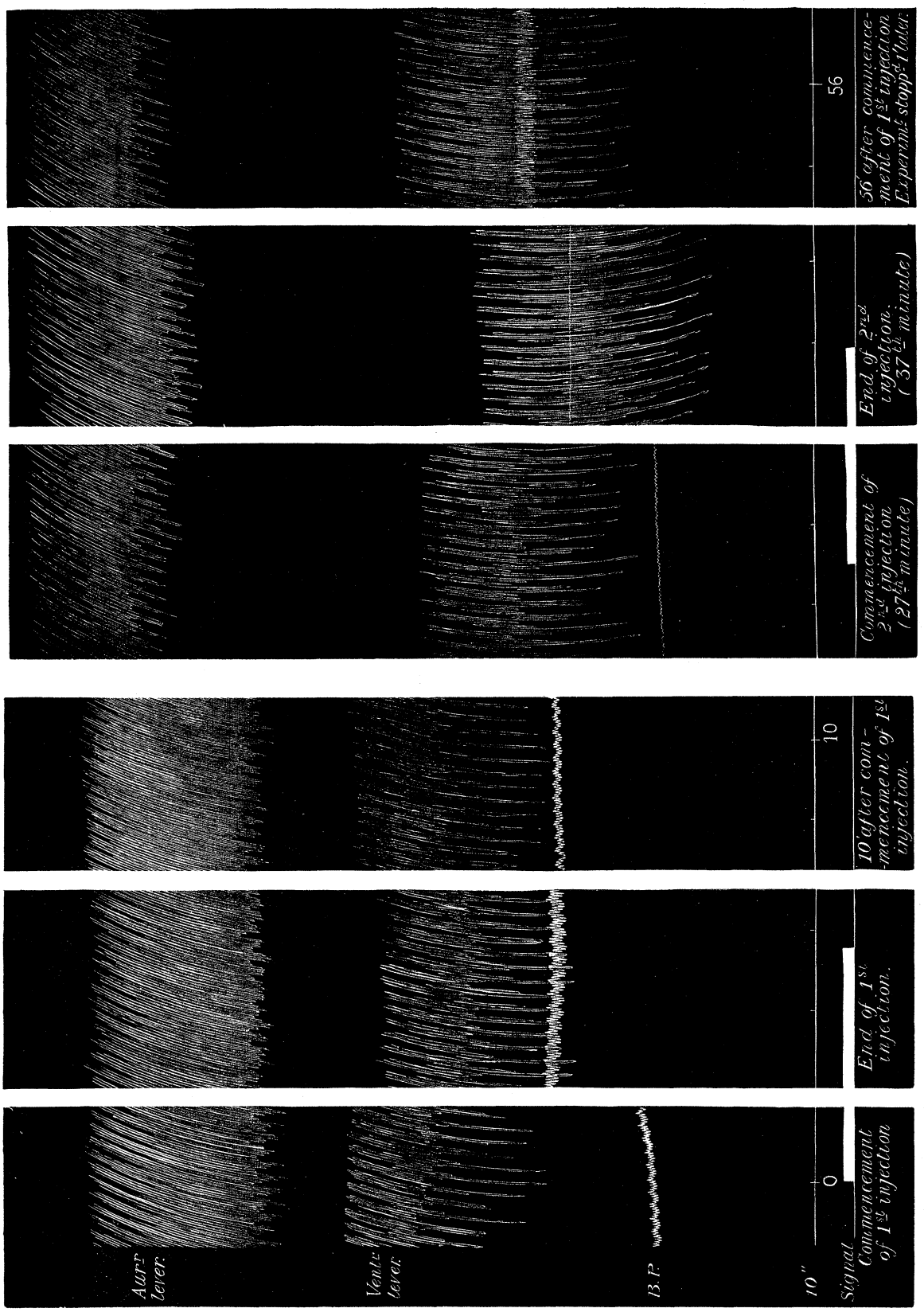
already shown, the venom of Enhydrina has no direct constrictive action on the arterial walls.

Experiment 4.—A cat, weighing 2 kilos., was etherised, and etherisation was maintained through a tracheal cannula. The blood pressure was taken in a carotid artery, and the auricular and ventricular movements were recorded by means of levers attached to the heart by hooks and silk threads, the chest wall being partly removed for the purpose. Two injections were made through an external jugular vein of five and ten times the subcutaneous minimum lethal dose, respectively, of *Enhydrina valakadien* venom.

The accompanying table (Table XIII.) shows the changes observed. The high rises of blood pressure after each injection are, doubtless, due to the volume of fluid injected. It will be observed that at the beginning of the second phase of the experiment, the pressure had fallen to its original level. The force of beat was lessened, but that is to be expected in operations so severe as this, especially in winter, even although, as in this experiment, all ordinary precautions to avoid shock are taken. The striking feature of the experiment is well shown on the accompanying extracts from the tracings (p. 272), and is the absence of all signs of inhibition, even though the doses of venom given were relatively very high, viz., five times the subcutaneous M.L.D. on the first administration, and ten times the subcutaneous M.L.D. on the second administration. Both the rate of beat and the amplitude of the excursions of the auricular and ventricular levers show by their constancy that this venom has no direct action on the heart, even when given intravenously in doses far above those which would be met with in snake-bite in man.

Experiment 5.—A cat, weighing 2·5 kilos., was etherised, and a cannula was placed in the trachea for the farther administration of the anæsthetic. Blood pressure was taken in the carotid, and respirations were recorded by means of a double stethograph (the movements of the lever are expressed in millimetres as some indication of the respiratory excursions). Injections were made by the external jugular vein with a fine hypodermic needle, and the venom solution consisted of ·001 gramme of *Enhydrina valakadien* venom, extracted from glands, dissolved in 1 cub. centim. of Ringer's fluid.

The subjoined table (Table XIV.) shows the results as recorded. In this experiment we were careful to reduce the bulk of our injections, and it is here clearly demonstrated that there is no rise of blood pressure apart from that attendant on progressive asphyxiation. Even then we do not notice any large rise, but, on the contrary, the pressure gradually falls towards death, the heart at the same time failing. A point of interest is the appearance, in this tracing also, of curves indicating a type of breathing like the Cheyne-Stokes type. These curves make their appearance directly after the second injection. The type of respiration is altered in another respect, as may be seen in the extracts from the tracing (p. 274). It will be observed that expiration immediately follows inspiration in the upper respiratory



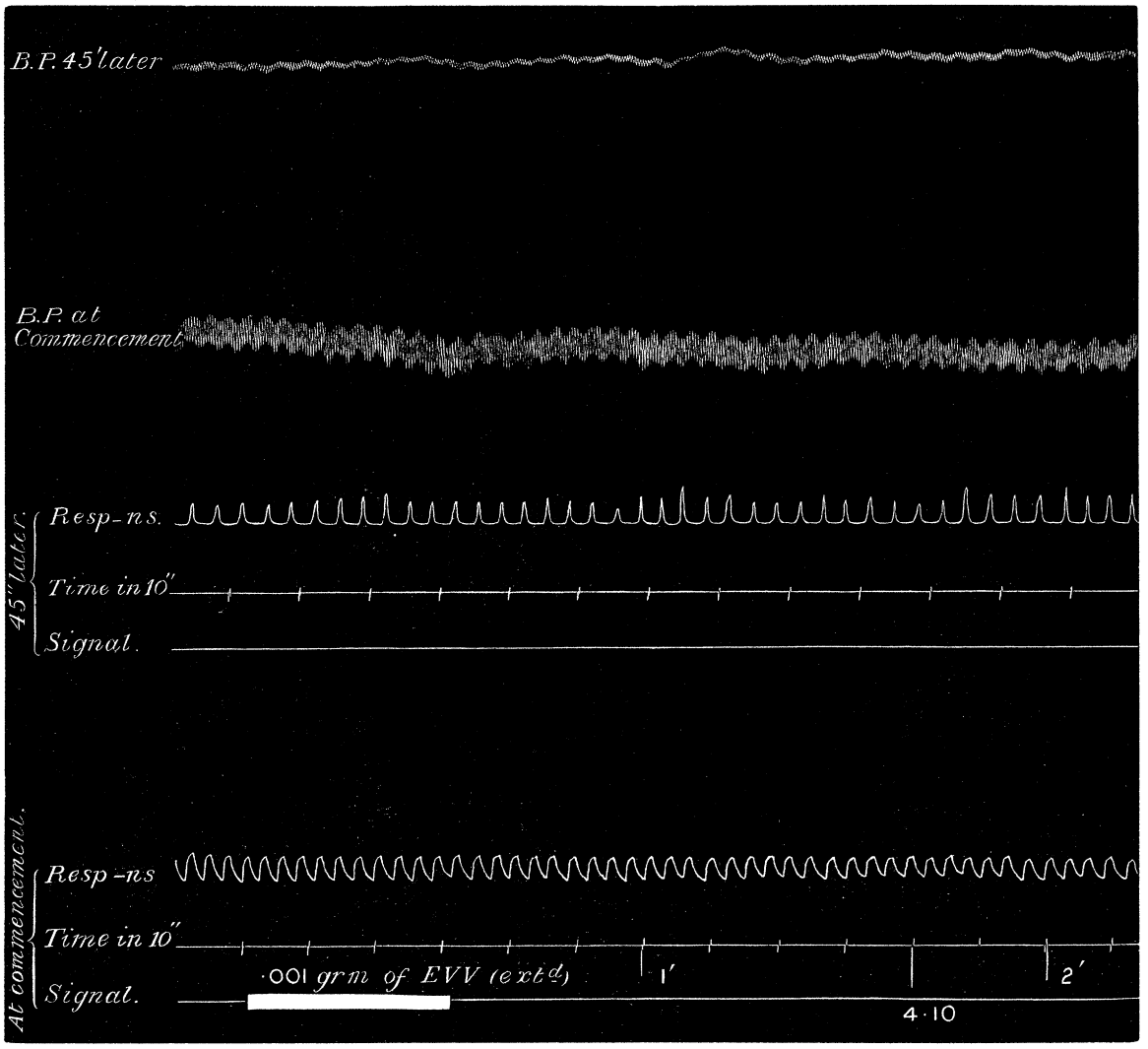
Experiment 4.—Extracts from Blood-pressure Tracing with heart exposed. Auricular and ventricular movements recorded by suspension method.

Table XIII.—Experiment No. 4.

Time.	Blood pressure.	Heart beats.	Auricular beat.	Ventricular beat.
	millims.	per min.	per min.	per min.
1 min. before injection . . .	84	30·5	28	14·5
Five subcutaneous M.L.D.'s injected intravenously in 5½ mins. (solution ·0001 gramme = 1 cub. centim.)				
1 min. after commencement of injection	110	30·5	29	14·5
2 ,, ,, ,,	117	29	29	14·5
3 ,, ,, ,,	122	29	28·5	14·5
4 ,, ,, ,,	122	29	28·5	14·5
5 ,, ,, ,,	124	29	31	14·5
6 ,, ,, ,,	125	29	29	14·5
7 ,, ,, ,,	120	29	31	14·5
8 ,, ,, ,,	122	29	31	14
9 ,, ,, ,,	122	29	31	15
10 ,, ,, ,,	124	29	32	15
11 ,, ,, ,,	127	29	29	14
12 ,, ,, ,,	134	29	28·5	16
Manipulation to remove clot (cannula opened).				
13 ,, ,, ,,	126	31	33	15
14 ,, ,, ,,	123	30	33·5	18
15 ,, ,, ,,	125	31	33·5	16·5
An interval of 12 mins., during which clot had to be twice run out, and auricular and ventricular levers were readjusted.				
Second injection of ten subcutaneous M.L.D.'s was made between 27th and 37th mins.				
27 mins. after commencement of injection	86	22	22	19
28 ,, ,, ,,	86	22	23	19
29 ,, ,, ,,	93	21	26	19
30 ,, ,, ,,	97	22	26	19
31 ,, ,, ,,	102	21	23·5	19
32 ,, ,, ,,	104	21	27	21
33 ,, ,, ,,	107	21·5	27	21
34 ,, ,, ,,	108	21·5	27	23
35 ,, ,, ,,	112	21·5	28·5	24
36 ,, ,, ,,	116	22	27·5	23
37 ,, ,, ,,	119	22·5	27·5	22·5
38 ,, ,, ,,	120	22	25	22
39 ,, ,, ,,	122	24	26	22
Manipulation to remove clot, and levers readjusted.				
45 ,, ,, ,,	119	24	23·5	22·5
46 ,, ,, ,,	—	—	—	—
50 ,, ,, ,,	122	24	22	22
55 ,, ,, ,,	136	23	22·5	26
57 ,, ,, ,,	136	23	20	25

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

trace, and that the pause between successive respiratory movements is greatly prolonged at the expense of the movements themselves.



Portions of two Tracings from Experiment 5 (*Enhy. val.* venom) are here partly superimposed. The lower one is at the time of first injection, the upper one is 45 mins. later.

Action on the Motor Nerve-ends.

If a very large dose of the Sea-snake venoms dealt with in this paper is injected intravenously into an animal, the animal dies in a few minutes, and asphyxial convulsions are absent or ill-marked. Rogers noticed this, and attributed it to the respiratory centre being completely paralysed "before the failure of the breathing had had time to render the blood sufficiently venous to produce respiratory convulsions." He adds, "Another possible explanation must, however, be considered: a paralysis of the end-plates of the motor nerves might cut off the peripheral

TABLE XIV.—Experiment No. 5.

Time.	Blood pressure.	Heart beats.	Respiration.	Resp. excursions.
	millims.	per min.	per min.	millims.
Before injection	151	25	3	5
·001 gramme (2½ subcutaneous M.L.D.'s) of extracted *Enhy. val.* venom dissolved in 1 cub. centim. of RINGER'S fluid injected intravenously in 30 secs.				
1 min. after commencement of injection	149	25	3·5	4
2 mins. ,, ,,	145	25	3·5	3
3 ,, ,, ,,	145	26	3·5	3
4 ,, ,, ,,	145	27	3·5	3
5 ,, ,, ,,	145	27	3·5	3
10 ,, ,, ,,	151	28	3·5	1·5 to 7
12 ,, ,, ,,	151	29	4·5	1·5 to 8
Artery cannula opened to detach clot twice over, thus causing fall in blood pressure.				
20 mins. after commencement of injection	122	26	2·5	1·5
25 mins. ,, ,,	122	26	2·5	1·25
30 ,, ,, ,,	126	25	2·25	1·5
35 ,, ,, ,,	129	25	2·75	2
37 mins. after first injection, a second injection the same as the first was given.				
37 mins. after commencement of first injection	121	25	2·5	2·5
38 mins. ,, ,,	122	25	2·5	1·5 to 2·5
39 ,, ,, ,,	122	26	2·5	1·5 to 2·5
40 ,, ,, ,,	125	26	3	2·5 to 3
41 ,, ,, ,,	125	26	2·75	2 to 3
42 ,, ,, ,,	125	26	2·75	1·5 to 4
After the second injection, Cheyne-Stokes breathing became well marked.				
45 mins. after commencement of first injection	127	26	3	2·5 to 6
50 mins. ,, ,,	127	26	2·5	4 to 6
52 ,, ,, ,,	119	24	2	5 to 6
54 ,, ,, ,,	105	23	2	4·5 to 7
55 ,, ,, ,,	94	22	2	2·5 to 8·5
56 ,, ,, ,,	54	16	2	1·5 to 6·5
57 ,, ,, ,,	14	10	0	0
60 ,, ,, ,,	Dead	—	—	—

muscles from the action of the respiratory centre, in spite of its over-stimulation by venous blood." He then proceeded to test the state of the motor nerve-ends in two legs, one of which was ligatured and one free, and he showed that those of the protected leg remained active, whilst those of the other became paralysed. He concludes that "it is therefore impossible to say how far the absence of convulsions

is due to this cause (paralysis of the phrenic nerve-ends), and how far to failure of the respiratory centre. To clear up this point he made two experiments, one on a cat and the other on a rabbit. The phrenic (left) was exposed in the neck, and stimulated at intervals, the results being graphically recorded. He was led to conclude that "the first and most important action of the poison appears to be its effect on the respiratory centre, although the paralysis of the phrenics speedily ensues, and is a very important feature of the action of the venom."

Rogers used a current with the secondary coil *at* 25 *millims.* Ragotzi* has stated that in order to obtain evidence of peripheral nerve-end paralysis in cobraism, one must avoid the administration both of large rapidly fatal doses and of too small doses. In fact, there must be a moderately large dose in circulation, and it must be allowed a sufficient time to act. One of us has recently shown that the phrenic nerve-ends remain fully sensitive (*a*) in animals which have been poisoned by large intravenous doses of Cobra venom, and (*b*) in those which have been killed by subcutaneous doses not greatly exceeding the minimum lethal. It was at the same time shown that the phrenic nerve-ends can be paralysed by giving lethal doses and then maintaining life by means of artificial respiration. The obvious inference was that in cobraism the respiratory centre is first attacked; and that this was not improbable was demonstrated by exposing the medulla oblongata and applying Cobra venom directly to its surface, when the centre speedily became paralysed, in one case death occurring in 12 mins.

In order to investigate more fully the action of Enhydrina poison on the various nerve-ends, and especially on the phrenics, three series of experiments were undertaken.

Series 1.—*Experiment* 1.—A rat weighing ·195 kilo. was etherised, and received an intravenous injection of *Enhydris curtus* venom. The dose was equivalent to 14 M.L.D.'s if given subcutaneously, and was dissolved in 1 cub. centim. of Ringer's fluid. Death took place in 4 mins. The chest was at once opened and the exposed phrenics were tested with the secondary current (Neave's hammer in circuit). No diaphragmatic contraction was observed even with a maximal stimulus. The vagi were next exposed and stimulated; again, even a maximal stimulus failed to produce a decided response, the heart being slowed but not stopped. A sciatic nerve was then tried and gave a response at 120 millims.

Experiment 2.—A rat weighing ·132 kilo. received intravenously seventeen times the subcutaneous M.L.D. of *Enhydris curtus* venom, and was dead in $2\frac{1}{2}$ mins. The left phrenic nerve again gave no response to maximal stimuli; the right responded by a feeble diaphragmatic twitch with the secondary coil at 70 millims. Even with a maximal stimulation the diaphragmatic twitch was very feeble. Stimulation of the vagi, with the secondary coil home, again failed to inhibit the heart

* Virchow's 'Archiv für Pathol. Anat. und Physiol.,' vol. 122, 1890, p. 201.

completely though it slowed it. The left sciatic responded at 180 millims. by producing a feeble muscular twitch.

Experiment 3.—A precisely similar experiment was made with a rabbit which received ten times the subcutaneous M.L.D. intravenously. A maximal stimulation of the phrenics produced no diaphragmatic response.

Experiment 4.—A rat was etherised and its trachea was ligatured. As soon as death had occurred, the nerve-ends were tested as before, with the following results—Phrenic response at 450 millims.; vagal response at 90 millims. Clearly, therefore, the nerve-end paresis we have noted was not accounted for by suffocation. Experiments on cats, rats and rabbits killed by a blow on the head have shown that stimulation of a normal phrenic nerve or of a sciatic with the secondary coil at 450 millims. produces marked muscular contraction. The exact strength of current to produce a complete temporary inhibition of the heart varied. In rabbits and cats such inhibition was usually got with the secondary coil at 100–140 millims., but in rats one had generally to bring the coil nearer, 80–90 millims. being a common but not always sufficiently approximated distance to cause definite inhibition.

It should be mentioned that even when the nerves of the poisoned animals above dealt with failed to give any response to a maximal stimulus, the muscles responded readily to a comparatively weak shock.

Series 2.—Whenever it was possible to do so, we tested the condition of the phrenic, sciatic and vagal nerve-ends immediately on death in the animals used for ascertaining the minimum lethal doses. In order to keep the conditions uniform, we watched each animal, and opened the thorax as soon as the corneal reflex had disappeared and respiration had ceased. We are unable to lay down any rule establishing a relationship between the amount of nerve-end paresis noted and the doses of venom used, but we found a distinct diminution of nerve-end activity so constantly present as to leave no doubt in our minds that we were dealing with cause and effect.

The direct Action of Enhydrina Venom on the Respiratory Centre.

Series 3.—We exposed the medulla oblongata of rabbits, by dividing the soft structures at the back of the neck, and cutting away the posterior occipito-atlantoid ligament. A large quantity (2–5 milligrammes) of Enhydrina venom was then dropped on to the medulla over the seat of the respiratory centre. The type of respiration was in each case quickly and profoundly affected, and the animals died with every evidence of respiratory failure, and the excitability of the phrenic and other nerve-ends was found at death to be distinctly impaired. We could not succeed in killing an animal with its nerve-ends still undamaged, as can be done when Cobra venom is applied in a similar way.

It would accordingly appear to be impossible with Enhydrina venom to kill a rabbit directly through its respiratory centre, before the poison has had time to enter the general circulation and thus affect the nerve-ends. On the other hand, the rapidity with which the type of respiration was altered, both in the above experiments and in those in which the venom was intravenously injected, appears to us to leave no room for doubt that the respiratory centre is directly affected by this venom. The conclusion would appear to be that both the centre and the nerve-ends are affected, and that this double action is responsible for the remarkable embarrassment of respiration which is characteristic of the action of Sea-snake venoms.

Summary of Conclusions.

(1) Enhydrina venom has no direct action on the walls of the arterioles, or at least has no such action in any strength of solution which could be met with in the blood of a human victim of Sea-snake bite.

(2) Enhydrina venom acts directly on the isolated frog-ventricle, producing a tonic and stimulating effect, but this action is produced only by very strong solutions (1 : 5000). The heart beat is quickened, and the result is, therefore, similar to that produced by very weak solutions of Cobra venom (1 : 1,000,000 or weaker).

(3) By experimenting with the mammalian heart exposed *in situ*, we have shown that Enhydrina venom has no direct action on the vagal cardio-inhibitory centre. This affords a striking contrast to the condition observed in Cobra poisoning. In the latter case, the powerful tonic and stimulant action of the venom on the heart muscle (or probably on its nerve-ends) is masked by equally powerful and direct stimulation of the cardio-inhibitory centre. In Enhydrina poisoning, on the other hand, the complete absence of cardio-inhibition leaves the feeble tonic action on the heart free to manifest itself, as appears to be displayed in several of our tracings. We cannot otherwise explain the increase in rate of the heart beats which we have not infrequently observed.

(4) Enhydrina venom has apparently no direct action on the vaso-motor centre. Rogers found that on the injection of a Viperine venom, or of the venom of the Colubrine Banded Krait (*Bungarus fasciatus*) into an animal, a marked fall of blood pressure occurred, which was of central origin. In collaboration with Drs. Sillar and Carmichael (of the Materia Medica Laboratory, Edinburgh), one of us has found that a similar and powerful action is exerted by the venom of the common Krait (*B. cæruleus*).* The absence of any such action in the Sea-snake venoms with which we are dealing is, therefore, worthy of note.

(5) The blood-pressure curve in Enhydrina poisoning is a remarkably steady one, provided that moderate doses are given and that care is taken to avoid the injection of large volumes of fluid into the blood-vessels. This is due to the fact that the

* Paper communicated to the Royal Society, June 9, 1904.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

blood pressure is exposed neither to the influence of the rival forces which act on the heart so strongly in cobraism, nor to the direct vaso-motor changes which we have dealt with in the preceding paragraph.

(6) The respiratory mechanism appears to be that which is chiefly affected by Enhydrina venom. If large lethal doses are employed, such as ROGERS appears to have confined himself to, respiration falls rapidly, and a considerable rise of blood pressure, asphyxial in origin, may precede death. The heart beat also quickly slows, and blood pressure falls with corresponding rapidity.

Obviously, these are simply the phenomena of rapid asphyxiation. If, however, smaller doses of venom are employed, no marked rise in blood pressure occurs. The ordinary level is maintained till near the occurrence of death; the beat then slows and the blood pressure falls. Here we have an expression of gradual cardiac failure, brought about by slowly progressive asphyxiation. The absence in slow Enhydrina poisoning of the large asphyxial rises of pressure, which are so characteristic of the final stages of Cobra poisoning, is readily explained by the fact that Enhydrina venom has no direct constrictive action on the walls of the arterioles, such as Cobra venom possesses.

(7) As to the part of the respiratory mechanism that is affected by Sea-snake venom, the rapidity with which respiration is modified, both when venom is injected into a vein and also when it is applied directly to the medulla oblongata, leaves no room to doubt that the respiratory centre is directly acted on by the venom. On the other hand, we have shown that some degree of motor nerve-end paresis is constantly present in animals dying from the effects of subcutaneous injections of this venom. We have also emphasised the fact that in our experiments carried out by dropping venom on the exposed medulla oblongata, we have failed to kill animals through the respiratory centre with the motor nerve-ends still undamaged. In this respect we have shown that Enhydrina venom differs in its action from Cobra venom. It would, therefore, appear that in poisoning with Enhydrina venom, motor nerve-end paresis plays a much greater part than it does in cobraism. It is not difficult to suppose that a blunting of the motor nerve-end mechanism, even though far from absolute, may seriously add to the embarrassment of a centre which has already been directly and gravely damaged. We hope to return to this and other points in a future communication.

In conclusion, we desire to express our sense of indebtedness to the India Office, to the Government of India, and last, but by no means least, to the Madras Government for the assistance and facilities which they have given us in the carrying out of this research.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES