A CONTRIBUTION TO THE PHARMACOLOGY

of the venom of

THE HAMADRYAD - NAIA BUNGARUS;

and

its action compared with that of

the venoms of

other serpents of the sub-family Elapinae.

by

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INTRODUCTION.

In recent years the poisons of many of the venomous ophidia have been examined by investigators and the results of their observations recorded in a manner which permits of their being compared one with another.

The first step in all such work is to be able toexpress the relative activity of the various poisons as determined by the minimal lethal dose on various animals.

Pharmacology attained a higher level of accuracy when this important point was recognised - an advance which was, I believe, first made by Fraser in his observations on the action of Physostigma (/,)

Some observations interesting in themselves as isolated facts are rendered comparatively useless for purposes of comparison by the neglect of recording the preliminary observations with regard to the activity of the particular specimen of the active substance used. This is more particularly the case when observations are being made on the action of substances of which the chemical composition is imperfectly known or which vary considerably in their relative activity.

In/

In order to illustrate this point I may take the case of a comparison between the lethality of the venom of the indian cobra determined by Fraser for frogs and found to be 0.0002 grs- per kilogramme. and that of a cobra venom used by Noguchi (2) when observing the effects of venoms on a large number of cold blooded animals. Noguchi He states that Rana catesbiana 95 grammes in weight recovered, only experiencing a slight temporary stupor, after the administration intraperitoneally of 0.001 gramme. He found that another of the same species 90 grammes in weight died in 3 hours and 42 minutes after 0.005 grammes of the venom had been administered in the same manner. The lethality therefore of Noguchi's specimen of cobra venom must have been between 0.01 gramme per kilo-gramme and 0.053 grammes per kilogramme showing therefore either that Rana catesbiana is much more resistant to the action of cobra poison than is R. temporaria or R. esculenta or that this specimen of poison was much less active than is usually the case. As however he does not state the minimal lethal dose either for warm-blooded animals or for other species of frogs we are left in some doubt as to which of these/

these explanations is correct.

The chief portion of this paper being the consideration of the action of the venom of the hamadryad I shall confine myself in contrasting its activity with the venom of other serpents to observations made on the venoms of the indian and african cobras, and the common krait as these have been investigated in the laboratories of this University. An additional reason on my part being that as I have assisted to a considerable extent in the numerous experiments performed with these venoms in the Pharmacological Laboratory, I may claim to possess a first-hand knowledge of the observations recorded.

These researches on the venoms of

(a) the indian cobra (naia tripudians) carried out by Elliot (3) of

(b) that on the african cobra (naia haie) by Prentice (4) of

(c) that on the common krait (Bungarous Coeruleus) by Elliot and Carmichael in conjunction with myself (5) and that on the

(d) and that on the venom of the hamadryad (naia bungarus) prove that there are certain features/

features possessed in common by these venoms, but that each possesses some peculiarities of action; so that by contrasting the less complicated with the more complicated we are enabled to understand more easily the mechanism of the action of the latter.

These four serpents all belong to the sub-family Elapinae of the family Colubridae of the Ophidia. The Indian and African cobras and the hamadryad belong, the to the genus Naia and the krait to the genus Bungarus. This classification is that given by Boulenger in the catalogue of the snakes of the British Museum. (6)

The Hamadryad-

The various names by which the hamadryad has been known and described are thus given by Boulenger Naia fasciata, Naia Elaps, Naia ingens, Naia tripudians variety Sumatrana, hamadryas hannah, hamadryas Ophiophagus, hamadryas elaps, trimeresurus ophiophagus, trimeresurus bungarus and Ophiophagus elaps. Fayrer admirably depicts the serpent under the last named title in his Thanatophidia (7)

It is one of the largest, if not actually the largest venomous serpent known and is no less distinguished/

distinguished for its size than for is ferocity .

It not only defends itself when attacked but attacks fiercely when disturbed and has been known to pursue its victim with great speed and determination, turning aside for no obstacle. It attains sometimes the length of 14 feet, inhabits trees and devours other serpents, from which characteristics it derives certain of its names (ingens, hamadryas, ophiophagus). Its activity is very great and its aim in striking very certain. It therefore enjoys a terrifying reputation, the effects of which are well described in a short sketch by Aitken (8) entitled "The King Cobra" to use its colloquial name. One can understand therefore the extreme difficulty of obtaining any considerable quantity of this venom.

The specimen of venom whose action is described in this paper was obtained by Sir Thomas Fraser from hamadryads which were for some time kept alive in the Materia Medica Department. They did not however eat sufficiently to keep themselves in good condition. I have been informed that it is advisable to feed these serpents by hand once every six days - which method however for obvious reasons was not attempted.

On one occasion it was interesting to see a hamadryad/

hamadryad swallow a serpent supplied to it for food - a grass snake (Ptropedonotus natrix) about eighteen inches in length. The hamadryad made a rapid dart across the cage and seized its prey about the middle of its body. By means of a lateral movement of its jaws the hamadryad caused the grass snake to be passed along until its head reached the larger serpents mouth. When all but three inches of the grass snake had been swallowed its tail was seen to have firmly grasped the neck of the hamadryad; it remained in this position for about three quarters of an hour in spite of all attempts on the part of the hamadryad to complete its meal and was then ejected and escaped apparently uninjured. When the cage was visited the next day however the grass snake had finally disappeared.

The poison of these hamadryads was extracted from the glands by Sir Thomas Fraser and given to me for examination by him. He has most kindly allowed me to use my observations for the purpose of this thesis.

The condition of the serpents, in confinement and through want of sufficient food would lead us to expect that the venom would be less active than that of/

of the venom of the serpent under more natural conditions. It was found to be considerably less toxic than the venoms of the other three serpents mentioned above.

Not very many observations have been made on the action of this venom, the researches of Rogers (9) and of Lamb (//) being the only ones known to me. Fayrer and Cunningham previously had likened its action to that of the cobra. Rogers found the minimal lethal dose for pigeons to be 0.0006 grammes per kilogramme and states that the symptoms produced were quite indistinguishable from those produced by cobra venom. He observed that there was little haemolytic action, that death was produced by paralyses of respiration and that this paralyses is due to an action both on the respiratory centre and on the peripheral ends of the phrenic nerve. He also observed that artificial respiration prolonged life for 24 minutes after 0.002 grammes per kilogramme had been injected intravenously into a cat during which experiment the natural respiration ceased twenty minutes after the injection.

He gives the minimal lethal dose for "a warm-blooded animal"/

animal" as 0.0006 grammes per kilogramme. He does not state however whether this is true for a cat as well as a rabbit or pigeon nor does he state whether he considers 0.002 gramme per kilogramme administered intravenously a large or a small dose for a cat. He also observed that with large doses the ends of the sciatic nerve are paralysed before death. The researches of Lamb (12) into the haemolytic action of the venoms of various serpents, included the hamadryad which he found to possess very little of such an action except under special circumstances.

The specimen of the venom was of an orange yellow colour in fine somewhat glistening fragments, very soluble in water, the reaction of the solution being neutral to test-paper-

The minimal lethal dose of this venom was first determined in frogs and found to be the same for Rana temporaria and Rana esculenta, but as the quantity of venom was not large and the toxicity not very great , entailing therefore the strictest economy of material, the minimal lethal dose is not very minutely determined. A very considerable difficulty was experienced also owing to the length of time which frogs remained paralysed without obvious sign of life- This led in some of the earlier experiments either to their being considered terminated unduly early, or to other factors being introduced which undoubtedly hastened a fatal result in cases where, had the animal been left undisturbed recovery might have taken place- A description of one experiment in which there was administered a dose very nearly the minimal lethal will serve to show the general course of poisoning which obtains under these conditions.

To a healthy male frog R. esculenta thirty nine grammes in weight, there was administered subcutaneously at 2-45 p.m. 0.29 C.C. of a solution of Hamadryad venom dissolved in Ringer's solution. The quantity of the poison was 0.000117 grammes which gave a dose of 0.003 grammes per kilogramme. Before the injection the respiratory movements of the throat were 21 in 10 seconds strong and regular- The abdominal movements were 15 per 10" and irregular. The cardiac impacts were 13 per 10" fairly easily seen through the thoracic wall. The lymph hearts were beating regular.-ly at the rate of 12 per 10" easily seen on both sides of the urostyle. The Reflexes were all acute.
10 minutes after the injection the respiratory throat movements had increased to 24 per 10", the abdominal had decreased to 7 per 10", the lymph hearts were 11 per 10", rather more difficult to see. The frog sat still while undisturbed but jumped very vigorously when touched. At 3-15 respirations were 23 per 10" the lymph hearts 5 per 10" and seen with great difficulty- The cardiac impact was still 13 per 10" At 3-30 abdominal respiratory movements had ceased, the throat movements were 22 per 10",still regular, but//

but shallower; the lymph hearts were no longer seen to be beating. The frog sat quite still but with head drooping.

At 4 o'clock the respirations had become irregular varying from 13 to 16 per 10" some few movements being deep but the greater number very shallow. The Reflexes elicited by tickling the nose or touching the eyes were quite acute, Stimulating the skin of the foot by an induced interrupted current with the secondary coil 150 mm. caused the animal to jump away very vigorously -

At 4.30 p.m- the respirations were 14 per 10" irregular, jerky and very shallow. The lower eyelid was beginning to rise over the eye. The same electrical stimulation caused the animal to jump, but not instantaneously.

At 4-45 P.m. the respirations were 10 to 16 per 10", the majority being shallow, one or two deeper movements being visible. The cardiac impact was still 13 per 10". No movement of the lymph hearts was seen and the frog needed a stronger stimulus to cause him to alter his position. After a jump the legs remained stretched out and were not drawn up to the normal position for a few seconds.

At/

At 5-15 p.m. the animal could be placed on its back and struggled feebly but was unable to regain its normal position. Stimulation with the coil at 130 mm. applied for several seconds produced one jump. There was however strong local contraction of the muscles over which the electrodes were applied.

At 5-20 the head was on the ground, the eyes were completely covered owing to the paralysis of the lower eyelids, but the eye and nose reflexes were still acute.

At 5-50 the respirations were very irregular, never exceeding 8 per 10" with pauses of from 30" to 45" in length, and the movements were extremely feeble, being indeed confined to the anterior portion of the floor of the mouth. The cardiac impact was easily seen, the rate of the heart having fallen to 9 per 10". The frog remained in any position in which it was placed, the legs could be extended and a pinch of the toes caused general feeble struggles, but the animal was unable to regain the normal position. Electrical stimulation with the coil at 100 produced strong local movements only. When the stimulus was applied over the spinal cord with the coil at 110 mm- both legs were extended but not instantaneously. Stimulation of/

of one leg caused movements of the arms and body and other leg. when the coil was at 90mm.

At 6-17 only two slight movements of the floor of the mouth were observed in 60". The cardiac impact showed the heart to be beating at 9 per 10" and the blood was seen to be circulating very actively when the web was examined under the microscope.

At 6-30 one respiratory movement was seen during 2 minutes and at 6.45 there was an absence of all respiratory movement during five minutes. The eyes were completely closed, paralysis appeared to be complete but the reflexes were not entirely abolished; touching the eye caused a slight movement of the head and a pinchof the toes caused general feeble movements. Stimulation over the spinal cord with the coil at 100 mm. caused both legs to be extended.

At 7-30 the cardiac impacts were 8 per 10", and the blood stream very active in the web. No respiratory movements were visible.

At 9-45 p.m. the cardiac impacts were 6 per 10" easily seen, the praecordia pulsating as though the heart were dilated. The animal was completely paralysed. No reflexes could be elicited by the strongest stimulation (with the coil at zero). The circulation of the blood/

blood in the web was however quite active.

At 9-30 a.m. on the following day the heart was beating at the same rate, the frog lay motionless, the blood circulation was active, but no reflexes could be elicited. Stimulation of the gastrocnemius muscle through the skin with the coil at 160mm. caused contraction of the muscle.

At 6-30 p.m. the cardiac impact was 6 per 10" the blood flow was active, and the muscles contracted when stimulated with the coil at 150mm-

On the third day the frog remained in much the same condition, paralysis was complete, stimulation over the cord with the coil at zero causing local contractions of the muscles; without any there was no extension of the legs.

On the fourth day the heart beat was barely perceptible owing to a certain increase of fluid under the skin but it could be made out to be at 4 per 10". The flow of blood in the web was quite good though less rapid. Electrical stimulation gave much the same results as on the previous day.

On the fifth, sixth and seventh days the animal remained in the same condition, no sign of life was visible with the exception of an extremely minute dimpling/

dimpling of the skin over the region of the heart, the rate was 6 per 10", the circulation was active in the web and the results of electrical stimulation remained the same.

On the ninth day slight respiratory movements were seen again, the rate 5 per 10", the amount however merely a dimpling of the anterior portion of the floor of the mouth- The cardiac impact 5 per 10" were very easily seen and on this day the lymph hearts were again observed to be beating very feebly at the rate of 5 per 10". The reflexes were still absent- stimulation with the strongest electrical current caused local contractions only. With the coil at 149 mm. the muscles contracted.

On the eleventh day the respirations were still very faint 6 per 10" , the cardiac impacts 5 per 10" and the lymph hearts also 5 per 10". Stimulation over the cord with the coil at 105 caused the legs to be slowly extended .

On the twelfth day the respirations were stronger. reflexes were elicited by the electrical stimulation of one foot causing faint movements in other parts of the body. A slightly weaker stimulus was required to/

to produce extension of the legs than had previously been the case. A similar improvement in the various functions was observed on the thirteenth and fourteenth days, the respiratory movements becoming much more easily seen-

On the fifteenth day the respiratory movements were 21 per 10" the cardiac impact 6 per 10" very easily seen and heaving in character. The circulation was very active in the web. A slight pinch of the toe caused vigorous movements of the fore arms and head , but the animal was unable to regain the normal position if placed on its back. The muscular power of the lower eyelid was recovering the membrane being withdrawn from the upper half of the eye. Stimulating electrically one foot with the coil at 130 mm- the leg was drawn away from the electrodes, with the coil at 100 the animal jumped. After the electrical stimulation it was observed that reflexes could be elicited by tickling the nose or touching the eyes which before the stimulation were apparently absent. The membrane was withdrawn from the eyes and respiratory movements were much deeper.

On the sixteenth and seventeenth days the reflexes were/

were more acute. Stimulation over the cord at 200mm- caused the frog to gather up the previously extended legs and to jump away normally; pinching the toe gently also caused a jump. The respiratory movements, cardiac impact and movements of the lymph hearts were observed to be as previously noted.

The animal moved voluntarily and appeared to have almost completely recovered.

On the twentieth day it appeared more sluggish moving about somewhat slowly. it could however regain its position with very little difficulty if placed on its back.

On the twenty-first day it appeared more vigorous, jumped well, but could still be turned on its back after several trials.

On the morning of the twenty-second day it was found dead. When the thorax was opened the heart was seen to be arrested in diastole. The nerves and the muscles responded to electrical stimulation.

In this case there was therefore partial recovery, the respiration which had been paralysed in about four hours and had remained in abeyance for eight days had recovered. The heart which had been slow did not however regain its normal rapidity and/

and the reflexes which had been abolished had recovered to a very considerable extent though they could not be said to be entirely restored to the normal condition. The motor power did not however recover to the same extent.

In another frog to which a slightly larger dose had been administered the motor power was however completely restored.

In the adjoining table the general results of the experiments to determine the m.l.d. are given, and the chief points observed.

There was a considerable difference observed with regard to the increase of weight of frogs to which had been administered doses somewhat similar in amount according to the conditions under which they were kept. When the frog remained on a moistened slate a moderate amount of oedema in the subcutaneous tissues occurred. If on the other hand the animal was kept on wet filter paper the oedema was considerable in amount and on gently squeezing the frog a quantity of from 2 to 3 C.C. of clear fluid could be expressed daily from the bladder. In the frog(whose condition has been detailed above)the weight increased from 39 grammes to 46 grammes, attaining this weight on the eleventh day, after which the weight diminished steadily until the weight was 35 grammes.

DETERMINATION OF MINIMAL LETHAL DOSE BY SUBCUTANEOUS INJECTION FOR FROGS

No. of Expt.	dose in gr. per kilogr.	weight of frog in kilogrs.	Quantity of venom in grammes injected.	Amount of Solution.	Result.	Remarks.
1	0.0001	0.025	0.0000025	in 0.13 C.C.	Recovery	Very slightly affected.
2	0.0002	0.024	0.0000048	in 0.24 C.C.	Recovery	Resp. & Lymph hearts affected.
3	0.0003	0.030	0.000009	in 0.45 C.C.	Recovery	Impairment of motility-
4	0.0004	0.032	0.0000128	in 0.64 C.C.	Recovery	Reflexes impaired.
5	0.0005	0.035	0.0000175	in 0.875 C.C.	Recovery	Impairment longer
6	0.00077	0.035	0.000027	in 0.27 C.C.	Recovery	
7	0.001	0.034	0.000034	in 0-085 C.C.	Death	Resp- ceased about 48 hours- Died 8th day-
8	0.002	0.054	0.000108	in 0.277 C.C.	Death	Resp. ceased 24 hours heart ceased 10th day
9	0.0021	0.029	0.000062	in 0.62 C.C.	Recovery	Resp. ceased 30 hrs. abolished during 10 days.
10	0.0025	0.027	0.00007	in 0.7 C.C.	Death	Resp- ceased 16 hrs. heart ceased 7th day.
11	0.003	0.039	0.000117	in 0.29 C.C.	Partial Recovery Death.	Resp. ceased 7 hrs. suspended 8 days. Died 22nd. day.
12	0.0035	0.036	0.000126	in 0.31 C.C.	Recovery	Resp. ceased 7 hrs. Abolished 12 days. Well on 25th day.
13	0.004	0.040	0.00016	in 0.32 C.C.	Death	Resp. ceased 12 hrs. Death 7 days.
14	0.005	0.051	0.000255	in 0.25 C.C.	Death	Resp. ceased 4 hours. Heart ceased 12 days.
15	0.01	0.041	0.00041	in 0.41 C.C.	Death	Resp. ceased 3 hours. Heart ceased 9 days.
16	0.02	0.046	0.00092	in 0.31 C.C.	Death	Resp. ceased 5 hours. Heart ceased 4th day.
17	0.03	0.04	0.0012	in 0.04 C.C.	Death	Resp. ceased 3¼ hours heart ceased 3rd day.

Analysis of those experiments to determine the M. L. D.
for frogs in which death resulted.

Dose in grammes per kilogramme	Respiration	Heart	Lymph Hearts.	Reflexes	Weight	Rise of lower eyelid.
0.001	Ceased in 48 hours	Ceased 8th day.	Ceased in 8 hours.	Gradual impairment abolished on 8th day.	Inc. from 34 to 60 grammes.	Complete in five hours.
0.002	Inc. 24 to 28 in 2 hours. Ceased in 24 hours.	Ceased on 10th day.	Ceased in 4 hours. Reappeared 7th day.	Abolished 8th day	inc. from 57 to 62 grammes.	Complete in 8 hours.
0.0025	Ceased in 18 hours	Ceased 8th day.	Ceased 5 hours. Reappeared 5th Day.	Abolished 5th day.	Inc. from 27 to 39 grammes.	Complete in 8 hours.
0.003	Inc. 21to 24 in 1 hour Ceased 4 hours. Reappeared 9th day Ceased 22nd day.	Ceased 22nd day.	Ceased 45 minutes. Reappeared 9th day.	Abolished on 2nd day.	Inc- from 39 to 46 grammes.	Complete in 2½ hours.
0.004	Ceased 6 hours.	Ceased 4th day	Not observed.	Abolished on 3rd day.	Inc.	Complete in less than 6 hours.
0.005	Ceased 4 hours.	Ceased 12th day	Ceased 1½ hour No reappearance.	Abolished on 3rd day.	Inc. from 51 to 70 grammes.	Complete in 6 hours.
0.01	Ceased 3 hours.	Ceased 10th day.	Ceased less than 1½ hours.	Abolished between 6 and 18 hours.	Inc. from 41 to 55 grammes.	Complete in 1½ hours.
0.02	Ceased 4 hours 20 minutes.	Ceased 4th day.	Not observed.	Abolished between 6 and 24 hours.		Complete between 2 and 3 hours.
0.03	Ceased 4 hours	ceased between 48 and 60 hours	Not observed.	Abolished in 6 hours.		Complete in 4 hours.
0.1	Ceased ½ hours	ceased 3 hours	Not observed.			Complete in 30 minutes.

Analysis of those experiments to determine the m.l.d. for frogs in which recovery occurred.

Dose in grammes per kilogramme	Respiration	Heart	Lymph Hearts.	Reflexes	Weight	Rise of lower eyelid.
0.0001	Slight diminution in rate, strength and regularity.	Slight diminution in rate.	Invisible in 3 hours reappeared in 24 hours.	unaffected	unaffected	Unaffected.
0.0002	Dim. from 18 to 10 in 3 hours. 28 in 15 days	Slight diminution in rate.	Invisible in 3 hrs. reappeared in 15 hours.	unaffected	unaffected	unaffected.
0.0003	Dim. from 24 to 4 in 63 hours.	Dim- from 13 to 5 in 63 hours.	Invisible in 3 hrs- invisible for three days.	Impaired from 3rd to 8th day.	unaffected	unaffected.
0.0004	Inc. 20 to 22- Dim. 20 to 8 in 48 hours.	Dim. from 12 to 4 in 67 hours.	Invisible in 3 hrs. Invisible for 72 hours.	Impaired for 14 days.	Inc- from 32 to 40	$\frac{2}{3}$ covered on 4th day.
0.0005	Inc. from 15 to 23. Dim. 22 to 5 in 4 days.	Dim. from 9 to 5 in 3 days.	Invisible for 5 days.	Impaired on 3rd. day to 8th day.	Inc- 35 to 49.	$\frac{4}{5}$ on 4th day
0.00076	Inc. from 17 to 24. Dim. from 24 to 6 in 24 hours.	Dim. from 8 to 6 in three days.	Invisible for 6 days.	Marked impairment	Inc. 38 to 42.	Nearly complete. on 3rd day.
.002	Inc. from 17 to 22. in 3 hours, ceased in 30 hours and during ten days, reappeared on 12th day	Dim. from 10 to 5	Invisible in 3 hours, remained for 4 days.	Strongly affected in 5 hours. Impaired for 13 days	Inc. 29 to 38.	Complete on 3rd day.
.0035	Inc- from 21 to 26 ceased 7 hours- Begun 18 days.	Dim. from 10 to 5.	Disappeared in 25 minutes. reappeared on 12th day.	Abolished in 24 hours Reappeared 18th day.	Inc. 26 to 60	Complete 24 hours.

From the table of doses given to determine the minimal lethal dose it may be observed that though death occurred after the administration of 0.001 grammes per kilogramme yet that recovery occurred after 0.0035 grammes per kilogramme had been given and that therefore we must look upon the dose of 0.004 asthe certain minimal lethal dose for frogs. At the same time the recovery from 0.0035 gr. P.K. is probably exceptional.

The symptoms of poisoning which follow the administration of from 8 to 10 times the minimal lethal dose by subcutaneous injection do not differ very markedly, except in point of duration of the symptoms, thosedetailed in the frog poisoned with aboutthe minimal lethal dose. A male frog (R. esculenta) of weight 40 grammes was injected at 10.45 a.m. with 0.03 grammes per kilogramme. The respirations previous to the injection were 14 per 10". At 11-10 the respirations were 16 per 10" and at 11-30 had increased to 17 per 10". The frog was sitting up with the head raised and the eyes well open .

At 11- 55 the respirations had fallen to 14 per 10"/

per 10" and were irregular-both in volume and rhythm. At 12-30 p.m. the respirations were 3 in 30 seconds and had ceased at 1.18 p.m. At 2-55 p.m. the head was resting on the table, the eyes completely closed and the frog could be laid on its back. If touched however it regained the normal position. The cardiac impact was seen to be heaving in character and of the rate of 6 per 10". At 4.30 p.m. the animal remained in any position in which it was placed. At 6.12 p.m. there was complete paralysis, the cardiac impact was still 6 per 10" and the circulation as seen in the web under the microscope very active. The condition remained practically unchanged during the two following days with the exception that the circulation was somewhat more sluggish through the vessels in the web. On the fourth day the circulation ceased, no cardiac movement was perceptible, and, when the thorax was opened, the heart was seen to be motionless in extreme diastole, purple, distended with dark fluid blood and responding to no stimulation. The gastrocnemius muscle gave a minimal contraction when stimulated directly by an induced current with the secondary coil 280 mm. from the primary. It contracted also/

also when the exposed sciatic nerve was stimulated with the coil at 60 mm.

In all the experiments performed to determine the minimum lethality of the venom on frogs the respiration was observed to be primarily affected. After a transient stimulation the respiratory movements gradually became lessened in force and irregular in rhythm, interrupted by long pauses until they ceased completely. The heart was comparatively slightly affected with the smaller doses, but during the cessation of respiration the rapidity of the rate was diminished. When sufficiently large doses were administered which arrested the heart it was invariably found to be arrested in a condition of more or less extreme diastole. The lymph hearts ceased comparatively early in the great majority of the experiments and remained in abeyance for approximately the same length of time as did the respiratory movements. The reflexes were always markedly interfered with and motor paralysis was generally complete. During the period of paralysis, while the respiration was abolished, with the exception of that through the skin, the circulation was always fairly active through the vessels in the web which were generally slightly dilated. The blood which

was/

was examined in many of these experiments both microscopically and by allowing the serum to separate in capillary tubes never presented any symptoms of haemolysis. The coagulability was neither delayed nor hastened. We have therefore to deal with a venom whose action is mainly exerted on the nervous system. The action on the heart, blood-vessels and blood being very slight in amount.

ACTION ON MOTOR NERVE ENDS.

It was thought advisable to investigate the activity of the ends of the motor nerves at various stages during the action of the poisoning. The condition of the frogs so closely resembled animals under the influence of a poison like curara that it appeared to be advisable to investigate the activity of nerve ends.

Experiment 18.

Seven frogs were weighed and to each as nearly as possible at the same time there was administered a quantity of venom by subcutaneous injection equivalent to a dose of 0.003 grammes per kilogramme. The frogs were killed by destruction of the cerebrum at different periods during the poisoning, the sciatic nerves were then exposed and the contraction of/

of the gastrocnemius observed when the nerve was stimulated electrically by an interrupted current. The observations in each case were made while complete motor paralysis supervened. The following table gives the results obtained; The figures representing the distance beyween the secondary and primary coils in millimetres. The weakest current requisite to produce complete contraction of the gastrocnemius was noted.

DURATION OF POISONING.

	2nd day	4th day	5th day	6th day	7th day	8th day.	18th day
Frog 1	285						
" 2		275					
" 3			170				
" 4				50			
" 5					70		
" 6						190.	
" 7							260

The three last frogs were slightly recovering from the effects of the poison, respiratory movements being again perceptible. Thus during the whole period of poisoning the sciatic nerve though distinctly impaired/

impaired did not appear to lose entirely its power of responding to electrical stimulation.

The quantity of venom circulating in the blood when a dose of about the minimal lethal is administered may be estimated at about 1 part in between 20,000 and 30,000 parts if we estimate the blood of the frog to be about one tenth part of the body weight.

Experiments were performed to determine the effect of the poison on the nerve ends when the tissues were kept in contact with solutions of the venom rather stronger than this amount.

During these experiments stimulation of the muscles at various periods did not seem to give much evidence that the muscles themselves were markedly deteriorated. Experiments were also performed to test the activity of a solution of the venom kept in contact with the muscles and similar solutions on the involuntary muscle in the walls of the blood vessels.

Experiment 19.

The gastrocnemius musclesof a frog were esposed and separated with as long a portion of the sciatic nerve attached as it was possible to get. One of these nerve muscle preparations was placed in normal saline solution 0.65 per cent . The excitability of both muscle and nerve was determined by observing the minimal contraction of the muscle first when the nerve was stimulated and secondly when the muscle was stimulated. The stimuli were supplied by breaking the current obtained from a Du Bois Raymond induction coil. The figures referring to the distance in millimetres between the primary and secondary coils.

July 4th 1904. Laboratory temperature 62° F- Frog rana temporaria, weight 30 grammes. Control.

Time	Nerve	Muscle		Time	Nerve	Muscle
11.47	345	190		5.35	350	190
12.15	370	210		6.58	350	185
1.12	330	180		7.55	350	195
2.23	320	220		8.20	360	165
2.58	325	210		9.25 pm 5th July	350	170
4.4	350	210		9.20 am	250	175
4.50	335	195		4.7 pm 6th July.	205	150
5.15	340	190		6.35	195	130

The other nerve muscle preparation of the same frog was treated in the same manner, the excitability of the nerve and muscle respectively determined and the nerve then removed from a normal saline solution and placed in a solution of hamadryad venom in 0.65 per cent saline of the strength of 1 part in 10,000.

Time	Nerve	Muscle	Time	Nerve	Muscle.
4th July					
11.35	450	220	5.0	360	190
12.13	450	230	5.18	355	190
1.5	450	230	5.30	385	200
1.18	420	240	6.58	360	180
2-23	425	235	7.45	340	180
2.46	nerve placed in poison		9.25 pm	350	175
			5th July 9.20 am	290	155
3.15	400	190	4.7 pm	270	145
3.50	380	205	6.35 "	270	150
4.35	370	190			

In this experiment therefore the conductivity of the nerve was not appreciably affected in 7 hours and only partially diminished during its immersion in the poison for 28 hours.

Experiment 20.

On the same day another frog weighing 32 grammes was killed and the gastrocnemius muscle with its corresponding sciatic nerve prepared in the same manner. In this case however the muscle was immersed in the poison solution the nerve and the muscle being repeated stimulated as before described

Time	Nerve	Muscle	Time	Nerve	Muscle.
12 .0	390	220	7.15	0.	180
12.20	410	190	8.10	0.	190
1.14	370	210	9.25 pm 5th July	0.	170
2-23	365	220 muscle placed in poison.	9.20 am	"	145
			11.25	"	150
			12.22	"	125
3.10	350	215	2.20	"	110
3.35	95	180	3.10	"	100
4.8	60	210	4.10	"	100
4.45	0	200	5.15	"	90
5.18	0	190	6.35	"	0
5.40	0	185			

In this experiment the muscle ceased to contract when the nerve was stimulated after its immersion for 2 hours; The muscle itself did not lose its contractility entirely , however, until it had been immersed for 28 hours.

We may conclude from these experiments therefore that in this strength of solution the nerve ends are the first to be affected, the muscle is also affected after a considerably longer period of time while the nerve trunk is practically unaffected.

With a solution twice the strength employed in the last experiment, the effectsproduced are not very different.

Experiment 21. 6th July 1905.

Two muscle nerve preparations of Rana temporaria weighing 29 grammes were treated in the same manner. After the normals had been taken the nerve of the one was poisoned and the muscle of the other.

Time	Nerve	Muscle	Time	Nerve	Muscle
11.52	330	240	4.22	430	200
12.	380	220	5.	425	200
12.12	415	230	5.35	425	200
12.43	415	230	6.10	360	195
12.55	400	250	9.10	320	185
2.20	375	235	10.35	360	190
2.40	nerve placed in poison		7th July 8. am	285	160
			9.35	280	210
			noon	280	200
3.	390	225			
3.55	440	225			

The readings of the nerve muscle preparation in which the muscle was placed in the solution of 1 in 5,000 are as follows;-

Time	Nerve	Muscle	Time	Nerve	Muscle.
10.50	295	170	12.25	360	160
10.58	390	180	12.38	360	160
11.5	370	160	12.47	80	160
11.30	380	185	12.49	40	160
11.33		muscle placed in poison	12.50	30	
			12.53	0	160
11.42	360	180	12.54	0	160
11.53	350	175	4.30	"	170
12.3	330	200	7.30	"	150
12.15	360	170			

The effect of this solution is therefore at first to slightly increase the excitability of the nerve which is poisoned, but that directly the poison reaches the nerve ends it appears to destroy their power of conducting the nerve impulses.

Practically no effect was produced on the muscle in 7 hours.

In these experiments we find that though the motor nerve ends are decidedly affected when kept in prolonged contact with a strong solution of the poison, yet that in conditions of poisoning when a dose has been administered which is nearly ten times the minimal lethal dose, or 30 times the amount of a dose which can, but not with certainty, produce death, that the motor nerve ends maintain their excitability till after respiratory failure, arrest of the heart and paralysis of motor and reflex excitability.

Experiment 22.

To a frog weighing 30 grammes 3 milligrammes of hamadryad venom were injected subcutaneously, dissolved in .5 cc- of Ringers solution.

This is equivalent to 0.1 grammeper kilogramme The respirations ceased in half an hour, the reflexes were abolished and the heart was arrested in three hours. The animal being dead, the sciatic was exposed and stimulated and the gastrocnemius was observed to contract with the coil at 370 mm. The contraction of the gastrocnemius was not sufficient to move the leg, but the stimulus was obviously therefore/

therefore conveyed to the muscle. We may conclude that even with such an enormous dose the motor nerve ends are not paralysed before death occurs as is stated by Rogers. ( )

In order to determine the action on the skeletal muscle more definitely than can be done by stimulating the muscle while it is lying in a solution which necessitates applying electrodes to various portions of the muscle it was arranged that automatic tracings should be taken. Two crank myographs were arranged the one above the other. To the cork plate of each was secured a glass tube large enough to hold a muscle without its touching the glass. One end of the tube was closed by a cork through which passed a platinum hook. The levers of the myographs the weights to be lifted and the stimulations supplied to the two muscles were as nearly as possible similar. Indeed in the case of the stimulations the current from the secondary coil was taken through both the muscles. In order to stimulate with sufficient frequency and regularity in order to elicit a comparatively rapid result the current was broken in the primary circuit automatically by a clock once every five seconds-/

seconds. A key was placed in the circuit to shut off all stimuli when necessary.

Experiment 23.

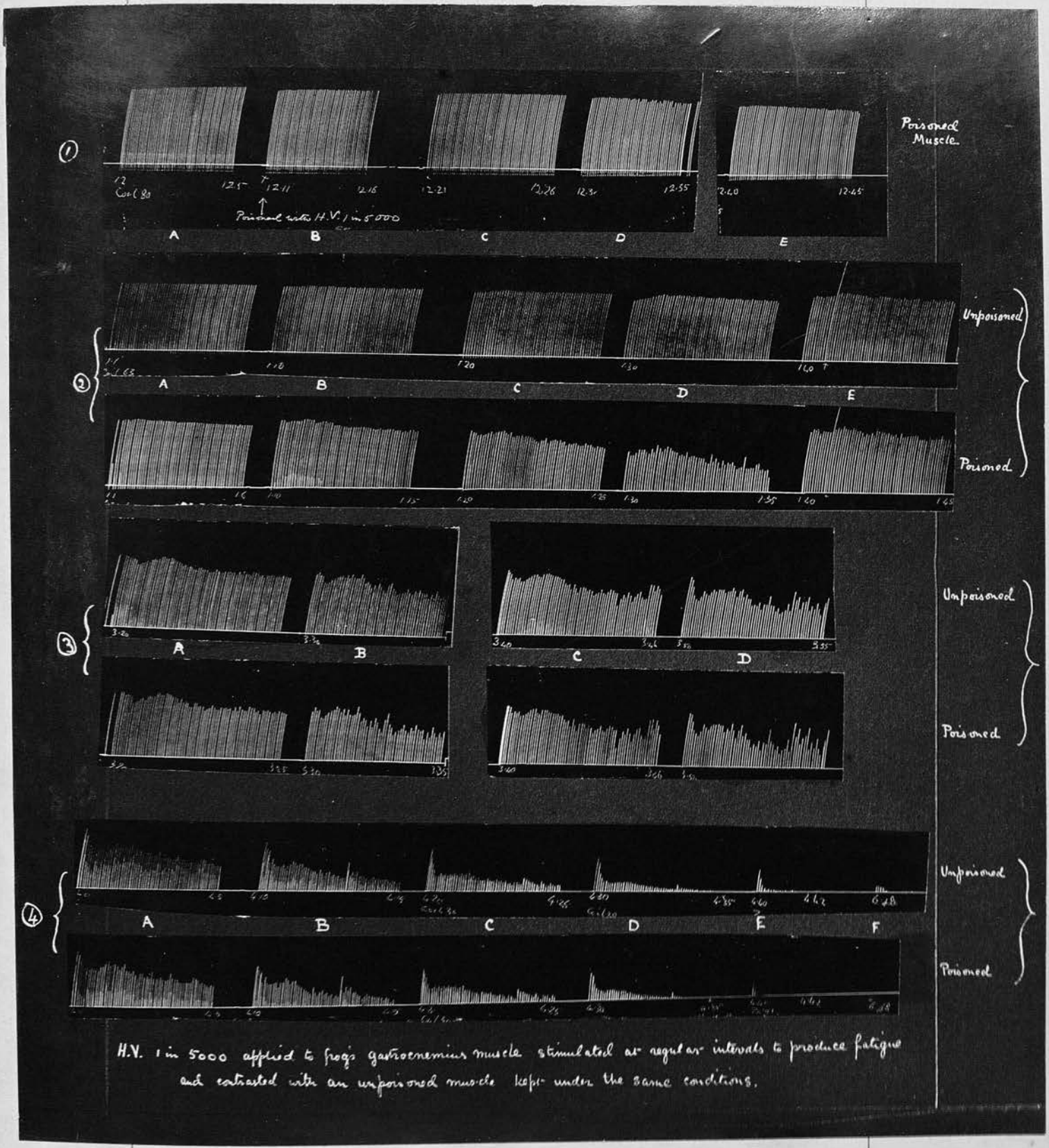
The gastrocnemii of a frog weighing 28 grammes were isolated from the animal having at the one end the tendo achillis and at the other a small portion of the femur. The muscle was held by means of this in the tube by the platinum hook. To the tendon there was attached a thread to be carried to the lever of the myograph. The tendon was also perforated by a fine platinum wire which served as the opposite electrode and the other end of which was connected with the platinum hook which held the second muscle. Simultaneous records could thus be taken of the two muscles under conditions as nearly as possible the same.

The shorter limb of the lever was 16 mm. in length and the longer limb 140mm. The weight of 5 grammes was suspended at the distance of 30 mm, from the fulcrum. The muscles were placed in Ringer's solution until a normal had been taken after which the Ringer's solution was removed from the lower tube and its place taken by a solution of the poison of the strength of 1 in 5,000.

On Plate 1 will be seen the modified fatigue curve of the two muscles under these conditions. The upper muscle was kept throughout the experiment in unchanged Ringer's Solution and is spoken of as the unpoisoned muscle. The lower muscle after having been stimulated for 5 minutes at intervals of 5 seconds with the secondary coil at 80mm. in order to obtain a record of its normal contractility was poisoned as has been described. The record of each twitch was taken on a slowly revolving cylinder, revolving at the rate of about 20 mm. in 5 minutes.

Fig I. A. shows the normal contractions at the beginning of the experiment before the poison was applied to the poisoned muscle. The normal contractions of the other,unpoisoned,muscle are not shown but were identical with those of the muscles as yet unpoisoned shown in this Figure. After the repeated stimulations a period of rest of 5 minutes duration followed during which time the poison was placed in the lower tube. At 12.11 the second series of stimulations was registered;(B) at 12.21 the third series (C); at 12.31 the fourth series (D) at 12.40 the fifth series (E) at 1.1 the strength of the stimulus/

PLATE 1.



H.V. 1 in 5000 applied to frog's gastrocnemius muscle stimulated at regular intervals to produce fatigue and contrasted with an unpoisoned muscle kept under the same conditions.

stimulus was increased the coil being moved to 63 mm. in order to elicit the maximum contraction without however causing a contraction to take plaee both at the making and breaking of the current. The upper line shows the contractions of the unpoisoned muscle the lower those of the poisoned.

Fig- 2 C. and D. at 1.20 and still more markedly at 1.30 the contractility of the poisoned muscle appeared to be lessened,that this was due however, to an interference in conduction rather than in power on contraction was seen by at 1.40 increasing the strength of stimulus to 55 mm.

Fig. 2 E. shows that the relative contractility of the two muscles is not very different. From 1.45 to 3.20 no stimuli were administered, but the muscle was during all this period in the poison. At 3.20 3.30, 3.40, 3.50 and 4.0'clock the muscles were again stimulated the strength of the stimulus being again increased at 4 o'clock to 40 mm.

Fig. 4. A. shows the curve obtained under these conditions. At 4.20 the stimulus was again increased to 30 mm. and at 4.30 to 20 mm. During all this period the muscles show evidences of fatigue not very markedly different the one from the other. The unpoisoned muscle however, being slightly the more efficient of the two.

At 4.30 the poisoned muscle ceased to contract a little sooner than the unpoisoned. At 4.40 when the stimulus was the maximum the coil being pushed down to zero the unpoisoned muscle was seen to contract when the poisoned had ceased and at 3.48 Fig 4. F. the unpoisoned muscle still gave a perceptible contraction while the poisoned gave none. This experiment shows therefore that the effect of the poison is to slightly hasten the normal death by fatigue of the muscle.

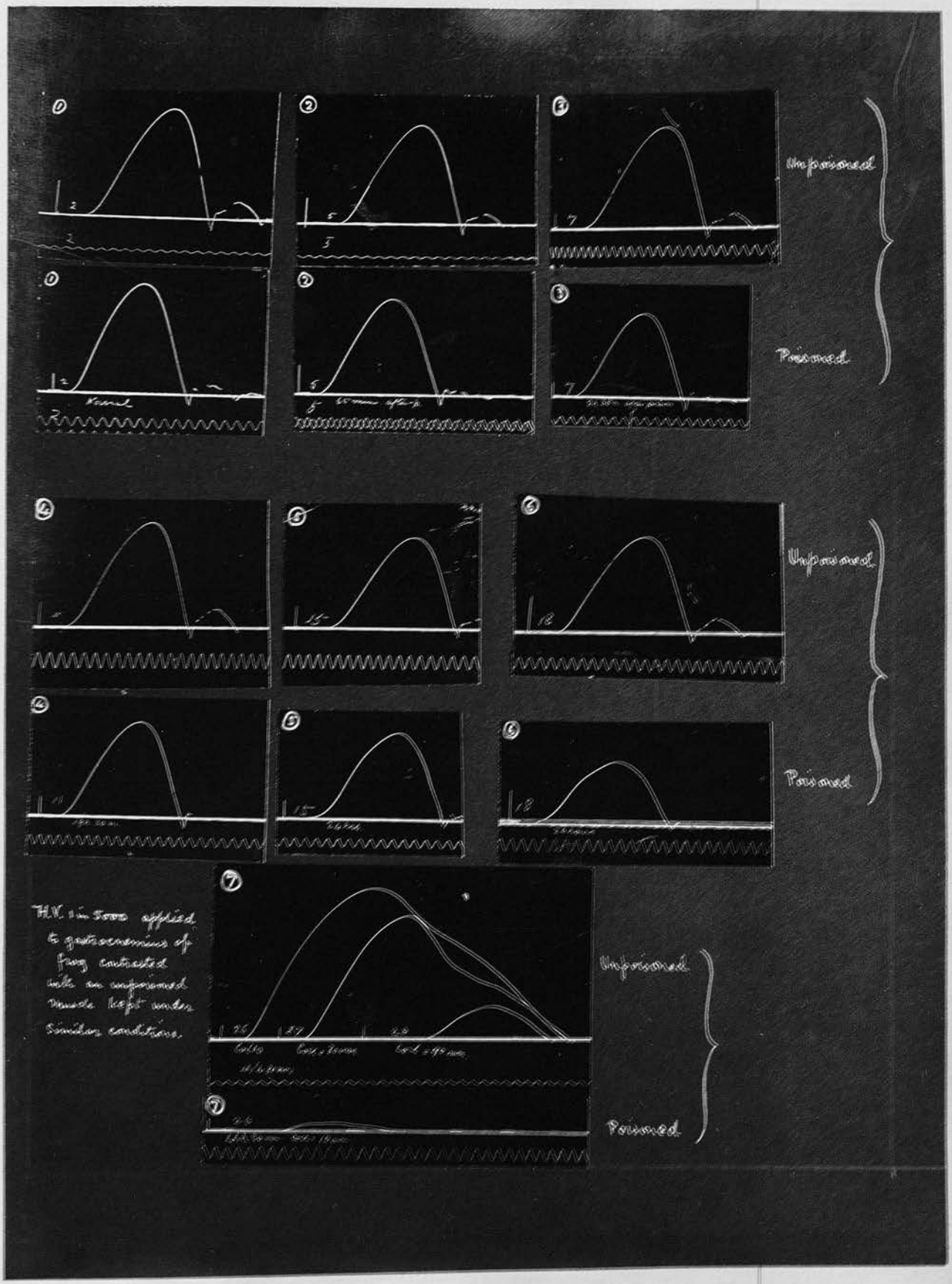
In the next experiment it was thought convenient to keep the muscle for a longer period of time in contact with the poison and to stimulate less frequently. The opportunity was taken therefore to record the individual contractions on a more rapidly revolving cylinder. The stimulations were produced by the breaking of the current by means of a key fixed to the axis of the revolving cylinder. The rate of the cylinder was about 200 mm. per second and the time was recorded by a tuning fork vibrating 100 times in the second.

Experiment 24- Plate II.

On 31st January 1906 the temperature being 58°F- at 2-30 p.m. the two gastrocnemii of Rana esculenta weighing 41 grammes were prepared. These were attached to the myographs in the same way as in the preceding experiment the levers and arrangement of weight was the same. The stimuli were provided by the induced current supplied from a bichromate cell and Du Bois Raymond coil.

At 3.22 the first stimulus was applied with the coil at 90 mm- The twitch recorded may be seen on Plate 2 Figure I. the upper of each pair of tracings being the unpoisoned muscle the lower the poisoned. At 4.10 the Ringer's fluid was removed from the lower tube and a solution of 1 in 5,000 hamadryad venom replaced it. At 5.15 that is 65 minutes after the poisoning a contraction was again registered. The poisoned muscle showing a very slight diminution in the height of the curve. Contractions were elicited at intervals as nearly as possible of an hour. Those recorded however being those at considerably greater intervals. At 10.40 a.m. on the following morning that is 18½ hours after

PLATE 2.


Unpoisoned
Poisoned
Unpoisoned
Poisoned
H.V. 1 in 5000 applied to gastrocnemius of frog curarised with an unpoisoned muscle kept under similar conditions.
Unpoisoned
Poisoned

after poisoning contractions were again registered and Figure IV. shows that produced by the same strength of stimulus as was employed up to this time At 11.40 that is 19½ hours after poisoning. At 6.10 26 hours with the same stimulus (Figure 6) the poisoned muscle contraction is distinctly less than that of the unpoisoned but the period of contraction practically the same. The muscle contracts rather less rapidly than does anormal muscle. At 9.15 p.m. the same stimulus produced very little response from the poisoned muscle, that is 29 hours 17 minutes after the poisoning had commenced. On the following morning (2nd Feb.) the poisoned muscle did not contract when stimulated with the coil at 60 mm. At 11.20 a.m. with the coil at 20 mm. the poisoned muscle gave but a small twitch and at 12.40 that is 44½ hours after poisoning with the coil at 10 mm. a barely perceptible contraction was produced . With this same strength of stimulus the unpoisoned muscle gave a very vigorous and prolonged contraction. With the coil at 30 and even at 90 mm. the unpoisoned muscle gave a good contraction taking into consideration the length of time it had been separated from any blood supply./

blood supply.

We may conclude therefore from this experiment that even in this strength of solution which represents approximately five times the minimal lethal dose that the action on muscle is barely perceptible in 24 hours and that the action is in the direction of simply hastening the normal death of the structure.

ACTION OF THE VENOM ON BLOOD VESSELS.

During the progress of poisoning though the circulation was not very strongly affected yet as there has been proved to be a slight action on the voluntary muscle it was thought necessary to investigate the action on involuntary muscle and to perfuse solutions of the venom throught the blood vessels of frogs, the nervous system of which had previously been destroyed. An additionale reason for these experiments being that in the case of the allied poisons of the cobras and of the krait distinct evidence of a constricting action had been obtained. Indeed in the case of the indian cobra Elliot had recorded an effect produced by extremely dilute solutions. He states that perfusion by a normal saline solution produces a dilating effect and he used therefore as his normal solution Ringer's fluid. I am not able to agree with his statement, my experience after having performed a very large number of such experiments being that when the conditions are carefully regulated with regard to the time ~~of performing the experiment~~ which is allowed to elapse between the destruction of the nervous system and the perfusion of fluid through the vessels and provided/

provided that the vessels are not in a state of active constriction at the beginning of the perfusion the effect is invariably one of slight constriction. In these experiments I have used as a normal solution a normal saline (0.65%) of pure sodium chloride in distilled water.

On Plate 3 I have recorded two experiments performed with the normal solution alone in which the solution was perfused for 140 minutes. In the first of these the flow was a somewhat rapid one in the second the flow was at the rate of about 2.5 c.c. per minute-at the commencement of the experiment and 1.5 c.c. at the end. This is the condition which most often obtains and is most satisfactory for eliciting either a constricting or a dilating action. The method of performing the experiments was to destroy the brain and spinal cord of a frog, to expose the heart by removal of the sternum, to cut the venae cavae across and to allow the blood to drain away. A period of at least half an hour (generally one hour) was allowed to elapse between the destruction of the nervous system and the exposure of the heart. An essential precaution is to most thoroughly destroy the whole of the spinal cord. Where this is too rapidly/

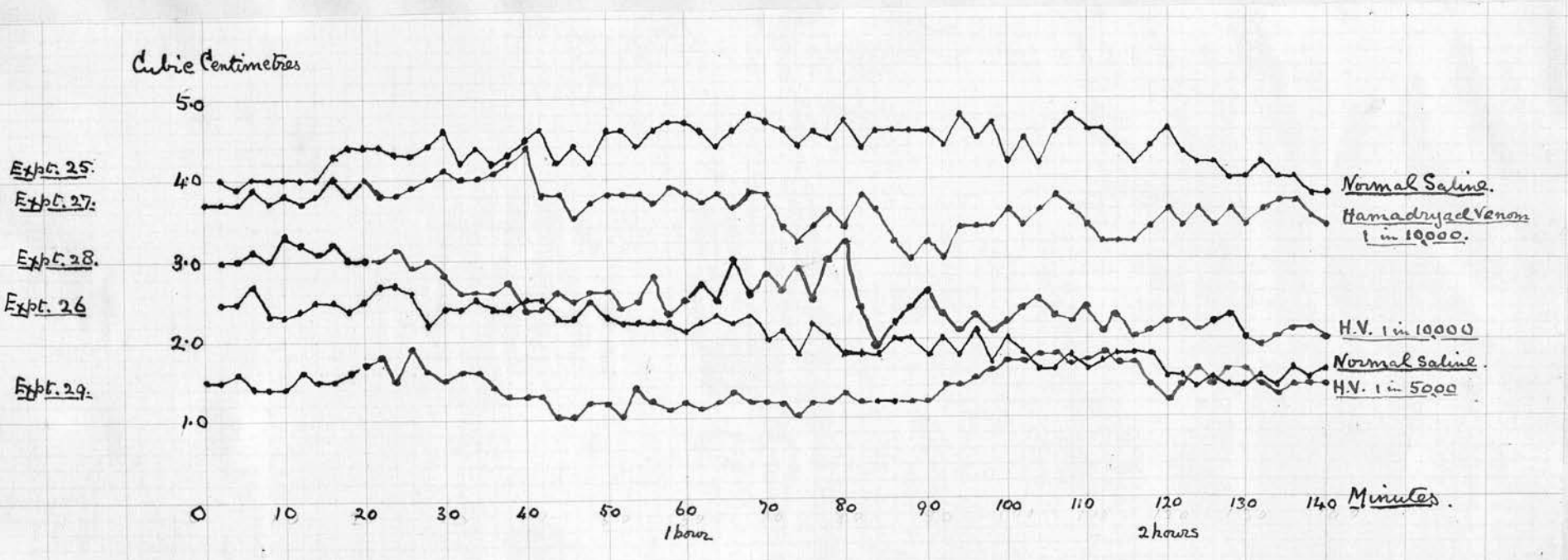


Plate III. Perfusion of frog's blood vessels with Normal Saline solution = black lines.
" " " " " Hamadryad venom " = red lines.
Flow registered in C.C. per minute.

PLATE III.

40 a.

rapidly done or perhaps incompletely discrepant results in the subsequent perfusion may be expected. A ligature having been passed under each aorta a cannula is inserted into the one and tied in position the other aorta being closed by its ligature. The fluids to be perfused are placed in Marriottes? flasks and these are connected with the cannula by means of glass tubes. The fluid escaping from the cut veins is collected in graduated measures the amount passing in each minute being accurately noted. The normal solution is allowed to perfuse until any blood remaining in the vessels is displaced. The reading of the flow is then commenced and a normal taken for 20 minutes.

Experiment 25.

On 27th of May 1904 a frog R. temporaria weighing 40 grammes the laboratory temperature being 65°F. was prepared as above described. The height of the fluid in the flasks was six and a half inches above the level of the heart. At 12.26 the flow was observed to be 4.2 c.c. per minute In 14 minutes, 32 minutes, 48 minutes, 74 minutes 92 minutes, 104 minutes, 116 minutes, and 132 minutes, the same flow was registered.

Certain intermediate readings showed slight oscillations but the general effect was to show that the calibre of the vessels remained practically unaltered .

Experiment 26.

In the second experiment No. 26 on 6th July 1904 on a frog weighing 27 grammes (laboratory temperature 68°F.) under precisely the same conditions with regard to apparatus and preparation the initial flow was 2.5 c.c. and remained at that level for 48 minutes. It fell to 2 c.c. in 70 minutes remained at that level till the 100th minute, fell to 1.5 c.c. in 120 minutes and remained at that level till the termination of the experiment.

The third experiment whose curve is plotted on the same plate (experiment 27) was one performed with hamadryad venom dissolved in the normal saline to give a strength of 1 in 10,000. As in this case the initial flow was the somewhat large one of 3.7 c.c. it should be contrasted with the first of the two normals. On the 13th July 1904 0.01 grammes of hamadryad venom was dissolved in 100 c.c. of 0.65% saline. The weight of the frog was 25 grammes and the temperature 72°F. The following are the readings observed.

HAMADRYAD VENOM 1 in 10,000.

Before Poison.

20	minutes	3.7 c.c.
18	"	3.7 "
16	"	3.7 "
14	"	3.9 "
12	"	3.7 "
10	"	3.8 "
8	"	3.7 "
6	"	3.8 "
4	"	4 "
2	"	3.8 "

Poison turned on

After 4. c.c.

2	minutes	3.8 "
4	"	3.8 "
6	"	3.9 "
8	"	4. "
10	"	4.1 "
12	"	4. "
14	"	4. "
16	"	4.1 "
18	"	4.2 "
20	"	4.4 "
22	"	3.8 "
24	"	3.8 "
26	"	3.5 "
28	"	3.7 "
30	"	3.8 "
32	"	3.8 "
34	"	3.8 "
36	"	3.7 "
38	"	3.9 "
40	"	3.8 "
42	"	3.7 "
44	"	3.8 "
46	"	3.6 "
48	"	3.8 "
50	"	3.8 "
52	"	3.4 "
54	"	3.2 "
56	"	3.4 "
58	"	3.6 "
60	"	3.4 "
62	minutes	3.8 c.c.
64	"	3.6 "
66	"	3.2 "
68	"	3.0 "
70	"	3.2 "
72	"	3. "
74	"	3.4 "
76	"	3.4 "
78	"	3.4 "
80	"	3.6 "
82	"	3.4 "
84	"	3.6 "
86	"	3.8 "
88	"	3.6 "
90	"	3.4 "
92	"	3.2 "
94	"	3.2 "
96	"	3.2 "
98	"	3.4 "
100	"	3.6 "
102	"	3.4 "
104	"	3.6 "
106	"	3.4 "
108	"	3.6 "
110	"	3.4 "
112	"	3.6 "
114	"	3.7 "
116	"	3.7 "
118	"	3.5 "
120	"	3.4 "

At the end of the exreriment the weight of the frog was 33 grammes showing a slight amount of oedema.

Experiment 28.

This experiment was performed under the same conditions excepting that the temperature of the room was considerably lower. The frog R. esculenta of weight 42 grammes was treated in exactly the same manner after a normal had been taken for 37 minutes a solution of hamadryad venom 1 in 10,000 was perfused for 2 hours. As the general result was almost identical with that of the last experiment it is unnecessary to detail the various readings. The flow commencing at 3 c.c.' per minute oscillated somewhat and at the end of two hours was passing at the rate of 2 c-c.' per minute. We see therefore that in this strength of solution the action of the venom as a vaso constrictor is little more if at all than that of the normal solution in which it is dissolved.

Experiment 29.

It was determined to perfuse the vessels with a solution of twice the strength of the foregoing but no greater result in the direction of constriction was observed.

The weight of the frog was 24 grammes and the laboratory temperature 66°F.

HAMADRYAD VENOM 1 in 5.000.

Before poison.

20 minutes	1.5	c.c.
18 "	1.5	"
16 "	1.6	"
14 "	1.4	"
12 "	1.4	"
10 "	1.4	"
8 "	1.6	"
6 "	1.5	"
4 "	1.5	"
2 "	1.6	"

Poison turned on

After	1.7	c.c.
2 minutes	1.7	"
4 "	1.5	"
6 "	1.9	"
8 "	1.6	"
10 "	1.5	"
12 "	1.6	"
14 "	1.6	"
16 "	1.4	"
18 "	1.3	"
20 "	1.3	"
22 "	1.3	"
24 "	1.0	"
26 "	1.0	"
28 "	1.2	"
30 "	1.2	"
32 "	1.0	"
34 "	1.4	"
36 "	1.2	"
38 "	1.1	"
40 "	1.2	"
42 "	1.1	"
44 "	1.0	"
46 "	1.3	"
48 "	1.2	"
50 "	1.2	"
52 "	1.2	"
54 "	1.0	"
56 "	1.2	"
58 "	1.2	"
60 "	1.4	"
62 minutes	1.2	c.c.

Saline.

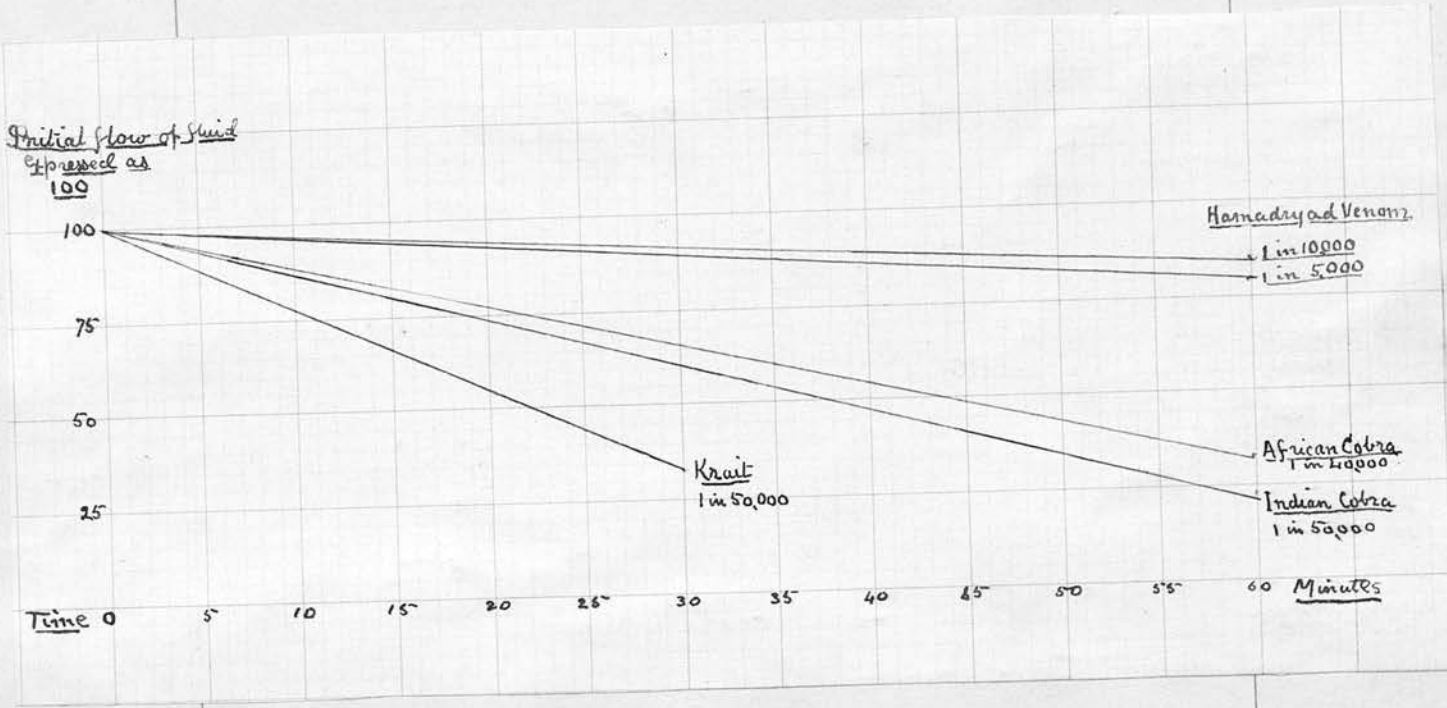
64 minutes	1.2	"
66 "	1.2	"

Venom resumed.

68 minutes	1.2	"
70 "	1.2	"
72 "	1.4	"
74 "	1.4	"
76 "	1.5	"
78 "	1.6	"
80 "	1.7	"
82 "	1.7	"
84 "	1.8	"
86 "	1.8	"
88 "	1.7	"
90 "	1.7	"
92 "	1.8	"
94 "	1.7	"
96 "	1.7	"
98 "	1.4	"
100 "	1.2	"
102 "	1.4	"
104 "	1.6	"
106 "	1.4	"
108 "	1.6	"
110 "	1.6	"
112 "	1.4	"
114 "	1.3	"
116 "	1.4	"
118 "	1.4	"
120 "	1.4	"

The weight of the frog at the end of the experiment was 30 grammes.

From these experiments we may conclude that the hamadryad venom in any quantity in which it is likely to be present in the blood of an individual bitten by the serpent is not likely to produce any effect by a constricting action on the walls of the vessels. In the accompanying diagram I have illustrated the difference between the effects produced by the hamadryad venom in the strength of 1 in 5,000, 1 in 10,000 and 1 in 50,000 and from the effects produced by the African Cobra in a strength of 1 in 40,000 the Indian Cobra 1 in 50,000 and the Krait 1 in 50,000. From this diagram we can easily see that the constriction produced by the African and the Indian Cobra venoms are practically identical and that in a similar strength the poison of the Krait constricts vessels rather more rapidly. At the same time it must be observed that weaker solutions of both African and Indian cobra venom produce a constricting effect which was entirely absent in the specimen of krait venom in similar strength.



Comparative effect of the four poisons on the walls of the blood vessels of the frog.

ACTION ON REFLEX CONDUCTIVITY OF THE CORD.

In the experiments in which the minimal lethal dose was determined the reflexes were observed to be abolished at intervals of time proportional to the size of the dose administered. In some of the experiments in which the sciatic nerve was exposed and stimulated after the reflexes had disappeared it was found to be still active. It was advisable to investigate specially this point taking care that the poison did not affect the motor ends by ligaturing the vessels of one leg.

Experiment 30.

On 21st May 1905 with a temperature 54°F. a male frog R. temporaria weighing 23.5 grammes was killed at 11.30 a.m. by destroying the brain. The cord was cut through behind the condyles. At 11.48 the frog was suspended by a hook through the chain and the reflex activity determined by dipping the feet alternately into a solution of dilute sulphuric acid of the strength of 1 in 1000. The time which elapsed before the foot was withdrawn was noted. The acid was instantly and completely washed away from the skin. The foot was immersed to/

to as nearly as possible the same extent on each occasion. At 11.50 each leg was withdrawn in 7 seconds. At 11.59 the withdrawal took place in 5 seconds. This remained constant till 12.30 when the vessels of the left leg in the thigh were exposed and occluded by a ligature. The web was examined after the operation and the circulation found to be conpletely arrested in the leg which had been ligatured but quite active in the uninjured leg. At 1 o'clock the reflexes were tested by means of the acid. At 1.5 the left leg was withdrawn in 6 seconds, the right in 5 seconds. At 3.15 both legs were withdrawn in 4 seconds. At 3.30 the left leg was withdrawn in 4 seconds, the right in 3 seconds. At 3.48 0.235 C.C. of normal saline solution containing 0.001175 grammes of hamadryad venom were injected subcutaneously into the dorsal lymph sac. This dose was equivalent to 0.05 grammes per kilogramme an amount which might be expected to affect the reflexes in 4 or 5 hours.

The effects observed will best be seen by the following table.

Time	Left Leg protected.	Right Leg. unprotected.
12.15	5 seconds	5 seconds.
12.30	Vessels ligatured	
3.15	4 seconds	4 seconds.
3.48	Poison injected subcutaneously.	
3.52	2 seconds	3 seconds.
4.	2 "	3 "
4.30	3 "	4 "
5.	3 "	4 "
5.30	3 "	4 "
6.	4 "	5 "
6.10	4 "	6 "
6.20	5 "	7 "
6.40	5 "	7 "
6.50	7 "	9 "
7.	8 "	10 "
7.10	5 jerked 12 withdrawn.	8 jerked 15 withdrawn.
7.20	5 jerked 14 withdrawn	5 jerked. 18 withdrawn.
7.30	5 jerked 15 withdrawn.	8 jerked 18 withdrawn.
7.40	5 jerked. 19 withdrawn.	8 jerked. 18 almost withdrawn.
7.50	5 jerked. 40 almost withdrawn	10 jerked. not withdrawn.
8.30	8 jerked 16 withdrawn	13 jerked not withdrawn.
8.55	9 jerked. not withdrawn	13 jerked not withdrawn.
next day 12 noon.	No response	No response.

At noon

the sciatic nerves were both exposed and stimulated the right gave no response with the exception of a slight contraction of the thigh muscles with the coil at zero. The sciatic of the protected leg caused the gastrocnemius to contract when stimulated with the coil at 330.

In this experiment we see that the reflexes diminished very nearly equally on both sides up till 7.40 p.m. that is about 4 hours after the injection After this period there was a loss of power to withdraw the foot to which the poison had had unrestricted access. 5 hours after poisoning neither foot was withdrawn though the sciatic nerve on the protected side had its ends intact. We see therefore that the abolition of the reflexes is due primarily to an action on the cord. The nerve ends are poisoned subsequently.

Experiment 31.

On the 1st December 1905 at 10 a.m. the brain of a frog R. esculenta weight 40 grammes was destroyed. The laboratory temperature was 58°F. At 11.20 all the vessels in the right thigh were exposed and ligatured. The circulation in the feet was examined and was found to be arrested in the right foot, active in theleft. The cardiac impacts were six per 10 seconds, the lymph hearts 5 per 10 seconds. At 11.45 when either foot was stimulated by an induced current with the coil at 130 both legs were moved. At 4.30 p.m. the same effects were observed/

observed. At 4.35 there was injected subcutaneously 1 mm.^{3} of hamadryad venom dissolved in ½ c.c. Ringer's solution. This was equivalent to about 6 times the minimal lethal dose. At 5 o'clock the animal lay with the legs normally drawn up, the cardiac impacts being 7 per 10 seconds and the lymph hearts 5 per 10 seconds. At 5.15 when either leg was stimulated both legs moved vigorously. Both legs were also moved when the stimulus was applied to other portions of the body. At 5.20 the lymph hearts had ceased. At 6.28 the cardiac impacts were 7 per 10 seconds and the circulation was still active in the left foot. Stimulation of the foot however caused only local contractions of the muscles of the leg of the side to which the stimulus was applied. No movement of the opposite leg was produced even when the stimulus was sufficient to throw the muscles of the leg to which the electrodes were applied into a condition of tetanus. That the muscles and nerves of both legs were still intact was proved by stimulating directly over the thoracic portion of the cord with the coil at 100 causing both legs to be rapidly and vigorously extended. At 7.7 no cross/

cross reflexes could be elicited even with the coil at zero; and to this stage of the experiment no difference in the behaviour of the two posterior limbs had been observed. When however the leg to which the poison had had access was stimulated its movements were slightly feebler than those produced by similar stimulus applied to the protected leg showing that at this stage the motor nerve ends were also becoming affected. When a strong stimulus was sent through the cord by stimulating directly over the cord with the coil at 30 mm. (a much stronger stimulus than had been required 39 minutes previously)both legs were extended but the left (poisoned) more slowly than the right (protected). At 9.35 p.m. stimulation over the cord with the coil at 40 caused extension of the right protected leg but no movement of the poisoned left leg showing that the motor ends in the poisoned leg were now paralysed and also that the cord had not altogether lost its power to conduct impulses which however required to be powerful. At 10.15 p.m. the sciatic nerves were exposed and stimulated with the result that the nerve to which the poison had access very faintly responded when strongly stimulated there being but a dimpling of the gastrocnemius when the coil was at 40 mm.

On the other hand when the protected nerve was stimulated with the coil at 200 a strong contraction of the gastrocnemius and of the toes was observed. On the following morning at 11.30 a.m. stimulation over the cord at zero caused no extension of the legs, nor was any effect beyond a slight dimpling of the thigh muscles observed when the poisoned sciatic was stimulated with the coil at zero. The muscles of the protected leg contracted when the sciatic was stimulated with the coil at 50 MM. so that the poison was probably reaching the nerve ends on this side also, perhaps by diffusion. At 1.20 p.m. the heart was still beating distended, dark and not emptying completely.

ACTION ON THE HEART-

In view of the pronounced actionof cobra venom on the heart in small quantities causing systolic arrest it was surprising to find the poison of an allied serpent apparently causing the heart not only to be affected very slightly but producing arrest in extreme diastole even when large doses were administered. The only occasions whan any approach to an arrest in systole were observed were those when an enormous dose was administered (30 times the minimal lethal dose or more); on these occasions the heart after its diastolic arrest passed rapidly into a condition of rigor.

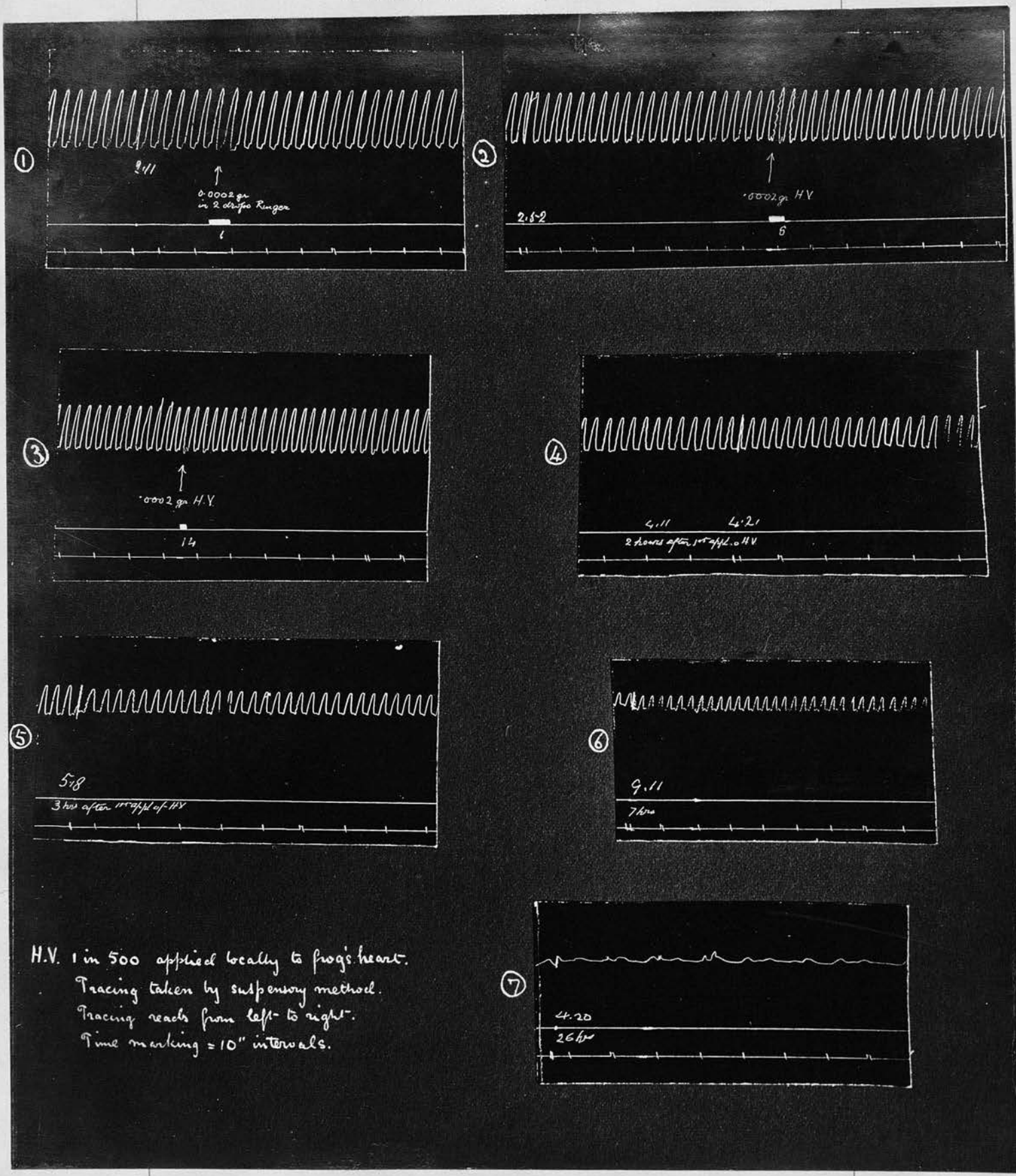
Experiment 32.

PLATE IV- A solution of hamadryad venom of the strength of 1 in 500 was applied to the exposed heart of a pithed frog. The heart was attached by a hook to a writing lever in order that the contraction might be registered on a revolving cylinder. 0.003 grammes of the venom dissolved in 1.5 c.c. of Ringers solution were applied to the surface of the heart in drops by means of a hypodermic syringe, at regular intervals of five minutes during a period of two hours. At the end of this time the whole had been/

been applied. Each application approximated to 0.0002 grammes. The modifications of the heart beat (see next page) may be seen on Plate IV- ∧where a selection has been made from a continuous tracing. When the heart contracted the lever described an up stroke so that extreme systole occurred at the apex of the curve. During the poisoning the heart slowly but gradually diminished with regard to its contractility. The shortening of the curve was seen to take place at the systolic portion. The action on the heart was ~~therefore~~ very slow and slight in amount complete arrest not having occurred in 28 hours though the heart had been beating with very little if any blood passing through it for 12 or 15 hours. The rate throughout was very little altered though a slight increase in rapidity was observed as the contractions grew weaker.

The action being therefore a very slight one and the amount of venom actually remaining in contact with the heart in such an experiment very doubtful a series of experiments was performed in which the poison was kept in contact with the heart. This was attained by perfusing through the isolated ventricle solutions of the venom of different strengths, the method in each case being the same. Schafer's Plethysmograph /

PLATE 4.



H.V. 1 in 500 applied locally to frog's heart.
Tracing taken by suspensory method.
Tracing reads from left to right.
Time marking = 10" intervals.

Plethysmograph was used to record the heart beats. The bulb of the instrument in which the heart was suspended contained Ringer's solution (Rusch's modification).(14) The metal cylinder in which the piston travelled was filled with oil. The reservoirs were filled with defibrinated ox blood and Ringer's solution, one part of the former to 2 parts of the latter. The mixture was thoroughly aerated and filtered and one portion was used as a solvent of the poison. The frog hearts were dissected with the greatest care to avoid injury, and great precautions observed to prevent the introduction of air into the heart during the manipulation. During the performance of the perfusion the air traps introduced by Elliot and Burnet (16) proved of the greatest service in preventing the admission of air to the heart. This advantage is especially appreciated when the duration of the experiment is for several hours.

Elliot observed (3) that with solutions of cobra venom in greater strength than 1 in 100000 the duration of the experiment is to be reckoned in seconds or at the outside in minutes. My experience with hamadryad venom is that with solutions more dilute/

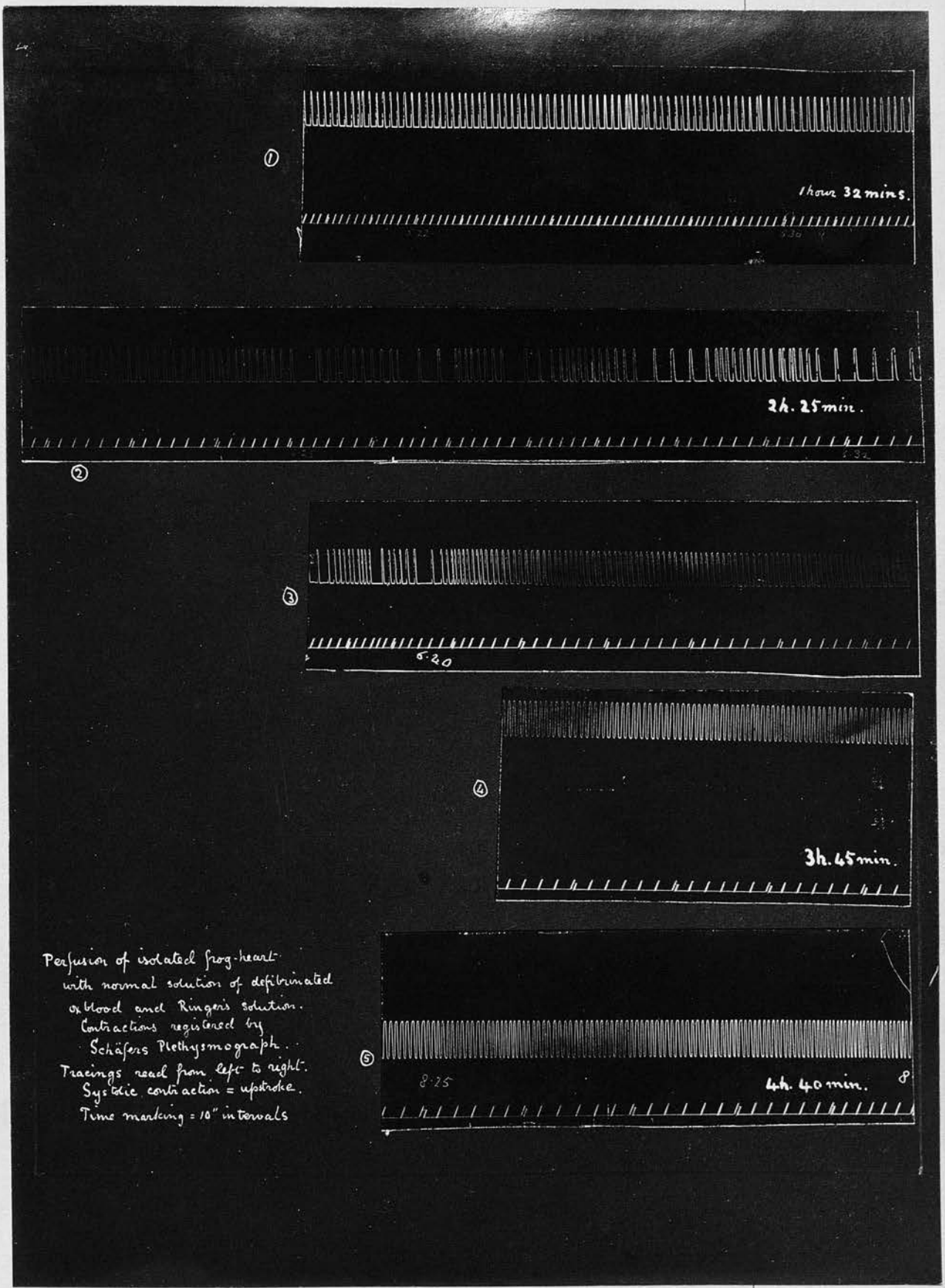
dilute than 1 in 1000 the duration of the experiment may be reckoned in hours. It was important therefore to have a control experiment under the conditions in which the poison was to be applied, but without this factor, in order to see the difference which the poison in the more dilute solutions might produce on the action of the heart.

Experiment 33- PLATE V-

27th February 1905. R. Esculenta 40 grammes. Laboratory temperature 62°F.

The heart was prepared at 3 p.m. and the cannula was tied into the ventricle including as little as possible of the auricle but avoiding the inclusion of any portion of the ventricle. The height of the fluid therein between 7⅓ and 10⅓ inches. At 3.50 the record was taken and continued thereafter uninterruptedly for five hours. Portions of the tracings at different times are shown on Plate V. A difficulty experienced during such lengthy perfusion experiments is due to the alteration of the position of the piston which tends to travel along the cylinder either towards the heart or away from it. This being possibly due to the inertia of the piston we might expect to find that the diastolic expansion of the heart aided by the pressure/

PLATE 5.



Perfusion of isolated frog-heart with normal solution of defibrinated ox blood and Ringer's solution. Contractions registered by Schäfers Plethysmograph. Tracings read from left to right. Systolic contraction = upstroke. Time marking = 10" intervals

pressure of the fluid within it which is in direct connection with that in the reservoir caused the piston to travel inperceptibly further at each stroke away from the heart than it returned. If a substance were used which weakened the heart and increased its expansion as well as diminished its contraction this tendency would be exaggerated. If on the other hand a sunstance were used which increased the systolic contraction rendering it more powerful at the same time causing a more gradual diastolic expansion the piston would tend to travel towards the heart. Both these phenomena have been observed, during the experiments with hamadryad venom on the one hand in which the piston invariably travelled away from the heart and on the other with solutions of strophanthin in which the piston travelled towards the heart. Whether this is the explanation or not, at various times during these experiments the piston required to be adjusted.

In this particular experiment where the fluid perfused was the nutrient solution without any admixture of poison and in which therefore there was no tendency to increased contraction of the heart the piston twice reached the extremity of the cylinder and was replaced.

The heart remained beating regularly both as to rate and amplitude of contraction and dilatation for more than two hours. Fig. I. shows the character of the tracing after 1½ hours of perfusion. After 2 hours and 20 minutes the diastolic pauses became slightly longer and the rate irregular. (Fig. 2) which condition was found to be due to the blood not being sufficiently aerated and the production therefore of fatigue in the heart.

When the blood solution was oxygenated by vigorously shaking it with air before returning it to the reservoir for renewed perfusion, this effect passed off and regularity was again established. The condition was unchanged and the heart still beat regularly with no loss of amplitude when the experiment was stopped after five hours.

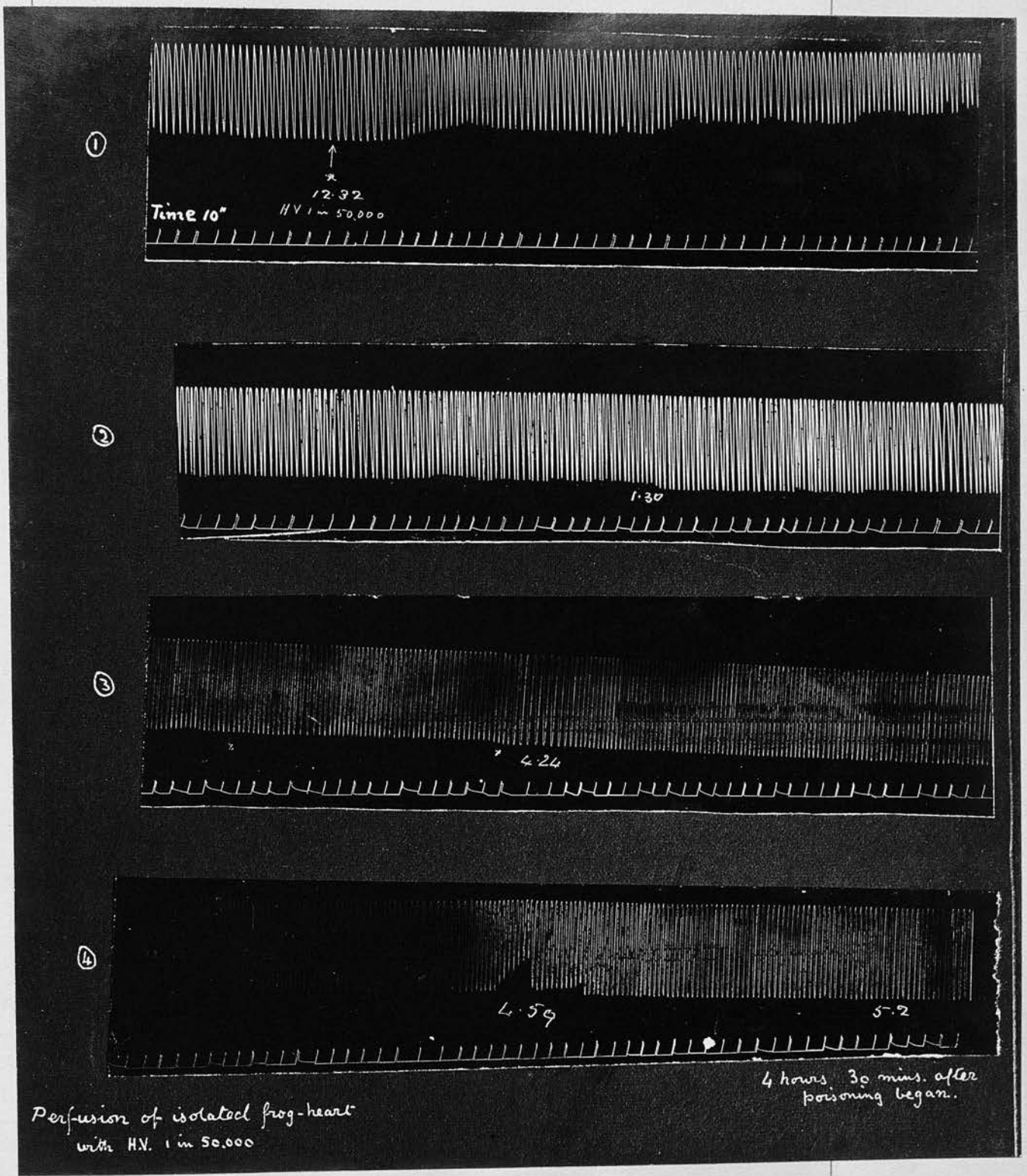
(page 63)

Experiment 34. Plate VI. ^ 16th January 1906. R. Esculenta weight 41 grammes. Temp. 57°F. The conditions were similar to those in the last experiment, with the exception that one reservoir was filled with a solution of the venom. 0.0003 grammes were dissolved in 150 c,c. of the nutrient mixture giving a strength on 1 in 50,000. When this had passed through the heart, it was collected, thoroughly aerated and returned to the reservoir to pass through again. At noon the record was begun and at 12.32 the poison was turned on. The rate of the heart was 17 per 60" and the amplitude of excursus 16 mm. With the exception of a diminution in amplitude to 10 mm. which took place in 5 minutes and which was accompanied by an increase of rate to 20 per 60" no distinct change took place for five hours. Fig. I shows the tracing at the time of poisoning with the subsequent irregularity. Fig. 2 at 1.30 p.m. shows the restoration/

EXPERIMENT 345

minutes before poison	rate per 60"	Amplitude of excursus.
30	16	8.5 millimeters
24	21	8. "
9	20	7. "
2	22	7. "
poisoning	21	7. "
14 minutes after	21	7. "
20	20	7. "
30	20	6 & 5 irregular
50	20	6 & 5 "
57	21	5 millimeters.
1 hour 18 minutes	22	6 "
1 hour 31 minutes	28	4 "
2 hrs- 32 "	32	3 "
2 hrs- 22 "	32	4 "
3 hrs.	31	3 "
3 hrs. 29 minutes	37	3 "
3 hrs. 40 minutes	diastolic pause for 30"	
3 hrs. 45 minutes	8	3.5 "
4 hrs.	10	3.5 "
4 hrs- 12 minutes	6	3 "

PLATE 6.



Perfusion of isolated frog-heart with H.V. 1 in 50,000

4 hours 30 mins. after poisoning began.

the restoration to regularity, the amplitude being the same as it was at first and the rate 24 per 60" Fig. 3 at 4.24 p.m. shows the amplitude unaltered and the rate 18 per 60" Fig-4 at 5.2 p.m. i.e. 4½ hours after poisoning shows the rate 21 per 60" and the amplitude 16 mm.

An important point observed during this and the preceeding experiment was the absence of any colouration of the Ringer's fluid in the bulb of the Plethysmograph, thus showing that there had been no passage of blood through the walls of the heart at any period of the experiment its termination. We may conclude from this experiment that in the dilution of 1 in 50,000 the action on the heart is infinitesimal.

p.p. 66 & 67.

Experiment 35. Plates VII and VIII. 17th January 1906. In this experiment 0.006 grammes of H.V. were dissolved in 150 c.c. of the fluid giving a strength of 1 in 25,000 R. esculenta 42 grammes temperature 58°F. At noon the record was begun and the poison was turned on at 12.48 (Fig-2). Fig. I shows the normal. There was a steady diminution of amplitude of excursus with a constant travel of the piston away from the heart. The heart emptied less and less during the whole experiment/

experiment. The first irregularity was seen at 1.32 (see Fig-3) which lasted for five minutes At 2.19 (Fig-4) the piston had reached the extremity of the cylinder and required readjustment. The force of the heart diminished gradually and the heart became more and more dilated, the systolic contraction becoming less and less complete (Fig- 5,6,& 7). The further progress in the same direction is seen in Figs- 8, 9, & 10 the piston having been again adjusted. At 4.28 long pauses in extreme diastole occurred lasting in some cases 30" the heart being much distended and globular. When the beats were resumed they were confined to a portion only of the base of the ventricle. This condition persisted till the experiment was stopped at 5.5 i.e. that is 4 hours 17 minutes after the commencement of the poisoning. The Ringer's fluid at the end of the experiment was quite clear and colourless. The following table shows the gradual diminution in amplitude with the accompanying increase in rate.

PLATE 7.

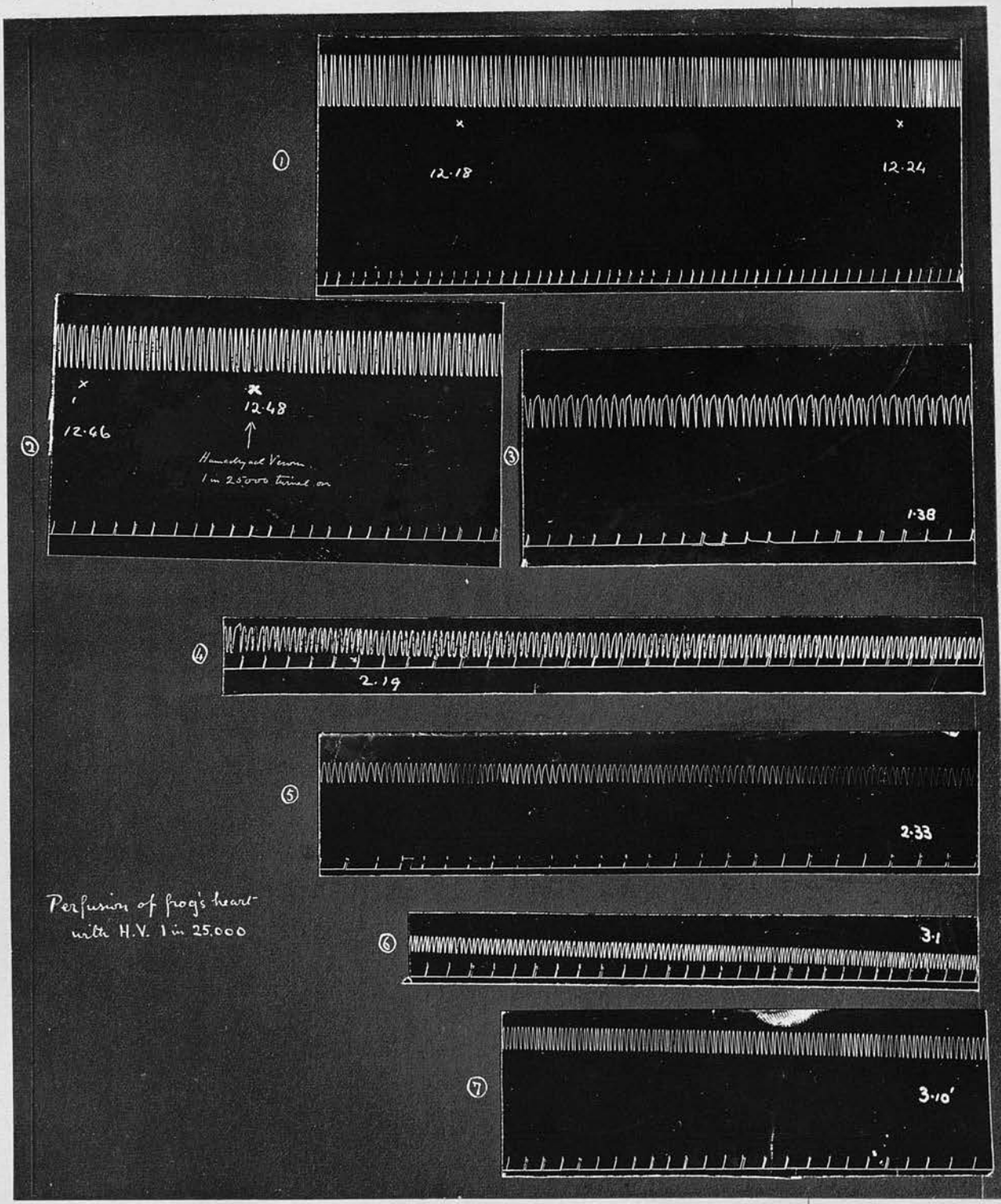
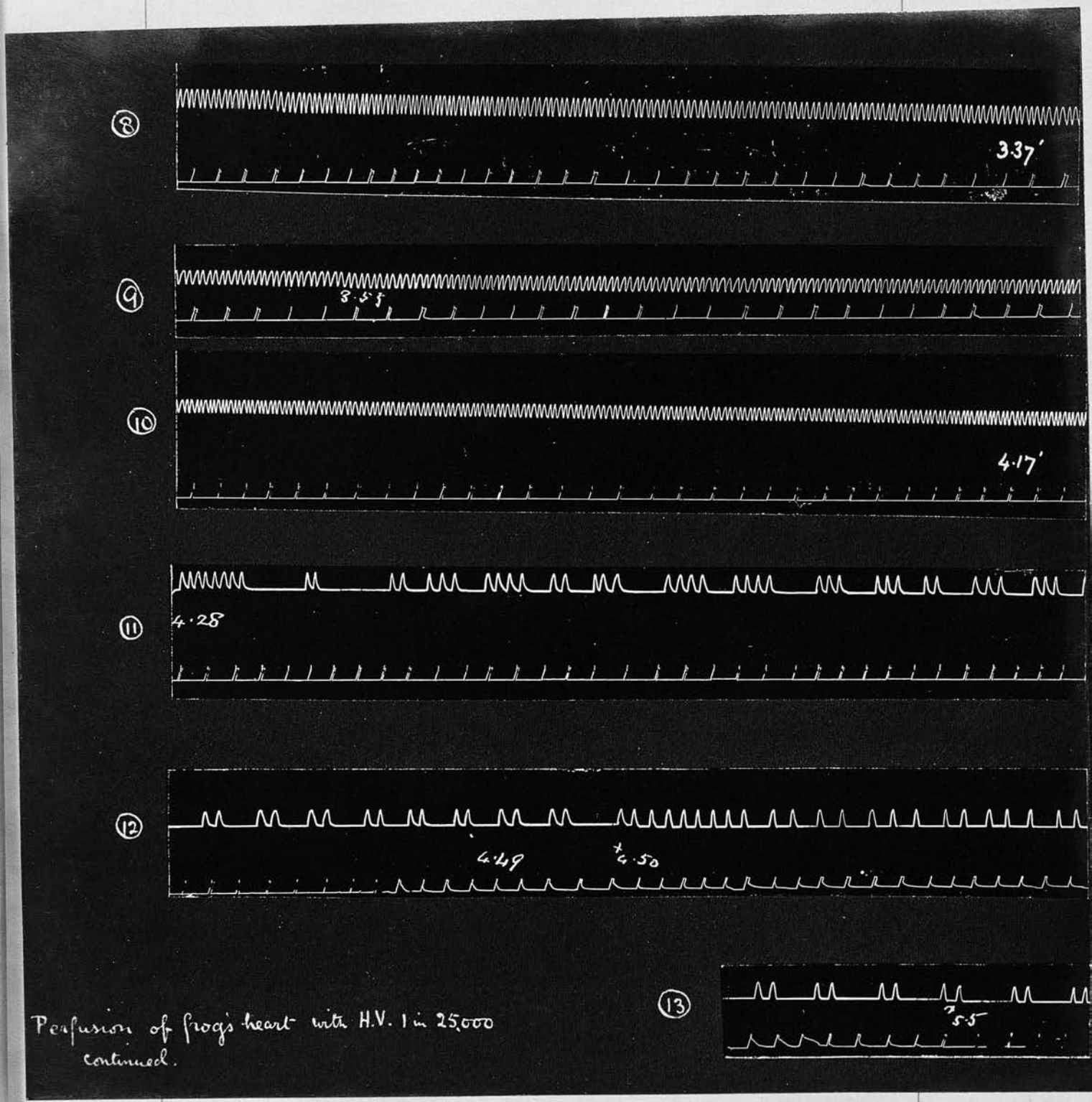

①
12·18
12·24
②
12·46
12·48
Homedyal Vernon
1 in 25000 tried on
③
1·38
④
2·19
⑤
2·33
Perfusion of frog's heart
with H.V. 1 in 25,000
⑥
3·1
⑦
3·10'

PLATE 8.



Perfusion of frog's heart with H.V. 1 in 25,000
continued.

EXPERIMENT 36

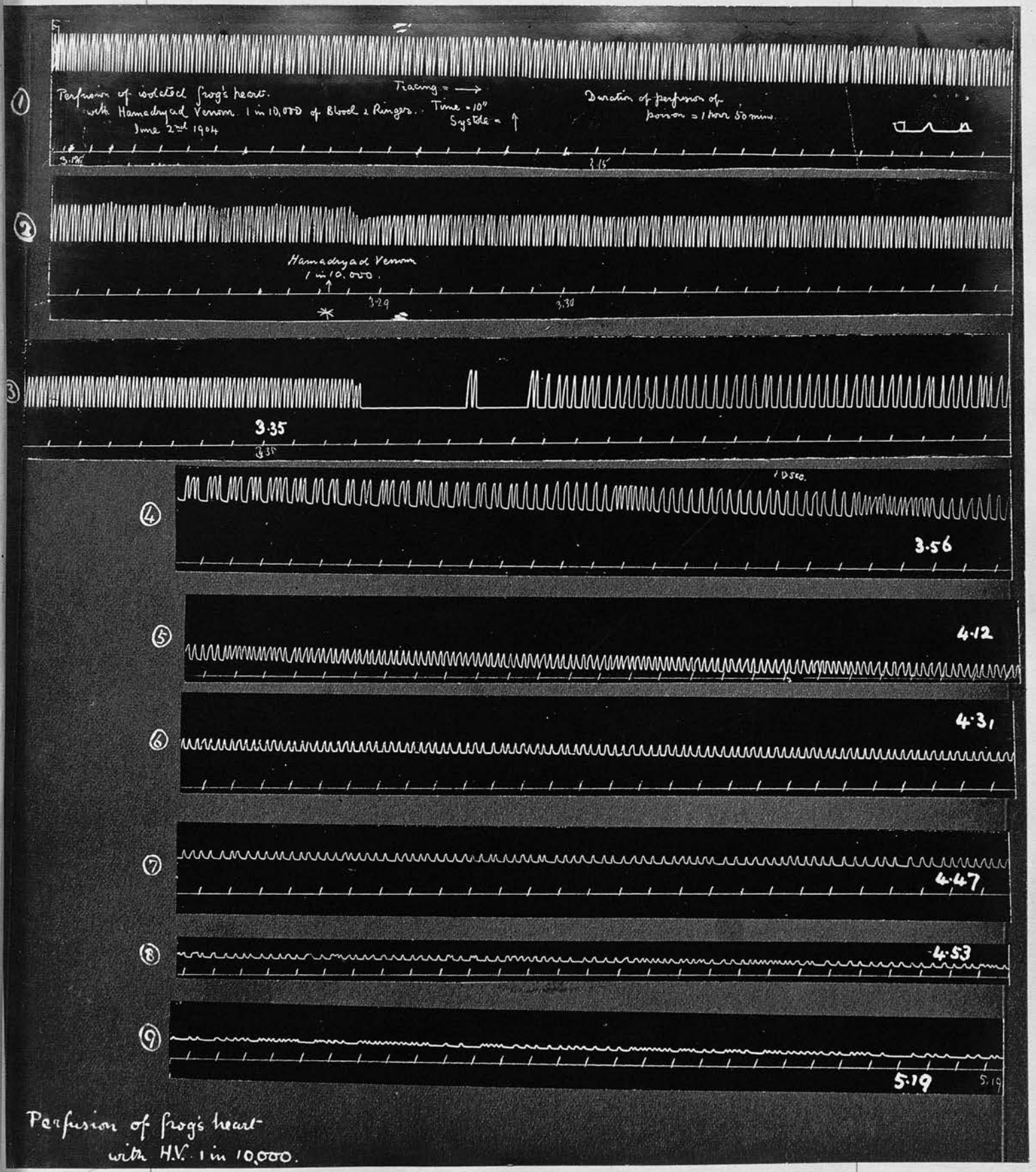
Plate IX 2nd June 1904. Temperature 64°F. R. (see p. 70) esculenta 30 grammes, strength of solution 1 in 10,000, height of the fluid 10 to 12 inches. The record was begun at 3.12, and ^ at 3.29 the poison was turned on. At 3.35 long diastolic pauses were the first symptom of poisoning (Fig. 3) After which the rate of the heart diminished owing to occurrence of the pauses- An irregularity in rhythm was observed occasionally alternating with these pauses (see Fig. 4). The subsequent course of the poisoning was seen in the gradual dilatation of the heart in the same manner as observed in the previous experiment- The heart was practically arrested in two hours. The following table gives (p.71) the alteration in rate per 60" in amplitude of excursus and in the amount of diastole measured by the distance of the systolic end of the curve from the time tracings as Abscissa- The increase necessitated by the readjustment of the piston has been added to the measurements which were taken Before the readjustment.

EXPERIMENT 36.

1 in 10,000.

Minutes before poison.	rate per 60"	Amplitude of excursus.	Distance from Abscissa.
17	47	8 millimeters	48 millimeters
14	48	8 "	47 "
9	46	8 "	45 "
4	47	7 "	44 "
1	46	7 "	43 "
poison turned on	45	6.5 "	42 "
minutes after poison			
1	46	6.5 "	41.5 "
2	46	6 "	41 "
3	46	6 "	41 "
4	46	6.5 "	40 "
5	46	6 "	40 "
6	pause for 50"		
8	20	7 "	38 "
9	21	6.5 "	38 "
11	22	6 "	38 "
13	25	5.5 "	37 "
17	38	4 "	36 "
18	pause for 60"		
21	44	4.5 "	34 "
22	30	5 "	33 "
24	23	5 "	32.5 "
25	20	5 "	32 "
	regular		
29	32	4 "	29 "
	irregular		
34	30	3 "	26 "
42	28	3 "	21 "
43	28	2.5 "	20 "
53	25	2 "	16 "
63	22	2 "	14 "
73	21	1.5 "	12 "
82	21	1.5 "	9.5 "
98	18	1 "	5 "
109	18	0.5 "	2.5 "

PLATE 9.



Perfusion of frog's heart with H.V. 1 in 10,000.

EXPERIMENT 37. Plate 10.

3rd June 1904- Temperature 64°F. Solution 1 in 5,000
Height of fluid 10 to 12 inches. (see p. 72)

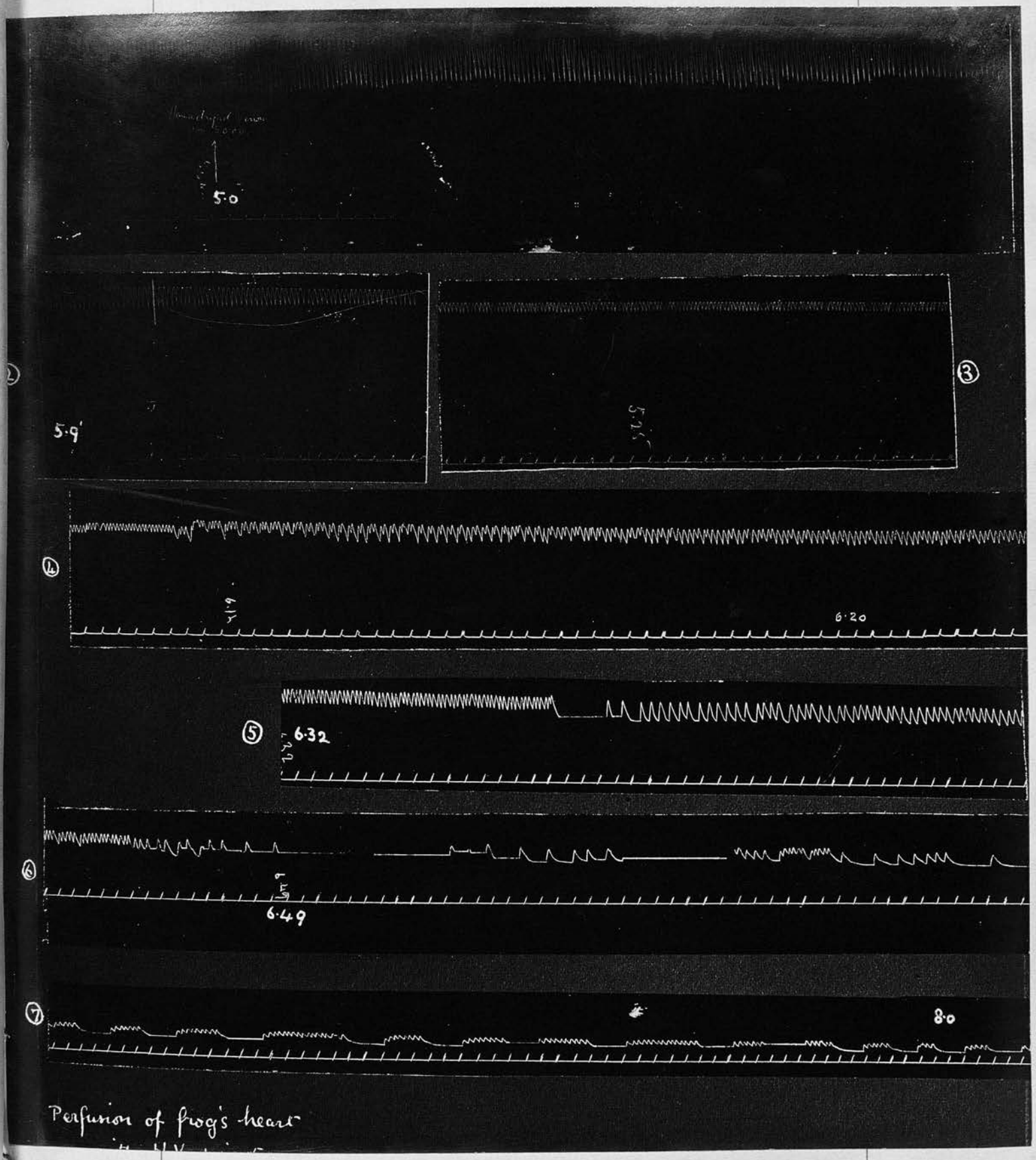
The heart was prepared at 4 p.m. the record started at 4.58 and the experiment was ended at 8 p.m. though the heart was not absolutely arrested. The poison was turned on at 5 p.m. This strength of poison represents approximately 5 times the mimimal lethal dose and there was observed a lessening in the amplitude of the excursus soon after the poison was turned on. This diminution was at the expense of the diastolic portion of the curve the heart not dilating quite so completely as it had been doing. The rate increasedas the excursus diminished (Figs. 1 and 2)- At 5.25 the heart was dilated and a portion of the ventricle was the only part of the heart to continue contracting (Fig. 3). At 6.14 the heart became more distended and balloon -like and beat irregularly. The relaxation increased and the contraction diminished (Fig. 4) At 6.34 a pause in diastole for 30" occurred. (Fig. 5). and the subsequent contractions of the heart were very feeble being limited to the base of the ventricle. The irregularity and increased diastolic pauses are seen in Figures 6 and 7. At the end of the experiment the Ringer's solution was observed to be coloured but the time at which this change began had not been observed.

EXPERIMENT 37.

1 in 5,000.

minutes before poison	rate per 60"	Amplitude of excursus.	Distance from Abscissa.
7	19	11 millimeters	33 millimeters
6	20	11 "	33 "
5	18	11 "	33 "
4	21	11 "	33 "
3	21	11 "	33 "
2	22	11 "	33 "
1	23	11 "	33 "
poison turned on minutes after poison.			
1	24	11 "	33 "
2	22	9 "	35 "
3	22	8.5 "	35 "
4	21	8.5 "	35 "
5	22	9 "	34 "
6	22	6 "	37 "
7	21	7 "	36 "
8	19	7 "	36 "
11	25	3.5 "	39 "
12	24	3.5 "	39 "
13	23	3.5 "	39 "
15	25	3 "	39 "
21	28	2.5 "	38.5 "
26	30	2 "	37.5 "
38	30	2 "	36 "
48	32	2 "	33 "
56	33	2 "	30.5 "
62	30 hitherto regular	2 "	29 "
71	30 irregular	2 & 4 "	29 & 26"
72	19	2 & 4 "	28 & 26"
87	22	2 "	25
92	regular diastolic pause for 30"		
94	11	4 "	20 "
107	diastolic pause for 110 seconds. 5 beats in 60" pause for 70" irregular.		
119	15	1.5 millimeters	12 millimeters.
131	9	2 "	9 "
138	12	1 & 2 "	7 "
139	2	2 "	7 "
153	9	1 "	5 "
169	9	2 "	0.5 "

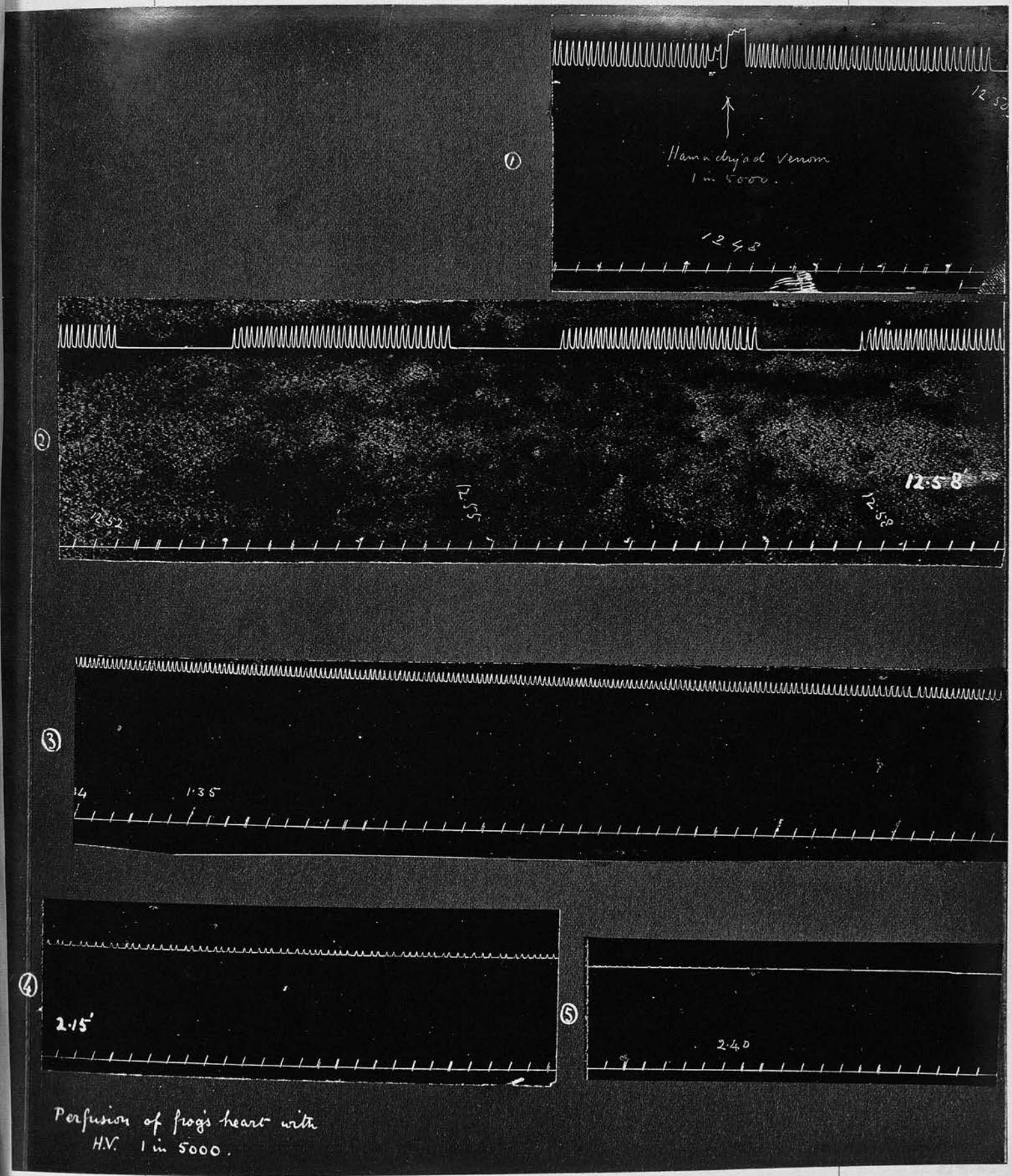
PLATE 10.



Experiment 38. Plate XI.

3rd June 1904. This experiment was performed on the same day and under the same conditions as Experiment 37 the strength of the solution of venom being the same, 1 in 5,000. The heart was somewhat weakened evidently at the commencement of the experiment and before the application of any poison. It was spontaneously arrested in diastole for short but varying periods, as the perfusion of the nutrient fluid was continued the heart became quite regular and had remained regular for 20 minutes before the poison was turned on. In 4 minutes after poisoning had commenced the diastolic pauses reappeared (Fig. 2). The heart lost its power of contracting in rather less time than in the previous experiment and stopped in diastole -distended- in two hours, (See Figures 3, 4, & 5). This experiment is interesting as it shows the characteristic effect of the poison in intensifying the already weakened condition of the heart.

PLATE 11.



Perfusion of frog's heart with
H.V. 1 in 5000.

EXPERIMENT 39.

19th January 1906. Solution of venom 1 in 2, 500. This experiment presented no exceptional features during the poisoning which ran a very similar course but rather more rapidly. There was the same loss of comtractility and increased dilatation of the heart. Particular attention however was paid to the time at which the staining of the Ringer's solution occurred. This appearance was very slight when the weaker solutions were used but became a marked feature when the strength of the solutions was increased. At 12.32 p.m. the poison was turned on. At 12.46 and until the Ringer's fluid was observed to be quite clear and colourless. At 12.47 a fine thread of reddish fluid was seen to ooze from the ventricle and fall through the clear liquid. This stream gradually increased in volume, collected at the bottom of the bulb and gradually coloured the solution which became red and less transparent. This increase in the amount of fluid surrounding the heart obviously became a factor in the displaeement of the piston which has been previously alluded to. The effect of this may be seen in the tracings in which the approach of the piston with the writing point to the time tracing becomes more rapid. I have not included the tracing of this experiment as the curve is identical in appearance with figures 1, 2, and 4 of Plate 12. These stronger solutions therefore/

therefore not only hasten the death of the heart and increase the distensibility of it, but so affect the wall as to prevent it retaining its contents under the pressure to which it is subjected in these experiments.

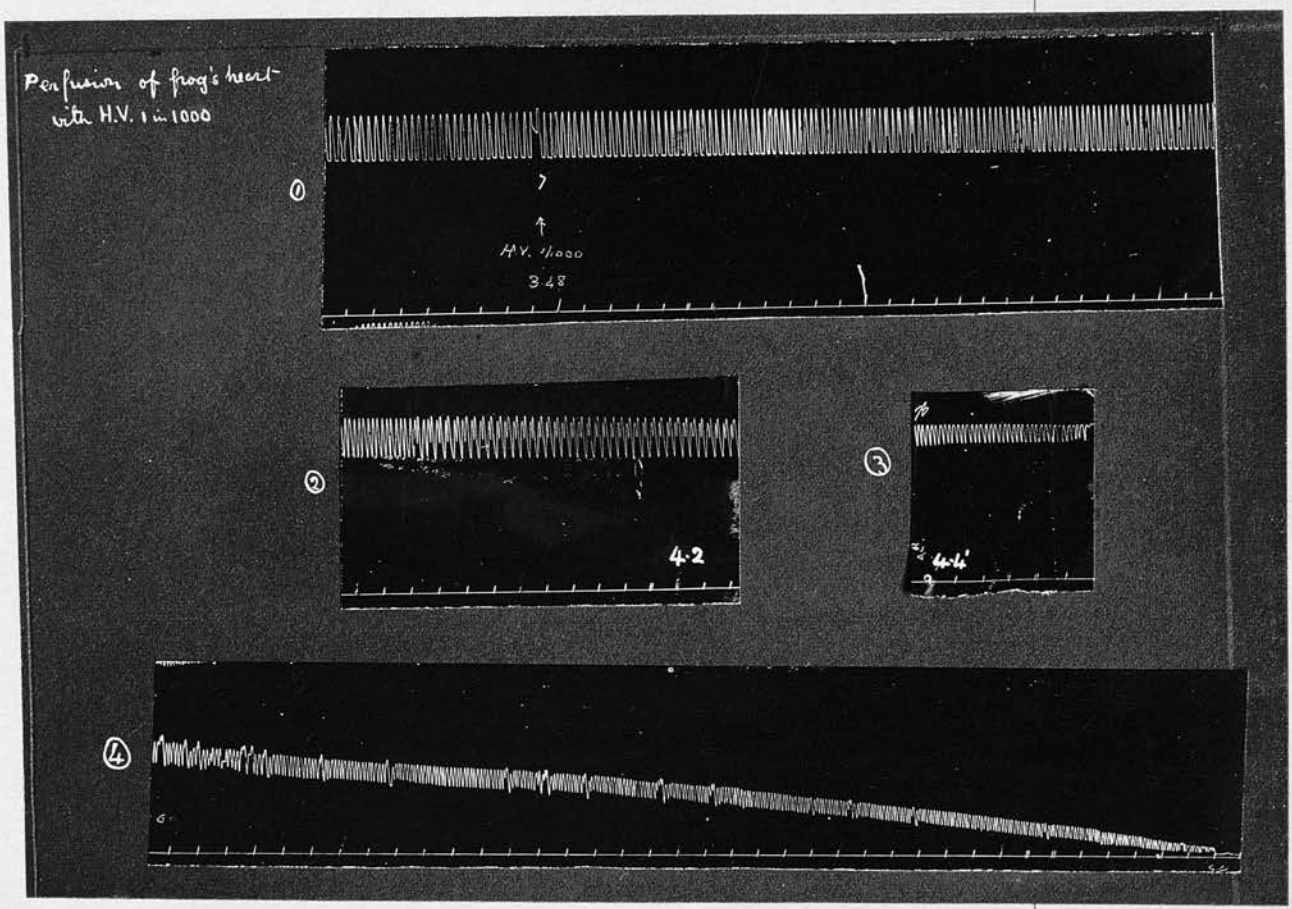
Experiment 40 Plate XII. p. 77.

1st July 1904. Temperature 68°F. Solution of venom 1 in 1000. In this experiment in which a solution was used representing approximately from 30 to 50 times the minimal lethal dose (according to the rapidity of absorption which we allow) 16 minutes after poisoning there was a short period in which the beats were irregular (Figures 1, 2 and 3) and this was followed by an appearance of arrest in systole. This phase passed off however, and in figure 4 we see the diminution in excursus as the heart rapidly passes towards the condition of diastolic arrest. It was finally arrested extremely distended. The passage of fluid through the wall of the heart was observed 16 minutes after the commencement of poisoning.

Experiment 41 Plate XIII.

Solution/

PLATE 12.


Perfusion of frog's heart-
with H.V. 1 in 1000
0
7
H.V. 1/1000
3.48
2
4.2
3
4.4'
4

Experiment 41. Plate XIII. p.79

1st July 1904 Temperature 68°F. Solution of venom 1 in 1000.

In order to see whether the height of the fluid was a determining factor in causing the distension of the heart and the consequent passage of fluid through the wall this experiment was performed with the reservoir lowered so that the height of the fluid varied between 6½ and 8½ inches. No tendency to arrest of the heart in systole was observed. The oozing of the coloured fluid was however noticed before any irregularity in the beat of the heart was observed (Fig. 2 B.)

Experiment 42. Plate XIV. July 3rd. 1904. Exp.80.

Solution of venom 1 in 1000.

In this experiment the pressure was again lowered the height of the fluid in the reservoir varying between 4 and 6 inches above the level of the heart. The same rapid progress towards death in diastole was observed and the oozing from the ventricle was observed 15 minutes after the poison had been turned on.

PLATE 13.

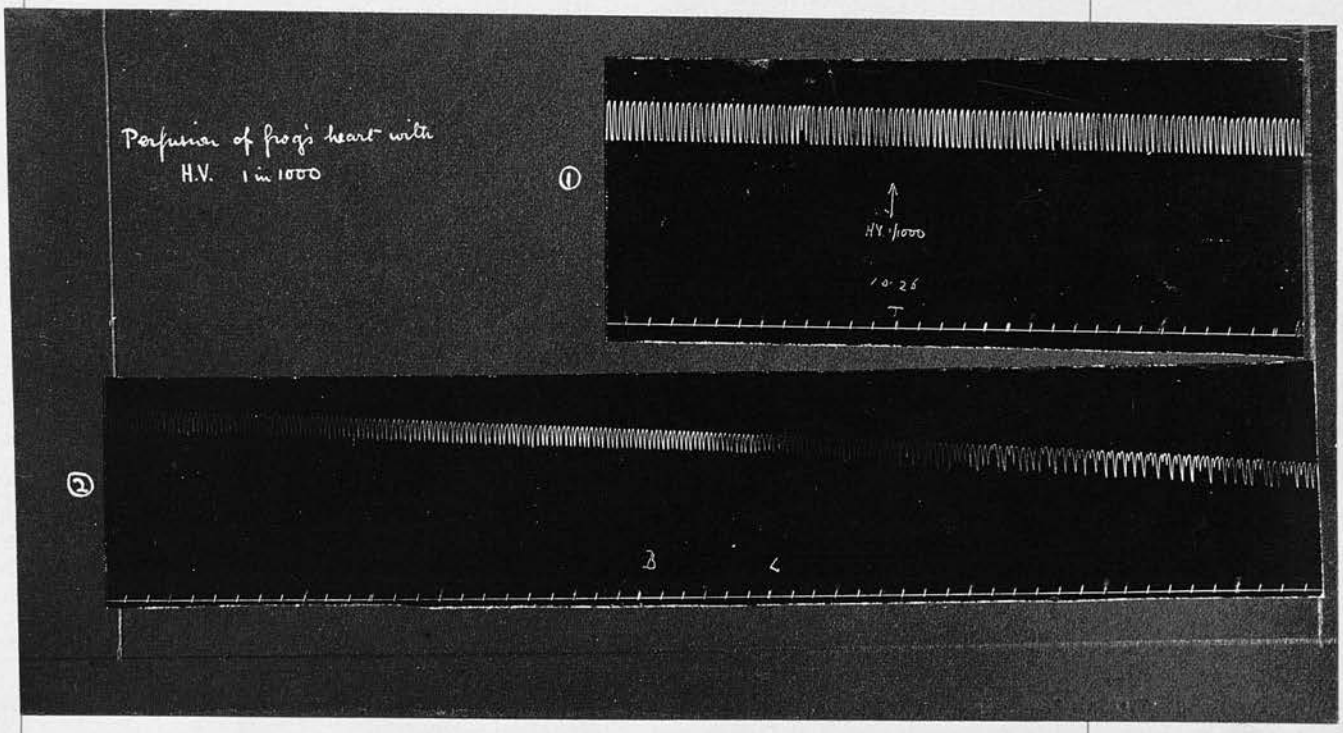
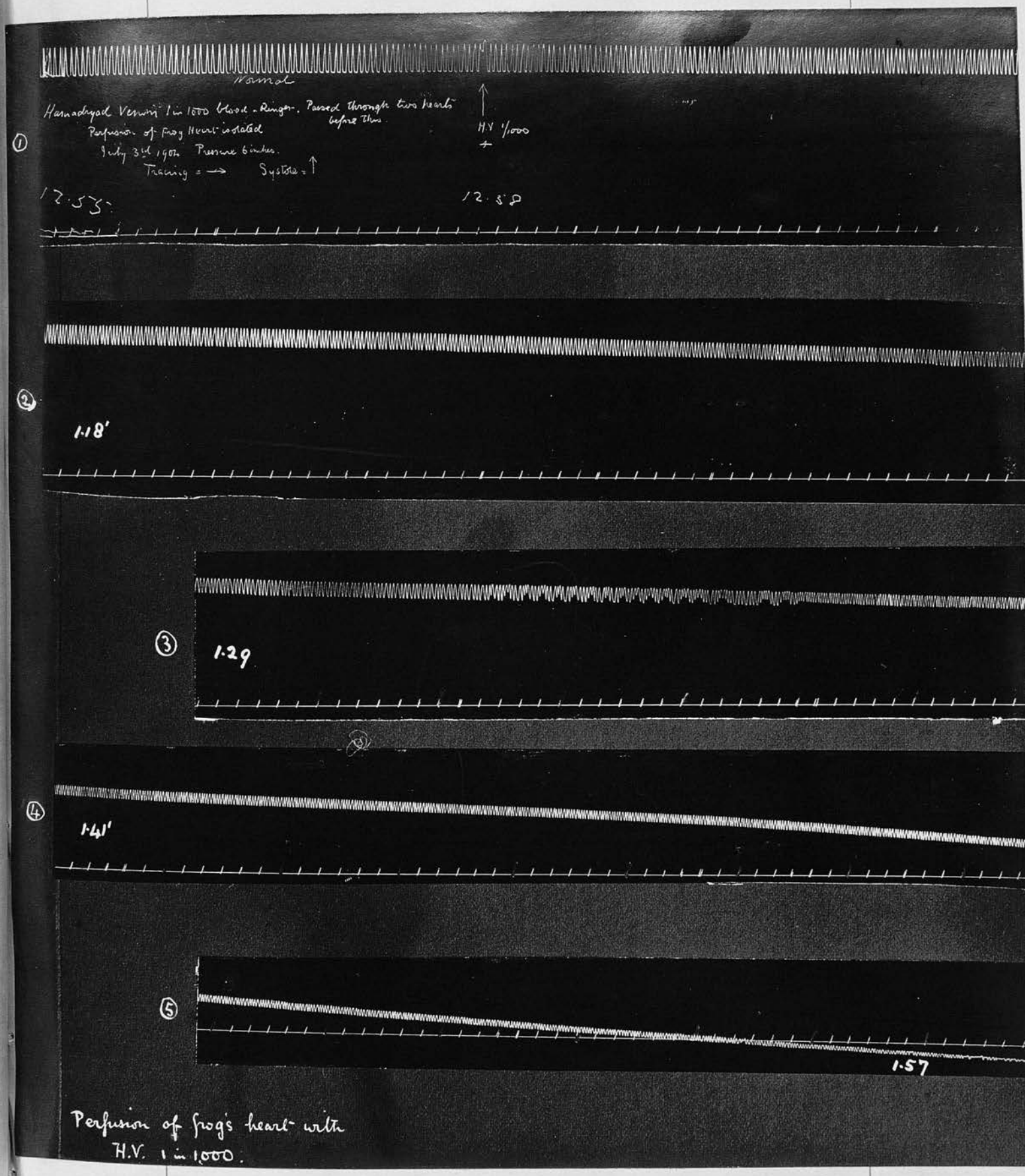

Perfusion of frog's heart with
H.V. 1 in 1000
①
H.V. 1/1000
10 25
T
②
B
L

PLATE 14.

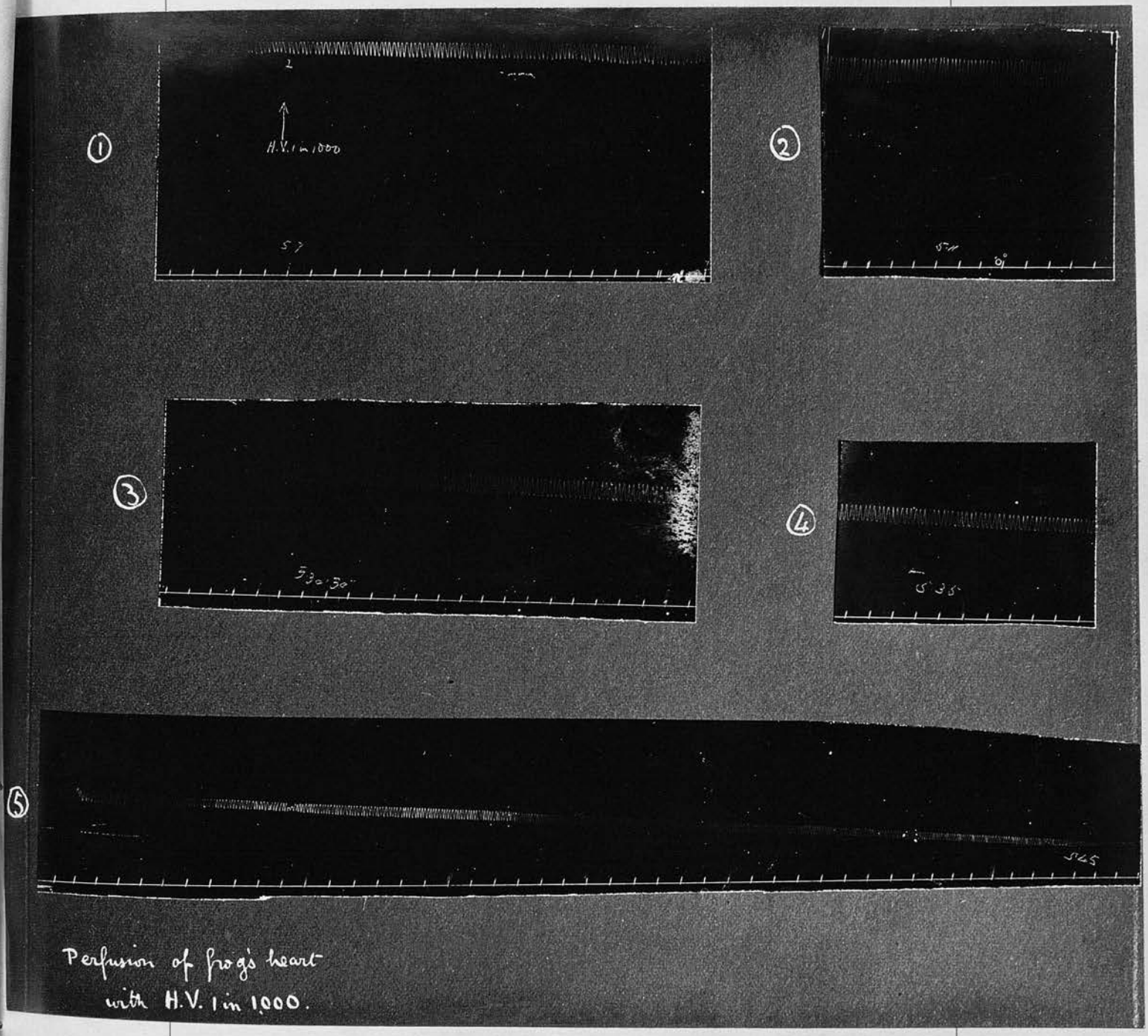


Perfusion of frog's heart with H.V. 1 in 1,000.

Experiment 43 Plate XV. 1st July 1904.
Temperature 68°F. Solution of venom 1 in 1000.
The heart which was observed during this experiment was at the beginning of the experiment apparantly about to pass into a condition of systolic arrest while it was being perfused with the normal nutrient solution. Under ordinary circumstances it would not have been used for an experiment on the action of a poison, but it was thought interesting to observe whether the poison which had apparently the orposite effect would counteract this condition. The heart was not only not arrested in systole but the expansion was distinctly lengthened (Fig.2) and the subsequent progress was in all respects similar to that of the other experiments.

We see therefore that the effect of hamadryad venom is quite unlike in its effects on the heart the venoms of the allied serpents and we are enabled possibly to explain the differences observed with regard to the length of time which frogs with lethal doses lived. If the animal possessed a heart which was already somewhat weakened its death would be very considerably hastened by the administration of a dose of poison which would be powerless to arrest a very active heart.

PLATE 15.



Perfusion of frog's heart with H.V. 1 in 1,000.

In several experiments the activity of the ends of the vagus nerves was tested, with the general result that there was little action, if any, until late in the poisoning. There was then a certain loss of excitability.

EXPERIMENT 44.

24th May 1904. Temperature 60° F.

R. esculenta, weight 64 grammes. At 6.25 p.m. there was injected subcutaneously into the posterior lymph sac 0.32 c.c. of Ringer's solution containing 0.00032 grammes H.V. equivalent to 0.005 grammes per kilogramme i-e- rather more than the minimal lethal dose.

At 9 p.m. the respirations were very shallow, the eyes closed and the animal was lying flattened with its head resting on the ground.

On the following morning at 8 a.m. paralysis was complete, the respirations absent and the cardiac impacts seen through the thoracic wall 7 per 10" the reflexes were absent. The brain was destroyed at noon and at 12.15 the sciatic nerve was exposed, stimulated by Faradic current and found to respond when the coil was at 285 mm. the result being contraction of the toes.

The heart was exposed and was beating regularly , filling and emptying well at the rate of 8 per 10"- At 12.23 the vagus on the left side was exposed and stimulated. With the coil at 40 the rate of the heart was slightly quickened,but with the coil at 20 mm. the heart was arrested for 10 seconds and resumed beating regularly when the stimulus was removed.

Experiment 45.

On the same day to another frog there was administered twice the dose received by the frog in the last experiment. R. Esculenta of weight 44 grammes was injected at 6.20 p.m. with 0.04 c.c. containing 0.00044 grammes equivalent to a dose of 0.01 gramme per kilogramme.

At 8 a.m. the next morning the frog was apparently dead, there were no reflexes and paralysis was complete, the heart was however, exposed and found to be beating regularly at the rate of 7 per 10" . The ventricle was not emptied completely at each contraction.

At 11.30 a.m. the heart was still beating but more feebly at the rate of 5 per 10 seconds. The left vagus was exposed and tested at 11.39 by means of the interrupted current with the coil at 80 mm.

As/

As this did not arrest the heart the coil was moved to 70 mm. with the following result. The rate of the heart was at this time 5 per 15"

11.42. 15"	5 beats	
30"	5 beats	
45"	0	Vagus stimulated for 15"
11.43.	4 beats	
15"	5 beats	
30"	5 beats	

Experiment 46.

26th May. 1904. R. temporaria. weight 35 grammes. Temperature 64° F-

At 2 p.m. the left vagus was exposed the pericardium opened the heart having been exposed- The rate of the heart was 9 per 10" and stimulation of the vagus with the coil at 60 caused arrest of the heart.

At 3.6 2 drops of a solution of the venom in Ringer's solution of the strength of 1 in 1000 were placed on the surface of the heart. The amount applied was approximately 0.0001 gramme. at each application and 0.0006 gr. altogether or 4 m.l.d.

The vagus was again stimulated and the application of the poison was repeated in the same way and the vagus stimulated with the following results:-

Time	Rate of heart-	Strength of Stimulus required to arrest heart.	Result
3.5	9 per 10"	V. Stim. 60 mm.	Arrest.
3.6	H.V. applied		
3-12	9 per 10"	V. Stim- 60 mm.	Arrest.
3.18	H.V. applied		
3.24	8 per 10"	V. Stim. 60 mm.	Arrest.
3.28	7 per 10"		
3.29	H.V. applied		
3.31		V. Stim. 60 mm.	Arrest-
3.40	7 per 10"		
3.41		V. Stim. 60 mm.	marked slowing not arrest.
		V. Stim. 40 mm.	Arrest
3.50	H.V. applied		
3.51		V. Stim. 40 mm.	Arrest
4.0	H.V. applied		
4.10		V. Stim. 40 mm.	Arrest
4.30	5 per 10" .	V. Stim. 40 mm.	Arrest.
4.35	H.V. applied		
4.45		V. Stim. 40 mm.	Arrest.
5.25	9 per 60"	V. Stim. 40 mm.	Arrest-
6.30	6 per 60"	V. Stim. 40 mm.	Arrest.

In these experiments there was no evidence that the slowing of the heart was due to increased excitability/

excitability of the vagal nerve ends nor was there evidence of early paralysis of the nerve.

SUMMARY ON THE ACTION ON FROGS.

The action on the frog may be briefly summarised then as follows:.

The certain minimal lethal dose of this specimen of the venom is 0.004 grammes per kilogramme, death occurring however sometimes with smaller doses. The most important effect is the paralysis of respiration; following on a preliminary stimulation of the function where the dose has not been excessive. This is followed by motor and reflex paralysis, due mainly to a direct action on the spinal cord. When a dose just sufficient to cause death has been administered the paralysis may last for days or even weeks during which period the circulation is maintained intact, gradually however becoming weaker. There is to some extent accumulation of fluid under the skin which also probably interferes with respiration during the period of paralysis. When its removal is aided by daily compression of the bladder and expulsion of its contents (which expulsion does not take place naturally during the paralysis) partial recovery may take place. The respiratory movements then reappear the/

the reflexes and motor power are restored and the animal may either recover absolutely (exp.t 12) or, after this stage is reached, subsequently become feebler and die (exp.t 11). The peripheral nerves and muscles are but slightly affected when minimal lethal doses are given and not till late in the poisoning when the doses are large. The blood is apparently unaltered when the poison is administered by subcutaneous injection, no evidence of haemolysis or destruction of corpuscles having been obtained where either large or small doses have been given.

The contrast between the action of the hamadryad venom and the venoms of the cobras and the krait is greatest when the action on the heart and blood vessels are compared. The comparatively rapid action on the heart which is seen when large doses of these other poisons are administered, and the appearance of the heart arrested in systole, are widely different from the slight and slow action of the hamadryad venom, and from the consistent appearance of the arrest of the heart in diastole. In fact the action on the heart of this venom may be described as a slight hastening of the natural death of the organ. The action on the blood vessels is also very much less than that of the other venom as/

as is also that on the voluntary muscles. The similarity is, on the other hand, very marked between the action of all the poisons on the nervous system. The paralysis of respiration and of the reflex function of the cord and motor conductivity are very similar. It would almost appear therefore as though there were in these snake venoms a neurotoxin and a muscle poison as well as a haemolysin, and that the second and third of these are present in very small quantities in the specimen of hamadryad venom examined, or that their activity is extremely slight. The difference of the action on the blood in the direction of haemolysis is noteworthy, my experiments confirming Roger's observation with regard to the very slight effect produced by this venom.

No further proof is needed now in view of all the recent investigations on these venoms that the respiratory paralysis and other nervous symptoms are secondary to changes in the circulation. Additional disproof of such a theory is to be seen in the great disparity between the time of these actions and the amount of the action displayed by this venom. For in the frog we see the nervous system profoundly affected for days before the circulation is interfered with to any marked extent.

In determining the minimal lethal dose it was nece necessary to discard some of the earlier experiments in which death had been assumed too hastily. In fact it was found that the only certain proof of death was the cessation of the flow of blood through the vessels of the web of the foot. When paralysis was complete and no sign of the beating of the heart could be made out the thorax was opened. In the cases in which the heart was still beating it was arrested in a much shorter time after this disturbance than would otherwise have occurred.

Again where marked oedema occurs and the fluid is not partly got rid off by expression of the bladder death occurs apparently from prolonged interference with the respiration.

Lastly where the respiration was on the point of recovery this result occurred much more rapidly where electrical stimulation was applied to the cord. This appeared to stimulate the respiratory movements greatly to increase the vitality of the animal

It was essential to leave the frogs quite undisturbed in order to eliminate these disturbing factors.

EFFECTS OF THE POISON ON WARM BLOODED ANIMALS.

A series of experiments to determine the minimal lethal dose by subcutaneous injection for white rats was undertaken. This was found to be 0.003 grammes per kilogramme. These experiments were not so troublesome to perform owing to the fact that if a quantity of the venom is administered sufficient to cause death the termination of the experiment is not delayed beyond some hours. With the smaller doses, from the administration of which the rat recovered, the animal remained drowsy and disinclined to move for a day, or perhaps, two, after which recovery was complete and perfect. In experiment 54 for example death was expected at any moment for 2 hours but recovery took place and was perfect. In the following table the general results of these experiments are expressed.

The account of Experiment 52 in which death nearly occurred, and the account of an experiment in which a larger dose was administered with fatal results will serve to give the symptoms of poisoning in these animals which symptoms were very consistent throughout the whole series.

DETERMINATION OF MINIMAL LETHAL DOSE BY SUBCUTANEOUS INJECTION FOR RATS.

No- of Expt	dose in gr. per kilogr.	Weight of rat in kilo. gramme.	Quantity of venom in grammes injected.	Amount of Solution.	Result	Remarks.
47	0.00025	0.140	0.000035	in 1.75 C.C.	Recovery	Drowsy for some hours.
48	0.00051	0.129	0.000065	in 0.065 C.C.	Recovery	Drowsy.
49	0.001	0.180	0.00018	in 0.18 C.C.	Recovery	Drowsy for 24 hours.
50	0.00211	0.128	0.00027	in 0.27 C.C.	Recovery	Respiration affected. Drowsy for 2 days.
51	0.0025	0.122	0.000315	in 0.315 C.C.	Recovery	Resp. strongly affected in 2 hrs impaired for 5 hrs- recovered in 48 hours.
52	0.00275	0.171	0.00047	in 0.94 C.C.	Recovery	Resp- affected in 2 hrs. Recovery began 4½ hrs after injection.
53	0.0028	0.147	0.000412	in 0.825 C.C.	Death	Resp. impaired in 1½ hrs. Died between 4 to 7 hrs
54	0.0029	0.172	0.0005	in 1 C.C.	Recovery	Resp- nearly ceased in 4 hrs. Condition lasted 2 hours. Gradual recovery.
55	0.003	0.172	0.000525	in 0.52 C.C.	Death	Between 3 and 10 hrs.
56	0.00326	0.187	0.00061	in 0.61 C.C.	Death	Resp, ceased in 2 hrs.
57	0.0035	0.167	0.000584	in 1.17 C.C.	Death	Resp. ceased 2½ hours.
58	0.005	0.160	0.008	in 0.8 C.C.	Death	1hour 3 minutes.
59	0.0125	0.120	0.0015	in 1.5 C.C.	Death	in 55 minutes.

Experiment 52. 20th May 1904. Temperature 64° F. Black and white male rat. weight 171 grammes. At noon the rat which had been kept without food for 24 hours to ascertain the true body weight, and which was very active, was breathing at the rate of 15 per 10" when at rest, and 29 per 10" when moving about and sniffing. The heart could be felt beating regularly and strongly through the thoracic wall at the rate of 46 per 10".

At 12.30 there was injected subcutaneously into the right flank 0.94 c.c. of Ringer's solution containing 0.00047 grammes of H.V. equivalent to 0.00275 grammes per kilogramme- There was no excitement or distress and the rat moved about freely sniffing and ate some rice which was given to it.

At 12.45 he was less active, but moved about occasionally more slowly and without the sniffing movements. The respirations were deeper and more uniform than before and were at the rate of 24 per 10", the cardiac impacts 44 per 10".

At 1.30 he sat quietly in the corner of the cage, but started at a sudden sound. The respiratory movements were from 18 to 24 per 10" not quite regular, some being deeper than others. The heart beats were 40 per 10".

When the eye was gently touched the eyelid was at once closed and the head moved away.

At 2 P.m. his condition was somewhat similar, he remained quietly in his corner, the eye reflex was acute but respiration was more laboured and occasionally audible, there being with inspiration slight whistling sound. The rate was 18 per 10", 3 or 4 only of the movements being deep, the others shallower but quite regular in rhythm. The cardiac impacts were 48 per 10" but were not quite so easily counted owing to the movements of the thoracic wall. They were however quite regular and apparently as strong as before.

At 2.30 the rat was no longer sitting up but reclined with his thorax and muzzle on the floor of the cage, breathing with apparently greater difficulty. The rate was still 18 per 10" and each inspiration could be distinctly heard being harsh and sounding as though drawn through moisture. The animal occasionally gave a jerk of the whole body, raising the head which, however, rapidly drooped again until it rested on the ground. This jerk or slight convulsive movement appeared to originate in the thorax. When disturbed he moved slowly and with an appearance of great caution, occasionally staggering, and after moving a short distance came again/

again to repose in the same position as before. Cardiac impacts were 46 per 10" apparently unchanged in character.

At 2.52 the respirations were still 18 per 10", jerky, and more distinctly heard; the cardiac impacts were 46 per 10" and the rat lay prone. It could be laid gently on its side but struggled, and with some difficulty regained the normal position and managed to crawl a few steps.

At 2.57 the respiratory movements were more irregular both in volume and rate varying from 15 to 18 per 10" shallower and more jerky. The pupils of the eyes were contracted, as they had been from the beginning of the experiment, but the eyes were insensitive when touched.

At 3 o'clock the cardiac impacts were 46 per 10" and the respiratory movements 18.; some urine was passed and a general spasmodic convulsion was witnessed.

At 3.5 the respirations were 16 per 10" of the same character and convulsive movements were more general and more frequent, occurring about every 2 minutes. The heart was beating strongly at 50 per 10".

At 3.27 the convulsions were more prolonged and the breathing more laboured and noisy, each inspiration being/acc

being accompanied by a rattling sound. The rat remained in this condition more or less unchanged till 4.12 the respirations varying from 15 to 18 per 10" sometimes shallow, but deeper after one of the convulsive seizures. The heart beat was regular and strong and about 48 per 10".

At 4.20 the head was resting on the ground and leaning over to one side, and the respirations were shallow and jerky - 16 per 10". The cornea was insensitive, the eyes were opened widely and the pupils were still contracted. The heart was rather irregular varying from 40 to 56 per 10" but beating strongly and easily felt. There were few general convulsions but the head was raised occasionally with a jerk and the mouth widely opened, the lower jaw jerking synchronously with the respiratory movements. At 4.50 he was weaker and lay on his side without attempting to move, the respirations being of a gasping character but still 18 per 10"- The heart was strong and regular and its rate 50 per 10".

At 5.30 he remained in much the same condition but did not seem worse and was laid in a box on cotton wool near the hot pipes.

At 10 p.m. the breathing was less jerky, not so noisy, and the cornea had recovered its sensitiveness. The rat had recovered its normal position but the muzzle/

muzzle was resting on the ground. The respiratory rate was 18 per 10" , the flanks heaving regularly and the heart was beating strongly and regularly at 56 per 10".

At 9 a.m. the next morning the rat was very much better but sat quietly in his cage with the eyes closed as if asleep. When disturbed he moved about freely, sniffing inquisitively for a short period, but soon came to rest again. The respiration was partly slow and deep alternating with rapid and shallow movements, the respective rates being 10 per 10" and 40 per 10" respectively. He ate some green food and except for the drowsiness and a slight amount of motor weakness he seemed to have recovered.

At 7 p.m. he still remained drowsy but when disturbed ran about with vigour.

On the following morning i.e. on the 22nd May he ran about quite recovered, to all appearance, a normal rat. The respirations were at the rate of 24 per 10" and the heart 38 per 10".

In this experiment therefore the respiration was strongly affected up to a point very nearly that of complete paralysis and the asphyxia was strongly marked. The reflexes were nearly abolished and there was great motor weakness.

Experiment 58. 23rd March 1906. Temperature 58° F. Black and white male rat. Weight 160 grammes.

At 10 a.m. the respirations were 15 per 10" when quiet, 22 per 10" when sniffing, and the heart was felt to be beating regularly at 40 per 10".

At 2.25 p.m. there was injected into the right flank subcutaneously 0.8 c.c. of Ringer's solution containing 0.0008 grammes of H.V. A dose equivalent to 0.005 grammes per kilogramme, nearly twice the m.l.d.

At 2.30 the respirations were 23 per 10" all full and deep and the rat sat quietly in the corner of the cage. The cardiac impacts were 46 per 10" strong and regular.

At 2.33 the respiratory rate was the same but the movements were shallower and they were interrupted by a choking sound or cough, the eye reflex was acute, the eyelids shutting promptly when the cornea was touched. The animal remained quiet but jumped when disturbed by a sudden sound. After this the rate of the respirations diminished gradually they were 21 per 10" at 2.35, 18 at 2.40, 19 at 2.43, 18 at 2.47, 17 at 3 o'clock, and 15 at 3.4.

At this time 3.4' he was sitting huddled in a corner of the cage breathing deeply, and noisily, but regularly; the cardiac impacts were 40 per 10" and the eye sensitive.

At 3.15 the respirations had become irregular, varying in rate from 13 deep movements per 10" to 20 shallower movements for the same period. The movements of the thorax were more perceptible, making it more difficult to count the rate of the heart, which was however about 39 per 10". The rat was restless, apparently uneasy, and walked slowly with a somewhat staggering gait. The head was raised and rested on a ledge at a higher level than the body; and sometimes the rat raised himself on his hind legs, resting the thorax in an angle, probably to remove the weight of the abdominal contents from the diaphragm and so to render the respiratory movements easier. The eyes were wide open and the cornea was less sensitive than before though the reflex was not entirely absent.

At 3.18 the respiration had fallen to 8 per 10" very forced, noisy and accompanied by general spasmodic contractions of the body, so that when the rat was standing on the ground he jumped from it approximateing the fore and hind limbs together.

At 3.22 the respirations were 4 per 10", deep and gasping, the mouth was opened spasmodically with each inspiration, and each was followed by a jerk of the body. When the rat was laid on its side it recovered its normal position but with some difficulty.

At/

At 3.25 respiration was in the same condition, the cardiac impacts were 26 per 10" strong and regular the pupils were contracted and the cornea insensitive. The animal could regain the normal position but with greater difficulty, and some urine was voided.

At 3.28 the respirations ceased, the pupils rapidly dilated and there were 2 or 3 convulsive jerks of the fore and hind legs. The heart was still beating regularly, but gradually became slower, and was felt distinctly during two minutes after the respirations had ceased.

At 3.30 the thorax was opened and the phrenic nerve very carefully exposed and placed on electrodes. With a single Daniell's cell and a Faradic current with the secondary coil at 410 mm. from the primary the diaphragm was observed to contract strongly.

At 3.47 the auricles were still beating rhythmically and the right ventricle was distended, dark in colour and filled with dark, fluid, blood The left ventricle was in moderate diastole and responded to a stimulus by giving a single slight contraction.

At 4 o'clock occasional beats of the auricles could still be seen and the sinus was contracting rhythmically. The liver and kidneys appeared slightly congested/

congested, the lungs were collapsed and rather dark, but no portion was solidified or sank when thrown into water. There was very slight injection of the vessels in the subcutaneous tissue at the seat of the inoculation; and the vessels of the intestine near this situation presented the same appearance. The morbid appearances however were all of them extremely slight.

Experiment 56. 22nd March 1906. Temperature 59°F. White buck rat. Weight 187 grammes.

At 3.25 p.m. 0.00061 grammes of H.V. dissolved in 0.61 c.c. of Ringer's solution was injected subcutaneously into the left flank. This dose was equivalent to 0.00326 grammes per kilogramme and produced very much the same symptoms, the respirations were at first increased in rate from 24 per 10" to 26 per 10" as well as in the amount of movement. After this preliminary stimulation they gradually diminished in rate to 6 per 10" at 4.14 when the rat was paralysed, the cornea insensitive, and the dyspnoea urgent.

At 4.15 the pupils dilated, urine was expelled and the respirations ceased. The thorax was immediately opened to expose the heart which was still beating, extremely/

extremely dilated, and not emptying completely. The vagus was also exposed in the neck and stimulated. With the coil at 180 the heart was arrested when the nerve was stimulated and the same effect was observed when the nerve was stimulated at 4.29 and at 4.35 p.m.

At 4.41 the strength of the stimulus had to be increased to arrest the heart, the coil being moved to 50 and at 4.43,28 minutes after the respiratory failure,when the nerve was stimulated with the coil at zero,the heart was slowed but not arrested.

At 4.55 the auricles were still contracting rhythmically 17 per 10" and at 5.35 one hour and 20 minutes after death they were still contracting at the rate of 10 per 30".

Experiment 59.

In this experiment where four times the minimal lethal dose was administered by subcutaneous injection respiration failed in 55 minutes accompanied by strong convulsions during which the rat was jerked off the ground. The phrenic nerve was tested soon after death and found to be active though not quite as sensitive as in the experiments where smaller doses had been given. The vagus was also stimulated and arrested the heart, the sciatic nerve,also,was found

to/

to be active. The blood was fluid but coagulated normally. Some was examined microscopically and presented no abnormality. Some was also collected in capillary tubes which were sealed and showed no trace of haemolysis in 48 hours, the serum separating out perfectly clear and colourless.

The rats die therefore, with a minimal lethal dose of 0.003 grammes per kilogramme, from respiratory failure practically uncomplicated. The circulation is quite efficient up to the time of death and the blood apparently normal. The phrenic nerve ends are quite active at death so that the respiratory paralysis is probably due to paralysis of the central mechanism in the medulla. In view of the statement that the phrenic nerves are impaired at death it is important to observe that in experiment 56 in which the dose was 0.00326 grammes per kilogrammea and in which death occurred in 2 hours the phrenic nerve was exposed most carefully to avoid the slightest injury in stretching or dragging it.

It was stimulated as soon after death as possible and good contractions of the diaphragm were observed to take place when the secondary coil was removed 650 mm. from the primary. It was therefore as sensitive as an unpoisoned phrenic nerve is and impresses one with the fact that accidental injury in exposing and stimulating/

stimulating the nerve may easily account for an apparent loss of sensitiveness. The nerve also very rapidly loses its vitality; and the element of time after death at which it is stimulated becomes of very great importance. In all cases in which the phrenic was stimulated the nerve was exposed where it is easily seen leaving the heart and where it can be placed on the electrodes with the least possible disturbance.

It is worthy of note that the preliminary stimulation of respiration is to be observed either by an increasing rate, or by an increase of volume (as evidenced by deeper abdominal movements) and sometimes by both of these factors simultaneously. The nerve ends of the sciatic and the vagus remain active after respiratory paralysis is complete.

ACTION OF THE VENOM ON RABBITS.

The minimal lethal dose was determined for the rabbit when it was administered by subcutaneous injection and also when it was injected into a vein. It was necessary to have this latter knowledge in order to have a basis from which to calculate the comparative lethality of the doses which were administered in this manner in those experiments on rabbits in which the blood-pressure was investigated.

The minimal lethal dose by subcutaneous injection was found to be 0.00251 grammes per kilogramme.

This is rather less than the dose for the rat (0.003 gramme per kilogramme) but is about four times the dose which was stated by Rogers (9) to be the minimal lethal dose for warm-blooded animals of the venom with which he worked.

The general results of the experiments which were performed in order to determine the minimal lethal dose by subcutaneous injection will be found im the table on the next page.

DETERMINATION OF MINIMAL LETHAL DOSE BY SUBCUTANEOUS INJECTION FOR RABBITS.

No. of Expt.	dose in gr. per kilogr.	Weight of rabbit per kilogramme.	Quantity of venom in grammes injected.	Amount of Solution	Result	Remarks.
60	0.0003	1.215	0.000365	in 0.73 C.C.	Recovery	Unaffected
	0.00051	1.362	0.000514	in 0.7 C.C.	Recovery	Unaffected.
	0.00102	1.469	0.0015	in .15 C.C.	Recovery	Unaffected.
	0.00151	1.514	0.0023	in 2.3 C.C.	Recovery	Slightly affected.
	0.0018	1.819	0.00327	in 1.83 C.C.	Recovery	Affected.
	0.00209	1.434	0.003	in 3 C.C.	Death	in 4½ hours.
	0.0025	1.650	0.0041	in 2.06 C.C.	Recovery	Very nearly died. ill for 24 hours.
	0.00251	.872	0.003	in 1 C.C.	Death	in 4 hours.
	0.00266	1.882	0.005	in 0.5 C.C.	Death	in 1 hour 35 minutes.
	0.0029	1.650	0.0048	in 0.48 C.C.	Death	in 1½ hours.
	0.003	2.380	0.007	in 2 C.C.	Death	in 1½ hours.

An account of the symptoms observed durigg the performance of experiment 67 will serve to shew the general course of th poisoning.

Expt. 67. 26th. May 1904. Temperature 62°F.

A buck rabbit wieighing 1650 grammes, whose respirations werefrom 23 to 27 per 10", whose heart was felt beating at the rate of 43 per 10", and whose rectal temperature

temperature was 102.2° F- received at 11.15 a.m. by subcutaneous injection into the right flank 0.00412 grammes of H.V. dissolved in 2.06 c.c. of Ringer's solution. This was a dose of 0.0025 grammes per kilogramme. He moved about freely and rather restlessly, and was very alert for about half an hour, and ate green food when it was offered.

At 11.45 the respirations were 22 per 10" fuller and deeper and the animal was quiet with head and ears erect. The heart, 40 per 10", was easily felt and the rectal temperature was 106.6- He remained in this condition until 12 noon when slight tremulous movements were observed affecting the lower jaw, and fore part of the body.

At 12.15 he still sat with head and ears erect, but had a tendency to allow the fore feet to slip from under him in front, so that he was rather resting the abdomen on the ground. Respiration 23 per 10" if he moved, 12 per 10" when quite quiet. Temperature 101.8°F. The cardiac impacts were more difficult to count owing to a muscular thrill or fibrillary trembling of the thorax but were at the rate of 38 per 10".

At 12.35 the head began to droop forwards and the eyes to lack their normal lustre, though the corneal/

corneal reflex was quite acute;and at 12.50 the respirations were 20 per 10", the cardiac impacts 44 per 10" and the temperature 102.2° F- An hour later at 1.50 the temperature was 102.8 and the respirations and cardiac impacts were 22 per 10" and 40 per 10" respectively, and the animal was more flattened in its attitude than before, the thorax now resting on the ground.

At 2.30 the muscular weakness as shown by the drooping head was more marked the respirations had fallen to 17 per 10" and the cardiac impacts to 32 per 10" but the temrerature had risen to 103° F.

At 5 o'clock the muzzle was resting on the ground the respiratory rate 13 per 10" and the heart 28 per 10". The respiration was more embarrassed and accompanied by audible sounds. The animal raised its head occasionally,but it soon fell again until it touched the ground. The temperature was 103.8.

At 10.10 the condition was unchanged excepting that the temperature was 103 .6 and the respirations 12 per 10".

27th May. At 9.30 a.m. on the following morning the attitude was unchanged the respirations were still 12 per 10" accompanied by whistling sounds and the animal/

animal was salivating. The eyes were almost insensitive, though a slight touch caused a minute movement of the eyelid. The cardiac impacts were strong and regular at the rate of 32 per 10". A considerable quantity of urine had been passed and the rabbit refused to eat green food when it was offered. The temperature had fallen to 100.3. It remained in very much the same condition during the whole of the day, but that at 2.30 the temperature was 102.4 and at 5.30 it was 103.2. On the 28th of May at 9.30 a.m. it had regained its normal position, was sitting up with head and ears erect. Its temperature was 101 the cardiac impacts 44 per 10" and the respirations 25 per 10". It ate green food readily and had apparently quite recovered and the recovery was permanent.

In Experiment 69 in which a white doe rabbit weighing 1650 grammes was administered by hypodermic injection 0.0048 grammes of venom equivalent to 0.0029 grammes per kilogramme death took place in one hour and a half. On the 27th June 1904 the temperature of the rabbit being 101.5 at 11.45 a.m. the respirations 15 per 10", and the cardiac impacts

37/

37 per 10" the poison was injected at noon.

At 12.20 the respiration was at the rate of 13 per 10" and the temperature was 99.2.

At 12.37 the respirations had fallen to 9 per 10" and the cardiac impacts were 38 per 10". The muscular weakness was evidenced by the sinking of the thorax and abdomen.

At 12.45 the temperature was 98.4 and the breathing laboured and noisy, the head was drooping and the cornea less sensitive than it had been.

At 12.50 the respiratory rate was 7 per 10'" and the animal lay on its side gasping, now and then making slight and fruitless efforts to regain its normal position. The breathing gradually became more and more difficult, the mouth being opened with each inspiration. The heart was slower and slightly irregular, the pupils contracted and the cornea quite insensitive.

At 1.35 the respirations ceased, the pupils dilated and the animal died with very slight spasmodic contractions of the fore and hind limbs. Immediately after death the phrenic nerves were exposed, stimulated by the faradic current and found to be active. There was a good contraction of the diaphragm observed when the coil was at 600 mm. The sciatic nerve was also exposed and/

and tested and responded with the coil at 340 mm. In order to ascertain the difference from the normal, if any, in the rate of the death of the nerve ends a control rabbit was killed by a blow at the back of the neck and its phrenic and sciatic nerves exposed and stimulated in the same manner as those of the poisoned animal. The comparison may be seen in the following table.

Time	Poisoned Phrenic	Sciatic	Control Phrenic	Sciatic.
4 minutes after death.	600	340	600	300
15 minutes	550	80	680	80
30 "	faint twitch at zero.	60	100	60
45 "	0	0	0	0

The Phrenic nerve therefore appears to die more rapidly after the poisoning than does the normal nerve. Practically no difference is however observed with regard to the sciatic nerve.

Blood was taken from the two rabbits and compared microscopically and in capillary tubes without any obvious difference between them being observed.

DETERMINATION OF MINIMAL LETHAL DOSE BY INTRAVENOUS INJECTION FOR RABBITS.

No. of Expt-	dose in gr. per kilogr.	Weight of rabbit per kilo gramme.	Quantity of venom in grammes injected.	Amount of Solution	Result	Remarks.
71	0.000562	1780	0.001	in 0.15 c.c.	Recovery	Rise of temperature.
72	0.000835	1705	0.00142	in 0.19 c.c.	Recovery	do.
73	0.000976	1680	0.00164	in 0.82 c.c.	Recovery	do.
74	0.0001	1570	0.00157	in 0.78 c.c.	Recovery	do.
75	0.000124	1505	0.00188	in 0.94 c.c.	Death	died in 5 hours.
76	0.00149	1745	0.0026	in 0.78 c.c.	Death	died in 1 hour.
77	0.0021	1425	0.003	in 0.5 c.c.	Death	died in $1\frac{1}{2}$ hours.

The relationship between the minimal lethal doses administered by subcutaneous injection and intravenous injection is therefore the proportion of 0.0025 grammes per kilogramme and 0.00124 grammes per kilogramme or approximately it may be expressed that the intravenous dose is one half the subcutaneous.

A rise in temperature of about 1 degree was observed in each of these experiments in which death occurred and slightly more than that in those in which death occurred.

EXPERIMENT 77. 13th February 1905. Laboratory Temperature 58°F. White doe. Weight 1425 grammes

At 2.14 p.m. the respirations were 29 per 10" the heart 45 per 10" and the temperature was 103.2. After these observations had been taken the hair of the right ear was cut short and the ear washed with ether until the veins had dilated. A clip was placed so as to compress the proximal end of the marginal vein. Into this was injected 0.85 c.c. of Ringer's solution containg 3 mg. of the venom and the syringe was rinsed with another quantity of 0.5 c.c. which was also injected into the vein.

At 4.3 8 minutes after the injection the respiration had fallen to 24 per 10" and the heart to 40 per 10".

At 4.32 the rabbit moved about restlessly for a few moments then sat quietly with the head drooping and the ears laid back, the respirations were 15 per 10" the cardiac impacts were 48 per 10" and the temperature 104.6.

At 4.50 the animal was sitting more flattened and appeared to move with a certain amount of difficulty.

At 5.3 its muzzle was resting on the ground the respirations were 11 per 10" and the heart 28 .

At 5.8 the rabbit lay on its side helpless, the eyes were insensitive and it responded to no stimulation. The respirations had fallen to 8 per 10" and were very embarrassed , the cardiac impacts had also fallen to 15 per 10" and were irregular.

At 5.17 the respirations were panting 7 per 10" the breathing being more of a thoracic type than of an abdominal and the animal was affected by slight convulsions.

At 5.20 the respiratory difficulty was greater, the mouth being opened with each inspiration,and the pupils were contracted strongly.

At 5.24 the respirations ceased and the pupils dilated widely. The thorax was opened and the heart was seen to be still beating.

At 5.39 the phrenic nerve was stimulated and observed to respond with the coil at 470.

At 5.44 it responded at 460

at 5.48 it responded at 380

at 5.54 it responded at 310

at 5.59 it responded at 290 and

at 6.4 with the current at zero,a minute fibrillary twitch was the only effect observed on the diaphragm.

At /

At 5.50 the auricles were beating regularly at the rate of 15 per 10" and the ventricles 2 per 10".

At 5.54 the auricles were 10 per 10" and the ventricles 3 per 10".

At 5.59 the auricles were 6 per 10" and the ventricle 2 per 15".

At 6.4 the auricles were 6 per 10" and the ventricle had stopped, i.e. 40 minutes after death. The auricles were observed to contract until 6.40 when the contractions were still at the rate of 4 per 10".

In Experiment 76 special observation was made with regard to the size of the pupils which had been observed to be generally constricted more and more powerfully as the asphyxial condition increased; and to be dilated at the moment of death. In this experiment a buck rabbit of weight 1745 grammes received into the marginal vein of its right ear .78 c.c. of Ringer's Solution with a dose of the venom equivalent to 0.00149 grammes per kilogramme.

Immediately after the injection and before the respirations were affected the measurement of the pupil was/

was 9 mm. In half an hour when the respirations were gasping, and the muzzle was resting on the ground, the pupils measured 8 mm. 36 minutes after poisoning the pupils had contracted to 7 mm. and at 38 minutes when the animal was lying on its side and the corneal sensitiveness was much impaired the measurement was 4 mm.

At 12.50 i.e. 40 minutes after poisoning, when the first convulsions were observed, the respirations having fallen to 6 per 10", the pupils measured 3 mm.

At 1.2 with the respirations 5 per 10" the same measurement was obtained and at 1.4 when the respirations ceased and general convulsions occurred the pupils rapidly dilated and measured 10 mm.

In this experiment as in most of the others poisoned by either intravenous or subcutaneous injection the phrenic nerves were found to be active the diaphragm contracting as a whole with the coil at 455. mm.

The symptoms of poisoning therefore ran the same course with about half the dose injected intravenously, as occurred when twice the amount of the poison is injected subcutaneously; but the incidence of the symptoms is rather more rapid.

When a lethal dose was administered the temperature was generally observed to fall gradually. In some cases however a slight preliminary rise was observed early in the poisoning.

In experiment 67 where the dose administered to a small buck was 0.0025 grammes per kilogramme the following observation on the temperature were taken.

Time	Temperature	Remarks.
10 am.	100.2	Before poisoning.
10.20		Subcutaneous injection of poison.
11.30	99.8	
12.30	100	Paralysis beginning.
12.45	99.2	Cornea insensitive.
1.17	98.8	Paralysis profound.
1.58	97.2	Severe dyspnoea.
2.38		Death.

Immediately after death in this experiment the phrenic nerve responded at 540 mm. and the heart was arrested when the vagus was stimulated at 100 mm. and the sciatic was observed to be active when stimulated at 215 mm.

In/

In experiment 64, in which recovery occurred, the symptoms of poisoning were comparatively slight but the temperature observations showed a distinct rise. The dose was 0.0018 grammes per kilogramme injected into a buck weighing 1819 grammes

	Time	Temperature
26th May.	10.0 a.m.	102.4
	11 a.m.	poisoned
	11.40	102.0
	12.20	101.4
	12.52	101.4
	2.0	103.
	2.45	103.4
	4.	104.6
	5.10	104.8
	6.20	104.8
	7.30	104.4
	10.10	103.8
27th May.	9.30 a.m.	103.
	2.30 p.m.	103.2
	5.30	103.4
28th May.	9.30 a.m.	103.
	2.30 p.m.	102.8
	6.0 p.m,	102.6

Asphyxia was the cause of death in both rabbit and rat. Its stages were observed by watching the pupil which contracted powerfully when stimulation by the venous blood of the centre on the floor of the aqueduct of Silvius took place, and dilated widely when paralysis occurred. The violent convulsions and expiratory spasms which mark the stage of exhaustion in that form of asphyxia not produced by a poison, are ,in the case of asphyxia produced by the venom comparatively slight. The motor centres of the rat indeed appeared to be more powerfully stimulated than those of the rabbit if one may judge by the greater vigour of the muscular contractions. On the other hand it may be that the conductivity of the cord in the rabbit is more readily paralysed than that in the rat, and that the muscles therefore receive less of the stimulation. Then again the fact that a larger dose in proportion is required to produce death in the rat points to its possessing greater resistance to the action of the venom.

EXPERIMENTS ON BLOOD PRESSURE.

Several experiments were performed in order to ascertain the action of the venom on the blood pressure both before and after the failure of respiration. These experiments were performed on rabbits, and the method followed was practically the same in all. The rabbit was kept without food for twenty-four hours to ascertain the true body weight, weighed, and anaesthetised with chloroform. When anaesthesia was produced, it was continued by means of ether until the end of the experiment. The trachea was opened and a cannula tied into it, through which the animal respired, and inhaled the anaesthetic. It was afterwards attached to the artificial respiration apparatus when natural respiration failed. The left carotid artery was exposed and a cannula inserted into it, which was afterwards attached to the manometer. A cannula was tied into the right internal or external jugular vein, and in some experiments one vagus

was exposed, and a thread passed under it. A double stethograph was attached by an elastic band to the thorax and was connected to a Marey's tambour in order to register the respiratory movements. All the tracings read from left to right, the respiratory curve being uppermost the blood pressure tracing immediately below it, and the time tracing below that again. The time marking was in intervals of ten seconds and except where otherwise noted was the abscissa of the blood pressure. The signal line was the lowest and served as the abscissa in one or two of the experiments. All the doses in this series of experiments are expressed as parts of or multiples of the minimal lethal dose when administered by intravenous injection.

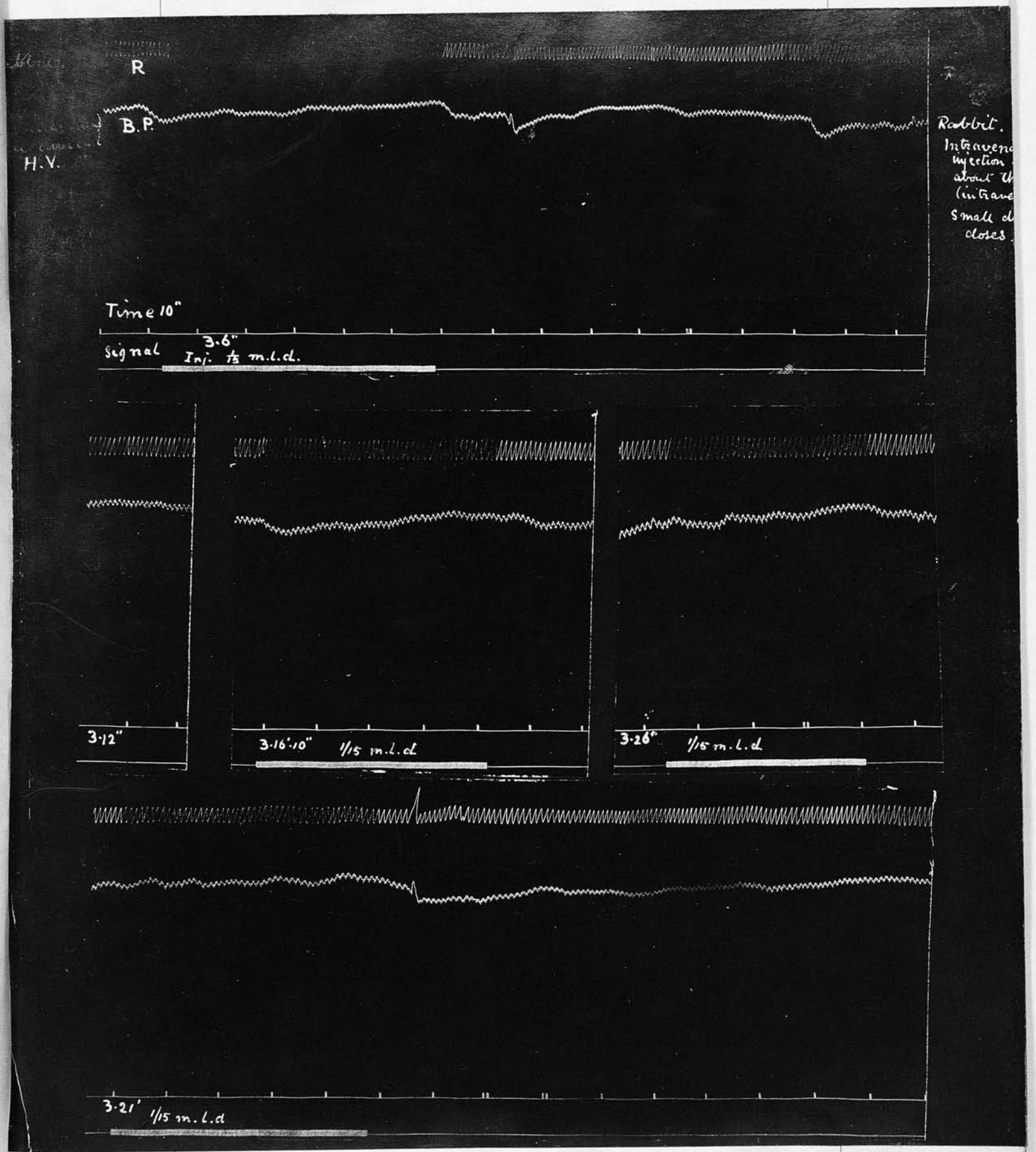
Experiment 78. Plate XVI. 4th June 1904. lab. temp. 64° F. Weight of buck rabbit 2524 grammes. The first solution used was 5 C.C. of Ringer's solution containing 1 milligram of the venom, the second 1 mg. in 2.5 C.C., and thirdly 2 mg. in 1.5 C.C, giving a total of 4 mg. in 9 C.C. This was equivalent to 0.00158 gr. per kilogram, or rather more than the minimal lethal by intravenous injection. The venom was administered in divided doses of 0.0002 grammes at a time (equivalent to about 1/15th of the M. L. D.

At two thirty p.m. the rabbit was anaesthetised

at two fifty-five was connected with the kymograph, and at three o'clock the record was begun. The following table will show the course of the experiment:-

Time.	Rate of Heart per 10"	Resp, Rate per 10"	Resp. Excursus.	Blood Pressure.	Remarks.
3.6p.m.	42.	10.	3 m.m.	95 m.m.	
3. 6,30"					Inj.0.0002 gr.
3.8	35	10.	3	96	
3.12					Fig. 2.
3.16	39	9	4	90	
3.16.10"					Injection repeated. Fig. 3.
3.18	37	10	3.5	92	
3.21					Injection repeated. Fig. 5.
3.26					Injection repeated. Fig. 4.
3.27	33	9	4.5	94	
3.30					Plate 17, Fig. 6.
3.34	35	8	4	96	Fig. 7.
3.46	39	8	2.5	89	Fig. 8. Inj. repeated. Total one third M.L.D.
3.46 10"					Injection repeated.
4.3	37	8	2.5	81	
4.3 30"					Inj. 0.0004

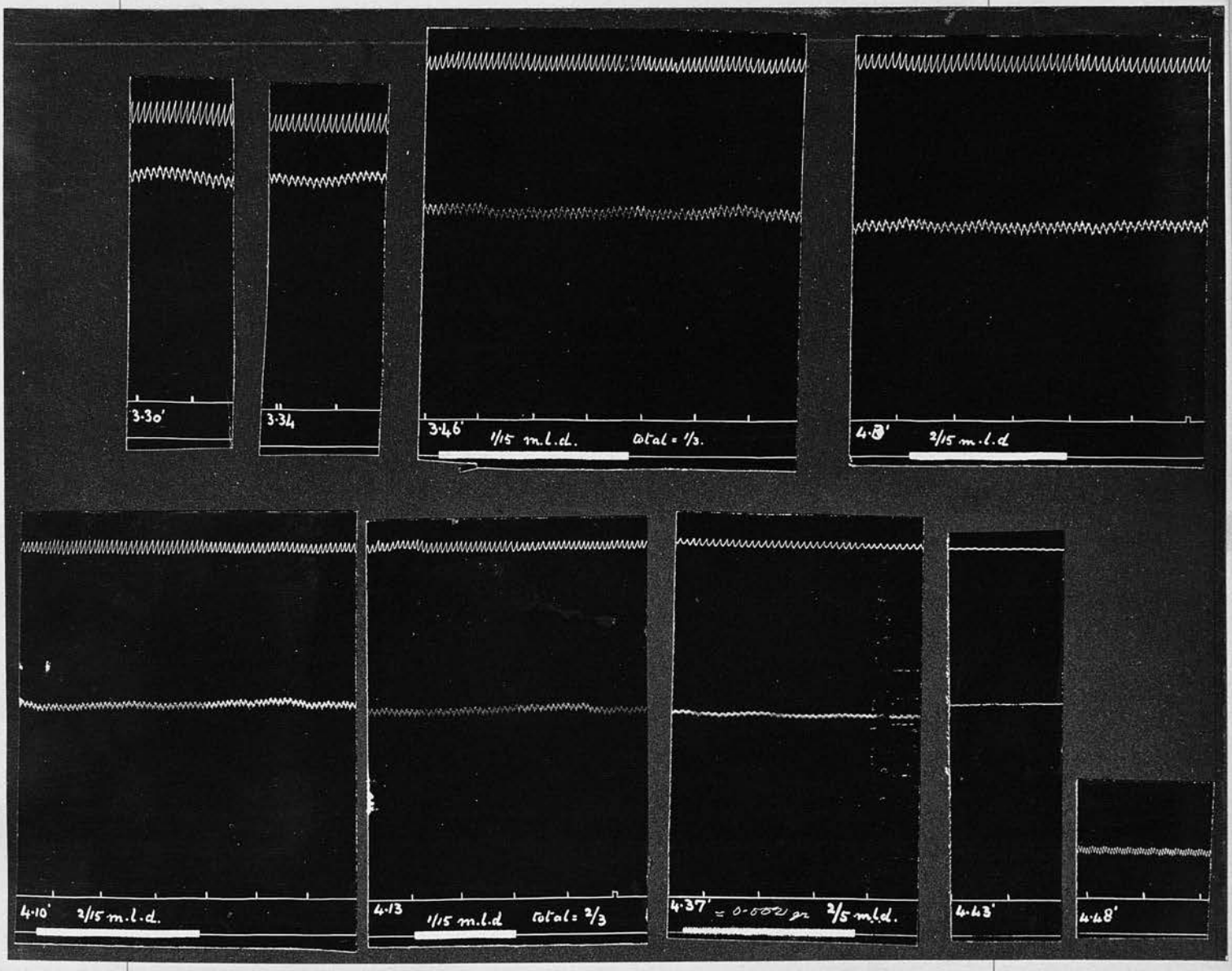
PLATE 16.


R
B.P.
H.V.
Rabbit.
Intravenous injection about th
(intrave
Small d
closes
Time 10"
3.6"
Signal
Inj. 1/5 m.l.d.
3.12"
3.16'.10"
1/5 m.l.d
3.26"
1/5 m.l.d
3.21'
1/5 m.l.d

Time.	Rate of Heart. per 10"	Resp. Rate per 10"	Resp. Excursus.	Blood Pressure.	Remarks.
4.8	35	7	3	81	
4.10					Inj. 0.0004 gr. Total three fifths M. L. D.
4.12	37	10	2	82	
4.13					Inj. 0.0002 gr. Total two thirds, M. L. D. Fig. 11.
4.20	39	9	1	78	
4.37	37	8	1	78	Inj. 0.002. Total one and a third M. L. D.
4.43		8	Imperceptible.	78	Fig. 13.
4.48					Fig. 14.

At four forty-eight a clot formed in the cannula, the heart was. however, beating regularly and well . At four fifty-seven its rate was twenty-one per ten seconds, and at four fifty-six it was ten per ten seconds. Stimulation of the right vagus arrested the heart, when the secondary coil was 160 m.m. from the primary. The phrenics responded at 320 m.m. and the sciatic at 250 m.m. This experiment shows after the first injections of one fifteenth M. L. D. a slight stimulation of respiration, increasing the amplitude

PLATE 17.


3·30'
3·34
3·46' 1/15 m.l.d. total = 1/3.
4·3' 2/15 m.l.d
4·10' 2/15 m.l.d.
4·13 1/15 m.l.d total = 2/3
4·37' = 0·002 gr 2/5 m.l.d.
4·43'
4·48'

of the excursus, though this was accompanied by a slight diminution in rate, The later doses, especially after two thirds of the minimal lethal dose had been given caused paralysis of the respiration. The blood pressure slowly and gradually fell during the experiment but rose when the respirations became deeper. There was a slight fall immediately after the injections, and a subsequent recovery. The rate of the heart was slowedimmediately after the injections, though very slightly, and the diminution was very transient.

The circulation therefore is but slightly affected with the small doses. The venous condition of the blood in the later stages has a distinct effect, however. The fall of the blood pressure is explained by the absence of any stimulating action on the heart and the absence of a constricting effect on the vessel walls. A very marked difference is seen in the action of this venom as contrasted with a similar size of dose in cobra poisoning, where the blood pressure, aided by these two factors, remains very high, or actually rises.

In the next experiment a dose of nine times the intravenous M. L. D. was administered and proved very rapidly fatal.

Experiment 79. Plate 18. 16th June 1904.

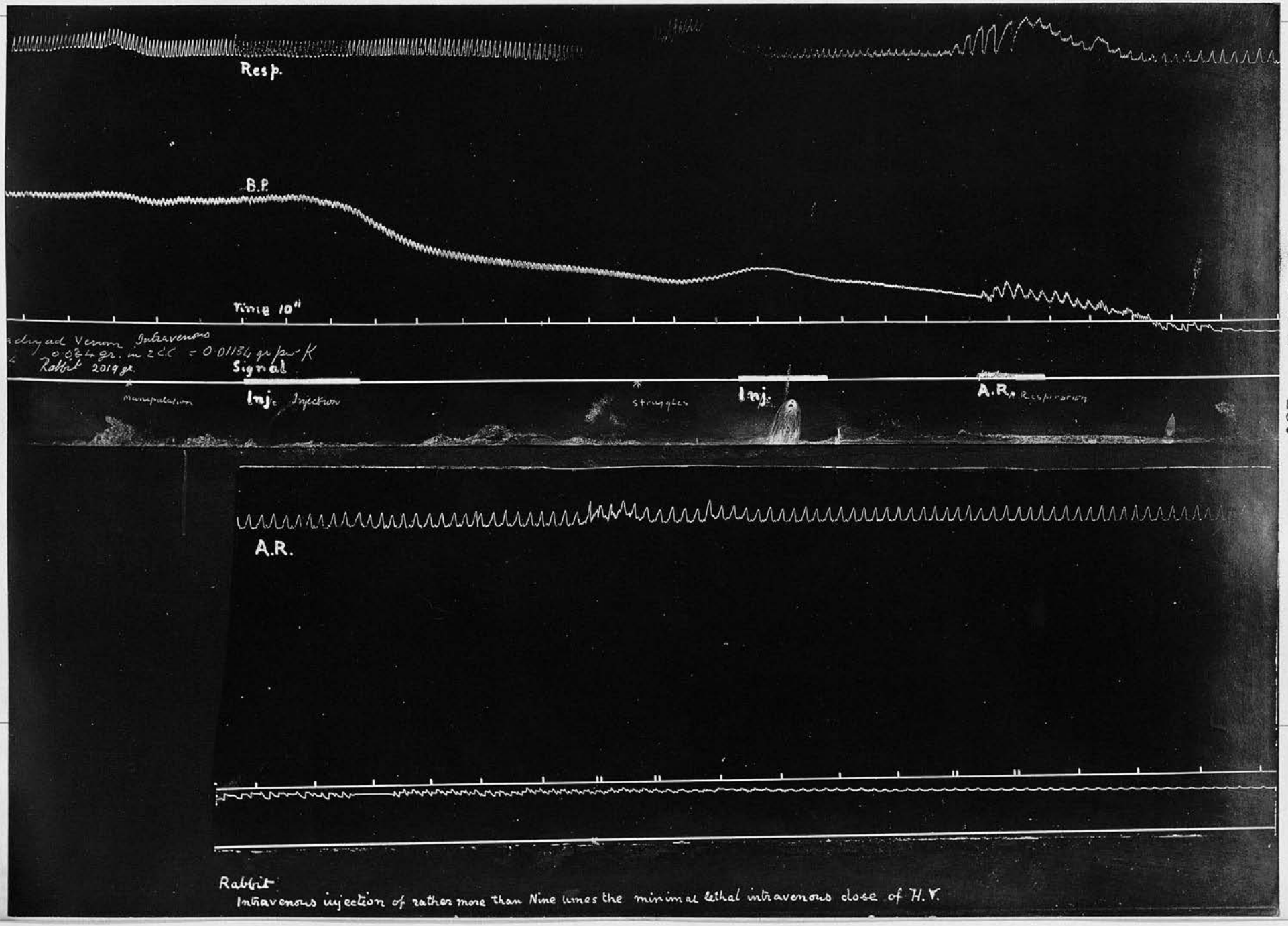
Weight of rabbit 2019 grammes.

Dose 0.024 grammes. of hamadryad venom dissolved in 2 C.C. Ringer's Solution. This was equivalent to 0.01134 grammes per kilogram. This dose thrown directly and rather rapidly into the circulation and reaching the heart almost in the strength of 1 in 200 paralysed the heart almost as rapidly as it did the respiration, so that the blood pressure fell steadily until death occurred, which happened eight minutes after the completion of the injection.

10 m l d.

Time.	Rate of Heart per 10"	Resp. Rate per 10"	Resp. Excursus.	Blood Pressure.	Remarks,
3.4 10"					
	39	11	4	79	
3.4 20"					
					Inj. begun.
3.4 35"					
					Inj. finished.
3.5	37	10	4	74.5	
3.5 50"					
	33	10	5	74.5	Resp. convulsions.
3.6 5"					
					Remainder of the Inj.
3.6 30"					
	31	8	2	42	
3.7	27		3	36	Artificial respiration.

At 3-12 the blood pressure had practically fallen to zero. The thorax was opened and the heart was seen fluttering, fully distended, and the contractions mainly auricular. They were arrested when the vagus



Rabbit
Intravenous injection of rather more than Nine times the minimal lethal intravenous dose of H.V.

PLATE 18.

128.

was stimulated with the coil at 120 m.m, The heart was opened and found filled with dark fluid blood. There was no evidence of clotting; some was collected in capillary tubes, and on examination 24 hours later was found to have undergone no haemolysis. The serum separated out perfectly clear and colourless, The blood examined microscopically showed no abnormal appearance.

Experiment 80. Plates 19, 20, 21, and 22. In this experiment the abscissa for the blood pressure in the plates is the signal.

13th June 1904. Temp. 66° F, Weight of rabbit 1,950 grammes.

In this experiment, which lasted nearly 9 hours from beginning to end, 3 mgs. of the venom. dissolved in 1 C.C. Ringer's Solution were injected. This was equivalent to 1.2 times the M.L.D. by intravenous injection. The respiration was paralysed in 13 minutes, and the animal was then kept alive by artificial respiration for 8 hours, at which time the heart was beating apparently as efficiently as at any time previously, and the blood pressure had fallen only to about three fifths of the height at which it was at the beginning of the experiment. The artificial respiration was intermitted at several periods to ascertain whether the natural respiration would recover. It did so partially,

the respiratory movements which did occur, however, were too slight to sufficiently aerate the blood. The circulation then began to fail and the artificial respiration was again resorted to:-

Time.	Rate of Heart per 10"	Resp. Rate per 10"	Resp. Excursus.	Blood Pressure.	Remarks.
11.14.50"	39	9	6m.m.	113	
11.15					Inj. .003 gr. during 30". Fig. 1.
11.17	33	11	6.5	86	
11.19	28	20	3		More ether given.
11.22	36	13	2		Fig. 2.
11.27	24	10	1		Fig. 3.
11.28				139	Artificial respiration.
11.34	31			97	Plate 20, Fig. 4.
11.37		Stopped A.R.		Rise.	
12 noon.	38	" "		103	No voluntary resp. movements.
12.27	36	" "		83	Fig. 5,
12.35				110	Fig. 6.
1.51	30			115	Fig. 7.
2.50		" "			No voluntary movements.
3.7	30			86	
3.20		" "			No voluntary movements. Fig. 8.
4.25	28			105	
4.35		" "			Slight Vol. movements. Fig. 9.
5.27	22			97	
5.35		" "			Slight vol. movement, Fig. 10.
6.19	20			83	
6.20		" "			Voluntary movements, Fig. 11. Plate 21.
6.22	10 before 16 after.	Artif. Resp.			
6.41	21			74	

PLATE 19.

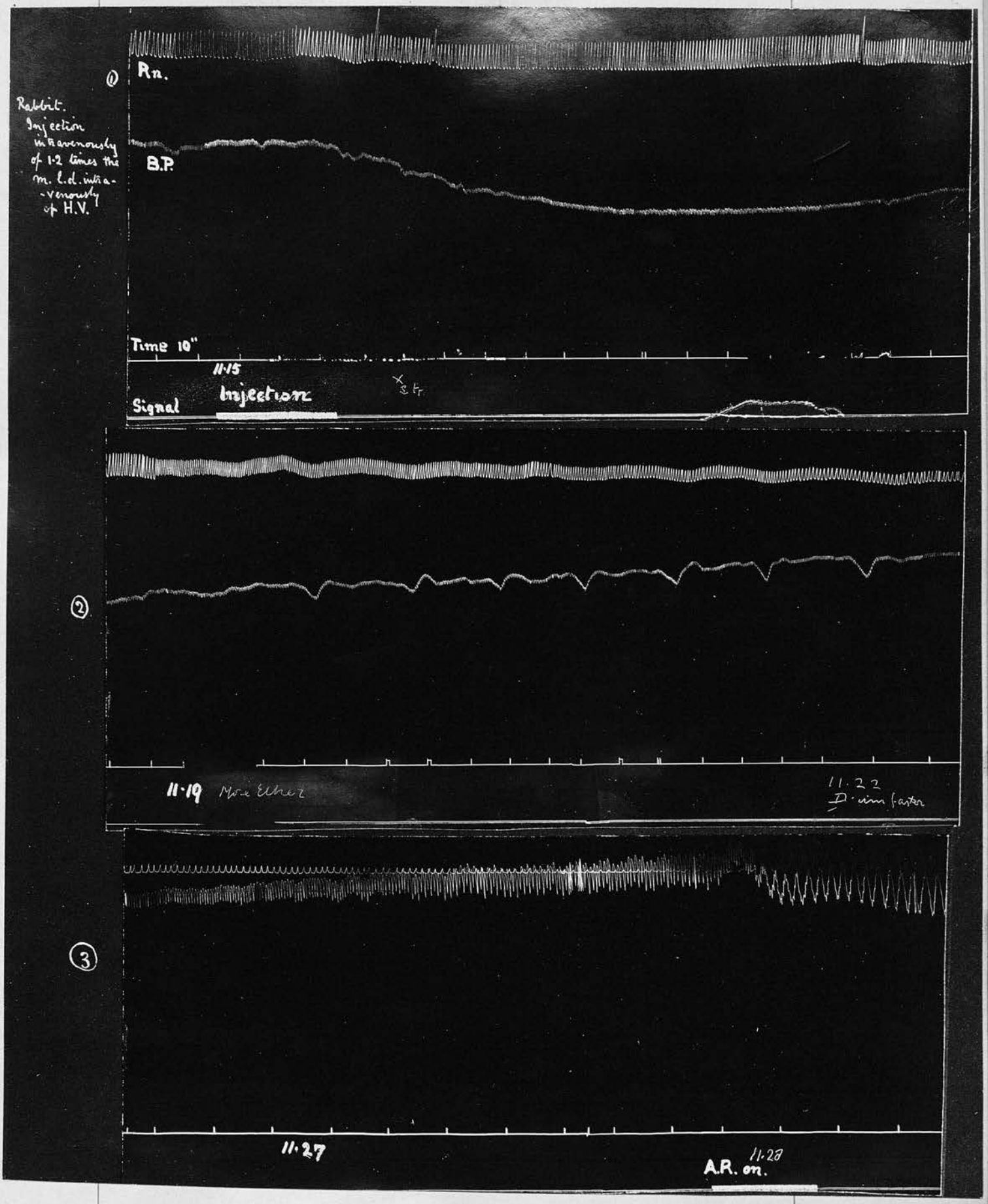
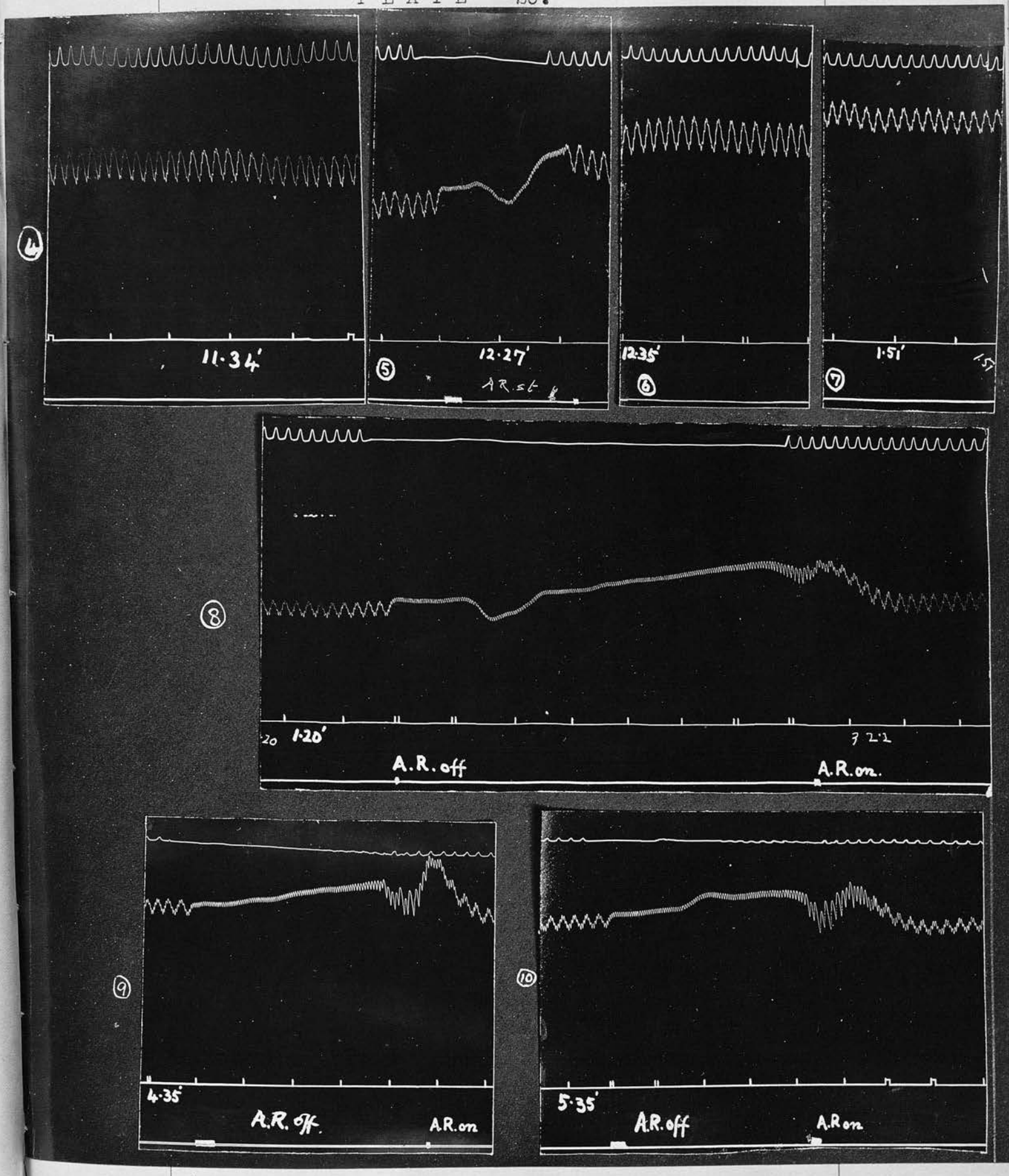

①
Rn.
Rabbit.
Injection intravenously of 1·2 times the m. l. d. intra-venously of H.V.
B.P.
Time 10"
11·15
Injection
Signal
②
11·19 More Ether
11·23 D. vim faster
③
11·27
11·28
A.R. on.

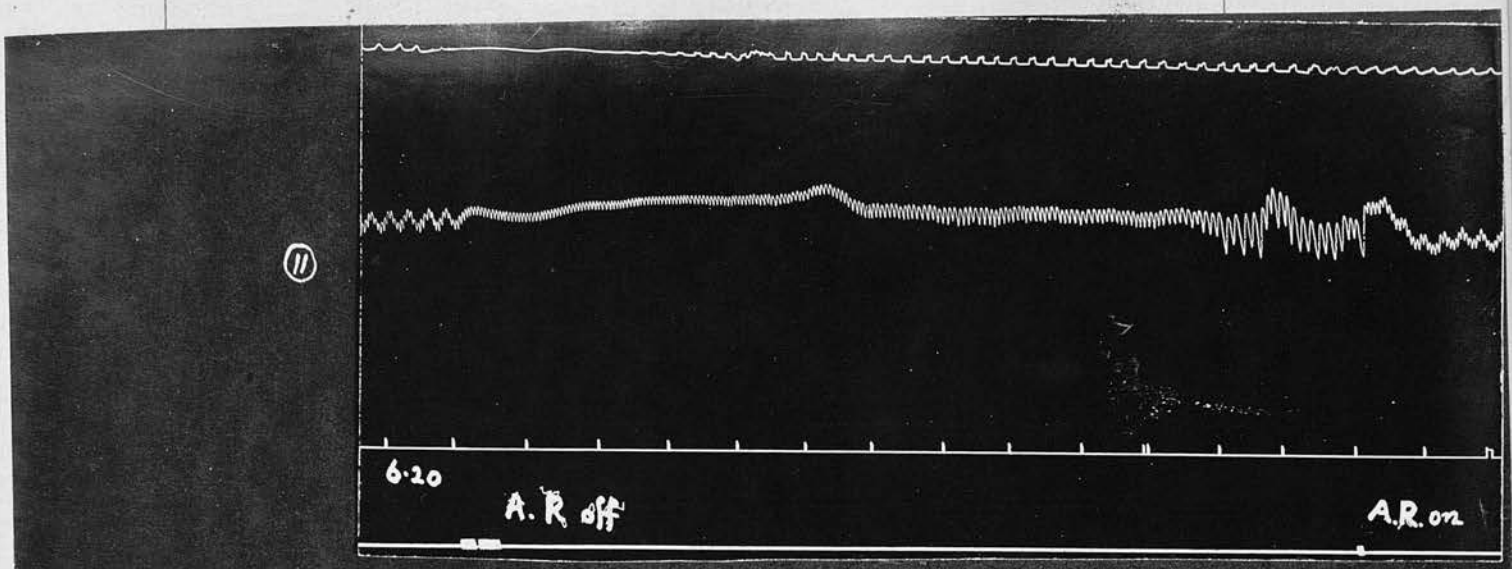
PLATE 20.

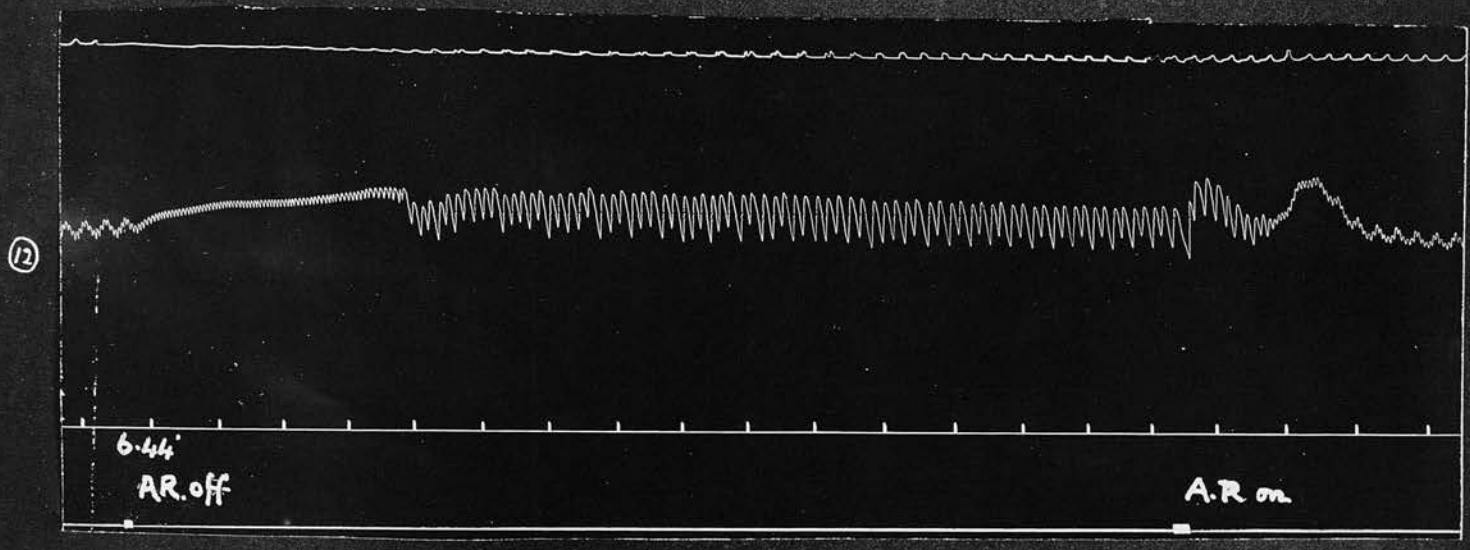

④
11·34'
⑤
12·27'
A.R. st
12·35'
⑥
⑦
1·51'
⑧
1·20'
A.R. off
A.R. on.
⑨
4·35'
A.R. off
A.R. on
⑩
5·35'
A.R. off
A.R. on

Time.	Rate of Heart per 10"	Resp. Rate per 10"	Resp. Excur-cus.	Blood Pressure.	Remarks.
6.44		Stopped A.R. for 160".			Blood very venous.
6.46	6				Fig. 12.
6.56		Stopped A.R. for 210".			Vol. resp. movements. Plate 22, Fig. 13.
6.59	5				
7.	19	A. R. on.			
7.10	17			92	Plate 21, Fig. 14. No vol. Movements. No asphyxial rise.
7.13	4 before,				
7.14	18 after.			79	
7.18	19			79	Stim. left vagus. Active at 100. Plate 22, Fig. 15.
7.25	19			79	
7.30	22			74	Fig. 16. Experiment stopped.

When the tracing was stopped the thorax was opened and the phrenic nerves were tested and found to be active with the coil at 240 m.m. We see therefore that with the minimal lethal dose there is to some extent a restoration of the natural respiratory movements by the prolonged use of artificial respiration, even when the animal is in such unfavourable conditions as were experienced here. These were the very rapid poisoning at the beginning, the extreme venosity of the blood at the end of the periods during which artificial respiration was suspended, and the effect of the anaesthetic continuously administered for hours. The nerve ends, however, even under these conditions are not paralysed.

PLATE 21.





for (13) see next plate.

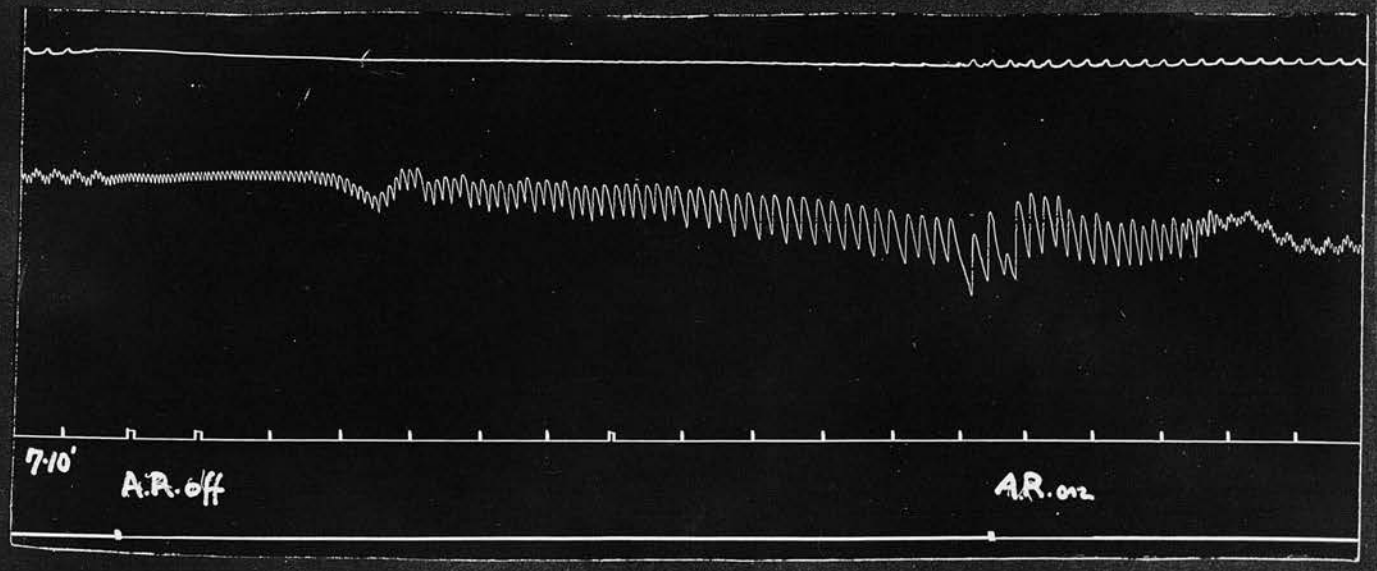


PLATE 22.

It was interesting to observe that in the earlier stages of the experiment the asphyxia caused a rise in the blood pressure, which was maintained for a considerable period after the artificial respiration was resumed. During the later period, however, this effect was less and less marked, until towards the end. when asphyxia caused no rise and the blood pressure steadily fell. This appears to point to a paralysis of the vaso-motor centres.

Experiment 81. Plates 23, 24, and [illegible]. 15th June 1905. Lab. Temp. 68° F.

To a buck rabbit of 1,990 grammes there was administered in the same manner, 6 milligrammes of the venom. This dose was equivalent to 2½ times the minimal lethal, and caused paralysis of the respiration in 7 minutes after the injection. Under artificial respiration the heart remained beating efficiently at the end of 4½ hours, the rate, however, being somewhat slower.-

Time.	Rate of Heart per 10"	Resp. Rate per 10"	Blood Pressure.	Remarks.
11.44	46	16	115	
11.46.30"			114	Inj. 6 mgrs, H.B. Plate 23, Fig. 1.
11.47.30"	55	24	98	Inj. completed.
11.50				Fig. 2.
11.51	54	7	138	
11.53	49	Art. Resp.	138	Fig. 3, Plate 24.
12.5	42		133	
12.25	42		103	Left vagus active at 150 m.m.
12.25	42		103	
12.38	47		100	
12.54	38		105	
1 .9	37		105	
1.56	39		88	
2 .20				Fig. 4.
2.33	34		89	
2.34				Left vagus active at 150 m.m.
3.43	30		74	
3.44				" " "
4.14	27		79	Fig. 5. Experiment stopped.

At 4-28 the thorax was opened, the phrenic nerves responded at 290 m.m., the vagus at 120 m.m. arrested the heart. When the sciatic nerve was exposed and stimulated, it responded at 150 m.m. The nerve ends were therefore intact 4½ hours after having been exposed to the poison, which was sufficiently active to paralyse the respiration in less than 10 minutes.

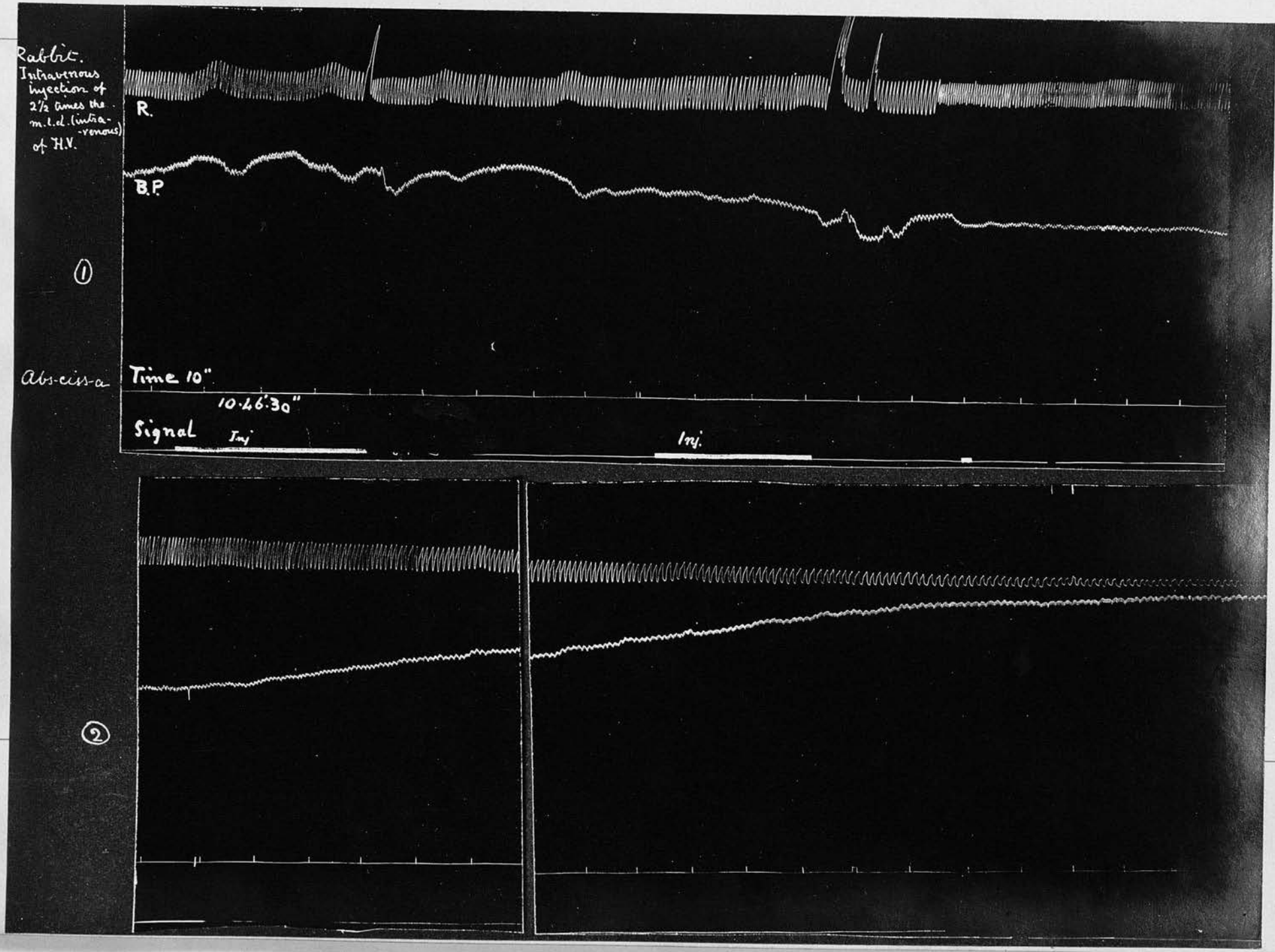
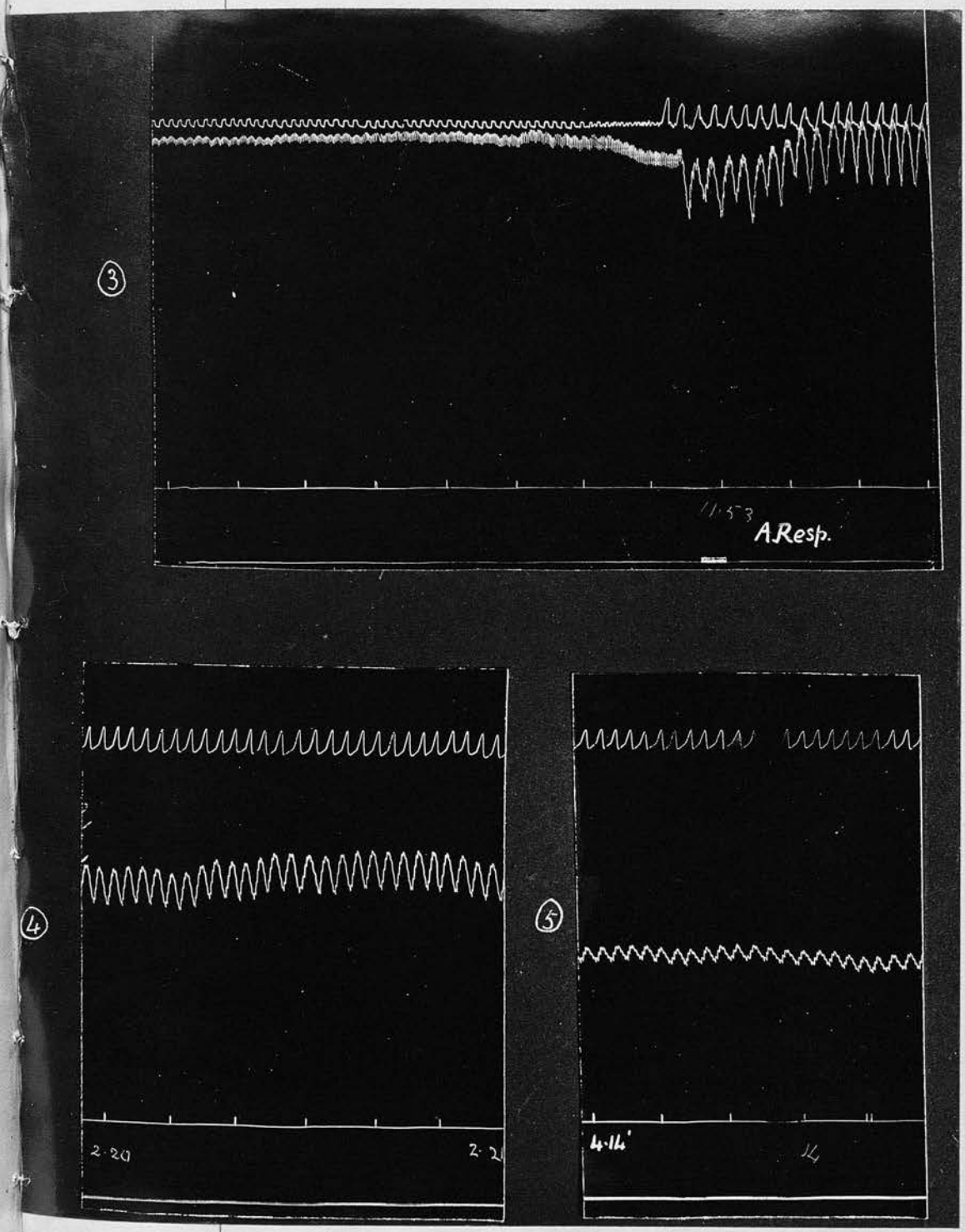

Rabbit.
Intravenous injection of 2½ times the m.l.d. (intra-venous) of H.V.
R.
B.P.
①
Abs-cis-a
Time 10"
10.46.30"
Signal
Inj.
Inj.
②

PLATE 23.

138

PLATE 24.



In the next experiment in which rather more than a lethal intravenous dose was given, and in which the dose was repeated in 45 minutes the vagus was stimulated before the poisoning and at various intervals after to see whether there was any increase or diminution in the excitability of the nerve ends. Fifty minutes after the paralysis of respiration, the animal having been kept alive by artificial respiration, it was killed, and the phrenic nerves found to be active. There was no evidence of the vagus nerve being markedly affected in the direction either of stimulation or of paralysis.

Experiment 82. 10th June 1904. Lab. Temp. 67° F,. Weight of rabbit, 1877 grammes. Dose 0.0021 gr. per kilo. repeated in 45 minutes. The total being 0.0042 grammes per kilo. The rabbit was injected at 4-30 p.m. The vagus responding to a stimulus of 150; at 5.44 the respiration ceased, the vagus having shown no change; at 6-15 the vagus responded at 140;;and at 6-35 at 130. At this time the phrenic nerve was exposed and found to be active with the coil at 370 m.m.

In the next Experiment a larger dose was given, about 4 minimal lethal doses, and this dose was repeated after 2½ hours, during which time the vagus

was repeatedly tested. The total dose was therefore about 8 times the M.L.D. by intravenous injection. and the animal was killed 3 hours after the completion of the second injection, at which time the nerves were found to be active.

In view of the fact that a slightly larger dose administered at one time, (Experiment 79) had caused a rapid and complete fall in the blood pressure it is interesting to observe the fall in the blood pressure which occurred after the second injection, which was, however, very much more gradual. The heart was beating 2 hours and 50 minutes after the second injection, which shows that the arrest of the heart in the former experiment was more influenced by the concentration of the dose than by its actual total amount.

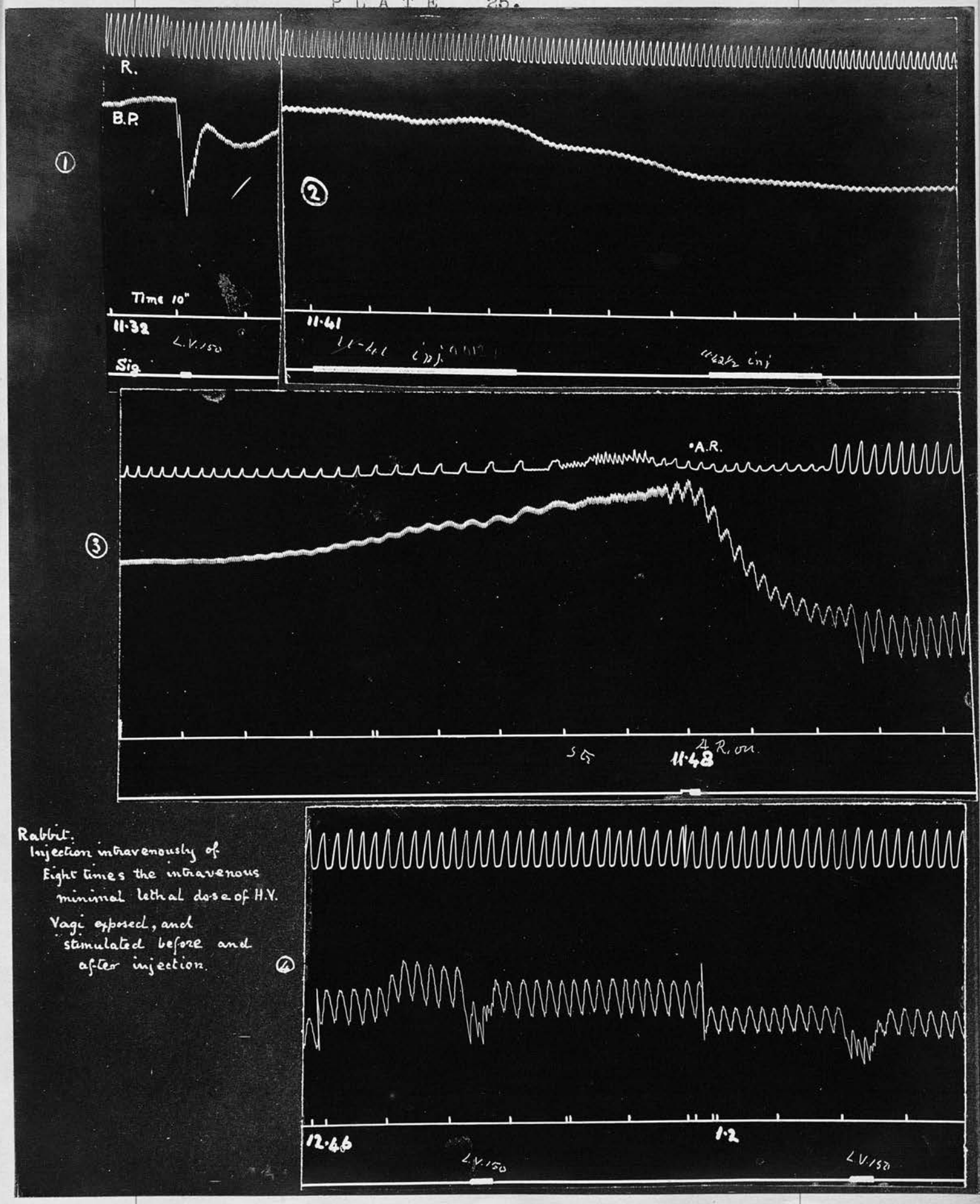
Experiment 83. Plates XXV and XXVI. 17th June 1904, Lab. Temp. 70° F. Weight of rabbit 2297 grammes. Dose 0.012 grammes, injected intravenously, and the same dose repeated in 2½ hours. The total dose was therefore 0.0104 grammes per kilo., or rather more than eight times the minimal lethal dose.

Time.	Blood Pressure.	Vagus Stimulation.	Remarks.
11.31	121 m.m.	150 m.m.	Plate 25, Fig.1.
11.41	110		Inj. of .012 grs. Fig. 2.
11.42	80		
11.47	105		Fig. 3, Resp. ceased.
11.49	70	150	
12.1	62	"	
12.15		"	
12.35		"	
12.46		"	Fig. 4.
1.2	66	"	
1.58	72	100	Plate 26m Fig. 5.
2.22	68		
2.23			Inj. 0.012 grammes.
2.40		100	
2.47	48		
2.56		110	Fig. 6.
3.44	44	"	Fig. 7.
4.49	30		
4.59	11	"	
5.12	38	"	Fig. 8.

At 5-13 the animal was killed, the phrenic nerves exposed and found to be active at 330 m.m.

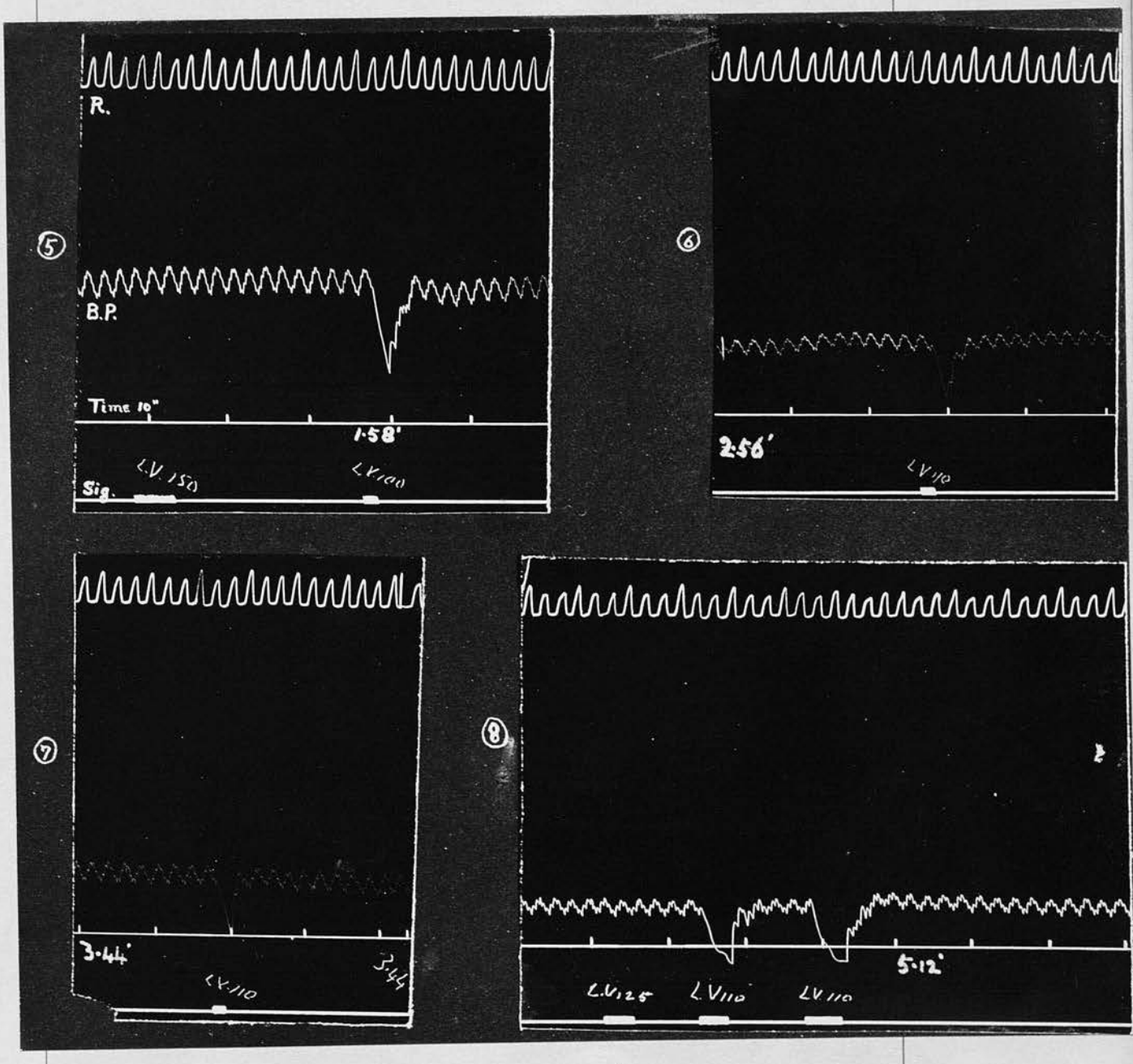
An interesting feature in the blood pressure curves is the initial fall, occurring immediately after the injection, which is checked by the failure of respiration and the production of asphyxia causing stimulation of the vasomotor centre by the venous blood. This initial fall is accompanied by a slight slowing of the heart, and is not due entirely to a stimulation of the vagal centre as is shown by the fact that a similar fall occurs after the vagi have been previously severed.

PLATE 25.



Rabbit.
Injection intravenously of
Eight times the intravenous
minimal lethal dose of H.V.
Vagi exposed, and
stimulated before and
after injection.

PLATE 26.


R.
B.P.
Time 10"
1·58'
L.V. 150
L.V. 100
Sig.
2·56'
L.V. 110
3·44'
L.V. 110
3·44
5·12'
L.V. 125
L.V. 110
L.V. 110

Experiment 84. 7th June 1904, Lab. Temp. 65° F, Weight of rabbit 2125 grammes. Practically the same dose was administered in this experiment as in experiment 79 – a dose that is of about 9 times the minimal lethal. In order to guard against the rapid paralysis of the heart by a similar local action, dilution was doubled, and the period of injection rather prolonged. The dose given was 20 mgrs. in 4 c.c. of Ringer's solution:-

Time.	Rate of Heart	Blood Pressure.	Remarks.
12.19	51	94	
12.20			Left vagus cut.
12.21			Right " "
12.24	48	161	
12.26			Inj. lasting 3
12.28	42	107	mins. 20 secs.
12.29	46	127	
12.30			Artificial resp.
12.31	45	148	
1.	38	75	
2.43	31	38	
3.41	28	33	
4.42	22	22	
5.			Heart arrested in diastole.

Death took place therefore in about 4½ hours after this injection; the symptoms following much the same course as those in the experiments with smaller doses in which the vagus was intact.

The post mortem appearances after this large dose and prolonged period of time were limited to slight congestion of the various organs. The blood showed no abnormality, and no trace of haemolysis after 24 hours.

In Experiment 85 the same large dose was given, after the vagi had been cut. The vagi were stimulated before and after the injection, and appeared to retain the same amount of activity. Towards the end of the experiment artificial respiration was intermitted for various periods, and in each case caused a rise in blood pressure until asphyxia was well established, after which the blood pressure fell. This rise took place when the artificial respiration was stopped 1 hour and 30 minutes after the injection of the poison. This shows that even with so large a dose the vaso-motor centre remains active, and it may be contrasted with Experiment 80, in which a smaller dose was given, and the animal kept alive for 8 hours, at the end of which time the rise in blood pressure was not seen.

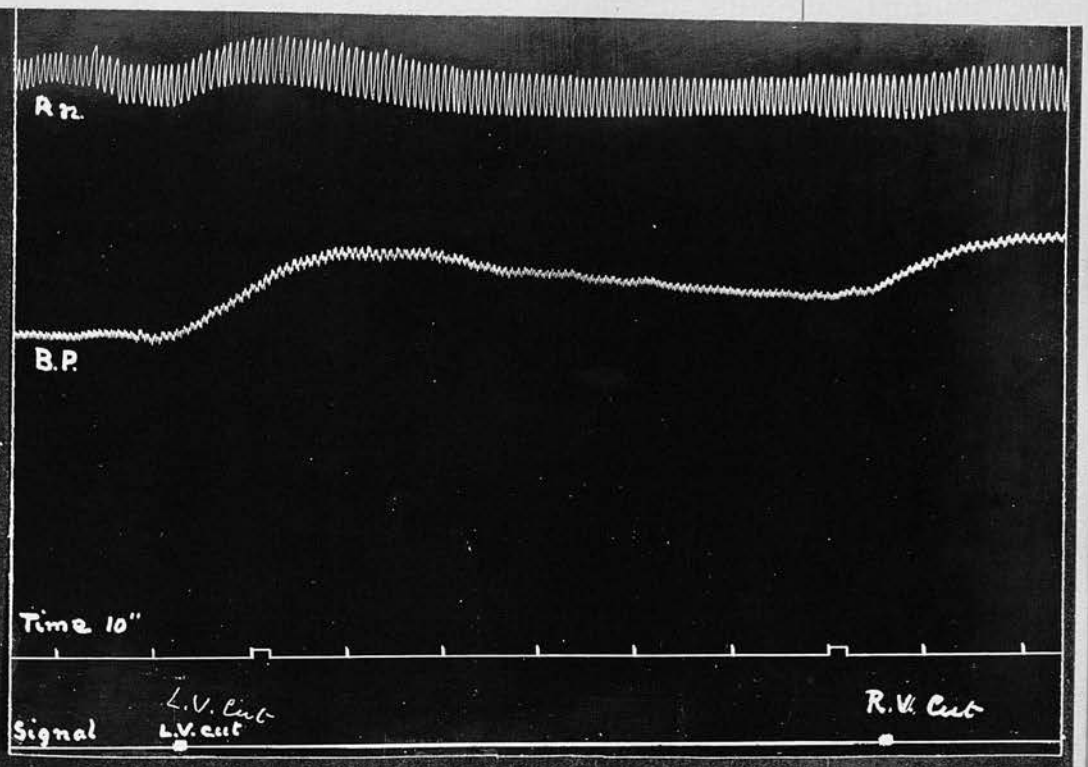
This difference shows that the element of time is of considerable importance in the poisoning of the nerve structures other than the respiratory centres. A smaller dose with a longer period of time in which to act produces an effect on the vasomotor centre greater than 9 times that dose will produce in a shorter period. The element of time being allowed sufficient to produce an effect on nerve ends has been observed by Ragotzi(/3) and other observers with regard to the action of cobra venom.

Experiment 85. Plates 27, 28 and 29. Dose 0.01 grammes per kilo. Weight of rabbit 1550 grs. Lab. Temp. 65° F, Both vagi divided:-

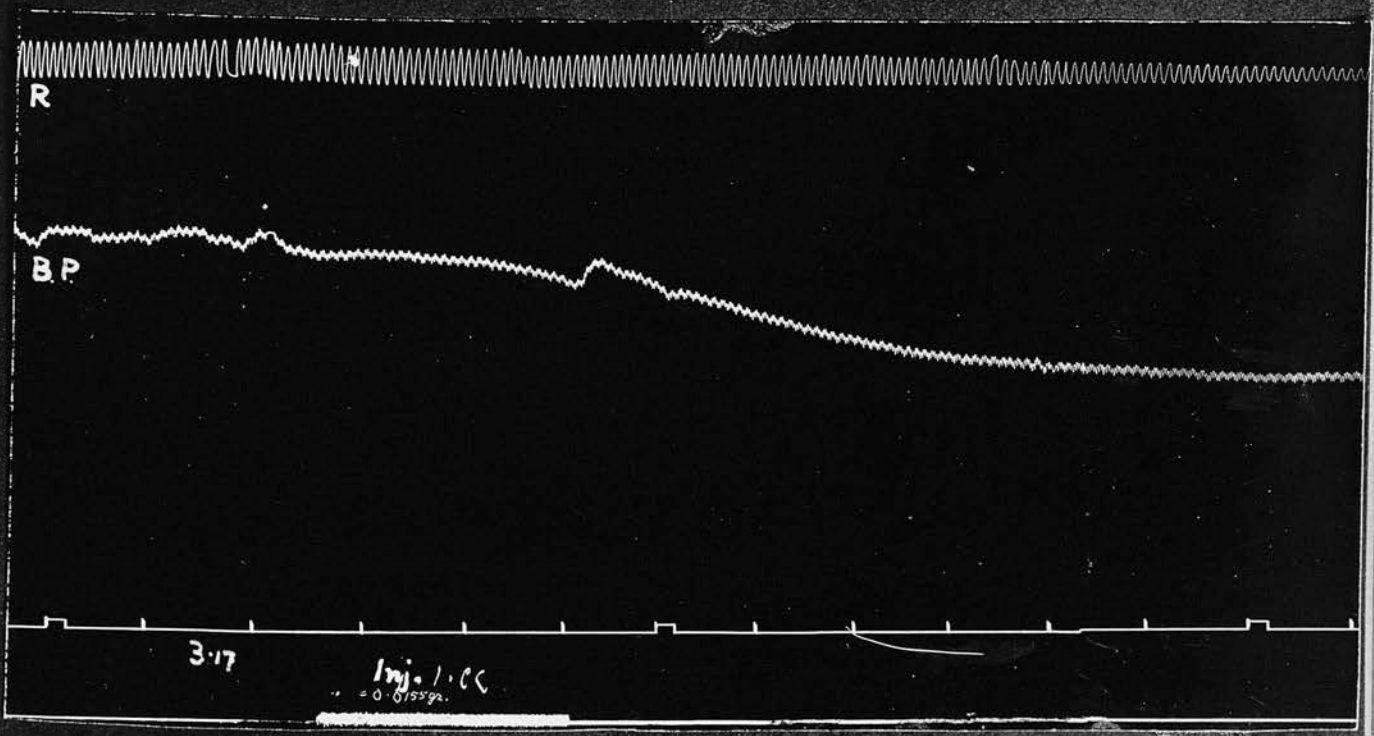
Time.	Rate of Heart. per 10"	Resp. per 10"	Resp. Excursus.	Blood Pressure.	Remarks.
3.13.30"	51	16	4 m.m.	96 m.m.	Abscissa for B.P. is signal line.
3.13.50"					Left vagus cut.
3.14.	49	15	5	106	
3.15					Right " " Fig. 1.
3.16	51	14	5.5	122	
3.16.30"					Vagus active at 150 m.m,
3.17	47	11	5	114	
3.17.25					Inj. .015 gr. Fig. 2.
3.19	48	11	2	90	
3.20.30"	48	Artificial Respiration.		128	Plate 28, Fig. 3.
3.21.33"		begun.		102	
3.29	30			91	Vagus active at 150 m.m.
3.40	41			55	
4.28					Vagus active at 100 m.m. Fig. 4.
4.31	29			66	
4.31.20"		Artificial respiration stopped for 130".			Fig. 5.
4.41		" " for 100".			Fig. 6.
4.47	15			14	

The heart appeared to be affected therefore in precisely the same way, whether the vagi were intact or severed.. with regard to the fall of the blood pressure, but with the vagi divided, the heart maintained the same rate of contraction.

PLATE 27.



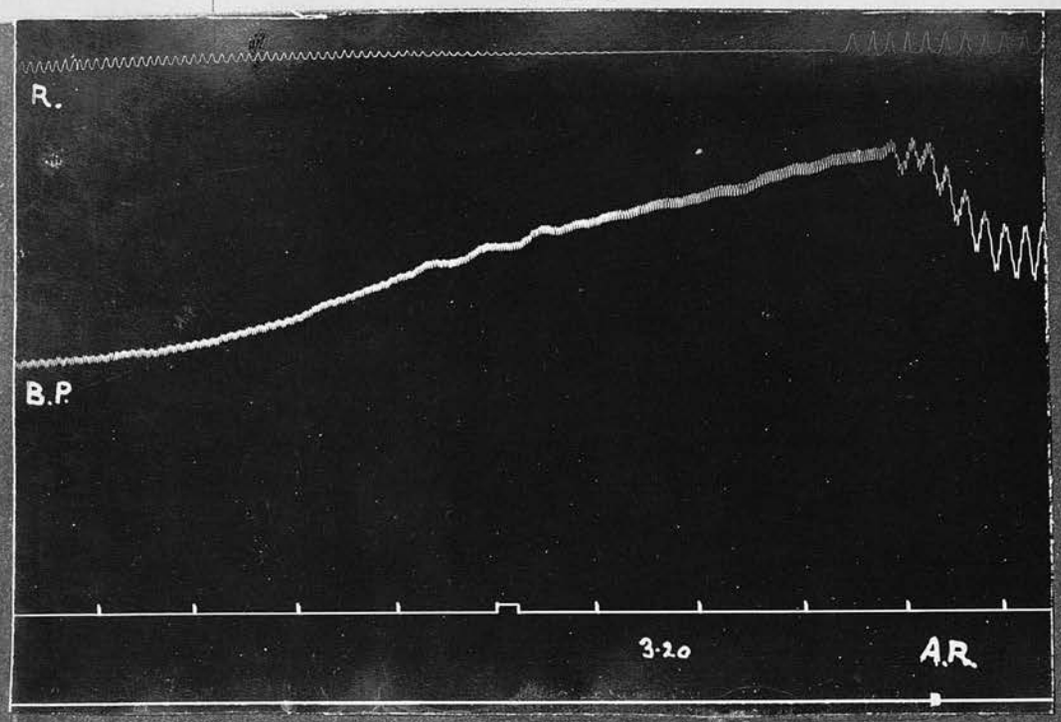
① Before Injection.



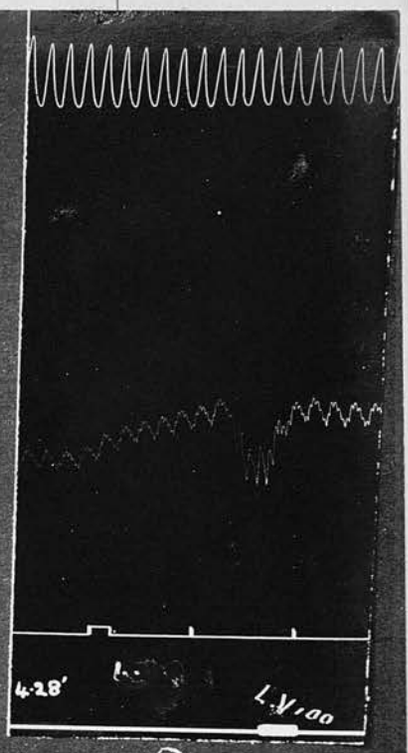
②

Intravenous injection of Eight times the minimal lethal intravenous dose for Rabbits of H.V. Both Vagi previously divided.

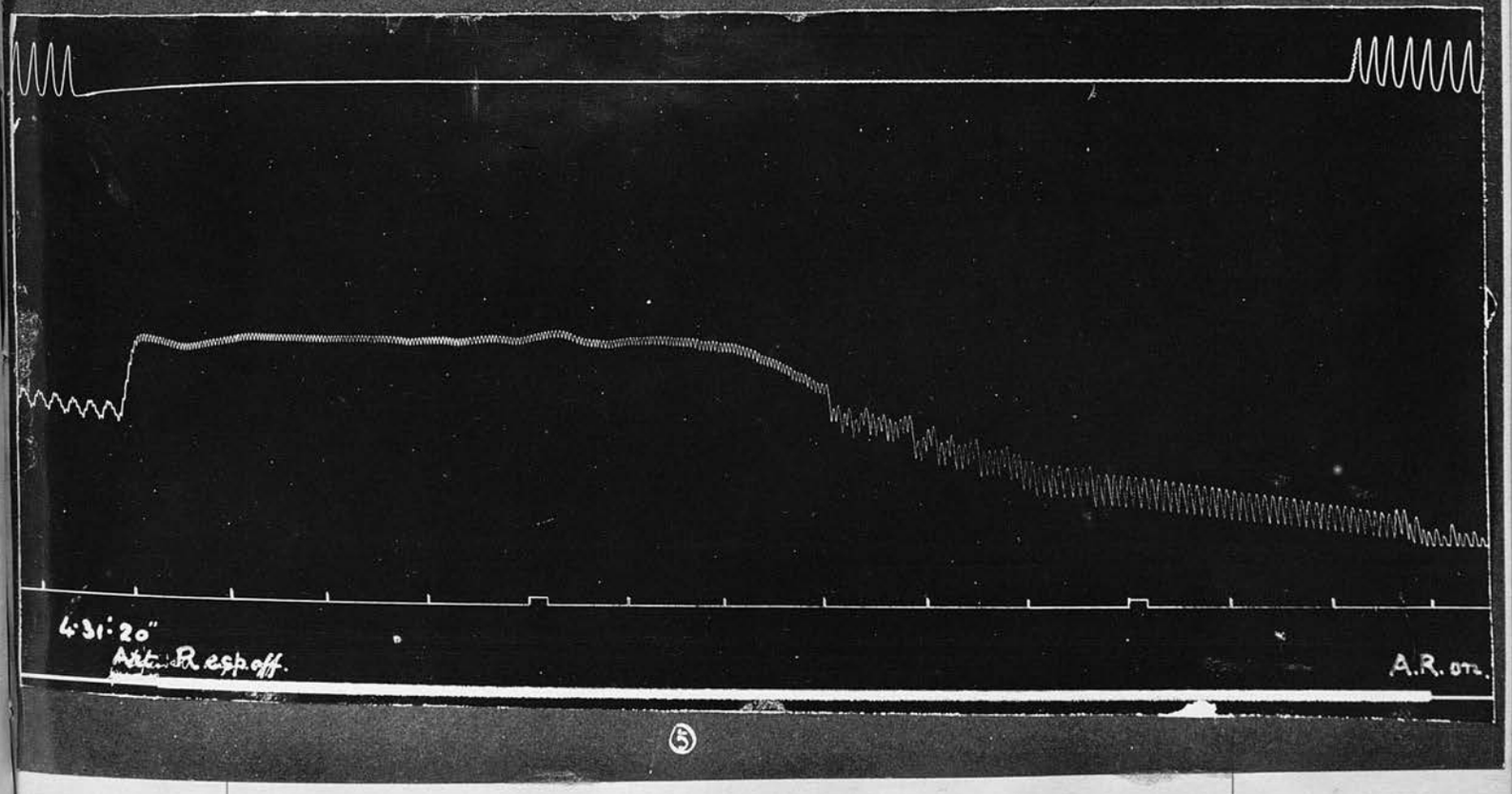
PLATE 28.



(3)

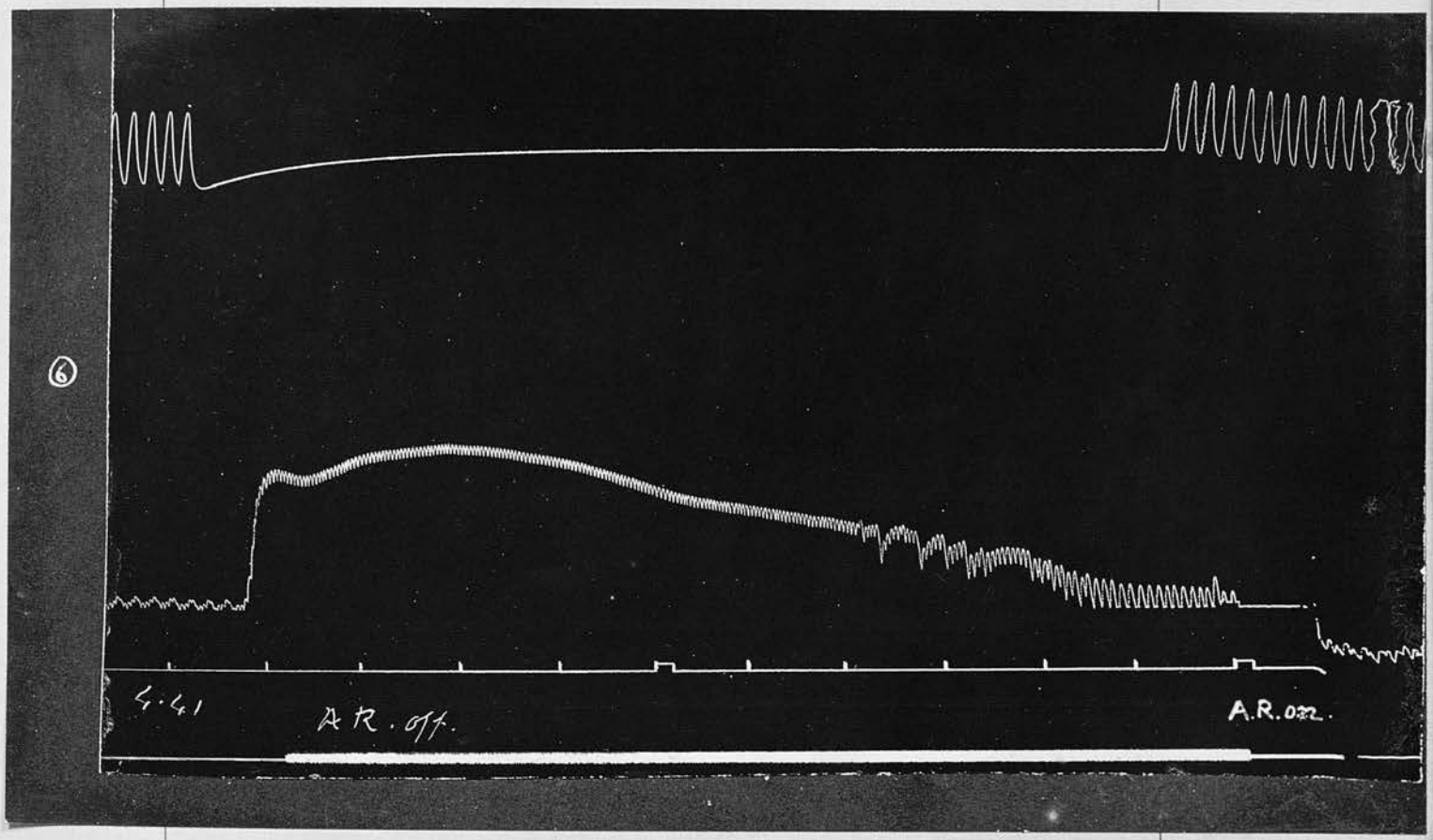


(4)



(5)

PLATE 29.



In order to see if possible the alteration produced on the contraction of the heart of such a large dose, a tracing was taken by means of levers attached to the right auricle and right ventricle respectively. The blood pressure was recorded at the same time. The rate of the heart. in this experiment, did not appear to be much altered, but the amount of auricular and ventricular contraction was distinctly affected. After the immediate effect of the injection upon the heart had passed off the heart recovered apparently completely.

Experiment 86. Plate 30. 8th January, 1906. Lab. Temp. 61° F. Weight of rabbit 2110 grammes. Dose 0.021 grammes in 1 c.c. of Ringer's Solution. This dose was equivalent to .01 grs. per kilo., and was injected slowly, the injection occupying a period of 80". At 2-10 p.m. the animal was anaesthetised, the respiratory tube tied into the trachea, the cannula tied into the left carotid artery, and a cannula tied into the right external jugular vein. The thorax was opened and the heart exposed. Hooks were attached to the right auricle and right ventricle and connected by means of silk threads to two levers, writing the one above the other. When the thorax was opened the respiratory tube was attached to the artificial respiration apparatus, which was started

at 3-10. At 3-25 the tube in the artery was connected to the manometer, and the tracing recorded. The time marker was the abscissa of the blood pressure:-

Time.	Rate of Heart per 10".	Amplitude of Ventricle,	Blood Pressure.	Remarks.
3.45	43	9	53	
3.45.30"	44	9	53	
3.46	44	9	53	
3.46.10"	43	9	53	
3.46.20"	42	9	53	Inj. begun.
" 30"	41	8	53	Plate 30, Fig.
" 40"	41	8	53	1.
" 50"	38	8	53	
3.47	41	9	50	
" 10"	38	9	50	
" 20"	39	8	48	
" 30"	38	6	46	
" 40"	40	8	42	Inj. ended.
" 50"	40	5.5	40.5	
3.48	39	5	34	
" 10"	39	5	34	
" 20"	38	4	32	
" 30"	38	4	31	
" 40"	39	4	30	
" 50"	40	4	30	
3.49	40	4	28	
3.49.30"	41			
3.50	39	4	28	
3.50.30"	41	4	28	
3.51	41	4	24	
3.54	40	5	26	
4.7	38	6	26	
4.14	35	6	28	Fig. 2.
4.15	35	6	28	
4.31	26	3	22	Fig. 3.

The effect of the venom on the heart in this large dose is therefore distinct though slight. The rate is very slightly slowed, but the fall in the

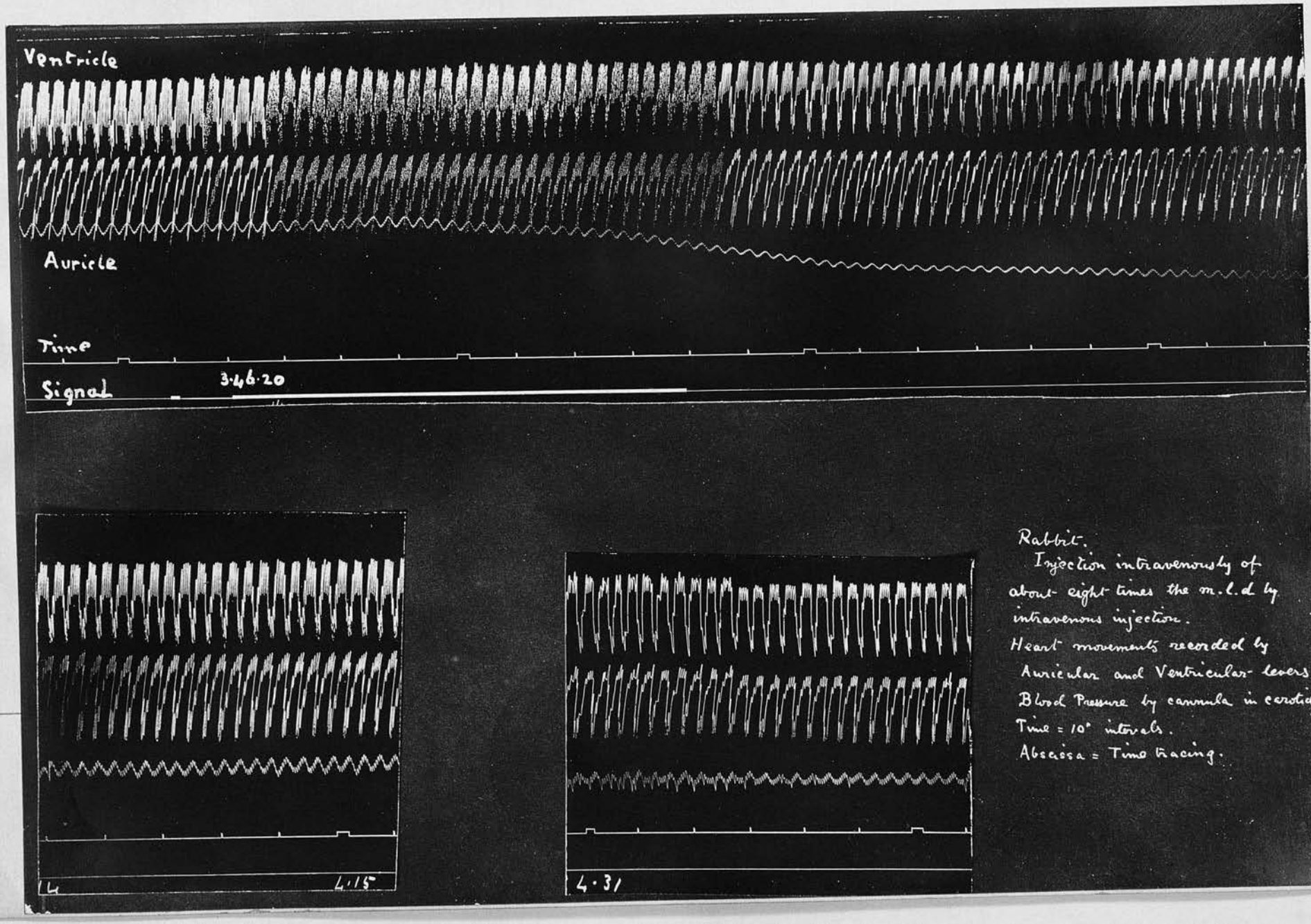


PLATE 30.

Rabbit.
Injection intravenously of about eight times the m.l.d by intravenous injection.
Heart movements recorded by Auricular and Ventricular levers.
Blood Pressure by cannula in carotid.
Time = 10" intervals.
Abscissa = Time tracing.

blood pressure is to be accounted for mainly by the diminished amplitude of the heart's contraction.

CONCLUSIONS FROM BLOOD PRESSURE EXPERIMENTS.

The conclusions to be drawn from these experiments are that the blood pressure gradually diminishes, this action being very slight with small lethal doses, more marked with large doses. A marked difference is observed therefore between this action and the action of the cobra venoms, which is distinguished by a high blood pressure. The heart is gradually slowed partly owing probably to an action on the vegal centre in the medulla, but mainly to the action of the venom on the heart itself, where present in sufficient quantity.

The action of these venoms being in certain respects so similar, we are enabled to confirm the conclusions arrived at by the investigators of the actions of the cobra venoms when they lay much stress on the action of the venoms on the blood vessels as an important factor in the maintenance of a high blood pressure. The Hamadryad venom being free from this action on the vessels causes therefore a consistently low blood pressure, especially where the dose given is sufficient to affect the heart directly.

DETERMINATION OF MINIMAL LETHAL DOSE BY SUBCUTANEOUS INJECTION FOR CATS.

No. of Expt.	Dose in gr. per kilogr.	Weight of cat in kilos.	Quantity of venom in grammes injected.	Amount of Solution.	Result.	Remarks.
87	0.0053	1.625	0.008	in 1 C.C.	Recovery.	Strongly affected.
88	0.0105	1.180	0.033	in 1 C.C.	Recovery.	Very ill for 24 hours.
89	0.02	2.305	0.046	in 1.5 C.C.	Death.	Died in 2 hrs. 50 mins.
90	0.03	1.805	0.054	in 1.5 C.C.	Death.	Died in 3 hrs.
91	0.05	1.698	0.085	in 1.5 C.C.	Death.	Died in 2 hrs. 50 mins.

The symptoms of poisoning in the cat may be seen in the description of experiment 90. In order to economise the venom, the larger doses were administered to half grown cats so that the effect is rather more pronounced than would be the case had a full grown animal been taken. The smaller lethal doses, however, were injected into full grown cats and the minimal lethal dose found to be between 0.01 and 0.02 grammes per kilo.

Experiment 90. 12th February 1906. Lab. Temp 56° F, Half grown male cat, of weight 1810 grammes. At 11-30 a.m. the cardiac impacts were 34 per 10", the respirationsll to 15 per 10", and the rectal temp. 102.4, at

2.25 p.m. 0.054 gr. of the venom, dissolved in 1C.C. Ringer's Solution was injected subcutaneously into the right flank and washed in with .5 C.C. of the solvent.

At 2.40 the cardiac impacts were 33 per 10", and the respirations 14. The animal, which had previously been very active, now preferred to sit quiet. The respirations were rather deeper than they had been at the time of the injection.

At 3 o'clock, the cardiac impacts were 36 per 10", and the respirations 14. The cat had remained quiet, and was now lying on its side coiled up with the head drooping.

At 3.30, the rate of the heart was 35 per 10", the respirations 10 to 14 per 10", and the temp. 101.2. The cat was sitting up, the eyes half closed and the respiratory movements were interrupted occasionally by a slight choking sound or cough.

At 4.12, the heart was felt to be beating at the rate of 42 per 10", strongly and regularly, but the respiration had diminished to 10 per 10" and were shallow and jerky. The animal sat quietly with its head resting on the table. When it was disturbed it could raise itself and walk about for a few seconds, but soon regained its former position.

At 4.30, the muscular weakness was more pronounced. The cat was unable to lift its head and lay on one side. The respirations were 5 per 10", gasping and irregular. When the eyeball was touched the eyelid moved slowly marking a diminution of sensitiveness. The heart was at the rate of 40 per 10", beating strongly and regularly.

At 4.50, it sat up for a moment, and then fell on its side, and remained in that position with its head resting on the table. The cornea was quite insensitive, the rectal temperature was 99.6. The respirations were 5 per 10", shallow and jerky.

At 4.57, it tried to rise to its feet but was unable. The heart was slower and more irregular, the rate varying from 25 to 28 per 10".

At 4.59, some urine was expelled, and at 5.3 there were slight convulsive movements. The pupils, up to this period, had become more and more contracted, and the respirations had diminished in rate to 4 per 10". They had also altered in character, the abdominal movement preceding the thoracic appreciably.

At 5.9, there were slight movements as if to shift the position, and the respirations were still 4 per 10", of a slightly gasping nature. There was no noise accompanying the movements, and no salivation.

At 5.15, the rate of the heart was 16 per 10", and the respirations 3 per 10". There was a slight convulsion, the hind and fore legs jerking.

At 5.19, the respirations stopped, and the pupil dilated. The heart was beating, gradually slowing in its rate and became imperceptible at 5.22. Artificial respiration was performed by alternately compressing and expanding the ribs, and at 5.23, the rate of the heart had risen to 25 per 10", and the pupils again contracted. Artificial respiration was performed until 5.48, during which time the pupil remained contracted so long as the respiration was efficient; when it was intermitted the pupil at once dilated and the heart slowed.

At 5.48, the nerves were exposed and tested, the phrenic responded at 1450, the vagus at 100, and the sciatic at 360.

The post mortem appearances were those of slight congestion, there being a little more irritation at the seat of injection and oedema than had been observed in the case of the rabbits. The blood, however, showed no abnormality.

The symptoms of poisoning in the cat are very like those in the rabbit and the rat, and are probably produced by precisely similar actions.

An interesting comparison can now be made with regard to the comparative lethality of these venoms, which we have been contrasting. The following table, in which the doses are expressed in grammes per kilogramme, presents these results:-

	African Cobra.	Indian Cobra, (Fraser.)(17)	Indian Cobra. (Elliot.)	Hamadryad.	Krait.
Rabbit.	.0003	.000245	.0006	.0025	.00008
Rat.		.00025	.0005	.003	.001
Frog.	.001	.0002		.004	.0005
Cat.		.005	.01	.02	

The proportions may be shortly expressed, taking the smallest dose in each case as unit in the following table:-

Rabbit	1	1	1	1	1
Rat.		1.1	1.2	1.2	12
Frog.	3.3	1		1.6	6
Cat.		20	20	10	

The relationship between the lethality proportions of the Hamadryad and Cobras are fairly consistent, and differ considerably from that of the Krait.

ACTION ON THE BLOOD.

The action of the venom on the blood is as we have seen very slight. In many of the experiments determinations were made of the numbers of the red and white blood corpuscles. The number of the former was found to be practically unaltered during all stages of the poisoning. The latter were, however, generally increased. The enumerations were made by means of the Zeiss-Thoma Haemocytometer.

In the cat for example, which received 0.03 grammes per kilo., and which died in 3 hrs. the leucocytes before poisoning numbered 14,800 per cubic millimetre. Two hours after poisoning they were increased to 15,600, and three hours after poisoning they numbered 17,000.

In the cat which received 0.01 grammes per kilo. and which recovered, the leucocytes before poisoning were 16,100, and three hours after poisoning were 18,300. In twenty four hours they were 20,400, and in forty eight hours 19,700. On the fourth day they were 18,500, on the sixth day 17,700, and on the fourteenth day 16,500. The increase appears to be mainly due to the increased number of finely granular eosinophile leucocytes.

With regard to the haemolytic action of the venom which was found to be non-existent in corpore in the quantities employed in the experiments and which has been investigated by Lamb(11) we may state that it is very much less than that possessed by the cobras and krait.

When washed corpuscles of frogs blood and of rabbits blood were mixed with the poison complete haemolysis occurred when the poison was present in the strength of 1 in 500, and 600; partial haemolysis was seen in the cases of strengths of 1 in 700 and 800 and none in solutions weaker than these.

PATHOLOGY.

In all the examinations made post-mortem the appearances other than those due to asphyxia were very slight, being limited to evidence of congestion of the kidneys, liver, etc. and the subcutaneous tissues at the seat of inoculation. And these appearances were very much less in amount than those which we are accustomed to see in cases of poisoning by the other venoms. At the same time the alteration in the cells of the anterior cornua of the cord described by Lamb and Hunter [1] as occurring in some monkeys poisoned with Indian Cobra venom, and by Prentice in frogs poisoned with African Cobra venom is /

is probably similar in the case of hamadryad venom.

The desquamative nephritis found by Fraser and by Prentice in the case of Indian and African Cobras respectively also but to a less extent probably occurs in hamadryad poisoning.

GENERAL SUMMARY.

Toxicity. This specimen examined possessed $\frac{1}{10}$ th of the activity of the cobra venom used by Fraser and $\frac{1}{4}$th probably of hamadryad venom used by Rogers. In this connection it is interesting to note that in another specimen of Hamadryad venom which I have examined but the results of which are not incorporated in this paper the activity much more nearly approximates in the toxicity for the rabbit to the figure given by Rogers for warm blooded animals.

Nervous System. Its action is mainly on the central nervous system, and it acts most rapidly on the Respiratory centre in the Medulla. The reflex conductivity of the cord is also affected and finally the peripheral ends of the nerves are affected, but much more slightly and much later during the course of the poisoning.

Circulatory System. The action is comparatively slight and is in the direction chiefly of lessening the activity of the heart. The heart is arrested in gradually//

gradually increasing diastole and its normal death is apparently hastened. There is in addition a primary inhibition produced which is to some extent of central origin, probably a stimulation of the vagal inhibitory centre.

The blood vessels are practically unaffected by any direct action on their walls; and the blood except for a leucocytosis and for the changes of an asphyxial nature practically unaltered.

Skeletal Muscle. The local action is comparatively slight and is in the direction of producing slightly more rapid fatigue and normal death.

Temperature. The venom causes a rise in the temperature in the cases of sub-lethal doses and to a slight extent with small lethal doses. A rapid fall is seen with large doses.

I must excuse the omission of several experiments which might be performed in order to render more definite our knowledge with regard to the action on the circulation through the nervous system, but the comparatively low toxicity coupled with limited amount of venom have prevented me from performing as many experiments as would otherwise have been desirable.

I /

I must in conclusion express my thanks to Sir Thomas Fraser for all the facilities afforded me and for his kind permission to use this material.

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