# SOME NOTES ON THE ACTION

of

# AFRICAN COBRA VENOM

(Naja Haja)

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The following research was conducted by me while holding the Vans Dunlop Scholarship in Materia Medica. Professor Sir Thomas R. Fraser suggested as a subject of enquiry the investigation of the action of the venom of the African Cobra (Naja Haja), and placed at my disposal an ample supply of this venom. He himself had obtained in 1895 and 1896 a number of the dried poison glands from Mr. J. W. Van Putten of the Clan William district of South Africa, and from them had extracted the venom in a nearly pure state.

I am also indebted to Sir Thomas Fraser for the interest taken in the progress of the work and for directions as to the points to be investigated, and the methods of their investigation. Sir Thomas Fraser had in 1897 already determined the minimal lethal dose of this venom for rabbits and the general character of the symptoms it produces, in connection with an as yet unpublished work on immunisation and on the antidotal influence of bile from various animals on this venom. He observed that the general effects and the lethality were very similar to those of the Indian Cobra Venom.

Beyond/

Beyond Sir Thomas Fraser's researches I have not been able to find that any other work has been done with the African Cobra Venom.

On the other hand, ~~many workers have investigated~~ the action of the Indian Cobra Venom and of a considerable number of other Venoms. *have engaged the attention of many investigators.*

Workers on the action of Indian Cobra Venom, with the exception of Cunningham, have established the fact that it has a profound action on the various parts of the nervous system.

Cunningham believes that the primary action of Cobra Venom is on the blood, and that the nervous phenomena observed, are a result of the altered state of this circulating medium. While other workers do not deny that cobra venom has an action on the blood, they hold that there is an action primarily on nerve tissue.

Lauder Brunton and Fayrer, who were among the first to conduct a systematic research on this subject, find that Indian Cobra Venom
(1) paralyses the termination of the motor nerve ends
(2) depresses the function of the spinal cord as a reflex centre, and as a conductor of motor impulses.

They/

They find that small doses act upon the motor nerve terminals, and that large doses affect the reflex mechanism in the spinal cord, but no definite dosage is given.

On the higher centres, they find that Indian Cobra Venom exerts an action in the shape of paralysing the respiratory centre.

They find that sensory nerves are not affected. As regards the action of the venom on the circulation Brunton and Fayrer find that, with the dose administered by subcutaneous injection, the heart is arrested in systole. This they also find to be the case when venom in solution is applied locally to the excised frog's heart. The cause of this systolic arrest is attributed by them to a local action of the venom on the motor ganglia, in the direction of stimulation. Besides the above actions on the nervous system, it was found by these workers that in some experiments crenation of the red blood cells occurred on injection of venom subcutaneously. The doses so injected were large, but the exact proportion of dose to body weight of the animal employed is not stated.

Brunton and Fayrer find that muscle tissue is profoundly affected by solutions of Indian Cobra Venom and that the change is in the direction of diminished irritability of the muscle with finally complete/

complete loss of contractile power on direct electrical stimulation.

The blood pressure they find to fall gradually.

Aron finds that Indian Cobra Venom kills muscle by a direct action. He denies any effect on the motor nerve ends, but agrees with Brunton and Fayrer in stating, that it paralyses the reflex centres in the spinal cord and also destroys the function of the cord as a conductor of motor impulses.

As regards respiration, Aron finds that Indian Cobra Venom causes a cessation of it by paralysing the respiratory centre of the medulla. On the circulation according to this worker, the venom exerts the same effects on the heart as are observed by Brunton and Fayrer, but he makes the additional statement that in small doses the ventricle stops in diastole. This result he considers due to paralysis of the vagal centre.

In his work the doses employed are large, certainly much above the minimal lethal dose, and no attempt was made either by him or by the previous workers to determine such a minimal lethal dose.

Wall finds the same results as the previous workers in so far as the effects of the venom on the conduction of motor impulses by the spinal cord and on the motor nerve ends are concerned. Respiration is/

is paralysed by the action of the venom on the respiratory centreand in Wall's book we find the interesting statements, that there is primarily an increase in frequency and depth of the respiratory movements. This is followed by a diminution both in the rate and excursus. The heart he finds beating after respiration ceases. As regards the action on the heart Wall finds there is a primary acceleration of the pulse rate.

The blood pressure he finds to fall. As regards the action of the venom on the red blood cells - crenation is noted post mortem. The liver is found by him to be much congested and to bleed freely on section, and the intestinal canal is also injected.

Ragotzi discusses the earlier literature, paying special attention to the work of Brunton and Fayrer and Aron, and while bringing the objection above noted as regards dosage against their results he is not without the same failing in his own work. He makes no attempt to determine an accurate minimal lethal dose although the doses he employs are not so large as those of previous workers. This investigator finds that with large doses the effect is mainly exercised on the heart and the characteristic effects on the nervous system are not obtained. Those latter effects, and especially the action of the venom on the nerve terminals, are, according to him, best obtained when small doses are employed. He does not however discuss/

discuss the effects of graduated doses on the various parts of the nervous system.

He finds paralysis of motor nerve ends to be a prominent feature in the action of Indian Cobra-venom. The reflex function of the spinal cord is, according to Ragotzi, not destroyed. The venom kills by paralysing respiration, this being brought about by an action on the ends of the nerves supplying the respiratory muscles and is not due to an action on the centre in the Medulla.

Ragotzi finds that Indian Cobra-venom is a muscle poison although the nervous phenomena are evident long before the muscles are affected. No curves, such as can be obtained by recording muscle twitches by means of a lever writing on a revolving drum, are adduced in support of this, and his results are inferred from the observation of the feebleness of contraction on stimulating the muscle. Ragotzi finds that in comparatively large doses there is no effect produced on the Vaso motor centre. He agrees with Aron that after small doses the heart stops in diastole of the ventricle and after large doses in systole of that organ. In the blood he observed haemolytic phenomena.

Weir-Mitchell and Reichert, who worked mainly on the action of Crotalus venom, have some interesting experiments on the action of Cobra Venom (Indian) on/

on the pulse rate, arterial pressure and respiration.

They find an increase in the pulse rate, which in the majority of their experiments was followed by a decrease.

On the arterial pressure there is found by these workers to be a primary fall followed by a rise, and the statement is made that the blood pressure may rise above normal as death is reached.

Respiration they find to be affected in the direction of a primary increase in the number and and depth of the individual respirations, followed by a diminution both in the number and depth . Much of Weir-Mitchell and Reichert's work is performed with the globulin and peptone that they separated from the crude poison, but later researches consider that the venom is really a proteose. Blyth separated what he considered to be Cobric acid from Cobra Venom but this was disproved by Norris Wolfenden . Myers says that Cobra Venom contains substances having two distinct actions, one a haemolytic agent and the other a nerve poison. Along with Stephens this opinion he is able to confirm and they consider that the haemolytic agent is a coagulable proteid and the nerve poison an incoagulable proteid. They also find that "in vitro" haemolysis can be obtained but "in corpore" there is no evidence of haemolysis: but in certain dosages there is delay in, or complete absence of clotting in the blood.

Vollmer supports the opinion that haemolysis can/

can be obtained "in vitro" and not "in corpore" , and Lamb and Hanna, who contrast the actions of Cobra and Daboia Venoms find that the former possesses no agent that causes intravascular thrombosis, whereas the latter is dependent for its effects on the organism upon this property.

Auché and Louis Vaillant in some experiments upon guinea-pigs performed with venom - the kind and dosage unfortunately not being stated, find that there is a marked leucocytosis. They also state that there is a rapid destruction of the red blood cells, but that if the animal recover, they soon return to normal numbers, and large nucleated corpuscles make their appearance.

Nowack finds that in animals killed by the action of snake venoms the liver and kidneys show marked changes when examined microscopically. He describes a condition in the kidney that resembles acute desquamative nephritis and finds in addition a condition of fatty degeneration in liver and kidneys. African Cobra Venom I have found to possess the power of producing acute nephritis and marked congestion of the liver but I have not been able to establish the existence of any fatty degeneration, either in kidney or liver.

In a very interesting preliminary note upon the action of snake venom on the nervous system, Lamb and Hunter find that with Indian Cobra Venom, degenerative changes are produced in parts of the motor/

motor neurones. They have been experimenting with monkeys and rats, and they realise that the time element is important, as they believe the animals died before there was time for extensive degenerative changes to occur. In some experiments on frogs that were performed in the early summer of last year, and which are incorporated in the present paper, the changes found in the anterior cornual cells are described. In the frog, time is allowed for the venom to exercise its action on the various parts of the nervous system, as the circulation continues long after the respiration is paralysed. This fact makes the frog a much more favourable animal in which to study such changes than is a warm blooded animal.

The sample of venom given to me by Professor Sir Thomas R. Fraser was in the form of a coarse powder of a bright yellow colour and freely soluble in water, the watery solution being neutral in reaction.

For the purposes of this research small quantities of the venom were dissolved in sterilised water, to which a little camphor was added, as required, and the effects were studied upon frogs and rabbits.

The/

The first point determined was the minimal lethal dose for frogs. Male frogs, (species Rana Temporaria), were employed. The injections were made subcutaneously in the back. The results are seen in the following table.

| No. of Expt | Wt. of Frog. | Amount injected | Amount per Gramme Wt. | Result |
|---|---|---|---|---|
| I. | 25 grammes | .015 milligramme | .0006 mg | Recovery, few symptoms |
| II. | 27 " | .02 " | .00073 " | " marked " |
| III. | 25 " | .025 " | .001 " | Death on 8th day |
| IV. | 30 " | .045 " | .0015 " | " " 8th " |
| V. | 28 " | .05 " | .0018 " | " " 9th " |
| VI. | 20 " | .1 " | .005 " | " " 9th " |
| VII. | 20 " | .2 " | .01 " | " " 3rd " |
| VIII. | 20 " | .3 " | .015 " | " " 3rd. " |
| IX. | 21 " | .4 " | .02 " | " " 5th " |
| X. | 25 " | .625 " | .025 " | " in 20 hours. |
| XI. | 25 " | .75 " | .03 " | " within 20 hours. |

The general symptoms, produced by the subcutaneous injection of African Cobra Venom, are well seen in the following experiments, selected from those in the above table.

Exp.VI.

March 10th 1903. Temperature of Laboratory 55°F. Frog (R Temp.) Male, Healthy, Weight, 20 grammes, Respirations 24 in 10 seconds. Heart beats, 9 in 10 seconds as seen through skin of pectoral region.

At 2.25 p.m./

At 2.25 p.m. .5 c.c. of a solution of African cobra venom in sterile camphor water (strength 1 mg in 5 c.c) was injected under the skin of the back. The total quantity of venom injected was therefore .1 mg, and the amount per gramme wt. of frog was .005 mg. Immediately after injection the frog was restless.

3.25 p.m. Animal is disinclined to move, but jumps well when stimulated.

4.15 p.m. Respirations are slower 18 in 10 seconds, and there are occasionally two or three deep gasps.

4.30 p.m. Animal sluggish: on pinching toe the frog moves leg away but does not jump : can still make voluntary movements.

5 p.m. Condition same.

7 p.m. There is weakness of anterior extremities so that thorax rests on the table. The respirations are feeble but regular: their rate is 12 in 10 seconds. Heart beats are visible but faint: their rate is 8 in 10 seconds.

7.20 p.m. moves spontaneously but sluggishly and after exertion takes two or three deep gasps. The reflexes are still active, and the frog turns quickly when laid on the back.

7.35 p.m./

7.35 p.m. All the ventral aspect is now resting on the table - animal is more sluggish.

7.45 p.m. Respirations are very feeble: rate is 7 in 10 seconds. On pinching skin of flank, the frog moves hind limb of same side, and foot is moved to part irritated. Movements occur in all the other limbs.

8.0 p.m. When laid on back frog finds great difficulty in turning on to belly. Convulsive movements are seen of abdominal muscles. While frog is lying on back, the heart is seen beating feebly at the rate of 7 contractions in 10 seconds. The respirations are exceedingly feeble and are at the rate of 7 in 10 seconds.

8.5 p.m. When frog was laid on back it remained in this position for three minutes and after much endeavour regained position on belly. The frog occasionally raises its thorax on fore legs but the head soon sinks down again. The respirations are exceedingly feeble: the reflexes are active: the animal can crawl sluggishly a little way and when stimulated by pinching a toe can jump. The heart beats are still regular and visible - rate 7 in 10 seconds.

8.30 p.m. When laid on back the frog remained in this/

8.30 p.m. this position after one or two endeavours to turn. The respirations in this posture were not visible. Heart beats still visible. When laid on belly, the respirations were seen to be very weak, their rate being 8 in 10 seconds. The animal makes straining movements with abdominal and throat muscles, simulating retching. Frog is disinclined to move, although it moves on pinching toe and very occasionally makes spontaneous movements.

9.30 p.m. Condition is same as at 8.30 p.m. Frog placed under inverted funnel in a little water.

11th March.

10.30 a.m. This morning the respirations are only occasional and very weak: two or three occurring in the 10 seconds. The heart is seen beating regularly and fairly strongly, its rate is 6 in 10 seconds. The animal makes very feeble attempts to regain ventral position when laid on the back. The reflexes are still active both to mechanical and electrical stimulation, and the animal can still make spontaneous movements, but the hind limbs do not resume their normal attitude of flexion for several minutes after movement.

12 noon. Respiration has ceased: heart still beating at/

12 noon at rate of 6 in 10 seconds: the reflexes are feeble.

3.15 p.m. Animal lying quite flaccid: very feeble and much delayed reflex movements are obtained in stimulation. Heart still beating regularly at rate of 6 beats in 10 seconds.

12th March.

Animal in same condition as yesterday.

13th March.

11 a.m. Frog is in same condition as yesterday. Reflexes which are exceedingly feeble and much delayed are obtainable on pinching toe or skin of abdomen. The heart is beating regularly but feebly at the rate of 5 in 10 seconds.

7 p.m. The animal remained in the same condition all day and at 7 p.m. the heart beats were with great difficulty made out: the rate being still 5 in 10 seconds. Very feeble reflexes are present in all limbs on pinching the skin.

14th March.

Animal flaccid: feeble reflexes still obtainable: heart beating regularly at rate of 6 in 10 seconds.

On the 15th and 16th of March animal remained in same condition.

17th March/

17th March. The reflexes are now confined to limb pinched. Electrical stimulation by means of an induced current causes contractions in muscles underlying the position of skin stimulated with the secondary coil at a distance of 80 mm.. from the primary. No reflex movement is obtained with this strength of stimulation. The heart is seen to be beating feebly at the rate of 6 in 10 seconds.

18th March Heart is very feeble: rate, 5 beats in 10 seconds. No reflexes are obtainable on pinching the skin, nor on strong electrical stimulation. The muscles are more irritable than yesterday and contract when secondary coil is at a distance of 140 mm from the primary.

19th March 11 a.m. Animal in same condition as yesterday is lying quite flaccid. The heart beats are made out with great difficulty: rate 4 in 10 seconds. No reflexes are obtainable: Muscles respond to stimulation with secondary coil at 120 mm from primary.

3 p.m. Heart has ceased to beat and on exposure the auricles were seen to be dark and dilated, the ventricle is moderately contracted. No response is obtained on stimulating the sciatic nerve even with the/

3 p.m. the secondary coil at 10 mm. Muscles respond well with secondary coil at 90 mm. Blood films and a capillary tubeful of blood were kept for examination, as were also the kidneys, which were much congested. A cut section of the muscles was alkaline to test paper.

Expt.VII. March 11th 1903. Temp. of Laboratory 56° F Frog male, healthy, Weight 20 grammes. Respiration 24 per 10 seconds. Heart beats 10 in 10 seconds.

11.30 a.m. Injected under skin of back .2 mg African Cobra Venom: this equals .01 mg per gramme weight of frog. The animal was much excited and jumped about wildly.

11.45 a.m. Respirations are irregular and quicker, the rate being 27 in 10 seconds with an occasional pause. The frog is sitting up in a natural condition.

11.55 a.m. On jumping there is slight delay in flexing the hind limbs after completion of jump. The respirations are now deeper, slower and very irregular, rate being 14 in 10 seconds.

12.40 p.m. Respirations are much weaker, rate being 12 in 10 seconds: they are irregular. The animal is disinclined to move but can jump well when stimulated. Animal can still sit up in natural attitude. Heart beats are now at rate of 8 in 10 seconds.

1.30 p.m. Condition is same as at 12.40 p.m. but there is some weakness and extension of the fore limbs.

2.15 Respirations are now very infrequent: occasionally deep gasps are made. Frog can still move about. The fore limbs are more extended. The reflexes to mechanical stimulation are active, and the animal can still jump well.

2.45 Frog is lying with whole ventral aspect, head included, on the table.

3.15 Respiration has now ceased. The heart is still beating regularly at the rate of 8 contractions in 10 seconds. The animal makes straining and retching movements with the abdominal and throat muscles. On pinching toe, reflex movements occur in all limbs and animal can make a feeble jump after which there is seen marked slowness in flexing the limbs, [illegible]. The frog is unable to turn when laid on its back although strong endeavours are made to do so.

3.45 Animal lies unresistingly on its back: the heart beats are slower and more feeble being now 6 in 10 seconds. General strong reflex movements occur on pinching skin of one limb. When laid on belly animal can make feeble spontaneous movements.

12th March/

12th March.

10 a.m. Animal appeared dead , is lying quite flaccid. No reflex movement is obtained in pinching skin or on the strongest electrical stimulation. No cardiac impact could be seen on the most careful examination, but on exposing the heart it was found to be beating feebly at the rate of 54 in the minute. Diastole of the ventricle is complete, systole not quite complete. The auricles and ventricle are beating at the same rate. The whole heart after 80 beats in one instance and 95 in another ceased in diastole for 2 seconds and then resumed beating. The sciatic nerve in the right leg was exposed. There was considerable oedema of the subcutaneous tissue and the muscles were very pale. Very feeble movement occurred in one toe on stimulating the sciatic nerve with an induced current, with the secondary coil at a distance of 10 mm from the primary. No reflex movement occurred even when stimulation of the sciatic nerve with the secondary coil at zero was employed. The muscles will respond to stimulation directly with the secondary coil at 120 m.m. The heart was kept moist with saline solution (·65% Sodium Chloride in water).

11 a.m.

11a.m. Systole of ventricle is feeble, the ventricle remaining dark at end of it.

11.30 Rate of heart is 36 per minute.

12 noon Heart's contractions very feeble- auricular contractions are more powerful than the ventricular, and each contraction of the auricles is followed by a contraction of the ventricle. Rate is now 35 per minute.

3 p.m. Condition is same as at 12 noon. The frog was kept moist by placing it on a pad of wool soaked in saline solution.

13th March.

11 a.m. The heart was found motionless in diastole though there is some mottling of the ventricle. The heart does not contract when pinched, and on electrical stimulation with secondary coil at 10 mm.. there is only slight dimpling between the points of the electrodes. The sciatic nerve was freshly exposed in the left leg, and on stimulation, even with the secondary coil at zero, no contraction was obtained in muscles of leg or foot. The muscles by direct stimulation respond with secondary coil at 90 mm. distance from primary. Blood films and a capillary tubeful of blood from heart were taken.

At 4 p.m. muscles still contract to stimulation with the/

the secondary coil at 90 mm. A cross section of muscle is neutral in reaction to litmus paper.

Kidneys liver and gut were much congested, and kidneys and portion of liver were removed for microscopic examination.

Expert.II. 19th March 1903. Temp of laboratory 56°F

Frog, Male, Healthy. Weight 27 grammes.

11.35 Respiration 24 in 10 seconds. Heart beats 19 in 10 seconds.

11.40 Injected .4 c.c. solution of African Cobra Venom in sterile Camphor water = .02 mg or .00073 mg per gramme weight of frog. Animal for rest of day showed bo symptoms.

20th March.

Animal is somewhat oedematous, weight being now 32 grammes, respirations regular, 20 in 10 seconds. Heart beats 9 in 10 seconds: reflexes active: can jump about.

21st March. Animal is markedly oedematous. Weight is now 40 grammes: respirations still active, rate being 18 in 10 seconds. Heart beats masked owing to the great oedema. Reflexes active: jumps well.

23rd March. Weight is now 43 grammes. Respirations are irregular, rate on average being 16 in 10 seconds with occasional deep respiration.

The/

The frog jumps more sluggishly: the reflexes remain active.

24th March. Weight is now 47 grammes. Respirations are regular in rate - 12 in 10 seconds - with a deep gasp every 3rd or 4th respiration. Can jump well: reflexes are active.

25th March. Weight 43 grammes. Respirations regular in time and nature of successive events. Rate 16 in 10 seconds - otherwise as yesterday.

26th March. Animal is recovering. Weight is now 35 grammes. Respirations regular in time and depth, rate being 20 in 10 seconds.

27th March. Animal quite recovered.

Expert IV. 19th March 1903. Temp. of Laboratory 56°F
Frog, Male, Healthy. Weight 30 grammes.

2 p.m. Respirations 24 in 10 seconds. Heart beats 7 in 10 seconds.

2.15" Injected .9 c.c. solution of Cobra Venom = .045 mg or .0015 mg to gramme weight of frog.

20th March. Frog lively: can jump well; reflexes to skin pinching are active. The animal resists attempts to lay it on its back. Respirations are now only occasional and occur after exertion. Heart is beating at rate of 7 in 10 seconds. Animal is slightly oedematous, weight being 32 grammes.

21st March/

21st March Animal is more oedematous, weight now being 35 grammes. Otherwise its condition is the same .

23rd. March Animal is very oedematous. Weight 45 grammes. Respirations occur only after exertion. Reflex movements etc are healthy.

24th March. Weight has increased to 47 grammes. Respiration has ceased. Animal is sluggish but can still jump. Reflexes are weak. There is no Corneal Reflex.

25th March. Weight 47 grammes: animal is much more sluggish and there is evident weakness of fore limbs.

26th March. Weight 48 grammes. Animal lies unresisting on its back. No reflexes are obtainable. Heart is beating feebly, rate 5 in 10 seconds.

27th March. Animal dead. Weight 50 grammes - a gain in weight of 20 grammes. There is some rigidity of fore limbs. Heart exposed and all chambers found in a state of diastolic arrest. There is great subcutaneous and intra-peritoneal oedema: fluid is colourless and slightly albuminous. The gut, kidneys and liver are much congested. Blood films and a capillary tubeful of blood were taken from blood in heart. The muscles respond to direct stimulation with secondary coil at 60 m.m. No response is obtained on the strongest stimulation of the sciatic nerve.

Summary/

## SUMMARY OF GENERAL EFFECTS ON FROGS.

From the above experiments it is seen that the first symptom of the action of the venom is an effect on the respiration. After a primary increase, we have a steady diminution in the rate with increase in the depth of the individual respirations, Later on, there is, the rate still continuing to fall, a great weakening in the depth and finally a complete paralysis. When the respiration is abolished the heart is seen to be little affected, both its rate and strength being well maintained.

In all the experiments motor weakness is well marked. This shows itself primarily in the fore limbs so that the thorax, and finally the nose come to rest on the table. During this time the reflexes are much diminished in activity, although in the frog they are obtainable long after respiration has ceased. The corneal reflex is the first to disappear. When the reflexes have disappeared the animal lies in a flaccid condition and is incapable of spontaneous movement. There is evidence of paralysis of the motor nerve ends while the muscles themselves are still capable of contraction when stimulation is applied directly to them. The heart is found to be beating up to the very end of life and after the motor nerve ends are paralysed. In small doses a marked feature of the poisoning is the great amount of oedema that occurs.

With/

With the larger doses this is not so marked, but it is still present. Post-Mortem we find great congestion of the kidneys and liver. The gut is not so markedly congested. The minute appearances in these and other organs will be described later on. The heart in an animal killed with small doses of venom is found in complete diastolic arrest of all chambers: with large doses the auricles are found dilated and dark and the ventricle moderately pale and contracted. The skeletal muscles are alkaline in reaction at moment of death.

In order to study the effects of the poison on the motor nerve ends the following experiments, showing the result of protecting one limb from the action of the poison, are instructive.

Results of protecting a limb from the action of the Venom.

Expert. XII. 17th March 1903. Temp of Laboratory 54°F Frog, healthy, male, weight 20 grammes, was pithed brain only, at 3.0 p.m. The right sciatic nerve was exposed and a ligature passed below it and tied tightly round the limb. Stimulation of the sciatic nerve induced contractions in the muscles of the leg and foot, when the secondary coil was at a distance of 100 m.m. from the primary.

At 3.20 p.m. when the frog had recovered from the shock and was able to move about, .5 mg of Cobra Venom was injected under the skin of the abdomen. This dose/

dose = .020 mg per gramme wt. Next morning (18th March) the frog was found motionless and all limbs except the ligatured one were slightly rigid.

Stimulation of the sciatic nerve of the ligatured leg produced contractions in the muscles of leg and foot with the secondary coil at 80 m.m. The muscles in this, the unpoisoned leg contract with the secondary coil at 60 m.m.

Stimulation of the sciatic nerve in the leg to which the poison had access had no effect in producing contraction; even with the secondary coil at zero. The muscles in the poisoned leg contract feebly with secondary coil at 40 m.m.

No reflex movements were obtained, even on strongest stimulation of the sciatic nerve of the poisoned leg, in the leg of the opposite side.

The heart was exposed and found motionless with auricles dark and dilated and ventricle moderately contracted.

Expert.XIII April 7th 1903. Temp of Laboratory 58°F Frog, male, healthy, Weight 27 grammes, was pithed - brain only - at 4 p.m. The right sciatic nerve was carefully exposed and a ligature passed round the limb beneath it and tied tightly round the limb. At 4.20 p.m. .7 mg of venom was injected subcutaneously This is equal to .026 mg per gramme weight of frog. At 11.30 a.m. .On April 8th. Stimulation of the sciatic/

sciatic nerve of protected limb with the secondary coil at a distance of 100 m.m. produced contractions in the muscles.

The muscles of the poisoned limb do not respond to to stimulation of the sciatic nerve even with the secondary at zero. The muscles of the protected limb respond to direct stimulation with the secondary coil at 100 m.m. The muscles of the non-protected limb respond at 60 m.m. No reflex movement is obtained in protected limb on stimulating the sciatic nerve of the poisoned leg.

No movement is obtained in either limb on stimulating a cut section of the cord.

Effect of the venom on the motor nerve ends.

From the above experiments we see that the muscles are little affected while the nervous mechanism is paralysed. In order to define more accurately the exact sphere of action of the poison the following experiments were made. A nerve muscle preparation was made consisting of the isolated gastrocnemius muscle with as long a length of the Sciatic nerve attached to it as possible. The muscle or the nerve, according as one wished to test the action of the venom on the nerve or on the nerve ends, was immersed in a solution of the venom in normal saline (.65% Sodium Chloride in water). The method employed was to place a solution of the venom in a watch/

watch glass over the edge of which was placed a microscope slide on which was placed a strip of filter paper which was kept moist with normal saline solution. Another strip of filter paper similarly moistened was in the intervals between stimulation placed over the nerve or muscle as the case might be. Before immersion of the nerve or muscle in the venom the minimal stimulation of the nerve and muscle, necessary to obtain a contraction was determined. Single opening shocks from an induction coil were employed and the figures represent the millimetres' distance of the secondary coil from the primary.

Experiment XIV. April 8th 1903 Temp of Laboratory 58°F.

Nerve muscle preparation as above.

Before immersion of muscle in Solution of Cobra Venom in normal saline, of a strength of 1 in 50,000, the muscle responded to direct stimulation with the secondary coil at 120 m.m. distance from the primary. The nerve on stimulation induced contraction in the muscle with the secondary at 200 m.m. the time being 3.10 p.m. The results of the various stimulations of the nerve and muscle are given in the table below.

| Time | Muscle (in solution of Venom) | Nerve (in Saline) |
|---|---|---|
| 3.30 | 110 mm. distance of secondary coil | 225 mm. distance of secondary coil |
| .40 | 100 " " " " " | 240 " " " " " |
| .50 | 95 " " " " " | 220 " " " " " |
| 4.0 | 90 " " " " " | 210 " " " " " |
| .10 | 85 " " " " " | 180 " " " " " |
| .20 | 83 " " " " " | 180 " " " " " |
| .30 | 75 " " " " " | 140 " " " " " |
| .40 | 75 " " " " " | 80 " " " " " |
| .50 | 75 " " " " " | 60 " " " " " |
| 5.0 | 70 " " " " " | 0 no contraction of muscle |
| 5.10 | 70 " " " " " | 0 " " " " |

Experiment XV. April 9th 1903. Temp. of Laboratory 59°F

Nerve muscle preparation as before. In this experiment the nerve was immersed in the solution of Cobra Venom (strength 1 in 50.000) and the muscle was kept moist with normal solution.

Before/

| Before Immersion of Nerve | | Nerve | Muscle |
|---|---|---|---|
| | 11.40 a.m. | 260 mm. | 120 mm. |
| After Immersion of Nerve | 11.55 | 260 | 120 |
| | 12.10 p.m. | 250 | 120 |
| | .25 | 250 | 115 |
| | .40 | 240 | 112 |
| | .55 | 260 | 115 |
| | 1.10 | 255 | 115 |
| | .25 | 260 | 110 |
| | .40 | 255 | 110 |
| | .55 | 260 | 105 |
| | 2.10 | 250 | 105 |
| | .25 | 240 | 108 |
| | .40 | 240 | 105 |
| | .55 | 240 | 105 |
| | 3.10 | 235 | 100 |
| | .25 | 220 | 100 |
| | .40 | 220 | 100 |

Experiment XVI. April 10th 1903. Temp. of Laboratory 58°F

A nerve muscle preparation was made as before. The muscle was immersed in a solution of Venom in normal saline of a strength of 1 in 100.000 and the nerve was kept moist with saline solution. Before immersion of the muscle in the solution of Venom the minimal stimulation necessary to induce contraction

was/

was determined. On direct stimulation of the muscle this was found to be 100 mm. The minimal stimulation of the nerve that induced contraction in the muscle was found to be that in which the secondary coil was 330 mm. distant from the primary.

| Time | Nerve (in normal saline) | Muscle (in Venom) |
|---|---|---|
| 12.15 p.m. Before immersion | 330 mm. Contraction of muscle | 100 mm, |
| 12.30 After immersion | 355 | 100 |
| .45 | 350 | 95 |
| 1. | 350 | 90 |
| .15 | 290 | 90 |
| .30 | 260 | 90 |
| .45 | 260 | 85 |
| 2. | 260 | 85 |
| .10 | 245 | 85 |
| .20 | 240 | 80 |
| .30 | 235 | 80 |
| .40 | 235 | |
| .50 | 200 | |
| 3. | 190 | 80 |
| .10 | 140 | |
| .20 | 90 | |
| .30 | 75 | 80 |
| .40 | 50 | 75 |
| .50 | 0 no contraction | 70 |
| 4.0 p.m. | 0 " " | 70 |

From the above table we see that after the muscle had/

had been immersed in the solution of venom for 3 hours and 25 minutes, stimulation of the sciatic nerve, even with the secondary coil at zero, failed to induce the slightest movement. The motor nerve ends therefore had become paralysed. In contrast to this, the results of the experiment XV where the nerve was exposed to the action of the Venom and the muscle kept moist in saline solution, we see that there is not more of a diminution in the excitability of the nerve or muscle than can be accounted for by the conditions under which they are placed. The two experiments XIV and XVI are also interesting in that they indicate that before the paralysis of the motor nerve ends there is a period during which their excitability is increased, since a weaker stimulation of the nerve than was necessary before the muscle was immersed in the venom is able to induce contraction in the muscle. This is well seen in the following experiment.

Experiment /

Experiment XVII April 11th 1903. Temp of Laboratory 60°F

Strength of solution of Venom 1 in 50,000.

| Time | | Nerve in Saline | Muscle in Venom. |
|---|---|---|---|
| 11. a.m. | Before immersion | 225 mm. | Secy coil 130 mm. |
| 11.10 | do. | 225 | 130 |
| .20 | After immersion | 225 | 128 |
| .30 | | 230 | |
| .40 | | 230 | |
| .50 | | 235 | |
| 12 noon | | 250 | 125 |
| .10 | | 260 | |
| .20 | | 240 | |
| .30 | | 235 | 125 |
| .40 | | 200 | |
| .50 | | 190 | |
| 1. p.m. | | 180 | 120 |
| ..10 | | 180 | |
| .20 | | 150 | |
| .30 | | 140 | 100 |
| .40 | | 100 | |
| .50 | | 80 | |
| 2. | | 30 | Secy coil at 80 mm. contraction |
| .10 | | Secy coil at 0 no contraction | 75 |
| .20 | | 0 | 75 |

Effect/

Effect on the Reflex Function of the Spinal Cord.

A marked feature in the action of African Cobra Venom is the ultimate loss of the reflexes. The following experiment shows the gradual lengthening of time that occurred before the animal responded to stimulation.

The experiment consisted in suspending a frog, of which the brain had been pithed, by means of a hook through its lower jaw. Stimulation was applied by means of a solution of Sulphuric acid of a strength of 1 in 1000. A foot was immersed at intervals of 5 minutes, care being taken that the same extent of skin was in contact with the acid on each stimulation; and the number of seconds that elapsed before the foot was withdrawn from the acid was noted.

Experiment XVIII April 12th 1903 Temperature of Laboratory 58 F. Frog, male, healthy, weight 18 grammes, was pithed, brain only, at 2 p.m. The reflex time was determined in the above noted method.

| Time | Secs elapsing before withdrawal of foot | | Remarks. |
|---|---|---|---|
| 2.30 | Right leg 3" | Left leg 3" | |
| .35 | " " 3" | " " 3" | |
| .36 | " " - | " " - | Injection of .5 mg. Venom subcutaneously. |
| .40 | " " 3" | " " 3" | |
| .45 | " " 3" | " " 3" | |
| .50 | " " 4" | " " 4" | |
| 3. | " " 4" | " " 4" | |
| .5 | " " 4" | " " 4" | |

Expert. XVIII contd

| Time | Secs elapsing before withdrawal of foot | | Remarks. |
|---|---|---|---|
| .10 | Right Leg 5" | Left Leg 6" | |
| .15 | " " 6" | " " 6" | |
| .20 | " " 10" | " " 11" | |
| .25 | " " 12" | " " 12" | Movement in left leg on stimulating Right, at 7 seconds. |
| 3.30 | " " 11" | " " 12" | |
| .35 | " " 15" | " " 13" | |
| .45 | " " 14" | " " 16" | |
| 4.0 | " " 20" | " " 17" | |
| .15 | " " - | " " - | At 20 secs attempts: no success within 60 secs. |
| .30 | " " - | " " - | At 17" in case of R.Leg and at 20" in case of L. leg attempts at removal of foot, but no success within 60" |

From this experiment we see that within two hours there is an almost complete abolition of reflex function. This method of experiment is however not very suitable, since the venom has, as has been shown, the power of paralysing the terminations of the motor nerves, and hence the inability to withdraw the foot might be due to this factor. That this is not so we have other evidence to adduce in the shape of the results obtained in Experiments XII and XIII. In these we find that stimulation of the sciatic nerve in the poisoned leg fails to induce movement in the same leg or even in the leg of which the motor nerve ends are protected from the action of the poison. Now experiment/

experiment XV shows that the nerve trunk is practically unaffected by being immersed in a solution of venom; and therefore the reflex arc must be interrupted somewhere in the spinal cord. In experiment XIII it is noted, as was also found in other experiments of a similar nature, that stimulation of the spinal cord induced no movement in either pelvic extremity, not even in the limb where the motor nerve ends were protected.

This points to the poison having affected the motor cells in the anterior cornua of the cord, so that they are no longer able to transmit impulses along the nerve and so induce a contraction of the muscle.

Evidence in support of this is obtained from the microscopic appearance of these cells. They are shrunken and the nucleus is smaller then that in healthy cells and is moreover vacuolated. This change is described by Clouston in his work Mental Diseases as occurring in degenerated cells. (Fig. I page I ).

Experiments to show that the Nervous Phenomena are not due to changes in the Circulation.

Although it has been noted that the heart continues to beat up to the moment of death yet the possibility/

possibility has to be entertained that its contractions are not powerful enough to drive the blood through the capillaries, and that the nervous changes are dependent on a failure of the circulation. That this is not so is proved by the following experiments.

Experiment XIX.

April 14th 1903. Temperature of Laboratory 60°F Frog, male, healthy, weight 16 grammes. The respirations were 22 in 10 seconds and the heart beats 9 in 10 seconds. At 12.30 p.m. .5 mg. of African Cobra Venom was injected under the skin of the back = .031 mg. per gramme weight. At 2 p.m. one and a half hours after injection, the respirations were gasping and occasional and the reflexes were very feeble and much delayed. At 3 p.m., two and a half hours after injection, respiration had ceased: the corneal reflex could not be obtained and exceedingly feeble reflex movements occurred on strongly pinching a limb. The heart was seen to be beating strongly and regularly at the rate of 9 contractions in 10 seconds. The web of a foot was spread out and examined under the microscope, when the circulation was seen to be vigorous.

At 5.30 p.m., five hours after injection, no reflexes could be obtained: examination of the web showed the circulation to be active. The sciatic nerve was exposed and on stimulation with the secondary/

secondary coil at a distance of 40 millimetres from the primary, contractions ensued in the muscles of the leg and foot. No reflex movement occurred with this or stronger degrees of stimulation.

At 8.30 p.m. the motor nerve ends were still active, although it now required a much stronger stimulation to induce contraction in the muscles: the secondary coil being at a distance of 20 mm. from the primary.

Next day 15th April at 10 a.m. the motor nerve ends were found to be paralysed, yet the circulation in the web was present although not so active as at 8.30 p.m. on the 14th of April.

Time Relation between paralysis of Reflexes and paralysis of Motor Nerve ends.

The above experiment, while it shows that the circulation is carried on after paralysis of the respiration, of the reflexes, and of the motor nerve ends, is also interesting in that it shows that the motor nerve ends retain their power for a considerable period after the reflexes are paralysed. The dose which the frog received was large and was calculated to kill within 20 hours. The same thing is observed in the experiment detailed below where a dose equal to .015 mg. per gramme weight was given.

Experiment XX

April 16th 1903. Temperature of Laboratory 60° F

Frog/

Frog, male, healthy, weight 34 grammes, received subcutaneously .5 mg. Cobra Venom = .015 mg. per gramme weight at 4 p.m.

At 6 p.m. the respirations were only occasional On April 17th at 10 a.m. feeble reflex movements could be obtained on stimulation. The heart was beating regularly at the rate of 8 in 10 seconds. The respiration had ceased and the circulation in the web was active.

At 2 p.m. no reflexes could be obtained. The sciatic nerve was exposed and on stimulation of it with the secondary coil at 80 mm. distance from the primary contractions occurred in the muscles of the leg and foot.

At 4 p.m. the motor nerve ends were still active. The heart's rate was now 6 in 10 seconds. Circulation in the web was active. The muscles respond to direct stimulation with the secondary coil at 100 mm.

At 6 p.m. Stimulation of the sciatic with the secondary coil at 50 mm. calls forth contraction in the muscles. The circulation in the web is still going on.

On April 18th at 10 a.m., (the frog having been kept moist on a pad of wool soaked in normal saline solution over night), the motor nerve ends were found to be paralysed but the muscles still reacted to direct /

direct stimulation with the secondary coil at 100 mm. At 2 p.m. the muscles still contract on direct stimulation with the secondary coil at a distance of 90 mm. from the primary.

In the following experiment a very small dose of Cobra Venom was administered.

Experiment XXI.

April 17th 1903. Temperature of Laboratory 59° F A frog, male, healthy, received at 3.30 p.m. .04 mg. of Cobra Venom subcutaneously. This dose is equal to .002 mg. per gramme weight of frog.

On April 18th The corneal reflex could not be obtained: the other reflexes were present but feeble and much delayed. Respiration had ceased. The circulation in the web was active.

On April 19th and 20th reflexes were still obtainable and the circulation in the web remained very active.

On April 21st. The reflexes were exceedingly weak at 10 a.m., and none could be elicited at 12 noon when the circulation in the web was active. The right sciatic nerve was exposed and on stimulation with a strong induced current - secondary coil at 10 mm. - a feeble twitch was obtained in the toes. No reflex movements were elicited with this or stronger degrees of stimulation. The same phenomena were obtained on stimulating the left sciatic nerve.

Further/

Further stimulation at intervals of five minutes failed to induce movement in the muscles of the leg or foot even with the secondary coil at zero. The muscles by direct stimulation contract to much weaker stimulation - secondary coil at 90 mm. This points to a practically simultaneous loss of the reflexes and of the motor nerve end activity with a small dose of Cobra Venom. Contrast a large dose where the reflexes are paralysed early and the motor nerve ends retain their activity for a considerably longer period of time.

Experiments to show that the Nervous Phenomena are not due to changes in the blood.

Cunningham, in the Scientific Memoirs of the Medical Officers of India pts. IX and XI, puts forward the theory that the symptoms of Cobra poisoning are due to changes in the blood . Such changes are in the direction of lessening or abolishing the respiratory capacity of the blood, according to Cunningham, who holds that the nervous symptoms are secondary in nature. That Cobra Venom has a marked effect on the blood cells is undoubted but I have not been able to find any change in the blood at the time that the other symptoms of Cobra-poisoning manifest themselves. It is convenient at this point to consider the changes that are found in the blood/

blood of a frog that has been killed by Cobra venom. Films and also capillary tubes of blood were taken from the blood of the heart in all the frogs which died during the estimation of the minimal lethal dose. The films and capillary tubes were prepared immediately upon the death of the animal.

The serum which separates from the blood of a frog killed by cobra venom is found to be stained with haemoglobin, in other words haemolysis has taken place. Control tubes from healthy frogs show that the serum is quite colourless or only slightly tinged with yellow: there is no trace of laking.

The changes in the elements of the blood are well seen in Figs 2 and 3 page (11) which are drawings of actual fields of microscopic preparations of healthy blood and of the blood of a frog killed by venom. The films were allowed to dry in air and were stained in Jenner's stain for four minutes. (This stain is a combination stain of Haematin and eosin). The films were then washed in distilled water for the same length of time, dried, and mounted in Xylol balsam.

In order to study the change in the blood - if any, that occur during the progress of Cobra poisoning the following experiments were performed.

Experiments 22, 23, 24, 25, 26 and 27.

Of/

Of these experiments the first three were performed on 18th May 1903. Temperature of Laboratory 64°F . Three frogs, male, healthy. Each weighing 23 grammes. Two A and B subcutaneously at 2 p.m. .5 mg. Venom = .02 mg per gramme weight of frog.

| Experiment XXII. FROG A. | Experiment XXIII FROG B. | Experiment XXIV. FROG C. |
|---|---|---|
| Respiration was paralysed at 5 p.m., i.e. three hours after injection. Blood films and a capillary tube-ful of blood were taken. The circulation in the web was very active. | Respiration paralysed 4.50 p.m. Reflexes paralysed 8 p.m. at which time contractions are obtained in muscles on stimulating the sciatic with secondary coil at 90 mm. Blood films were taken. The circulation in the web was vigorous. | Received injection at 4 p.m. of .02 mg. gramme weight at 8 p.m. respiration had ceased. The reflexes were then active. On 19th at 10 a.m. the reflexes and the motor nerve ends were paralysed. Circulation in web was still going on at 10 a.m. Blood films and capillary tube-ful of blood were taken. |

Experiments XXV, XXVI, XXVII performed on June 22nd 1903 were similar in nature except that the dose was larger being equal to .03 mg. per gramme weight.

The examination of the films and capillary tubes show that the poison must act on the nervous system primarily. In no case was the serum which separated in the capillary tubes stained with haemoglobin. The films showed a leucocytosis, but histologically the/

the cell elements were not altered and chemically they exhibited the same reaction to Jenner's stain that healthy frog's blood does.

Haemolytic Action of African Cobra Venom on Frog's blood in vitro.

Since, as has been already noted, the serum, which separates from the blood taken at death from a frog killed with African Cobra Venom, is stained with haemoglobin - an indication that haemolysis has occurred: and since the appearance of a blood film taken at a similar time supports this: it seemed desirable to test the haemolytic action of the venom on Frog's blood in vitro.

A 5% suspension of defibrinated frog's blood was made by adding 1 cc of the blood to 19 cc of .3% Saline Solution. This strength of saline was employed since Stephens and Myers in the Journal of Pathology 1898 showed that this strength is isotonic for frog's blood. As will be seen from the following table no haemolysis was obtained when unwashed corpuscles were employed. The strength of the solution of venom was 1 in 500.

Experiment XXVIII.

| 5% suspension frog's blood in 3% Saline: Unwashed. | Cobra Venom 1 in 500 | Result. |
|---|---|---|
| 1 c.c. | 1 c.c. | No haemolysis |
| 1 c.c. | .75 " | " " |
| 1 c.c. | .5 " | " " |
| 1 c.c. | .25 " | " " |
| 1 c.c. | .1 " | " "" |
| Control 1 c.c. + 1cc Saline | 0 " | " " |

Since the frog is a cold blooded animal the tubes containing the above quantities were not incubated but were kept at a temperature of 50° F. No haemolysis occurred within 24 hours

In the table below, washed corpuscles freed from their serum by repeated centrifuging were employed. The tubes were allowed to stand for 24 hours.

| 5% suspension frog blood corpuscles in .3% saline washed free from serum. | Cobra Venom 1 - 500 | Result. |
|---|---|---|
| 1 c.c. | 1 cc | Complete Haemolysis |
| 1 " | .75 " | " " |
| 1 " | . 5 " | " " |
| 1 " | .25 " | Not quite complete Haemolysis. |
| 1 " | . 1 " | Slight Haemolysis. |
| Control 1 " - 1cc.3% saline | 0 | No Haemolysis. |

Action of the Venom on Muscle.

Although it is found that the muscles respond to stimulation when the whole nervous system is paralysed and that they are alkaline in reaction at death, facts which point to the venom having little effect on the muscle, experiments were performed to furnish graphic records of the contractions in poisoned muscles.

Since the motor nerve ends are paralysed by the venom, arrangements were made to stimulate the muscle directly. These were made as follows. The isolated gastrocnemius muscle of a frog was suspended in a glass/

glass cylinder closed at its lower end by a cork through which passed a fine copper wire one end of which was passed through the muscle and the other end was connected to one of the terminals of an induction coil. The upper end of the glass cylinder was left open. A copper hook was passed through the muscle at its upper end and connected with the other terminal of the induction coil and the upper end of the muscle was attached to a lever so that the contraction could be recorded on a smoked revolving cylinder.

Experiment XXX.

April 29th 1903. Temperature of Laboratory 60°F

The gastrocnemius muscle was isolated and connected as above described. The cylinder was filled with normal saline solution (.65%) at 12.55 p.m. A muscle twitch was recorded at 1 p.m. At 1.5 p.m. .05 mg. of Cobra Venom was added to the Saline and records of the contractions taken at intervals thereafter. Break shocks with the secondary coil at a distance of 100 mm. from the primary, were employed. The cylinder contained 4.5 cc. and the strength of the solution of venom was therefore 1 in 9,000.

The results are shown in Figure 4 page (III)

An examination of the tracing shows that beyond an impaired amplitude in the contraction, a slowness in contraction, and a slowness in the relaxation of the/

the muscle, there is very little effect. Indeed these changes can be very well explained by the Cobra Venom inducing an earlier onset of the phenomena observed in fatigue of a healthy muscle.

That a muscle which has been exposed to the influence of Cobra Venom is more readily fatigued than a healthy muscle is seen in the following experiment. No fibrillary twitches occurred in any of the muscles experimented upon.

Experiment XXXI.

May 4th 1903. Temperature of Laboratory 62 °F At 6 p.m. a healthy male frog weighing 18 grammes received .4 mg. African Cobra Venom subcutaneously, after the right thigh, excluding the sciatic nerve, had been ligatured.

At 10 a.m. on May 5th the motor nerve ends were paralysed in the non protected limb. Curves - on a fastly revolving cylinder - were taken of the protected and non protected gastrocnemius of the. The muscles were stimulated directly and the secondary coil was at a distance of 100 mm. from the primary.

See Fig. 5 page (IV).

Tracing I is that of the protected muscle.

Tracing II is that of the poisoned muscle.

Experiment XXXII.

In order to study the incidence of fatigue, curves/

curves were taken of the protected gastrocnemius and of the poisoned gastrocnemius. It was found that on stimulating these muscles directly with single break shocks at intervals of 5 seconds: the strength of the current being that obtained with the secondary coil at a distance of 70 mm from the primary: fatigue came on much more quickly in the poisoned muscle than in the non-poisoned. Contractions were obtained in the first case ( non poisoned muscle) up to 16 minutes from the commencement of the stimulation, while in the case of the poisoned muscle no contractions occurred after 7 minutes. The individual contractions in this latter case show great variation, the muscle occasionally not responding to stimulation and at other times only a weak response being obtained.

Typical portions of the fatigue curve obtained from the poisoned muscle are seen in Figs 6 and 7 page ( IV.).

Action of African Cobra Venom on the Blood Vessels.

In order to investigate the action of Cobra venom on the blood vessels, solutions of the venom of various strengths were perfused through the blood vessels of a frog. The frog was pithed so that both the brain and spinal cord were destroyed: the heart was exposed and a glass cannula was inserted into/

into one aorta and securely tied there: the other aorta was ligatured and an opening was made in the sinus venosus to allow of the escape of the fluid after it had circulated through the capillaries. An opening was also made in the loin of the frog, which rested on a grooved zinc plate down which the fluid ran, to be collected in graduated measures. The cannula in the aorta was connected by means of bent glass tubes with beakers in which were inverted flasks. The beakers and flasks contained the solutions experimented with, and the fluid was kept at the same atmospheric level in each beaker and at a distance of 6½ inches above the level of the aorta of the frog.

In all the experiments, before the solution of venom was allowed to flow through the vessels, normal saline solution - prepared by dissolving sodium chloride in distilled water, the strength being .65%, was allowed to flow in order to wash out all the blood, and to establish a normal reading. Contraction of the vessels is indicated by a diminution in the amount of fluid which flows through the vessels. The amount flowing in each successive minute was noted.

Experiment XXXIII.

April 21st 1903. Temperature of Laboratory 60°F

Frog/

Frog, male, healthy, weight 22 grammes was pithed at 2 p.m. Both brain and cord were completely destroyed. The blood vessels were perfused at 2.35 p.m. The strength of the solution of venom was 1 in 20.000 parts of Saline (.65%).

| Mins. | No of cc. per min | Mins. | No of cc. | Mins. | No of cc. | Mins. | No of cc. | Mins. | No of cc. |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Saline again perfused. | |
| 1 | 2 | 28 | 1.3 | 55 | .8 | 82 | .35 | 109 | .2 |
| 2 | 2 | 9 | 1.2 | 6 | .6 | 3 | .35 | 110 | .2 |
| 3 | 1.9 | 30 | 1.2 | 7 | .6 | 4 | .4 | 1 | .2 |
| 4 | 2 | 1 | 1.2 | 8 | .7 | 5 | .3 | 2 | .25 |
| 5 | 2 | 2 | 1.1 | 9 | .7 | 6 | .4 | 3 | .25 |
| 6 | 2 | 3 | 1.2 | 60 | .6 | 7 | .3 | 4 | .2 |
| 7 | 1.9 | 4 | 1.2 | 1 | .5 | 8 | .35 | 5 | .2 |
| 8 | 2 | 5 | 1.2 | 2 | .5 | 9 | .4 | 6 | .2 |
| 9 | 2 | 6 | 1.1 | 3 | .5 | 90 | .35 | 7 | .2 |
| 10 | 2 | 7 | 1.1 | 4 | .5 | 1 | .3 | 118 | .2 |
| 1 | 1.9 | 8 | 1.2 | 5 | .5 | 2 | .3 | | |
| 2 | 1.9 | 9 | 1.1 | 6 | .4 | 3 | .35 | | |
| 3 | 2 | 40 | 1.1 | 7 | .5 | 4 | .3 | | |
| 4 | 2 | 1 | 1.1 | 8 | .4 | 5 | .3 | | |
| Solution of venom perfused | | 2 | 1.1 | 9 | .4 | 6 | .3 | | |
| 15 | 2.1 | 3 | 1 | 70 | .5 | 7 | .35 | | |
| 6 | 2 | 4 | 1 | 1 | .4 | 8 | .3 | | |
| 7 | 2 | 5 | 1 | 2 | .4 | 9 | .3 | | |
| 8 | 1.8 | 6 | .8 | 3 | .4 | 100 | .25 | | |
| 9 | 1.6 | 7 | .9 | 4 | .4 | 1 | .25 | | |
| 20 | 1.4 | 8 | .9 | 5 | .5 | 2 | .25 | | |
| 1 | 1.3 | 9 | .8 | 6 | .4 | 3 | .3 | | |
| 2 | 1.4 | 50 | .8 | 7 | .4 | 4 | .3 | | |
| 3 | 1.3 | 1 | .8 | 8 | .35 | 5 | .25 | | |
| 4 | 1.3 | 2 | .8 | 9 | .35 | 6 | .25 | | |
| 5 | 1.3 | 3 | .7 | 80 | .35 | 7 | .2 | | |
| 6 | 1.2 | 4 | .7 | 1 | .4 | 8 | .2 | | |
| 27 | 1.2 | | | | | | | | |

There was considerable oedema produced, the frog weighed 40 grammes at the end of the experiment having gained 8 grammes.

From this experiment we see that Cobra Venom in a strength of 1 in 20,000 has a powerful effect in the direction of diminishing the calibre of the vessels.

After the solution of venom has flowed through the vessels for 29 minutes we find that the flow has diminished from 2 cc per minute to 1 c.c. per minute a diminution of one half. After 50 minutes the flow is .5 cc. or one quarter of the amount that flows when normal saline is being perfused. At 108 minutes saline solution was turned on and failed to bring back the vessels to the condition they were in prior to the perfusion of the solution of Venom.

Experiment XXXIV.

April 23rd. 1903. Temperature of Laboratory 62°F Frog, male, healthy, weight 22 grammes, pithed brain and cord at 2 p.m. Perfusion was started at 2.30 p.m. and in this case the solution of venom was of the strength of 1 in 40.000.

| Min. | No of CC. | Min. | CC. | Min. | CC. | Min. | CC. | Min. | CC. | Min. | CC. | Min. | CC. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Saline | | Venom turned on. | | | | | | | | | | | |
| 1 | 2.8 | 11 | 3.3 | 21 | 2.7 | 31 | 2.1 | 41 | 1.6 | 51 | 1.6 | 61 | 1.5 |
| 2 | 2.8 | 12 | 3.2 | 22 | 2.7 | 32 | 2.1 | 42 | 1.6 | 52 | 1.7 | 62 | 1.5 |
| 3 | 3 | 13 | 3.1 | 23 | 2.7 | 33 | 2.1 | 43 | 1.7 | 53 | 1.7 | 63 | 1.5 |
| 4 | 3.1 | 14 | 3.1 | 24 | 2.6 | 34 | 1.9 | 44 | 1.7 | 54 | 1.7 | 64 | 1.4 |
| 5 | 3.2 | 15 | 3.1 | 25 | 2.7 | 35 | 1.9 | 45 | 1.7 | 55 | 1.6 | 65 | 1.5 |
| 6 | 3.1 | 16 | 2.9 | 26 | 2.7 | 36 | 1.8 | 46 | 1.6 | 56 | 1.5 | 66 | 1.4 |
| 7 | 3.1 | 17 | 2.9 | 27 | 2.7 | 37 | 1.9 | 47 | 1.7 | 57 | 1.5 | 67 | 1.4 |
| 8 | 3.2 | 18 | 3 | 28 | 2.6 | 38 | 1.9 | 48 | 1.7 | 58 | 1.5 | 68 | 1.4 |
| 9 | 3.1 | 19 | 2.8 | 29 | 2.5 | 39 | 1.9 | 49 | 1.7 | 59 | 1.5 | 69 | 1.4 |
| 10 | 3.1 | 20 | 2.8 | 30 | 2.1 | 40 | 1.8 | 50 | 1.6 | 60 | 1.6 | 70 | 1.3 |

Oedema was again marked the gain in weight amounting to 9 grammes.

From this experiment we see that venom even in a strength of 1 in 40.000 is capable of markedly constricting the frog's blood vessels.

Further experiments were performed in order to find at which strength the venom loses this power of contracting the frog's blood vessels.

Experiment XXXV.

May 12th 1903. Temperature of Laboratory 64°F A frog, male, healthy weight 20 grammes was pithed, both brain and spinal cord being destroyed at 1.30 p.m. A solution of Cobra Venom of a strength of 1 in 100,000 was perfused through the vessels at 2.10 p.m.

| Min. | No. of CC. Saline. | Min. | CC. | Min. | CC. | Min. | CC. | Min. | CC. | Min. | CC. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 11 | 1.4 | 20 | 1.5 | 39 | 1.4 | 58 | 1.2 | 77 | 1.4 | 96 | 1.3 |
| 2 | 1.3 | 1 | 1.5 | 40 | 1.4 | 9 | 1.2 | 8 | 1.3 | 7 | 1.4 |
| 3 | 1.5 | 2 | 1.4 | 1 | 1.4 | 60 | 1.3 | 9 | 1.4 | 8 | 1.3 |
| 4 | 1.6 | 3 | 1.4 | 2 | 1.2 | 1 | 1.2 | 80 | 1.3 | Saline 9 | 1.5 |
| 5 | 1.6 | 4 | 1.4 | 3 | 1.3 | 2 | 1.3 | 1 | 1.2 | 100 | 1.6 |
| 6 | 1.6 | 5 | 1.5 | 4 | 1.3 | 3 | 1.3 | 2 | 1.3 | 1 | 1.5 |
| 7 | 1.5 | 6 | 1.4 | 5 | 1.4 | 4 | 1.3 | 3 | 1.4 | 2 | 1.6 |
| 8 | 1.5 | 7 | 1.4 | 6 | 1.3 | 5 | 1.3 | 4 | 1.4 | 3 | 1.5 |
| 9 | 1.5 | 8 | 1.3 | 7 | 1.4 | 6 | 1.3 | 5 | 1.4 | 4 | 1.5 |
| 10 | 1.6 | 9 | 1.3 | 8 | 1.3 | 7 | 1.3 | 6 | 1.4 | 5 | 1.5 |
| 1 | 1.5 | 30 | 1.4 | 9 | 1.3 | 8 | 1.3 | 7 | 1.2 | 6 | 1.6 |
| 2 | 1.5 | 1 | 1.3 | 50 | 1.1 | 9 | 1.3 | 8 | 1.3 | 7 | 1.6 |
| Venom turned on 3 | 1.6 | 2 | 1.4 | 1 | 1.2 | 70 | 1.4 | 9 | 1.3 | 8 | 1.6 |
| 4 | 1.5 | 3 | 1.4 | 2 | 1.2 | 1 | 1.4 | 90 | 1.4 | 9 | 1.5 |
| 5 | 1.4 | 4 | 1.4 | 3 | 1.3 | 2 | 1.3 | 1 | 1.4 | 110 | 1.6 |
| 6 | 1.5 | 5 | 1.3 | 4 | 1.4 | 3 | 1.2 | 2 | 1.4 | 1 | 1.6 |
| 7 | 1.5 | 6 | 1.3 | 5 | 1.3 | 4 | 1.2 | 3 | 1.4 | 2 | 1.5 |
| 8 | 1.5 | 7 | 1.3 | 6 | 1.4 | 5 | 1.3 | 4 | 1.4 | 3 | 1.6 |
| 9 | 1.6 | 8 | 1.3 | 7 | 1.3 | 6 | 1.3 | 5 | 1.4 | 4 | 1.6 |

The frog weighed after this experiment 26 grammes, so that the gain in weight was 6 grammes. Here we find that the contraction of the vessels though it still occurs is not marked, the fall in the number of cubic centimetres that flow in one minute being only/

only from an average of 1.55 c.c. to 1.2 c.c.

As a control the following experiment was performed. The saline solution employed was prepared by dissolving pure Sodium Chloride in distilled water and the effects of such a solution on the vessels of a frog were tested.

Experiment XXXVI.

June 17th 1903. Temperature of Laboratory 65° F

The brain and cord of a healthy male frog weighing 24 grammes were destroyed at 2.10 p.m. Perfusion of the above saline solution .65% Sodium Chloride in distilled water, a solution of the same composition as had been used in all the experiments, was commenced at 2.40 p.m.

From the subjoined table we see that this Saline Solution has, after it has perfused the vessels for some minutes, an effect in the direction of causing dilatation. Since the Cobra Venom was dissolved in a solution of this strength it cannot be doubted that in sufficiently concentrated solution it causes contraction of the frog's blood vessels since it can overcome this dilatative effect of the saline and impose upon it a further contracting effect.

In the three tables which follow, the first shows the effect of .65% Saline: the second the effect /

effect of a solution of Cobra Venom in .65% Saline of a strength of 1 in 300,000., when we see that there is practically no alteration in the rate of flow, and the third shows that with a strength of 1 in 1,000,000 ( one in a million) the dilatative effect of the saline becomes evident.

Experiment XXXVI. Effect of "Normal Saline" i.e. .65% Sodium Chloride in distilled water (for data see above).

| Min. | CC. | Min. | CC. | Min. | CC. | Min. | CC. | Min. | CC. | Min. | CC. | Min. | CC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.4 | 19 | 1.5 | 37 | 1.9 | 55 | 2 | 73 | 2 | 91 | 2.1 | 109 | 2.2 |
| 2 | 1.3 | 20 | 1.5 | 8 | 1.9 | 6 | 2.1 | 4 | 1.9 | 2 | 2.1 | 110 | 2.2 |
| 3 | 1.3 | 1 | 1.5 | 9 | 1.9 | 7 | 2 | 5 | 1.9 | 3 | 2.1 | 1 | 2.3 |
| 4 | 1.3 | 2 | 1.6 | 40 | 1.8 | 8 | 1.9 | 6 | 1.9 | 4 | 2 | 2 | 2.2 |
| 5 | 1.3 | 3 | 1.5 | 1 | 2 | 9 | 1.9 | 7 | 1.9 | 5 | 2.1 | 3 | 2.1 |
| 6 | 1.3 | 4 | 1.6 | 2 | 2 | 60 | 1.9 | 8 | 1.9 | 6 | 2.2 | 4 | 2.3 |
| 7 | 1.3 | 5 | 1.6 | 3 | 1.9 | 1 | 1.9 | 9 | 2 | 7 | 2.2 | 5 | 2.4 |
| 8 | 1.3 | 6 | 1.6 | 4 | 2 | 2 | 1.8 | 80 | 2 | 8 | 2 | 6 | 2.2 |
| 9 | 1.3 | 7 | 1.7 | 5 | 2 | 3 | 1.9 | 1 | 1.9 | 9 | 2 | 7 | 2.2 |
| 10 | 1.3 | 8 | 1.6 | 6 | 2.1 | 4 | 1.9 | 2 | 1.9 | 100 | 2.1 | 8 | 2.3 |
| 1 | 1.4 | 9 | 1.6 | 7 | 2 | 5 | 1.9 | 3 | 2 | 1 | 2 | 119 | 2.2 |
| 2 | 1.4 | 30 | 1.7 | 8 | 2 | 6 | 2 | 4 | 2 | 2 | 2 | | |
| 3 | 1.4 | 1 | 1.7 | 9 | 2 | 7 | 2 | 5 | 2 | 3 | 2 | | |
| 4 | 1.5 | 2 | 1.8 | 50 | 2.1 | 8 | 1.9 | 6 | 2 | 4 | 2 | | |
| 5 | 1.5 | 3 | 1.8 | 1 | 2 | 9 | 1.9 | 7 | 2.1 | 5 | 2.1 | | |
| 6 | 1.5 | 4 | 1.8 | 2 | 2 | 70 | 1.9 | 8 | 2.1 | 6 | 2.1 | | |
| 7 | 1.5 | 5 | 1.9 | 3 | 1.9 | 1 | 2 | 9 | 2 | 7 | 2.1 | | |
| 8 | 1.5 | 6 | 1.8 | 4 | 1.9 | 2 | 2 | 90 | 2 | 8 | 2.2 | | |

Weight of frog after experiment 28 grammes: gain in weight by oedema 4 grammes.

The dilatative effect is evident in 30 minutes and is well marked at 41 minutes, and continues so for the rest of the two hours during which observations were taken.

Experiment XXXVII.

Effect of a solution of Cobra Venom in .65% saline solution of a strength of 1 in 300,000

June 19th 1903. Temperature of Laboratory 66°F A frog, male, healthy, was pithed, the brain and cord being destroyed at 1.30 p.m.: perfusion of the above solution of venom was begun at 2 p.m.
Weight of frog 25 grammes.

| Min. | CC. | Min. | CC. | Min. | CC. | Min. | CC. | Min. | CC. | Min. | CC. | Min. | CC. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Saline | | | | | | | | | | | | | |
| 1 | 1.8 | 19 | 1.9 | 37 | 2 | 55 | 2.1 | 73 | 2.1 | 91 | 2.1 | 109 | 2.1 |
| 2 | 1.8 | 20 | 1.9 | 8 | 1.9 | 6 | 2 | 4 | 2.1 | 2 | 1.9 | 110 | 2 |
| 3 | 1.8 | 1 | 1.9 | 9 | 2 | 7 | 2 | 5 | 2 | 3 | 1.8 | Saline 1 | 2 |
| 4 | 1.8 | 2 | 1.9 | 40 | 2 | 8 | 2 | 6 | 2 | 4 | 1.9 | 2 | 1.9 |
| 5 | 1.9 | 3 | 2 | 1 | 2 | 9 | 2 | 7 | 1.9 | 5 | 1.9 | 3 | 2 |
| 6 | 1.8 | 4 | 1.9 | 2 | 2.1 | 60 | 1.9 | 8 | 2 | 6 | 1.9 | 4 | 1.9 |
| 7 | 1.8 | 5 | 1.9 | 3 | 1.9 | 1 | 1.9 | 9 | 1.9 | 7 | 1.9 | 5 | 1.9 |
| 8 | 1.8 | 6 | 1.9 | 4 | 1.9 | 2 | 2 | 80 | 2 | 8 | 1.9 | 6 | 1.9 |
| 9 | 1.9 | 7 | 1.8 | 5 | 2 | 3 | 2 | 1 | 2 | 9 | 2 | 7 | 2 |
| 10 | 1.9 | 8 | 1.9 | 6 | 2 | 4 | 2 | 2 | 2.1 | 100 | 2 | 8 | 2 |
| 1 | 1.9 | 9 | 2 | 7 | 2 | 5 | 1.9 | 3 | 1.9 | 101 | 2 | 9 | 2 |
| m turned on. | | | | | | | | | | | | | |
| 2 | 1.9 | 30 | 2 | 8 | 2 | 6 | 1.9 | 4 | 1.9 | 2 | 2.1 | 120 | 1.9 |
| 3 | 2 | 1 | 2 | 9 | 2 | 7 | 1.9 | 5 | 1.9 | 3 | 2 | | |
| 4 | 1.9 | 2 | 2 | 50 | 2 | 8 | 1.9 | 6 | 1.9 | 4 | 2 | | |
| 5 | 1.9 | 3 | 2 | 1 | 1.9 | 9 | 1.9 | 7 | 2 | 5 | 2 | | |
| 6 | 2 | 4 | 1.9 | 2 | 1.9 | 70 | 2 | 8 | 2.1 | 6 | 2 | | |
| 7 | 2 | 5 | 1.9 | 3 | 1.9 | 1 | 2 | 9 | 2.1 | 7 | 2.1 | | |
| 8 | 2 | 6 | 2 | 4 | 2 | 2 | 2.1 | 90 | 2.1 | 8 | 2.1 | | |

Gain in weight by oedema was 6 grammes. In the above table we see that the rate of flow is practically unaltered and therefore in a strength of 1 in 300,000 Cobra Venom is only able to just counteract the dilatative effect of .65% Saline Solution.

Experiment XXXVIII.

This experiment shows that in a strength of one in a million (1 in 1.000.000) cobra venom has practically no action and the dilatative effect of the .65% Saline Solution becomes manifest.

The brain and cord of a healthy male frog were destroyed at 2.30 p.m. on June 20th. The temperature of Laboratory was 68°F. Perfusion was commenced at 3 p.m. Weight of frog 22 grammes.

| Min. | CC. | Min. | CC. | Min. | CC. | Min. | CC. | Min. | CC. | Min. | CC. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Saline | | | | | | | | | | | |
| 1 | .9 | 21 | 1.1 | 41 | 1.3 | 61 | 1.4 | 81 | 1.6 | 101 | 1.6 |
| 2 | .9 | 2 | 1.1 | 2 | 1.3 | 2 | 1.5 | 2 | 1.6 | 2 | 1.5 |
| 3 | .9 | 3 | 1.2 | 3 | 1.3 | 3 | 1.5 | 3 | 1.6 | 3 | 1.5 |
| 4 | 1. | 4 | 1.2 | 4 | 1.3 | 4 | 1.5 | 4 | 1.5 | 4 | 1.5 |
| 5 | .9 | 5 | 1.2 | 5 | 1.3 | 5 | 1.6 | 5 | 1.6 | 5 | 1.6 |
| 6 | 1. | 6 | 1.2 | 6 | 1.2 | 6 | 1.5 | 6 | 1.6 | 6 | 1.6 |
| 7 | 1. | 7 | 1.2 | 7 | 1.3 | 7 | 1.6 | 7 | 1.6 | 7 | 1.7 |
| 8 | 1. | 8 | 1.2 | 8 | 1.4 | 8 | 1.7 | 8 | 1.6 | 8 | 1.7 |
| 9 | .9 | 9 | 1.3 | 9 | 1.3 | 9 | 1.7 | 9 | 1.6 | 9 | 1.7 |
| 10 | 1 | 30 | 1.2 | 50 | 1.3 | 70 | 1.7 | 90 | 1.5 | 110 | 1.6 |
| Cobra Venom | | | | | | | | | | | |
| 1 | 1 | 1 | 1.2 | 1 | 1.4 | 1 | 1.6 | 1 | 1.5 | 1 | 1.6 |
| 2 | 1 | 2 | 1.3 | 2 | 1.4 | 2 | 1.7 | 2 | 1.5 | 2 | 1.6 |
| 3 | 1 | 3 | 1.3 | 3 | 1.4 | 3 | 1.6 | 3 | 1.5 | 3 | |
| 4 | 1.1 | 4 | 1.3 | 4 | 1.4 | 4 | 1.6 | 4 | 1.6 | 4 | |
| 5 | 1.2 | 5 | 1.2 | 5 | 1.3 | 5 | 1.6 | 5 | 1.6 | 5 | |
| 6 | 1.1 | 6 | 1.3 | 6 | 1.4 | 6 | 1.6 | 6 | 1.6 | 6 | |
| 7 | 1.1 | 7 | 1.3 | 7 | 1.4 | 7 | 1.6 | 7 | 1.6 | 7 | |
| 8 | 1.2 | 8 | 1.3 | 8 | 1.4 | 8 | 1.5 | 8 | 1.6 | 8 | |
| 9 | 1.2 | 9 | 1.2 | 9 | 1.4 | 9 | 1.6 | 9 | 1.6 | 9 | |
| 20 | 1.1 | 40 | 1.3 | 60 | 1.5 | 80 | 1.6 | 100 | 1.5 | 120 | |

Weight of frog after experiment—28 grammes: the oedema caused therefore a gain in weight of 6 grammes.

EFFECT OF COBRA VENOM ON THE HEART.

From a study of the general effects of African Cobra Venom in frogs we see that the heart beats up to the moment of death and for a considerable period after the more marked nervous phenomena present themselves. The rate is much slowed, but the fact that the contractions of the heart are visible through the chest wall shows that the power of the heart is not much affected.

Arrangements were made to record the contractions of the heart in a graphic manner. In some instances the ordinary method of suspension by means of a hook through the apex of the ventricle of the frog's heart was employed. In other cases tracings were obtained by means of levers connected with pads resting on the auricles and ventricle.

Experiment XXXIX.

June 21st 1903. Temperature of Laboratory 69° F Frog, male, healthy, weight 25 grammes received subcutaneously at 2.30 p.m. .75 mg of Cobra Venom. This equals .03 mg per gramme weight. The reflexes were found to be paralysed at 5.30 p.m. The brain was destroyed as bloodlessly as possible and the heart was exposed and was found to be beating at the rate of 5 contractions in 10 seconds. (Before injection/

injection the rate was 9 in 10 seconds). The auricular contraction was feeble. Small pads of cork attached to levers were allowed to rest on the auricles and on the ventricle and their contractions were recorded by means of the levers upon a smoked drum which was made to revolve at the rate of one inch in five seconds.

Figures 8 and 9 page (XI) show portions of the curves obtained. The tracings read from left to right and the downstroke in each individual curve represents the contraction, the upstroke the diastole of the auricles and ventricle.

In the tracing seen in Figure 8 page (XI) we have to note the slow heart beat, with the comparatively powerful systole of the ventricle, (indicated by the down stroke). Note also the long diastolic pause before another contraction takes place. The heart is beating regularly. But it is found that, at a later period in Cobra Poisoning, the heart becomes irregular in its rate of contraction. A portion of tracing of the same heart showing this is seen in figure 9 page (XI)

The auricular beat is seen to be very feeble in these curves but as the frogs were small some difficulty was experienced in getting the pads in place.

Experiment/

Experiment XL. June 23rd. 1903. Temperature of Laboratory 66°F.

In this experiment the suspension method of taking heart tracings was employed, and the antagonistic effect of Atropine Sulphate was investigated. A frog, male, healthy, weight 30 grammes received subcutaneously at 9 a.m. .9 mg Cobra Venom = .03 mg per gramme weight. The curve was taken at 2 p.m. i.e. 5 hours after injection. The reflexes were completely paralysed. We see here the same slow powerful heart beat and after the application of Atropine Sulphate (.2% solution in Normal Saline) we find a slight increase in the rate of the heart beat.

In figure 10 page (XII) is seen the slow powerful heart beat. The rate is 5 in 30 seconds.

In figure 11 page (XII) we find that the heart is now beating at the rate of 7 in 30 seconds. i.e. Atropine has to some extent increased the rate.

In order to localize the action of Cobra Venom, further experiments were performed. Experiments XLI and XLII show the effect of direct application of a solution of Cobra Venom to the heart muscle.

Experiment XLI

27th May 1903. Temperature of Laboratory 69°F

The brain of a frog, male, weighing 22 grammes

was/

was destroyed bloodlessly at 10.15 a.m. at 10.30 a.m. a tracing was taken by means of levers attached to pads resting on the auricles and ventricle, the pericardium having been removed. A solution of Cobra venom of a strength of 1 in 10,000 (normal saline being used as the solvent) was applied at intervals so that the heart muscle was bathed in a solution of the venom. See Figure 12 page (XIII).

Experiment XLII.

June 2nd 1903. Temperature of Laboratory 62° F Frog weight 25 grammes, male, healthy was pithed, brain only at 10 a.m. Heart tracings by means of pads on auricles and ventricle were taken. Cobra venom dissolved in Saline solution was applied at intervals. In all, 1 mg dissolved in 1 c.c. of saline was applied, and even this strength of 1 in 1000, had little effect by direct application to the heart.

See figures 13 and 14 page (XIV).

Experiment XLII a.

In this experiment the effect of the venom on the cardiac muscle, by direct application of it to the exterior of the heart from which the pericardium was removed, was observed. The number of pulsations in succeeding half minutes was observed, and all unneccessary interference with the heart in the shape of /

of suspension of it was avoided.

A frog, male, weight 25 grammes, was pithed, brain only, at 2.30 p.m. the pericardium was carefully removed from its ventral aspect and the pulsation of the heart noted. A solution of cobra venom in normal saline solution was applied at intervals . The results are shown in the accompanying table. The strength of the solution was 1 in 5,000.

| Time | No of Heart Beats in 30 seconds. | Remarks. |
|---|---|---|
| 2.34 | 27 | Systole of Ventricle and auricles complete. |
| .35 | 26 | |
| .36 | 26 | |
| .37 | 26 | |
| 2.38 | | 2 minims of solution applied. |
| .39 | 26 | |
| .40 | 26 | |
| .41 | 26 | |
| .43 | 26 | Systole still good. |
| 45 | - | 1 m. of solution applied. |
| 45.15" | 26 | |
| 47 | 26 | |
| 50 | 26 | |
| 50'30" | - | 2 m. of solution applied. |
| 51 | 25 | |
| 53 | 25 | |
| 54 | 25 | |
| 57 | 26 | |
| 57'15" | - | 2 m. of solution applied. |
| 2.58 | 24 | |
| 2.0 | 24 | |
| .4'30" | 24 | |
| .9'30" | 24 | |
| .10 | - | 2 m. of solution applied. |
| .11 | - | Struggles of frog. |
| .12 | 25 | |
| .16 | 24 | |

Exper. XLIIa cont.

| Time | No. of Heart Beats in 30 seconds. | Remarks. |
|---|---|---|
| .16'30" | - | 2 m. of solution applied. |
| .17' 30" | 24 | |
| .20 | 24 | |
| .25 | 24 | |
| .30 | 24 | |
| .30'10" | - | Struggles of frog. |
| .31 | 24 | |
| .31'30" | - | 2 m. of solution applied . |
| .32 | 24 | |
| .37 | 23 | Systole and diastole are still unimpaired. |
| .42 | 23 | |
| .47 | 23 | |
| 4.0 | 23 | |
| 4.13 | 22 | |
| 4.24 | 23 | Systole not quite so complete but still regular in time |
| 4.50 | 22 | |

Here we see that a heart that is kept continuously moist in a solution of venom which is applied to the exterior of the heart is practically unaffected.

Experiment XLII.b.

In order to acertain if the venom have any local action on the cardiac muscle when it is allowed to come into more intimate relation with it, the effect of it in the excised frog's heart was observed. In this form of experiment the solution of Cobra venom comes into contact with the endocardial aspect as well as with the exocardial: the heart in fact in its contractions, which take place in the solution of/

of venom, is constantly allowing the venom to circulate through it. The table quoted below is a typical one from several experiments that were performed. The frog being pithed, the heart was carefully excised and placed first of all in normal saline solution in order that the normal rate of its pulsations might be determined. Venom was then added so that the strength of the solution was 1 in 5,000, and the pulsations were again recorded.

| Time | Contractions in 30 seconds. | REMARKS. |
|---|---|---|
| 3.32 | 19 | Heart in Saline. |
| .33 | 18 | " " " |
| .34 | 18 | " " " |
| .35 | 18 | " " " |
| .36 | 18 | Heart in Solution of Venom |
| .37 | 18 | " " " " " |
| .38 | 19 | " " " " " |
| .39 | 18 | " " " " " |
| .40 | 17 | " " " " " |
| .42 | 17 | " " " " " |
| .44 | 17 | " " " " " |
| .46 | 18 | " " " " " |
| .48 | 16 | " " " " " |
| .50'30" | 15 | " " " " " |
| 3.52 | 16 | |
| .54 | 15 | |
| .56 | 12 | |
| .57 | 8 | Contraction irregular with frequent pauses in complete systole. |
| .59'to 4.0 | 9 & 7 | Irregularity marked as above. |
| 4.3 | 7 | Whole heart beating. |
| .6 | | 13 contractions of sinus alone: auricles and ventricle in systole. |
| . | | |
| . | | |

Experiment XLII b. Contd

| Time | Contractions in 30 seconds. | Remarks. |
|---|---|---|
| .9'30" | 7 | Whole heart beating |
| .11 | 6 | " " " |
| .13 | 6 | " " " |
| .15 | | 5 feeble beats of sinus alone. |
| 4.20 | | Whole heart ceased in condition of systole. |

Experiment XLII. c.

As a control an excised heart was placed in saline, and at the end of two hours it was found that the rate of beat had diminished from 27 in 30 seconds to 25 in 20 seconds, while the rhythm had remained unaltered.

Experiment XLII. d.

In the following experiment the effect of stimulation of the medullary centre, of the vagus nerve and of the sinus, upon a heart under the influence of African Cobra Venom was investigated. The vagal centre in the medulla was reached by cutting off the frog's head just behind the eyes and in front of the tympanic membranes. Figures 15 and 16 page (XV) show the effect of stimulation of the medullary centre and of the vagus nerve in a decerebrate frog. In figure 15 page (XV) we see that marked inhibition is obtained on stimulating the medulla with such a current as was obtained from a coil with the secondary coil at a distance of 60 mm.

mm./

mm. from the primary. In figure 16 on the same page the inhibitory effect of stimulation of the vagus nerve with the same strength of stimulation is seen.

The above tracings were obtained by the suspension method. The frog weighed 30 grammes and had been decapitated at 2 p.m. At 2.15 p.m. 1.5 mg of African Cobra Venom was injected intra-peritoneally. This dose equals .05 mg per gramme weight.

Further tracings were taken at 2.50 p.m. when the reflexes were found to be paralysed. The heart, which before injection was found to be beating at the rate of 6 contractions in 10 seconds, was now beating at the rate of 3 in 10 seconds. Stimulation of the vagus nerve with the secondary coil at a distance of 60 mm from the primary caused inhibition of the heart as is seen in figure 17 page (XV). Later on, stimulation of the Medulla showed that stimuli, which caused inhibition in an unpoisoned heart, are powerless to affect a heart when the frog is under the influence of venom, although strong stimuli still cause slight inhibition - see figure 18 page (XV).

Stimulation of the sinus has the same result, in a frog under the influence of Cobra Venom, that it has in a healthy heart. One finds augmentation with weak stimuli and inhibition with strong stimuli - see figure 19 page (XV).

These/

These results point to the intrinsic nervous mechanism and the vagal nerve ends being left intact by Cobra Poison, and to the venom having an influence on the cardio-inhibitory centre in the Medulla.

Experiment XLIV.

June 6th 1903. Temperature of Laboratory 67° F Frog, male, healthy, weight 25 grammes, received subcutaneously .75 mg. of Cobra Venom. Sixteen hours later a heart tracing was obtained. The rate of beat was found to have fallen from 8 beats in 10 seconds to 3 in 10 seconds. The motor nerve ends were at this time paralysed. In this experiment the effect of Atropine in causing a quickening of the heart beat and the effect of stimulation of the sinus before and after the application of Atropine are well seen. The tracing was obtained by the suspension method.

The portion of tracing seen in figure 20 page (XV) shows the heart beating at the rate of 3 contractions in 10 seconds, with the heart under the influence of the venom (normal is to distinguish from the curve under the influence of Atropine), and the effect of stimulating the sinus. We see that the heart was inhibited for a period of 68 seconds.

The portion of tracing seen in figure 21 page (XVI) shows that the application of a solution of/

of Atropine, (.2% Sulphate of Atropine in normal saline), is able to cause an increase in the rate of the heart beats from 4 in 20 seconds to 8 in 20 secs.

Stimulation of the sinus after the application of Atropia is powerless to arrest the heart.

From a consideration of these experiments one is lead to the conclusion, that the slow heart beat that it observed in frogs under the influence of African Cobra Venom, is due, in part to the action of the venom on the central nervous system - the vagal centre in the medulla -, and in part to the action of it on the heart muscle.

There must be at first a stimulation of the vagal centre resulting in a slowing of the heart. The vagal nerve ends are not paralysed, since stimulation of the vagus nerve arrests the heart, and we also find that the application of Atropine to the heart is capable of quickening the heart beat, by paralysing the vagal nerve ends, and releasing the heart from the increased inhibition that is being exerted by the vagal centre. While this is so, we find that the increase is not brought about to such a degree as one would anticipate if the action were purely nervous, and experiment XLII b. shows that part of the slowing is due to the direct action of the venom on the cardiac muscle. We find also that when the medulla becomes nearly paralysed/

paralysed - as is shown by the fact that stimulation of it requires to be very powerful to cause slowing. (Experiment XLIII), we are constrained to find an explanation of the slow heart beat that still persists in the local action of the venom on the heart muscle. The results of Experiment XLIV show that the intrinsic nervous mechanism of the heart is left intact by African Cobra Venom.

Experiment XLV. In order to determine whether African Cobra Venom had any effect on the lymph hearts of a frog, a frog weighing 19 grammes was pithed (brain only) at 11.30 a.m. and the posterior lymph hearts exposed. Their rate of contraction was then 10 in 10 seconds. At 11.35 a.m. .57 mg. Cobra Venom solution was injected subcutaneously, and immediately after injection the rate increased to 12 in 10 seconds. At 11.50 the rate was 8 in 10 seconds. At 12.15 p.m 6 in 10 seconds. At 12.30 p.m. there was an occasional contraction and at 12.45 p.m. i.e. one hour and ten minutes after injection the lymph hearts had ceased to beat.

As a control to the above experiment the following experiment was performed. A healthy male frog had its brain destroyed at 12.45 p.m. The lymph hearts were exposed and found to be beating at the rate of 10 contractions in 10 seconds. At. 2.45 p.m (two/

(two hours after pithing), the rate was 8 in 10 seconds. At 5 p.m. the lymph hearts were still pulsating at the rate of 8 in 10 seconds.

## GENERAL EFFECTS ON RABBITS.

In order to study the general effects on rabbits, graduated doses were administered subcutaneously under the skin of the flank. The minimal lethal dose was determined, and the effects on rabbits are seen in the following experiments, selected from those on the table showing the determination of the lethal dose. In all the experiments the blood was examined and the effect of the venom on the blood was investigated. The effect upon temperature was also noted, the temperature being taken per rectum.

### Experiment LI.

A rabbit, - a black doe - weighing 1750 grammes received subcutaneously in the right flank at 2 p.m. .55 mg. of African Cobra Venom. This is equal to .3 mg. per kilo weight of animal.

Before injection the temperature, as determined per rectum, was 101.2°F : the respirations were 40 in 30 seconds, and the heart beats were 34 in 10 seconds.

Up to six p.m., i.e. till four hours after the injection, the animal shewed no symptoms - but at this/

this time its respirations became laboured and irregular, their rate was on an average 36 in 30 secs: the expiration was abrupt. The animal's temperature at this time (four hours after injection) was 101.4°F. The rabbit was able to move about. At 6.20 p.m. signs of motor weakness began to show themselves. The animal was unable to support its head, and the nose rested on the table. Respiration was irregular and slower, its rate being 20 respirations in 30 seconds. The cardiac impacts numbered 32 in 10 seconds and were regular and powerful.

At 6.40 p.m., (four hours and forty minutes after injection), great motor weakness was seen; the animal became unable to stand and the belly and chest rested on the table, all the limbs being extended. The respirations were at the rate of 18 in 30 seconds; the cardiac impacts numbered 22 in 30 seconds and the temperature had fallen to 99° F.

At 7 p.m. there occurred slight convulsions ; the animal lay on its side: the cornea was insensitive: the respirations were 12 in 30 seconds, very irregular and feeble, with an occasional deep gasp. The heart at this time (five hours after injection) was beating at the rate of 10 in 10 secs.

At 7.3 p.m. respiration ceased: the heart was beating feebly at the rate of 10 contractions in 10 seconds, and it ceased to beat at 7.6 p.m.

A/

A post-mortem examination was made when the rigidity had become marked at 7.40 p.m. There was no free fluid in the abdomen: no peritoneal ecchymoses: gut not congested. Kidneys were slightly congested. The liver contained a great deal of blood which exuded from it on section.

The heart was found with the right side dilated: the auricle and ventricle contained loose dark clot which extended into the superior and inferior venae cavae.

The left side of the heart was contracted and contained but little blood. The lungs were healthy.

Some urine was obtained from the bladder and was found to contain a trace of albumin: no blood was present.

Experiment XLVII.

A rabbit, fawn buck, weighing 1475 grammes, whose respirations were 20 in 10 seconds and whose cardiac impacts were 28 in 10 seconds received under the skin of the right flank at 10.10 a.m. 1 mg of Cobra Venom. This dose is equal to .65 mg of Cobra venom per kilo gramme weight of rabbit. At 11.45 a.m. i.e. one hour thirty five minutes after injection, the respiration became markedly affected, in that it was slower and more laboured. There were now only 14 respirations in the 10 seconds and the cardiac impacts had fallen from 28 in 10 seconds to 18 in 10 seconds. The temperature, which before injection/

injection was 102.4° F was now 102.6° F. The rabbit was unable to support the head and the nose was found to be resting on the table. When placed on its side the animal remained in this position after some feeble attempts to get on to its feet.

At 11.50 the respirations were now at the rate of 12 in 10 seconds and the cardiac impacts while strongly felt had slowed considerably in rate, only 12 being counted in 10 seconds. The pupil at this time was found to be contracted and did not react to light: the corneal reflex could not be obtained.

At 11.55 slight convulsive movements occurred; the head was retracted. Respiration became very irregular and occasionally a deep gasp was taken. The heart was felt to be beating at the rate of 24 contractions in 10 seconds. The pupil was found to be dilating and at 12 o'clock was widely dilated

At 12.5 p.m. i.e. 1 hour 55 minutes after injection, respiration ceased: the heart was still felt to be beating, although feebly and irregularly at the rate of 6 beats in 10 seconds, and at 12.7 p.m the heart ceased to beat: the pupil was small.

On post-mortem examination, no free fluid was found in the abdomen. There were no peritoneal ecchymoses. The gut appeared a little congested as did also the liver and kidneys. The lungs were healthy. The left side of the heart was contracted and empty: the right ventricle and auricle contained some/

some dark blood clot.

Experiment LIV.

In this experiment the effects of a sub-lethal dose are well shewn, especially as regards the changes in temperature and in the white cells of the blood.

A white buck rabbit was employed: its weight was 1690 grammes. The respirations were 20 in 10 seconds, and the heart beats 30 in 10 seconds. Examination of the blood shewed that the haemocytes numbered 4.800.000 per cubic millimetre, the leucocytes 9.500, and the percentage of haemoglobin was 60. The temperature as ascertained per rectum was 102° F

At 12.20 p.m. .4 mg of African Cobra Venom dissolved in sterile camphor water was injected under the skin of the flank. This dose is equal to .237 mg per kilogramme weight of rabbit.

At 4.20 p.m. - four hours after injection- there was no gross change observable. It was found however that the temperature had risen to 104.2°F i.e. a rise of 2.2° F in four hours. The respirations were 22 in 10 seconds and the heart was beating at the rate of 28 contractions in 10 seconds.

The haemocytes were 4.200.000

The Leucocytes " 9.500

The haemoglobin was 58%.

Next/

Next day the animal was found to be quite lively. The temperature had fallen again to 102° F at which spot it had been before injection. The weight was 1615 grammes a loss of weight of 75 grammes. The respirations were now 22 in 10 seconds, and the heart was found to be beating at the rate of 26 contractions in 10 seconds. In the blood evidence of leucocytosis was obtained since the number of leucocytes was now 14.200 - an increase of nearly 5.000 in 24 hours. The haemocytes numbered 4,000,000 and the haemoglobin was 56%. Next day the weight had decreased to 1590 grammes: the leucocytosis was more marked, there being now present 14,800 leucocytes. . Otherwise the animal was healthy.

On the third day after injection the animal's weight was 1610 grammes and it steadily increased in weight and recovered completely. The number of leucocytes had fallen to 10.600.per cubic millimetre.

The records of the above experiments are obtained from three of those made during the determination of the minimal lethal dose, the results of which are tabulated below.

Determination/

Determination of minimal lethal dose of African Cobra Venom for Rabbits.

| Expt. | Sex | Colour | Weight | Amount injected | Amount per kilo | Result. |
|---|---|---|---|---|---|---|
| XLVI | F | Slate | 2050 g | 1.5 mg. | .75 mg. | Death in 4 hours |
| XLVII | M | Fawn | 1475 | 1 mg. | .65 | " " 2 " |
| XLVIII | F | Black White | 1920 | 1 mg. | .52 | " " 3¼ " |
| XLVIIIa | M | White | 1500 | .6 mg. | .4 | " " 5 " |
| XLIX | F | Black | 1750 | .55 " | .3 | " " 5 " |
| L | M | White | 1695 | .5 | .29 | Recovery. |
| LI | F | Black | 2130 | .6 | .27 | " |
| LII | F | White | 1360 | .39 | .26 | " |
| LIII | M | White | 1880 | .47 | .25 | " |
| LIV | M | White | 1690 | .4 | .237 | " |

In all the above experiments the animals were kept in the same condition as regards feeding etc. The injections were all subcutaneous.

From these experiments we see that the minimal lethal dose of African Cobra Venom is .3 milligramme of Cobra venom per kilogramme weight of rabbit: a dose which is slightly in excess of that for Indian Cobra Venom. In sub-lethal doses it is remarkable that there are practically no external symptoms of the action of the venom; and that in doses that are only very slightly below the minimal lethal dose the recovery is so complete. But it is found that effects are produced on the blood, on the temperature and on the general condition of the animal/

animal in so far as it can be determined by ascertaining the changes in weight.

General effects on Rabbits.

Before passing to a consideration of those changes in detail, it may be opportune to summarise the general effects on rabbits of African Cobra Venom.

The first symptom, that attracts attention is that the breathing becomes embarrassed. The respiration becomes irregular, laboured and after a transient increase in rate, progressively slower until death, which appears to be due to a paralysis of the respiratory centre.

Motor weakness is next displayed: the rabbit is unable to support its head and finally it lies on its belly with all limbs extended. The heart is slowed but still continues to beat after respiration has ceased. Evidence of involvement of the reflexes is obtained in the disappearance of the corneal reflex some time before death. Convulsions, which are not marked in degree, occur before death, with the gradual failure of the respiratory centre.

Effect of the Venom upon Temperature.

It has been noted in the three experiments selected from those in the table of minimal lethal doses, viz. Experiments XLVII, LI and LIV that changes take place in the temperature of the rabbits. This/

This is especially well seen in the case of the rabbit in Experiment LIV. The temperature was taken at stated intervals after injection, the thermometer being inserted into the rectum for this purpose.

The following table shows the changes in Temperature which followed the injection of the venom. The first column shows the temperature before injection: the second the dose per kilo weight of rabbit, and the succeeding columns show the temperature at the stated times.

| | Temp before injection | Dose per kilo wt. | Hours after injection with temp. at them | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1½ | 2 | 3 | 4 | 6 | 24 | 48 | |
| I. | 102.4° F | .65 mg. | 102.6° | Death in two hours. | | | | | | |
| II. | 101.8° F | .52 | | 102.8° | 100° | Death in 3¼ hours. | | | | |
| III. | 101.2° F | .4 | | 102.4° | | 103.8° | Death in 5 hours. | | | |
| IV. | 101.2° F | .3 | | 102.2° | | 103? | at 4.40 | 99? | Death in 5hr | |
| V. | 102.° F | .29 | | 102.6° | | 103.2° | 103.2° | 100.2° | 101.9° | R |
| VI. | 102.8° F | .27 | | 103.6° | | 104.6° | | 101? | 102.6° | " |
| VII. | 100.6° F | .26 | | 101.8° | | 102.6° | | 100? | 100.8° | " |
| VIII. | 101.6° F | .25 | | 101.2° | | 103.4° | | 100.8° | 101.4° | " |
| IX. | 102° F | .237 | | 103° | | 104.2° | | 102° | 102.2° | " |
| | | | | 2 | 3 | 4 | 6 | 24 | 48 | " |

Hours after Injection.
R = Recovery.

This table shows that following the injection of the/

the venom a rise of temperature takes place. This begins to show itself in two hours, and when the temperature is taken four hours after injection , the rise is marked. Twenty four hours after injection on the temperature is found to be lower than it was before injection, but by the second day after injection it has regained its normal level.

In cases where the rabbit had received a large lethal dose of the venom and where the temperature was taken shortly before death, it was found that the temperature had fallen to a very low point, indicative of collapse. (See No.IV in table above)

Effect of the Venom on the Blood of Rabbits.

It has been noted that a leucocytosis is a feature in the symptoms that follow the injection of Cobra-venom (see Experiment LIV.)

The blood as has been already stated was examined in all the rabbits used in determining the minimal lethal dose of the venom. Some of the rabbits however died before a second estimation could be made, and the best results are seen in the cases of those rabbits which recovered.

It may be stated at the outset that in no case did the blood shew evidence of haemolysis: for in no case was the serum, which separated from the blood, which was collected in capillary tubes, tinged with haemoglobin/

haemoglobin. That is to say, African Cobra Venom when administered to rabbits by subcutaneous injection does not produce destruction of the red blood cells.

That it stimulates the organism to produce an increase in the number of white cells circulating in the blood, is well seen from the following table.

The first column shows the amount of venom per kilogramme weight of rabbit, that was injected subcutaneously: the second column the number of leucocytes per cubic millimetre before the injection and the remaining columns the number at the stated times after injection.

TABLE SHOWING LEUCOCYTOSIS.

| | Amount per kilo injected | No. of leucocytes before injection. | No. in 3 hrs. | No. in 4 hrs | No. in 24 hrs | No. in 48 hrs | No. in 72 hrs |
|---|---|---|---|---|---|---|---|
| I. | .52 mg | 7.000 | 10.500 | died in 3¼ hours | | | |
| II. | .3 mg | 7.000 | 9.200 | (died in 5 hours) | | | |
| | | No. before injection | | No. in 4 hrs | No. in 24 hrs | No. in 48 hrs | No. in 72 hrs |
| III. | .29 mg | 12.000 | | 12.200 | 17.200 | 17.200 | 14.600 |
| IV. | .27 mg | 7.200 | | 9.600 | 12.400 | 12.800 | 8.200 |
| V. | .26 mg | 8.800 | | 12.200 | 16.600 | 17.000 | 10.000 |
| VI. | .25 mg | 7.400 | | 9.200 | 11.400 | 12.000 | 8.200 |
| VII. | .237 mg | 9.500 | | 10.100 | 14.200 | 14.800 | 10.600 |
| | Amount per kilo injected | No. of leucocytes before injection | | No. of leucocytes 4 hrs. after injection | No in 24 hours | No. in 48 hours | No. in 72 hours |

Here we see that the leucocytosis is evident in 4 hours and reaches its height forty-eight hours after injection. After this period the number is found to diminish.

As regards the varieties of the leucocytes which are increased, an examination of films of the blood taken when the leucocytosis was established, shows that the increase is due mainly to the increased number of finely granular eosinophile and coarsely granular basophile leucocytes. The lymphocytes appear to be diminished.

Examination of the blood films is also instructive in that it affords confirmatory evidence of the fact that the red blood corpuscles are unaltered by the action of the venom. They retain their circular shape, and exhibit the same reaction to Jenner's stain that healthy rabbit's blood does.

Besides the rise in temperature, and the leucocytosis, evidence of the action of the venom is seen in the loss of weight that occurs after the injection of the venom. The figures below are typical of the loss in weight that occurs in rabbits.

| Dose of Venom per kilo. | Weight before Injection. | Days after Injection. 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| .237 mg. | 1690 g. | 1615 | 1590 | 1610 | 1655 | 1680 |
| .29 mg. | 1695 g. | 1635 | 1615 | 1630 | 1670 | 1690 |

From/

From this we see that the body weight diminishes for the forty-eight hours succeeding injection and that thereafter the animal begins again to increase in weight and has nearly attained the weight it was before injection on the fifth day after injection.

## BLOOD PRESSURE EXPERIMENTS.

The blood pressure experiments were made upon rabbits, an ordinary Ludwig's Kymopaph being connected with one of the carotid arteries. Tracings were taken showing the uncomplicated action of the venom and also the effect of stimulating the vagus and depressor nerves; and the effect of stimulating the central end of the sciatic nerve. The effects of division of the vagi nerves and the injection of atropine were also investigated.

After some preliminary experiments it was found that the best results were obtained with large doses given by intravenous injection. Illustrative portions of the tracings are appended to this paper. The portions so taken are indicated by the "time" in the tables being underlined.

### Experiment LV.

The rabbit used in this experiment weighed 2000 grammes (2 kilos) and the dose injected was 1mg of African Cobra Venom in two divided doses into the left jugular vein. Ether was used as the anaesthetic.

Experiment LV.

| Time | Amount injected | Average blood pressure in M.M. | Pulse rate per 10 secs | Resp. per 10 secs | Notes. |
|---|---|---|---|---|---|
| 9.46 | | 98 | 39 | 9 | Respiration waves well marked regular. Pulse movements 1.5 to 2 m.m. |
| 9.48 | | 97 | 40 | 9 | Do. Do. |
| 9.48.30 | African Cobra Venom .25 mg per kilo .5 mg in 1cc Saline into left jugular vein. | | | | |
| 9.49 | | 97 | 41 | 8 | Respiration waves deeper. |
| 9.55 | | 95 | 41 | 7½ | Respiration waves very well marked. regular and deeper than normal. Pulse movements larger 3 m.m. |
| 9.59 | | 98 | 42 | 7½ | Do. Do. |
| 10.26 | | 98 | 37 | 6 | Pulse movements now much more evident being occasionally 5 mm. |
| 10.40 | | 104 | 32 | 5 | Respiration still regular pulse movements larger 6 mm. |
| 10.52 | | 104 | 32 | 5 | Respiration slightly irregular but waves very well marked. |
| 11.11 | | 112 | 32 | 5 | Pulse movements very well marked 7 mm. |
| 11.25 | | 110 | 30 | 5 | Do. Do. |
| 11.28.30 to 11.29 | African Cobra Venom in Saline. .5 mg into Vein. | | | | Respiration irregular for a little after injection. |
| 11.30 | | 110 | 33 | 5 | Pulse movements still better marked. |
| 11.40 | | 113 | 30 | 4 | Respirations becoming irregular. |
| 11.50 | | 110 | 28 | 4 | Respiration regular. |
| 12 | | 112 | 21 | 4 | Cardiac impacts are becoming exceedingly clear with the slowly beating heart. |

Experiment LV continued:

| Time | Amount injected | Average blood pressure in M.M | Pulse rate per 10 secs. | Resp. per 10 secs. | Notes. |
|---|---|---|---|---|---|
| 12.6 | | 116 | 16 | 3½ | The slowly beating heart exhibits in the pulse movements a large expansion often attaining 16 m.m. There are frequent diastolic falls. |
| 12.36 | | 116 | 15 | 4? | Respiration waves not easily distinguishable and very irregular. Pulse movements large. |
| 12.46 | | 108 | 9 | ? | Respiration very shallow with occasional convulsion and corresponding rise of blood pressure. Pulse movements few and very large. |
| 12.50 | | 100 | 8 | ? | Respiration as above. |
| 12.52 | | 66 | 9 | ? | Respirations very slow and shallow. 2 in 10 secs 12 in 60 seconds. |
| 12.53 | | 42 | 6 | 0 | Respiration ceased. Pulse movements very large. Fall in pressure continued but after |
| 12.55 | | 46 | 7 | | two minutes, pulse movements which had ceased reappeared. |
| 12.57 | | 0 | 0 | | Heart ceased finally at 12.57. |

From/

From the above experiment we see that the blood pressure is but little affected. There is a rise to a slight extent after injection of the venom, and the rise becomes more marked at the time that respiratory failure comes on.

The pulse rate is slightly quickened after the injection for a little time, but the main effect on the pulse rate is a decided slowing with the appearance of large pulse waves.

The pressure remains constant despite the fluctuations in the pulse movements. In this experiment the respirations are seen to become progressively slowed and they cease before the pulse movements are obliterated in the blood pressure curve.

Experiment LVI.

Rabbit, weight 1750 Grammes. 2 mg African Cobra Venom in two doses ( dissolved in Saline ) injected into left jugular vein. In this experiment the effect of stimulation of the vagus nerve was noted: as also the effect of division of the vagi.

Experiment LVI.

| Time | Substance injected and Dose. | Average blood pressure in M.M. | Pulse rate per 10 secs. | Resps 10 secs. | Notes. |
|---|---|---|---|---|---|
| 2.40 | | 102 | 40 | 5 | Respiration waves not very distinct. Pulse movements small (1 mm.) |
| 2.41 | | 100 | 40 | 5 | Do. Do. |
| 2.42 to 2.43.18 | Injection 1. 25 mg Venom intravenously. | | | | |
| 2.43.50 | | 102 | 42 | 6 | Pulse movements still small Respiration waves more marked. |
| 2.44 | | 102 | 44 | 6 | Pulse rate quicker. |
| 2.50 | | 100 | 40 | 6 | |
| 3 | | 98 | 38 | 6 | Respiratory waves still better marked than at 2.43.50. |
| 3.16 | | 94 | 38 | 6½ | Pulse movements larger. |
| 3.19 | | 96 | 36 | 6½ | |
| 3.22 | | | | | Stimulation of Left Vagus secondary at 120 mm. Fall in blood pressure and slowing of heart temporarily |
| 3.32 | | 90 | 34 | 7 | Respiratory waves well marked. |
| 3.33 to 3.33.40 | Cobra Venom .75 mg into Vein. | 90 | 34 | 7 | |
| 3.34 | | 94 | 35 | 8 | Respiration quicker. Pulse waves larger - being now 2 mm. |
| 3.47 | | 98 | 36 | 8 | |
| 3.51 | | 90 | 30 | 6 | Respiratory waves undistinguishable. Respiration slower and more feeble. |
| 4.2 | | 92 | 26 | ? | Pulse rate much slower, movements still larger (2.5 mm.) |

Experiment LVI. continued:

| Time | Substance & amount injected. | Average blood pressure in M.M. | Pulse rate per 10 secs. | Respns 10 secs. | Notes. |
|---|---|---|---|---|---|
| 4.16 | | 90 | 20 | ? | Pulse waves larger 4 mm. |
| 4.18 | | 93 | 19 | 6 | Respirations feeble, respiratory waves indistinguishable. Traube-Hering Curves evident. |
| 4.21.30 | | 96 | 17 | ? | |
| 4.22. | | | | | Stimulation of Right Vagus, secondary at 150 mm. marked slowing of heart and fall in blood pressure temporarily. |
| 4.26 | | 94 | 15 | | Pulse movements very large. 7 mm |
| 4.26.12 | | | | | Right Vagus cut. |
| 4.26.30 | | | | | Left Vagus cut. Immediate rise in pressure and increase in rapidity of pulse rate. |
| 4.27 | | 109 | 26 | 6? | Respiration waves indistinct |
| 4.30 | | 116 | 24 | 5 | Pulse waves smaller 3 mm. Respiration laboured and at rate of 5 in 10 seconds. |
| 4.36 | | 110 | 22 | ? | |
| 4.41 | | 110 | 24 | 4 | Respirations shallow. |
| 4.47 | | 106 | 21 | | |
| 4.59 | | 100 | 23 | | Pulse waves still small, (2 mm.) |
| 4.59.5 | | | | | Stimulation of lower end of left vagus with secondary at 120 mm. Immediate fall of pressure, and marked slowing of heart, temporarily. |
| 5.1.20 | | 100 | 24 | 4½ | Respiratory waves a little more distinct. |

Experiment LVI continued:

| Time | Substance and dose injected | Average blood pressure in M.M. | Pulse rate per 10 secs. | Respirations per 10 secs. | Notes. |
|---|---|---|---|---|---|
| 5.1.30 | | | | | Stimulation of lower end of left vagus with secondary at 120 mm, Immediate fall of blood pressure and complete inhibition of heart. |
| 5.12.35 | | 90 | 22 | 4 | Respirations gasping Pulse movements small- 2 mm. |
| 5.16 | | 88 | 19 | ? | Respirations 24 in 60 seconds, of a Cheyne Stokes type. |
| 5.18 | | 50 | 16 | 0 | Respiration ceased. Pulse movements 1.5 mm. |
| 5.21.30 | | 0 | 0 | 0 | Blood Pressure has reached the Abscissa line line. |

This experiment shows that the blood pressure, as in the previous experiment, remains at a fairly constant level, and this even though the pulse rate as seen in the blood pressure line is much slowed while the individual pulse movements are much enlarged. Stimulation of the vagus nerve at this time shows that there is no paralysis of the vagal nerve ends. Section of the vagi at once liberates the heart from the increased inhibition evidently exercised by the vagal centre in the medulla and there results a great increase in the number of the pulse beats and a diminution in their amplitude. The blood pressure is seen to be raised. Stimulation of the vagus nerve, even at so late a period in the action of the venom as 20 minutes before death, still calls forth the usual response and indicates that the vagal nerve ends are still active.

But while section of the vagi nerves results in an increase in the rate of the heart beat, this increase is not such as to bring the number of heart beats back to what it was before the injection of the venom. This therefore points to a local action of the venom on the cardiac muscle as is found to be the case in frogs.

The respiratory curves show the same changes as in the previous experiment. The rate is after each injection of venom, increased, but the final effect of the venom is to cause a paralysis of respiration.

Experiment LVIII.

Experiment LVII.

In this experiment the effect of the injection of Atropine was investigated. All interference with the vagi either mechanically or by electrical stimulation was avoided. The rabbit employed weighed one kilogramme: the dose of venom was 1 mg and the strength of the Atropine solution was .030 in 6 cc. (strength used by Fraser and Tillie in Paper on Acocanthera ).

| Time | Substance injected and dose | Average B.P. in m.m. | Pulse rate per 10 secs. | Respirations per 10 secs. | Notes. |
|---|---|---|---|---|---|
| 2.28 | | 86 | 40 | 7 | Pulse movements 1 mm. Respiratory waves well marked. |
| 2.29 | | 86 | 40 | 7 | do. do. |
| 2.30.30 | 1 mg.cobra venom in saline into vein. | | | | |
| 2.32 | | 90 | 45 | 8 | Pulse rate quicker, movements small (1 mm). Respiratory movements not so well marked. Respiration more rapid. |
| 2.36 | | 86 | 44 | 9 | Pulse rate still faster than normal, movements smaller .75 mm. Respiratory rate increased, waves well marked. |
| 2.38 | | 86 | 44 | 10 | |
| 2.50 / | | | | | |

Experiment LVII. continued:

| Time | Substance and dose injected | Average B.P in mm. | Pulse rate per 10 secs | Respns per 10 secs. | Notes. |
|---|---|---|---|---|---|
| 2.50 | | 94 | 36 | 7 | B.P. higher. Pulse rate slower, waves more marked (1.75 mm). Respiration slower and deeper in character. |
| 2.54 | | 97 | 30 | 6.5 | Pulse movements still larger (2 mm). |
| 2.58 | | 100 | 28 | 6 | |
| 2.59 | Injection .6 cc. Atropine sulphate solution. | | | | |
| 3 | | 104 | 42 | 7 | Effect of Atropine has been to increase the blood pressure, the rate of heart beat. The amplitude of pulse movement is much diminished being now 1 mm. Respiratory rate increased. |
| 3.0'30" | | 60 | 30 | ? | Blood pressure fell rapidly, as also rate of heart beat. Amplitude of pulse movements is greater being now (2 mm). Respiration is irregular and feeble. |
| 3.0'45" | | 38 | 20 | ? | |
| 3.2'30" | | 18 | 15 | 0 | Respiration ceased. |
| 3.5 | | 0 | 0 | 0 | Blood Pressure line has fallen to zero. |

In this experiment the characteristic effects of Atropine on the circulation and on the respiration are noticed, but the heart is evidently so affected by the venom that the changes induced by Atropine are only transitory and both the heart and the respiration are finally paralysed. In this experiment the marked increase in the number of the respirations that ensues for a short time upon the injection of the venom is well seen and points to a primary stimulation of the respiratory centre before its final paralysis.

While the characteristic effect of Atropine is obtained in the experiment, the doses of the venom and of the Atropine are evidently such, that the animal did not survive long enough to afford a good tracing, and the following experiment was performed in which a smaller dose of venom was administered, and the injection of Atropine was used at an earlier stage, while the animal was not so profoundly under the influence of the venom.

Experiment LVIII.

In this experiment the rabbit used weighed 1880 grammes. The total amount of venom injected into the jugular vein was 0.9 mg., and the dosage of the solution of Atropine Sulphate was as is stated in the accompanying table.

Experiment LVIII continued:

| Time | Substance & amount injected. | Average B.P. in M.M. | Heart beats per 10 sec. | Respns per 10 secs. | Notes. |
|---|---|---|---|---|---|
| 10.28 | | 102 | 34 | 8 | Pulse movements 1.5 mm. Respiratory waves not well marked. |
| 10.29 | | 102 | 34 | 8 | do. do. |
| 10.30 | .5 mg Cobra Venom in Saline into vein. | | | | |
| 10.31 | | 106 | 36 | 9 | Pulse quicken. Respiration quicker. Pulse movements still 1.5 mm. |
| 10.32 | | 108 | 32 | 10 | Pulse slower, movements larger 3 mm. Respiration quicker. Waves not well marked. |
| 10.34 | | 106 | 29 | 7 | Pulse slower, movements now 4 mm Respiratory waves well marked, and rate much diminished. |
| 10.35 | .25 cc. solution of Atropine Sulphate into vein. | | | | |
| 10.36 | | 108 | 36 | 7 | Pulse quicker, movements (3 mm.). |
| 10.38 | | 100 | 32 | 8 | Respiration quicker and waves well marked. |
| 10.41 | | 106 | 31 | 8 | Pulse movements 4 mm. |
| 10.47 | | 103 | 34 | 8 | Pulse movements 2 mm. |
| 10.49 | | 100 | 36 | 8 | Pulse movements 1.75 mm. rate quicker. Respiratory waves well marked and regular and respiration, since injection of Atropine, faster. |

Experiment LVIII. continued:

| Time | Substance & amount injected. | Average B.P. in M.M. | Heart beats per 10 sec. | Respns per 10 secs. | Notes. |
|---|---|---|---|---|---|
| 10.50 | .4 mg Cobra Venom in Saline into vein. | 100 | 36 | 8 | |
| 10.51 | | 100 | 38 | 8.5 | Pulse rate a little quicker. movements still small: Respiration faster. |
| 10.53 | | 98 | 39 | 7 | Heart beats still faster- pulse movements small 1.75 mm. Respiration slower and deeper. |
| 11. | | 98 | 33 | 7 | Pulse slower. Movements larger 2.25 mm. |
| 11.6 | | 100 | 31 | 7 | |
| 11.12 | | 128 | 22 | 6 | Pulse slower, movements very large 8 mm. Respiration slower and very deep occasionally. |
| 11.15 | | 130 | 21 | 5 | Pulse movements still very large (9 mm.) |
| 11.15'30" | .5 cc solution Atropine Sulphate into vein. | 130 | 21 | 5 | |
| 11.17 | | 130 | 24 | ? | Respiration irregular, much embarrassed. |
| 11.18 | | 132 | 28 | ? | Pulse faster, movements smaller 5 mm. Respiratory waves indistinguishable. |
| 11.19 | | 130 | 30 | ? | do. do. |
| 11.19'15" | | 110 | 26 | 0 | Respiration ceased. |

Experiment LVIII. continued:

| Time | Substance & amount injected | Average B.P. in M.M. | Heart beats per 10 sec. | Respns per 10 secs. | Notes. |
|---|---|---|---|---|---|
| 11.21 | | 98 | 24 | 0 | |
| 11.21'30" | | 70 | 24 | 0 | Pressure rapidly falling, Pulse slow. Pulse movements. 4 mm. |
| 11.22 | | 20 | 12 | 0 | |
| 11.22'15" | | 0 | 0 | 0 | Blood pressure tracing has reached abscissa line. |

A consideration of the above table shews the same phenomena occurring as in the previous experiment-LXVII. We note that after the injection of the venom there is an increase in the pulse rate and in the rate of the respiration. Both these are later on however, slowed and on the injection of Atropine the pulse rate is quickened and the amount of movement of the individual beats is lessened; the respiration is slightly quickened.

On a second injection of venom the pulse rate is still further quickened temporarily as is also the respiratory rate. Both the pulse rate and the respiratory rate become quickly affected and the further injection of Atropine is now not so potent in calling forth the usual response.

The/

The blood pressure remains remarkably constant and the greatest elevation of pressure corresponds to the final embarrassment of respiration. Respiration however ceased before the pulse beats are obliterated and while the blood pressure is comparatively high.

Experiment LIX.

In order to obtain a graphic representation of the changes in respiration that are induced by injections of African Cobra Venom arrangements were made to record the variations in the respiratory movements with a lever attached to the xiphisternum which had been previously isolated from the sternum but left attached to the two slips of the diaphragm which we inserted into it.

The lever attached to the xiphisternum was made to record its excursion on smoked paper fixed to a revolving drum in order to lessen the friction. The rabbit used in this experiment weighed 2.000 grammes, and the dose of venom that was injected intravenously was .5 mg per kilo weight.

Experiment LIX. continued:

| Time | Average blood Pressure in M.M. | Number of heart beats per 10 secs. | Respiration per 10 secs. | Respiratory excursions in M.M. | Notes. |
|---|---|---|---|---|---|
| 2.35 | 112 | 35 | 11 | 2.5 | |
| .37 | 114 | 35 | 11 | 2.5 | |
| .39 | 113 | - | - | - | Injection of .5 mg to kilo intravenously. |
| 2.39.30" | 120 | 36 | 15 | 3 | Blood pressure raised a little. Heart beats more frequent. Respiration a little quicker and deeper. |
| 2.41 | 118 | 38 | 17 | 5 | Heart faster, respiration is quicker and deeper. |
| 2.49 | 118 | 39 | 10 | 8<br>18<br>12 | Respiratory excursus much larger and a little irregular. |
| 2.56 | 120 | 37 | 8 | 20 | |
| 2.59 | 114 | 35 | 7 | 22 | |
| 3.4 | 114 | 33 | 6 | 20 | |
| 3.14 | 120 | 32 | 6 | 10 | Extra weight hung on lever as excursus was large. |
| 3.21 | 116 | 30 | 6 | 14 | |
| 3.25 | 114 | 30 | 7 | 16 | Respiration a little quicker, excursus deeper. |
| 3.28 | 110 | 30 | 7 | 16 | |
| 3.33 | 110 | 29 | 6 | 20 | |
| 3.40 | 108 | 32 | 7 | 22 | |
| 3.44 | 110 | 26 | 6 | 20:12:8 | Respiratory excursus very irregular. |
| 3.46 | 160 | 18 | 6 | 30:6 | Larger figure represents excursus of lever at commencing convulsions. |
| 3.48.30 | 140 | 16 | 5 | 3. | Respiration feeble and slow with occasional convulsive gasp. |
| 3.51 | 68 | 10 | 3 | 2 | |
| 3.51.30 | 60 | 11 | 0 | 0 | Respiration ceased. |
| 3.53 | 0 | 0 | 0 | 0 | |

In the above table we find that the injection of the venom produces a marked increase in the rate of respiration along with an increase in the depth of the individual respirations. This preliminary augmentation however gives place to a slower but still powerful respiration. The blood pressure line shows the same changes as are seen in previous experiments. The rate of heart beat is at first augmented but evidence of the slow heart with large pulse movements soon becomes apparent. In this table also the fact that the highest point of pressure corresponds to the commencement of respiratory failure is well seen.

Experiment LX.

In this experiment the effect of the venom on the vaso-motor centre was investigated by means of stimulation of the Sciatic and Depressor Nerves.

It was found as is seen in the accompanying table, that stimulation of the sciatic nerve caused a rise in the blood pressure and of the Depressor nerve a fall in the blood pressure.

The Vaso-motor centre, which is on the reflex arc involved, is seen to be in state of irritability up to practically the end of life; but it is found that in order to obtain the reaction stronger stimulation is required . At 4.24'10", for instance, when as can be seen from the tracing the animal/

animal was under the influence of the venom, the strength of stimulation necessary, was such as can be obtained with the secondary coil at a distance of 200 millimetres from the primary; at 6.21 the secondary coil had to be moved to a distance of 90 mm. from the primary, and at 6.34!30" the secondary coil was at a distance of only 40 mm. from the primary one.

So with stimulation of the Depressor nerve. At 4.29' the reaction was obtained with the secondary coil at a distance of 120 mm. from the primary coil, while at 6.27 the secondary coil had to be advanced to a distance of 90 mm. from the primary one and at 6.33'.30" to a distance of only 40 mm. to obtain the same phenomena.

This experiment is also interesting in that it shows the reaction previously described, on section of both vagi nerves.

The rabbit employed weighed 1650 grammes. Ether was used as the anaesthetic as in the previous experiments.

Experiment LX.

| Time | Average blood Pressure in m.m. | No. of Heart beat in 10 secs. | Respirations per 10 secs. | Notes. |
|---|---|---|---|---|
| 4.10 | 112 | 32 | 8 | |
| 4.12 | 112 | 32 | 8 | |
| 4.15 | - | - | - | Injection intravenously of .5 mg to kilo. |
| 4.16 | 116 | 34 | 9 | Heart beating more quickly. Respiratory rate is also increased. |
| 4.17 | 116 | 33 | 10 | |
| 4.18 | 120 | 30 | 12 | Heart slower. Respiration quicker pulse movements larger. |
| 4.24 | 120 | 28 | 12 | |
| 4.24.10 | 150 | - | 14 | Stimulation of Central end of Sciatic nerve. Immediate rise in blood pressure with convulsive respiratory movements and temporary increase in the rate. |
| 4.28 | 118 | 28 | 10 | |
| 4.29 | 90 | - | - | Stimulation of Depressor Nerve. Immediate fall of pressure. |
| 4.30 | 114 | 27 | 9 | |

Up till 6 p.m., during which interval stimulation of the depressor nerve on two occasions caused a fall in the blood pressure, the tracing maintained the usual characters. The number of heart beats however increased while the pulse movement became smaller. The respiration was also gradually diminishing in rate - being on an average 7 in 10 seconds. Notes on the tracing after 6 p.m. are appended/

| Time | Average blood Pressure in M.M. | Number of Heart beat in 10 secs. | Respirations in 10 secs. | Notes |
|---|---|---|---|---|
| 6.1 | 100 | 32 | 7 | |
| 6.5 | 102 | 32 | 7 | |
| 6.10 | | | | Injection of .25 mg. to kilo. |
| 6.11 | 106 | 30 | 7 | |
| 6.15 | 104 | 27 | 6 | |
| 6.20 | 110 | 20 | 5 | Respiration feeble. Heart slow. |
| 6.21 | 130 | - | 7 | Stimulation of central end of Sciatic nerve - respiration deeper and faster. |
| 6.26 | 108 | 14 | 5 | Heart profoundly affected, many dropped beats. Respiration feeble and slow. |
| 6.27 | 80 | | | Stimulation of Depressor Nerve |
| 6.29 | 100 | 10 | - | Respirations infrequent. |
| 6.29'20" | 70 | | | Stimulation of Depressor Nerve. |
| 6.31 | 80 | 10 | 4 | Gasping respiratory movements. |
| 6.31.30 | | | | Left Vagus Nerve cut. |
| 6.31.40 | | | | Right " " " |
| 6.32 | 90 | 21 | 2 | Heart beats increased in rate. amplitude of pulse waves is much smaller. |
| 6.33 | 60 | 18 | 2 | Respiration very feeble. |
| 6.33'30" | 30 | 12 | 0 | Respiration ceased. Stimulation of Depressor Nerve. |
| 6.34 | 20 | 5 | 0 | |
| 6.34!30" | 25 | 8 | 0 | Stimulation of Central End of Sciatic Nerve. |
| 6.35'30" | 0 | 0 | 0 | Blood Pressure had reached the Abscissa line. |

## Conclusions from Blood Pressure Experiments.

The conclusions one draws, from the above series of experiments, are that the blood pressure in an animal under the influence of African Cobra Venom remains high till very near death: and that there is a rise above the normal level when the extreme respiratory embarrassment occurs.

The heart is, after a primary quickening in rate, progressively slowed. This slowing is in part due to an action on the Vagal centre in the Medulla and in part to the action of the venom on the heart itself. That it is not wholly due to a nerve action is proved by the fact that on section of the vagi nerves, an increase in the rate of the heart's contractions occurs, but this increase is not so great as would occur were the heart itself unaffected. Evidence in support of this is obtained from the results of local application of the venom to the frog's heart and especially from the experiments where the venom had access to the interior of the heart as well as to the exterior of that organ.

That the Vaso-motor centre is affected, is seen from the results of the last experiment-LX. and in the experiment-LVI the appearance of Traube-Hering Curves is noted which points to a condition of failure of this centre. The contractile effect of the/

the venom on the walls of the vessels is no doubt a great factor in the preservation of the arterial pressure. That there is such an action exercised is seen from the results of the experiments performed in perfusing the blood vessels of the frog.

## Some Pathological Effects produced by African Cobra Venom.

In post-mortem examination of animals killed by the action of this venom, it has been noticed throughout the paper, that there was to the naked eye an appearance of congestion of the kidneys, gut and liver. Microscopic sections of the kidneys, liver and spinal cord of the frog were prepared by the usual paraffin method. The appearance of the anterior cornual cells has already been referred to, and is well seen in figure 1 page ( ).

The appearances of the stained sections of kidney justify the conclusion that an acute desquamative nephritis is set up by the action of the venom. In the sections of liver, marked evidence of congestion with degeneration of the liver cells is seen.

The microscopic appearances of healthy kidney and liver, and of kidneys and liver of an animal killed by the action of African Cobra Venom are shewn/

shewn in figures 22, 23, 24 and 25 pages ( XXXII & XXXIII, ).

## SUMMARY OF RESULTS.

African Cobra Venom is primarily a nerve poison. Evidences of its action on the nervous system are obtained, while the blood is unaffected, and while the circulation is still carried on vigorously. Experiments XIX, XX, XXI: XXII, XXIII, XXIV, XXV, XXVI, XXVII.

### Action on the Nervous System:-

As regards the action on the Nervous System it is found that African Cobra Venom paralyses the motor nerve ends, after a transient stimulation. See experiments on the determination of the minimal lethal dose in frogs and also experiments XII, XIII, XIV, XV, XVI, XVII.

It paralyses the reflex function of the spinal cord and the part of the arc affected is the anterior cornual cell. Experiments XVIII, XII, XIII. With large doses it is found that the reflexes are paralysed before the motor nerve ends - experiment XX., and with small doses there is a practically simultaneous paralysis of the reflexes and of the motor nerve ends. Experiment XXI.

After a transient stimulation of respiration there is a depressant action exercised and the respiratory/

respiratory centre in the Medulla is finally paralysed. This is the cause of death.

There is a stimulant action on the Vagal centre in the medulla. Experiment XLIId. etc. That this stimulant effect is exercised for a considerable time after the respiratory centre is paralysed is probable - see Experiment XXXIX etc. This points to the venom having a special action on the respiratory centre.

The final effect of the venom is to depress the vagal centre in the medulla and finally to paralyse it.

The Vaso-motor centre is not so profoundly affected as the other medullary centres but the appearance of Traube Hering Curves in the blood pressure tracing in Experiment LVI and the result of the stimulations of the Sciatic and Depressor Nerves seen in Experiment LX point to a depressant action being also exercised upon this centre by African Cobra Venom.

Action on Muscle (skeletal).

African Cobra Venom induces an earlier onset of the phenomena of fatigue, and also irregularity in the amount of contraction towards the end of the fatigue curve. Experiments XXX, XXXI and XXXII.

Action on the Heart and Blood Vessels:-

Slowing of the heart is produced. This is in part due to the action of the venom on the vagal centre in the medulla and in part to the action on the heart itself. Experiments XXXIX to XLII d. inclusive.

The results of the perfusion experiments performed on frogs, (XXXIII to XXXVIII inclusive) point to a powerful constrictor effect being exercised by African Cobra Venom , if the strength of solution be sufficient. The pulse rate is, after a primary increase in rate, steadily slowed till death. Experiments LV to LX inclusive.

Action on the Blood.

No haemolysis occurs "in corpore" in rabbits.

When the action on the various parts of the nervous system in frogs is manifest the blood shews no evidence of haemolysis.

"In vitro" in frogs haemolysis can be obtained Experiments XXVIII and XXIX.

In frogs at death haemolytic phenomena are found, see experiments on the determination of the minimal lethal dose for frogs and figure 3 page ( ).

A marked leucocytosis is found in rabbits after subcutaneous injection of African Cobra Venom: see Experiments detailed on page ( ).

## Action on Temperature.

In rabbits a rise in temperature is found in cases of slow absorption. Where on the other hand the dose has been large there is a marked fall near death - an algid state of the animal : see experiments detailed on page ( 76 ).

## Effect on Metabolism.

In so far as this case be gauged by the loss of weight in the animal, we see from experiments detailed on page ( 79 ) that metabolism must be affected.

## Pathological Effects.

Desquamative nephritis is produced, as also great congestion of the liver with degeneration of the liver cells. Nowack describes fatty degeneration of the kidney and liver as a sequance to the injection of snake venom but African Cobra Venom does not appear to possess this action.

## Toxicity of sample employed in this research.

Minimal lethal dose for frogs, .001 milligramme per gramme weight.

Minimal lethal dose for rabbits, .3 milligramme per kilogramme weight.

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BIBLIOGRAPHY. consulted.


| | |
|---|---|
| BRUNTON & FAYRER | Transactions of the Royal Society of London 1873 & 1875. |
| ARON | Archives fur Pathologie und Pharmacologie 1880. |
| WALL | Indian Snake Poisons 1896. |
| RAGOTZI | Virchow's Archives Band 122 S 232. |
| WEIR MITCHELL & REICHERT | Smithsonian Contributions to General Knowledge XXVI. |
| BLYTH | Journal of Pathology 1885. |
| NORRIS WOLFENDEN | Journal of Pathology 1886. |
| CUNNINGHAM | Memoirs of Medical Officers of India pts. IX and XI. |
| MYERS | Lancet 1898. |
| STEPHEN & MYERS | Journal of Pathology and Bacteriology 1898. |
| LAMB & HANNA | Journal of Pathology and Bacteriology 1903. |
| AUCHE & VALLIANT | Comptes Reudus de Societe de Biologie 1902. |
| NOWAK | Pasteur's Annalen 1898. |
| VOLLMER | Archives fur Pathologie und Pharmacologie 1892-93. |
| CALMETTE | Annalen Pasteur 1892. |
| FEOKTISTOW | Memoirs Academie des Science de St. Petersburg. Vol. VII. S XXXVI. |
| MARTIN | Clifford Allbut's System of Medicine. |
| ELLIOT | British Medical Journal 1900. |
| CLOUSTON | Mental Diseases |
| LAMB & HUNTER | Lancet 1904. |
| FRASER | British Medical Journal. |


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DIAGRAMS and TRACINGS

Illustrative of Paper

on

# A F R I C A N  C O B R A  V E N O M

W. H. PRENTICE, M.A., M.B., Ch.B.

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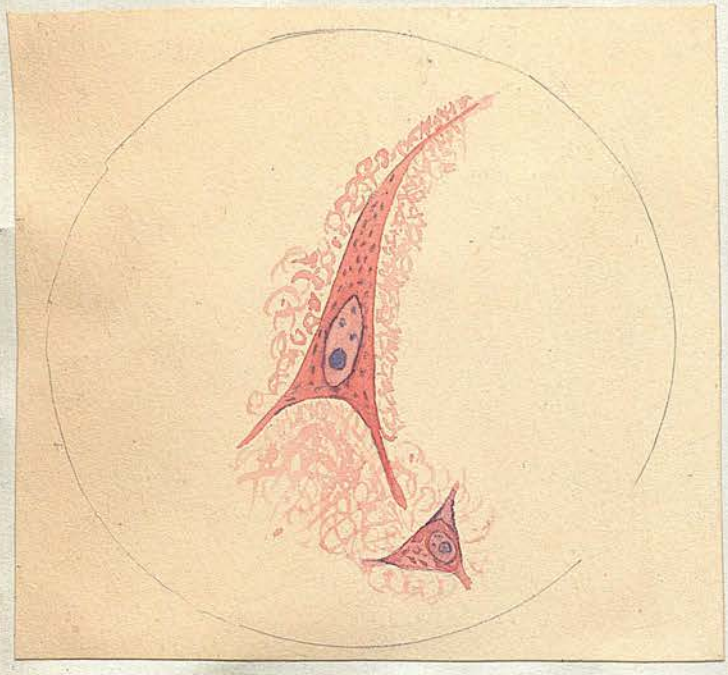
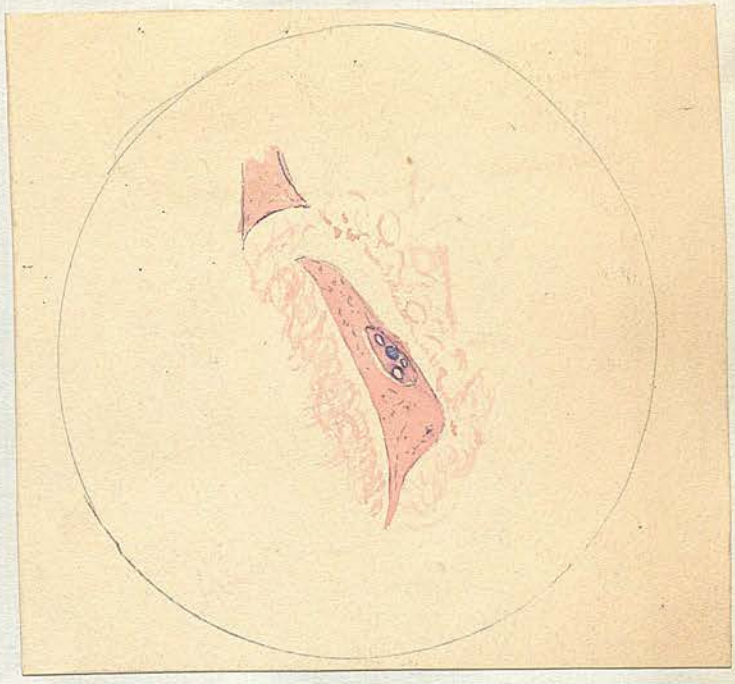
Cells in anterior cornua of Spinal Cord of Frogs.



Ia



Ib.

Fig. Ia shews the appearance of a healthy nerve cell in the anterior cornu of the lumbar part of the cord.

Fig. Ib shews the appearance of a nerve cell in the anterior cornu in the lumbar part of the spinal cord of a frog which died on the 9th day after the subcutaneous injection of .0018 mg of African Cobra Venom to the gramme weight of animal.

Note that the poisoned cell is shrunken as a whole, also that the nucleus is smaller and is vacuolated. The chromatin granules also stain much more faintly and irregularly than in the healthy cell. Degeneration of processes of cell?

Magnified - 750 times. Stain:- Haematoxylin and Eosin.

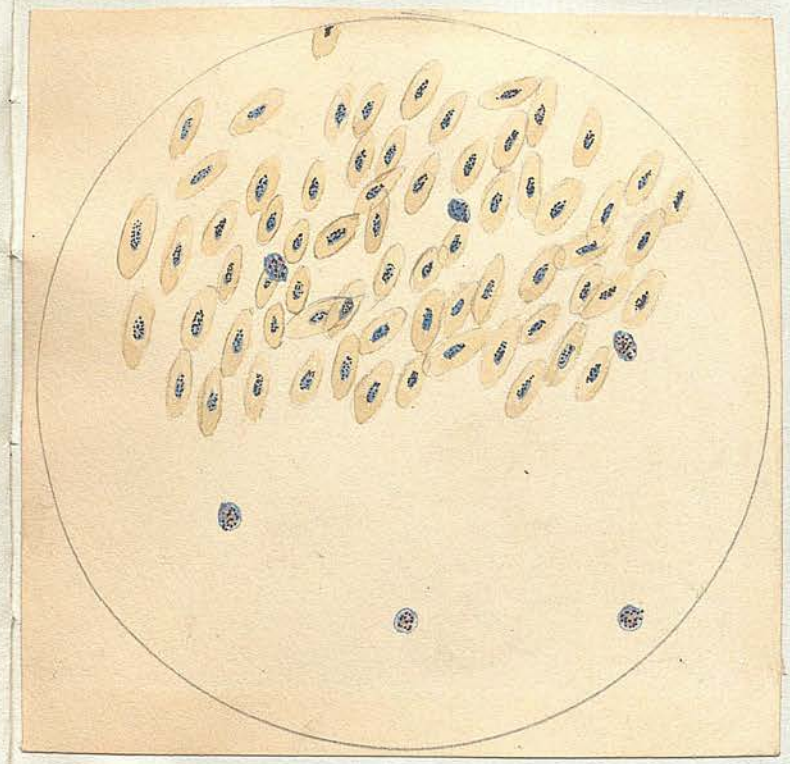


Fig. 2.

Blood from heart of healthy frog.

Note the regularity of the blood cells and the deep staining, of the nuclei of the red cells, and of the leucocytes.

Magnified 600 times

Stain :- Jenner's.

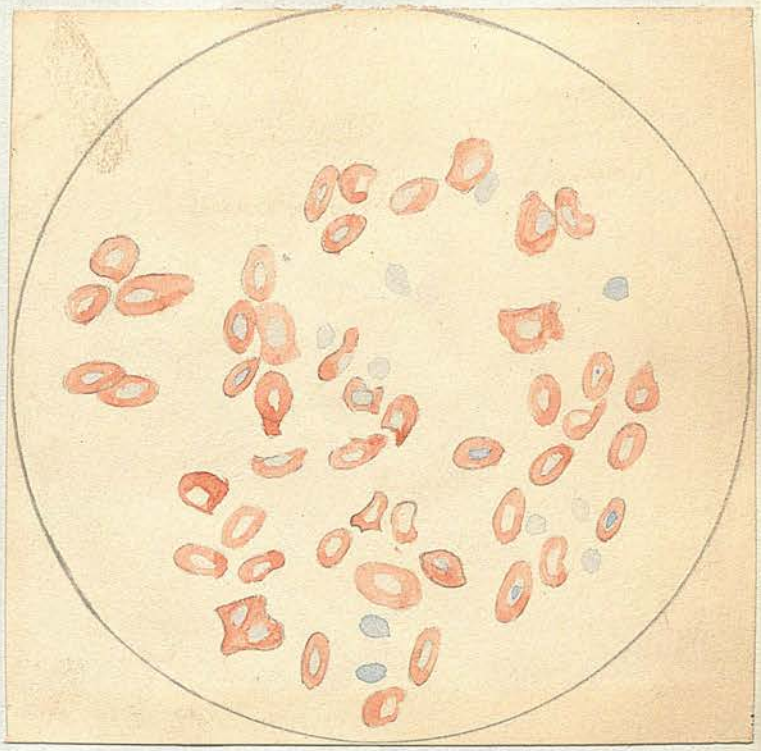


Fig. 3.

Blood from heart of frog IX. on table of estimation of the minimal lethal dose. Taken from heart at death from the injection of .02 mg of venom per gramme weight.

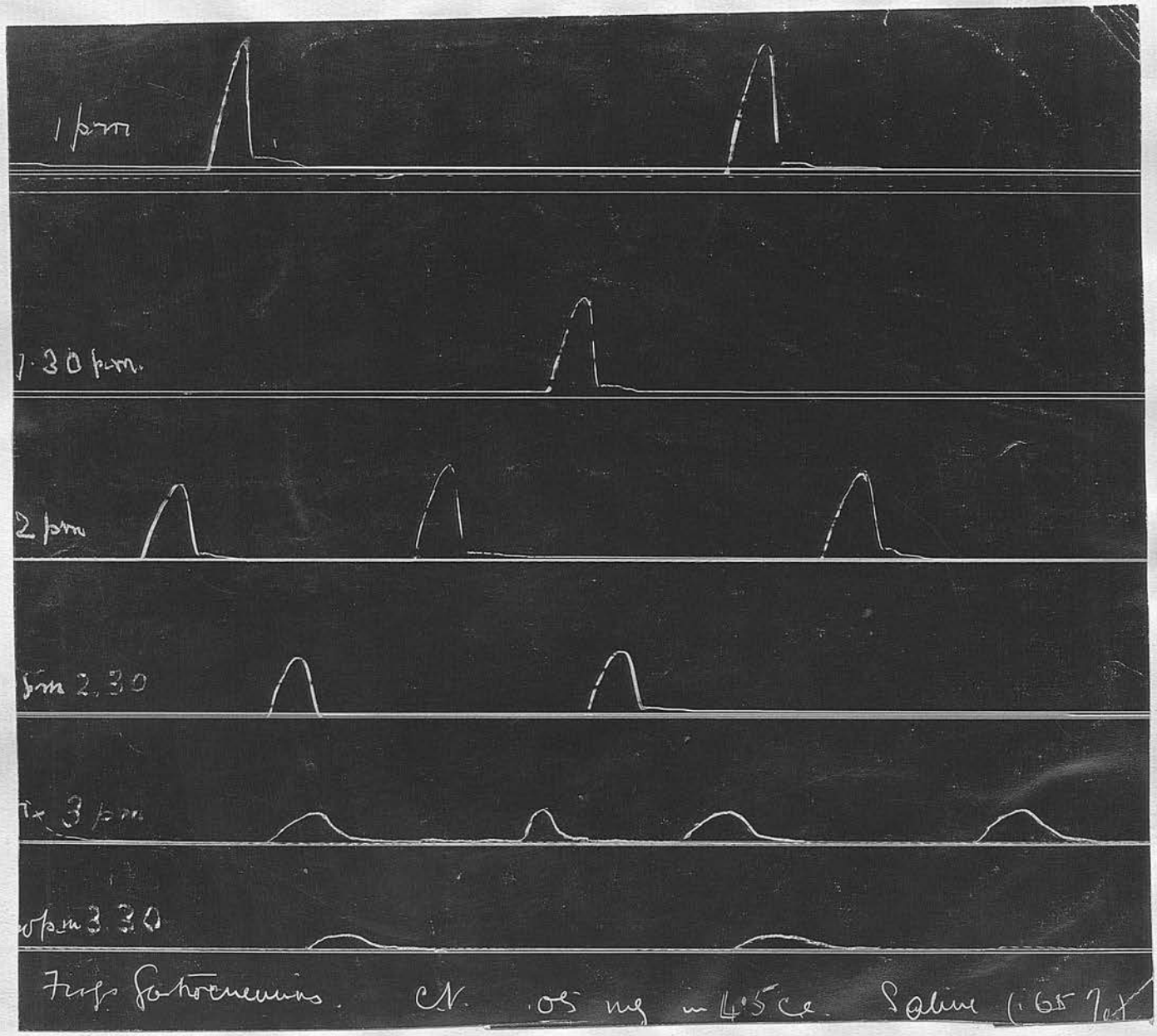
Note:- leucocytosis.
- poikilocytes.
- shadow corpuscles.
- faint staining,
- altered staining of haemoglobin of the red cells.

Magnified 600 times.

Stain :- Jenner's

Tracing shewing the effect of African Cobra Venom on muscle.

Fig. 4.




At 1 p.m. contractions that can be obtained from the muscle before it is influenced by the venom are seen. From 1.30 p.m. to 3.30 p.m. inclusive, the curves shew the contractions obtainable from the muscle immersed in a solution of venom.

Stimulation of Muscle directly. See page 44.

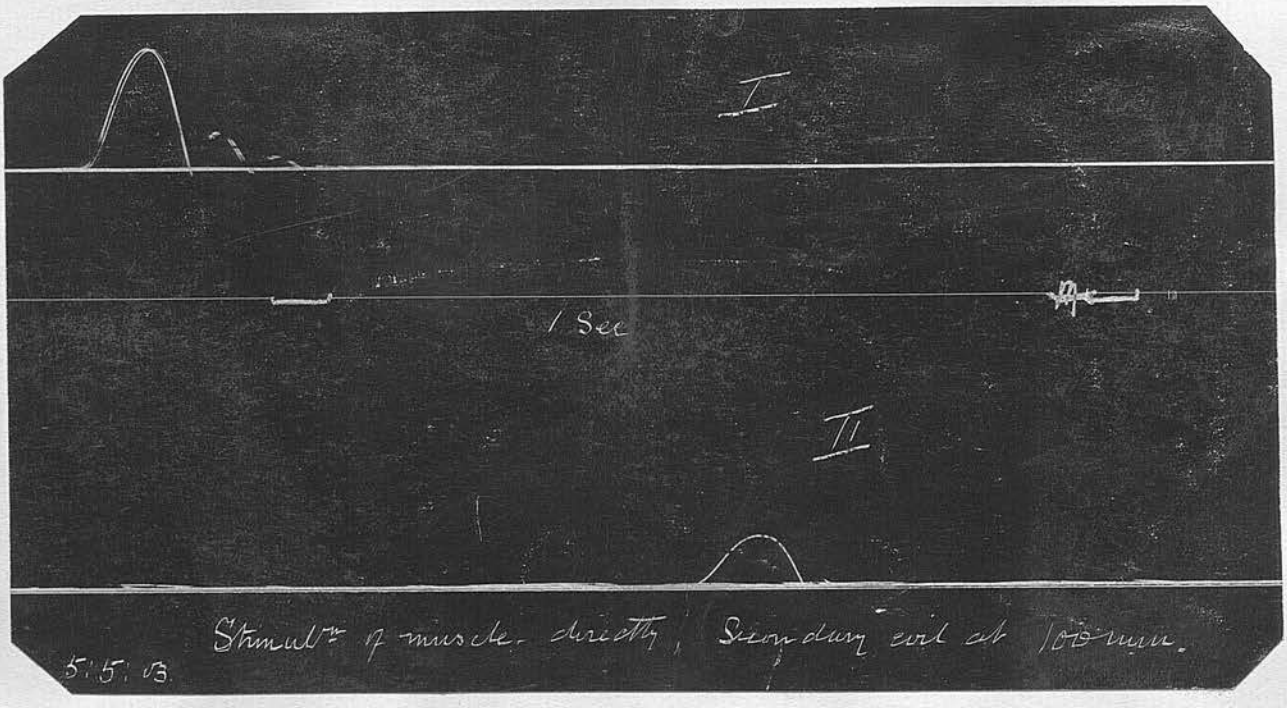
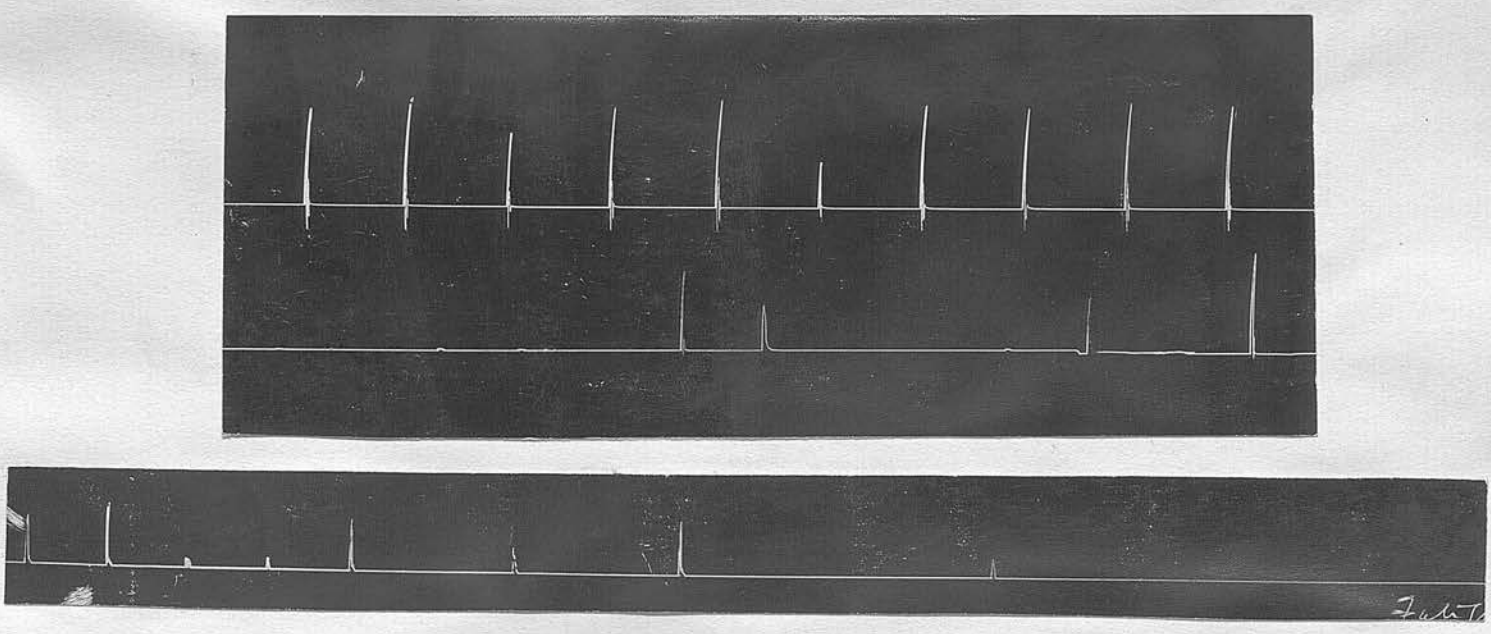



Fig. 5. Tracing above shews in I the amount of contraction obtainable from a gastrocnemius muscle in a leg of a frog, which is protected from the action of the venom: in II the amount of contraction obtainable from a poisoned muscle. See page 45.

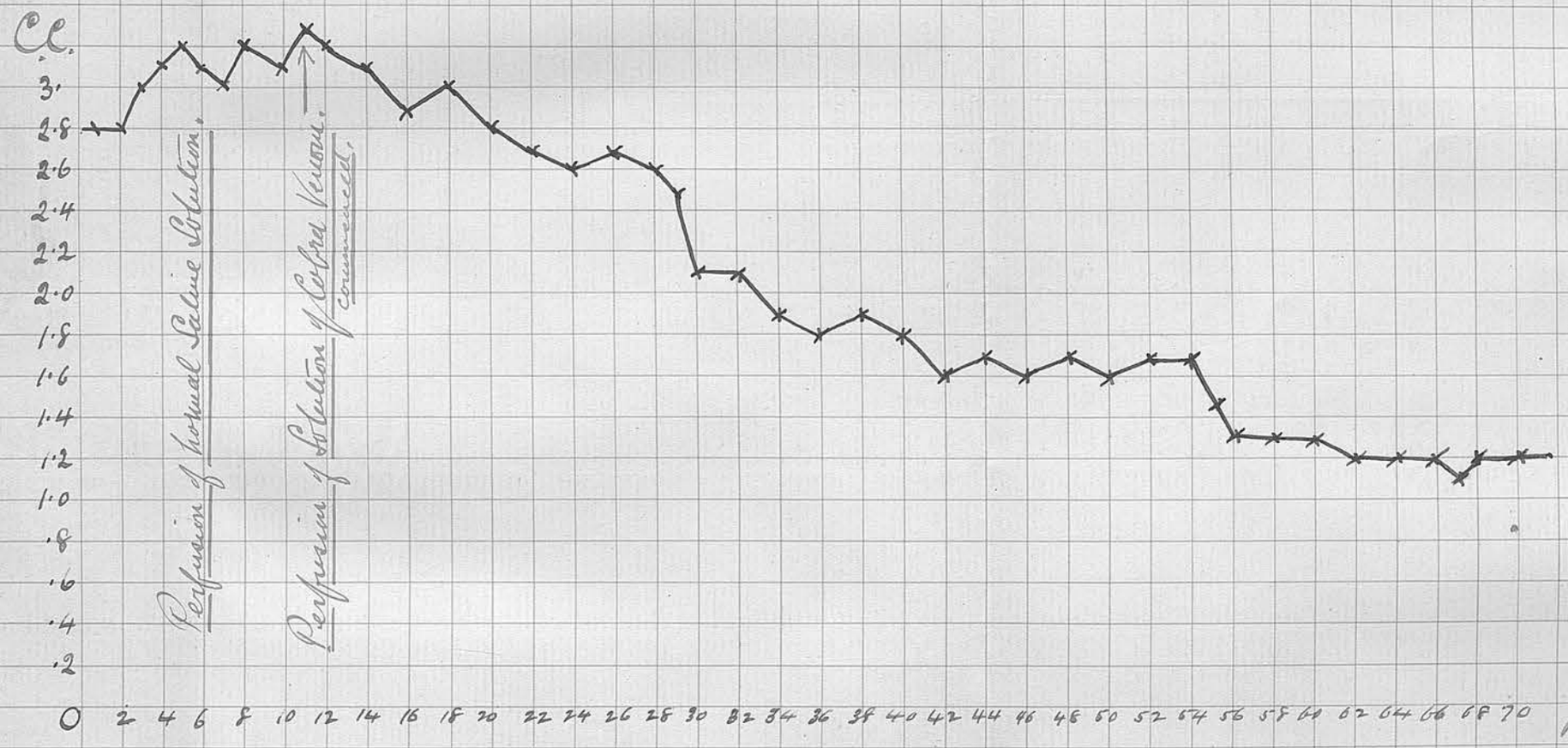


Figs. 6 & 7, above, shew the variations in the individual contractions of a gastrocnemius muscle to which the venom had access. See page 46.

Curve Illustrating Expt XXXIII; see table on page 48.

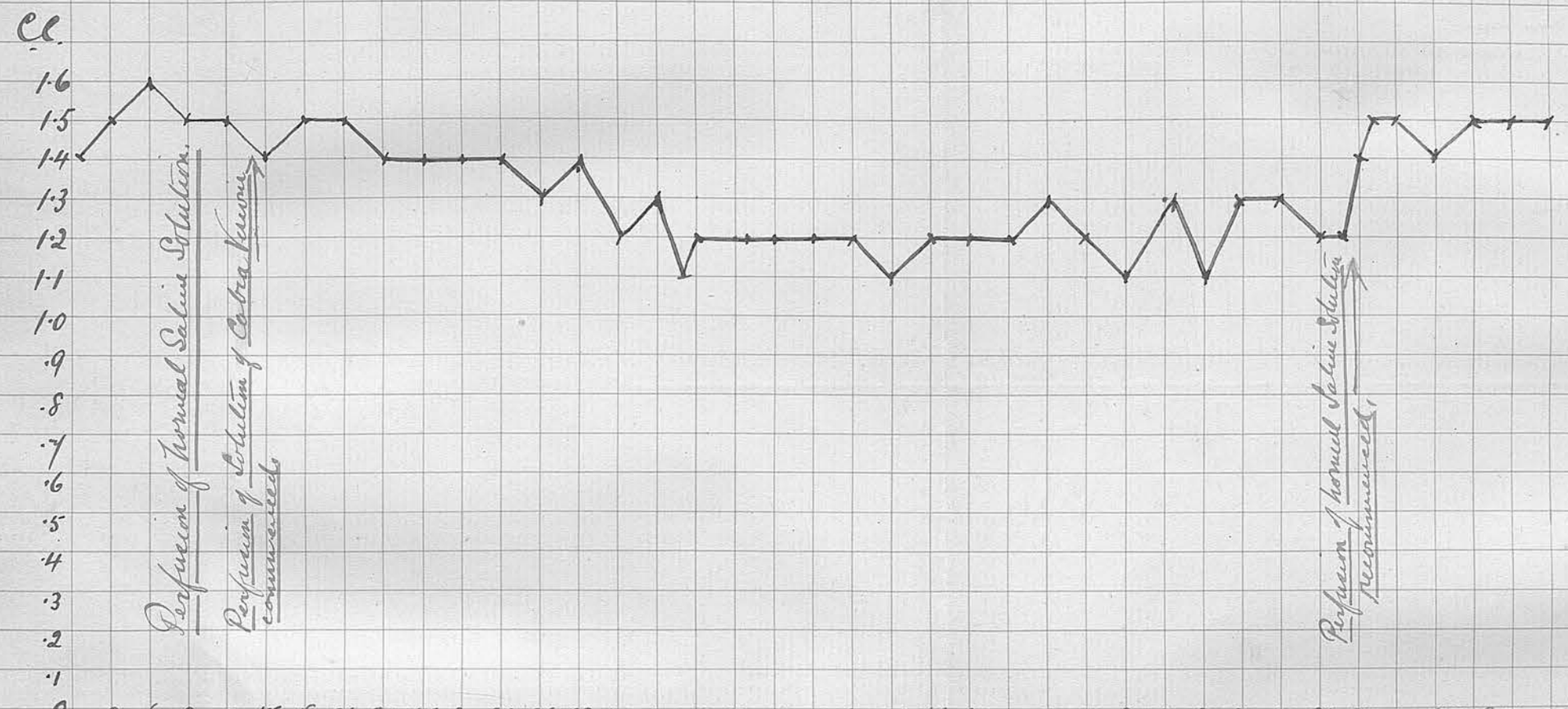

The Curve shews the marked constricting effect on the vessels of a solution of African Cobra Venom in a strength of 1 in 20,000.

Curve Illustrating Exp^t. ~~XXXIV~~ as table on page 60.



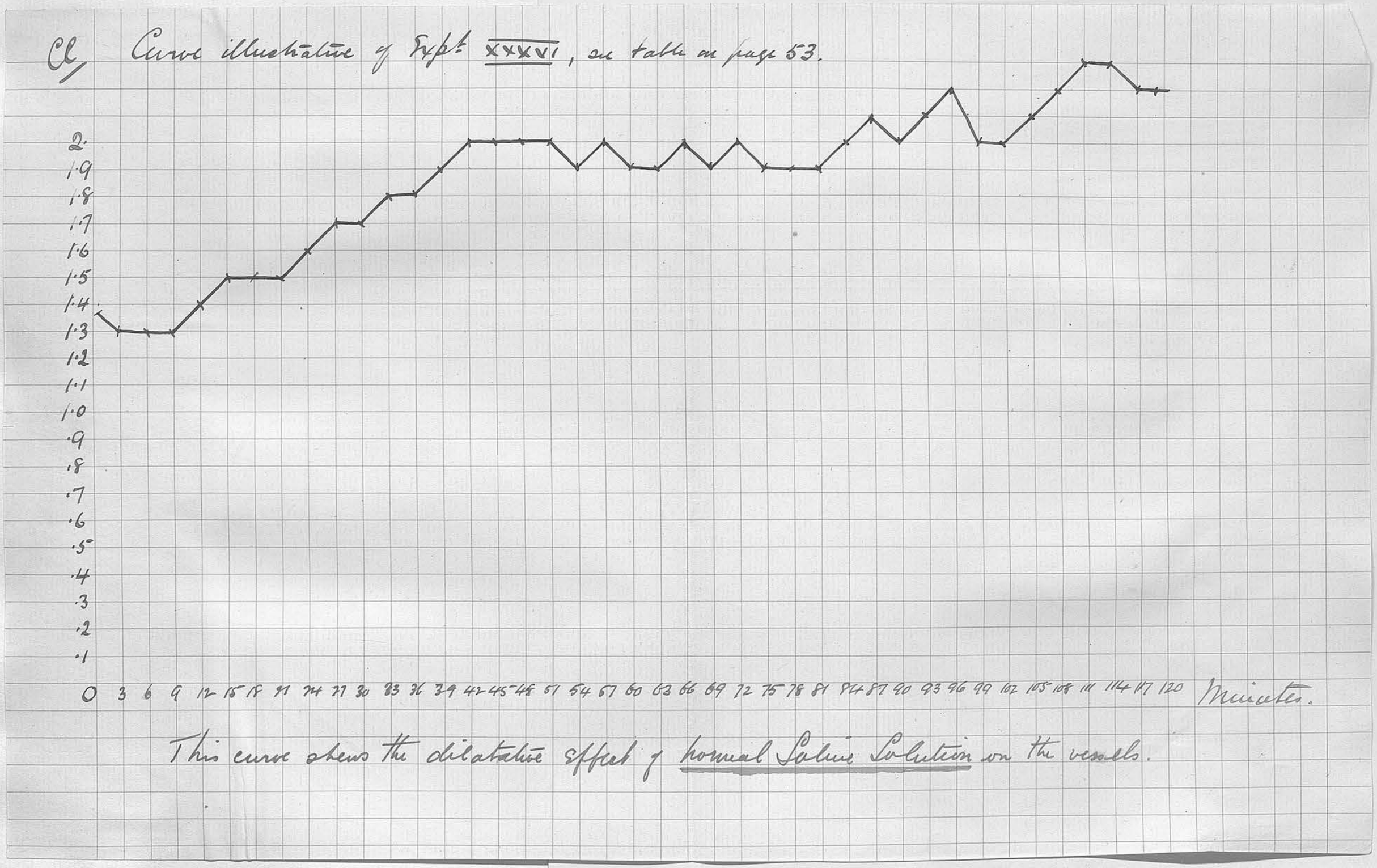

This Curve shews the effect on the vessels of a solution of African Cobra Venom in a strength of 1 in 40.000.

Curve Illustrating Expt. XXXV : see page 51.



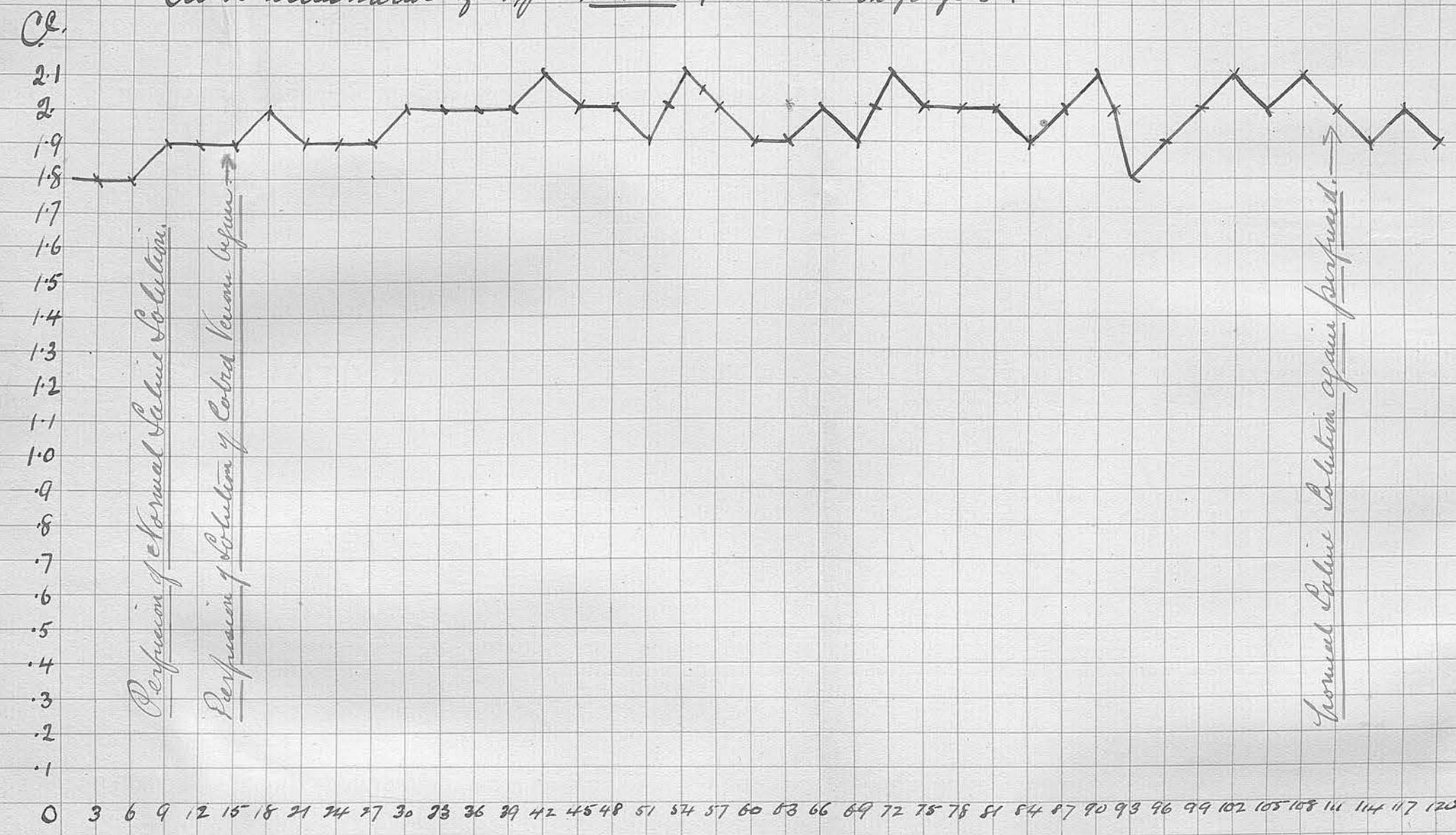

This Curve shows the effect of a solution of Cobra Venom on the vessels in a strength of 1 in 100.000. Note also the recovery after Normal Saline Solution is again perfused.

Cl Curve illustrative of Expt. XXXVI, on table on page 53.



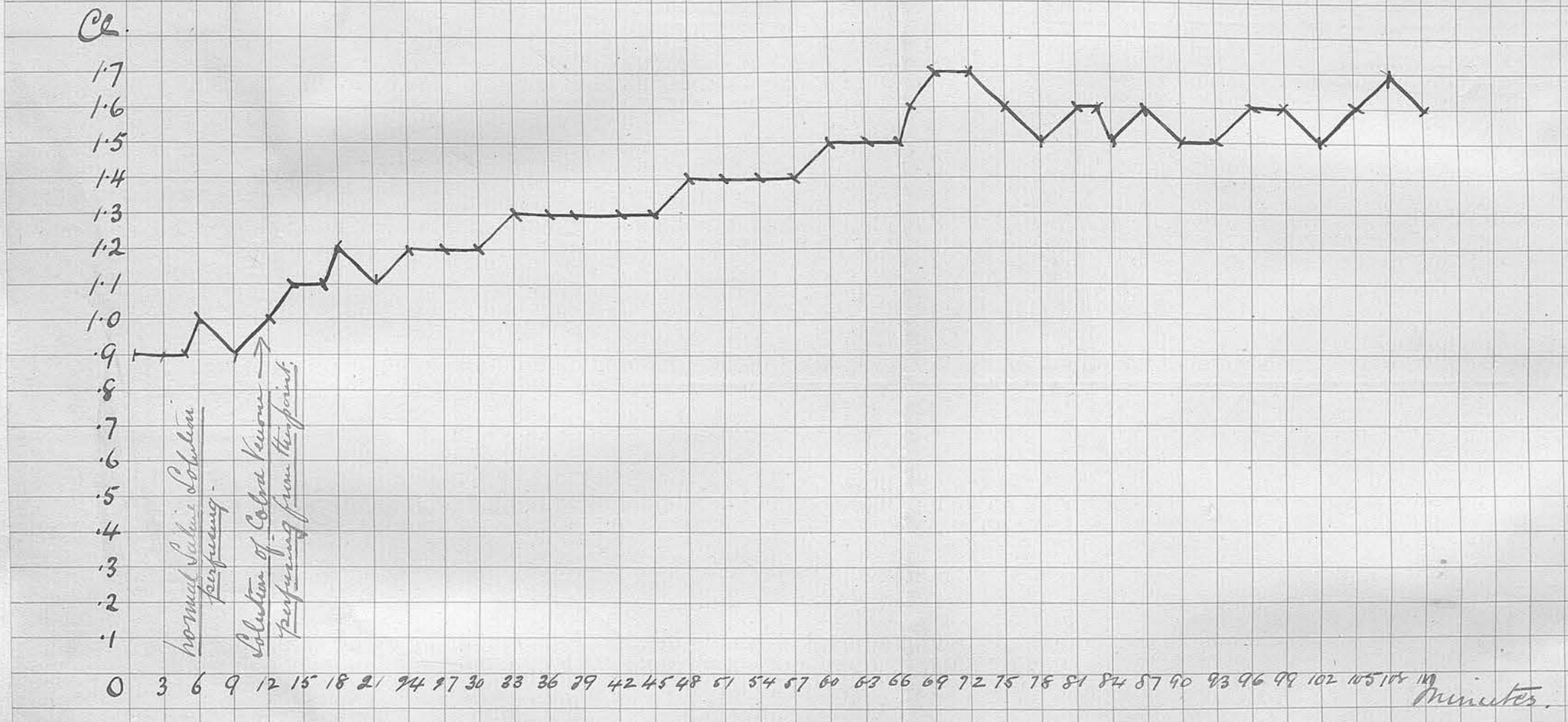

This curve shews the dilatative effect of Normal Saline Solution on the vessels.

Curve illustrative of Expt XXXVII, on table on page 54




This curve shows the effect on the vessels of a solution of Cobra Venom in a strength of 1 in 300,000.

Curve illustrative of Expt. XXXVIII, see Table on page 55.




This Curve shows the effect on the vessels of a solution of Cobra Venom in a strength of 1 in 1,000,000.

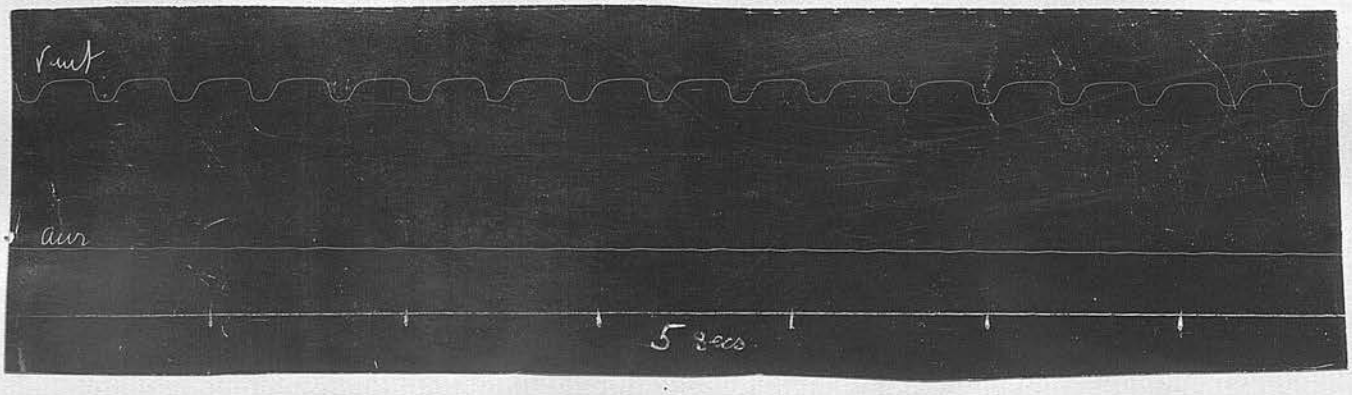



Fig. 8

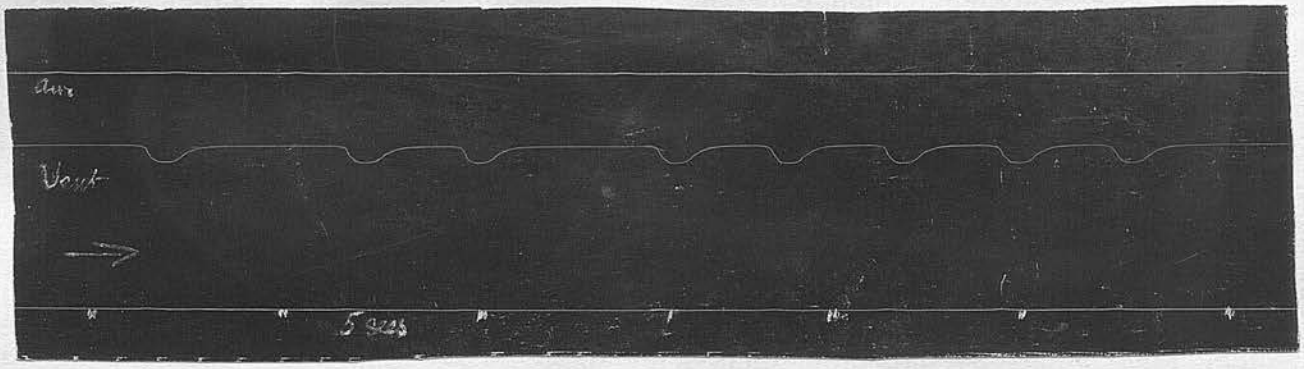



Fig. 9

The above figures represent the tracings obtainable from a frog's heart by means of levers resting upon the auricles and ventricles, and are illustrative of Experiment XXXIX pages 56 and 57.

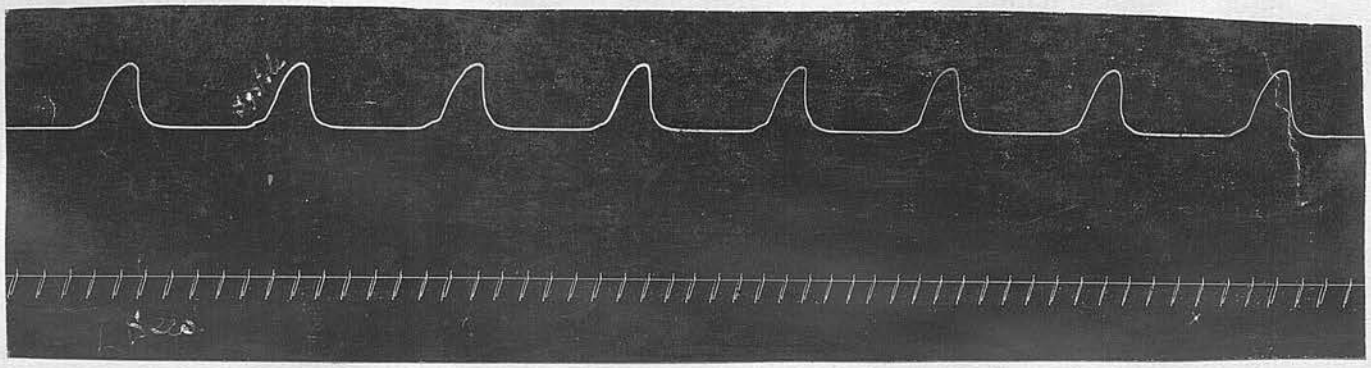


Fig. 10

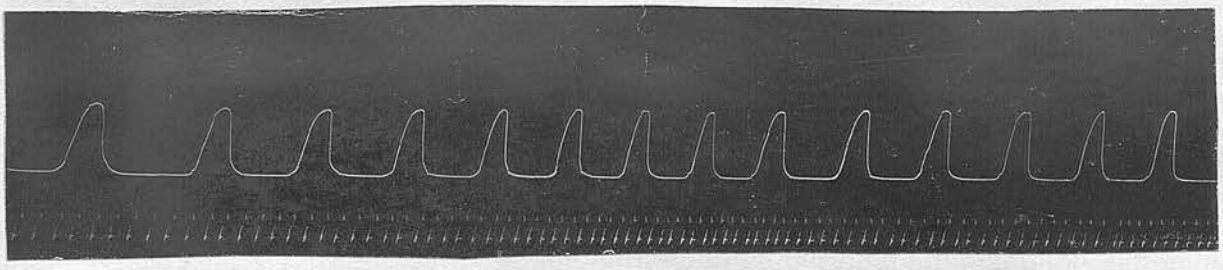


Fig. 11

Tracings showing the contractions of a frogs heart obtainable by the suspension method.

Fig. 10 shews the slow heart beat that occurs in a frog under the influence of African Cobra Venom.

Fig. 11 shews the effect of a solution of Atropine Sulphate in occasioning an increase in the number of heart beats in such a frog.

See page 58 :- Experiment XL.

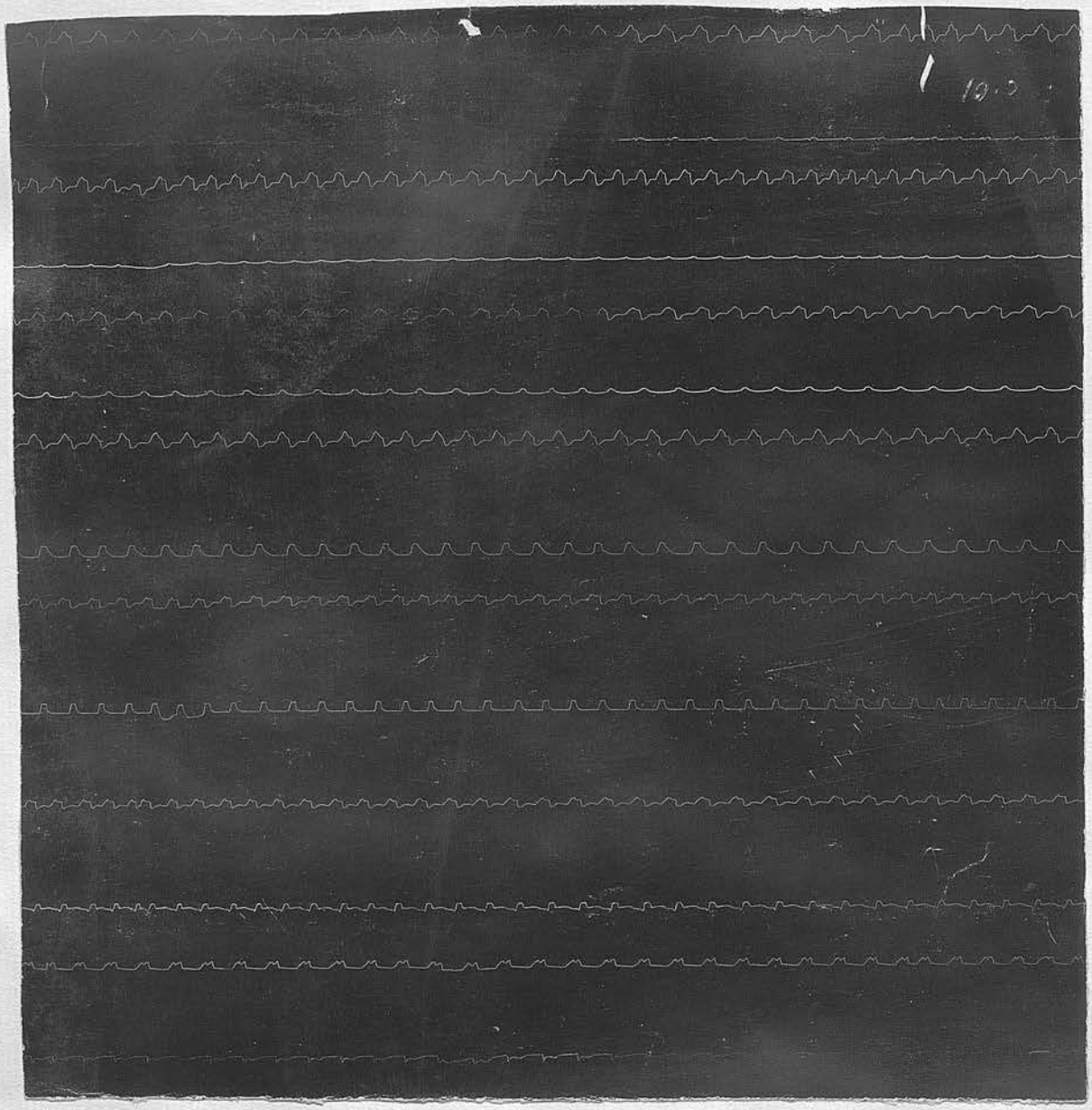



Fig. 12.

Tracing shewing that a solution of African Cobra Venom in a strength of 1 in 10,000 has practically no effect on the heart when applied locally to the exterior of that organ.

Curve reads from left to right. Vent = Ventricle. Aur = Auricle. At 10.30 a.m. tracing of heart before venom was applied is shewn at 11 a.m. and intervals thereafter solution of venom in the above strength was applied, and curves taken at times noted. The auricular pad was readjusted at 11.40.

Drum revolving at rate of 1 inch in 5 secs.

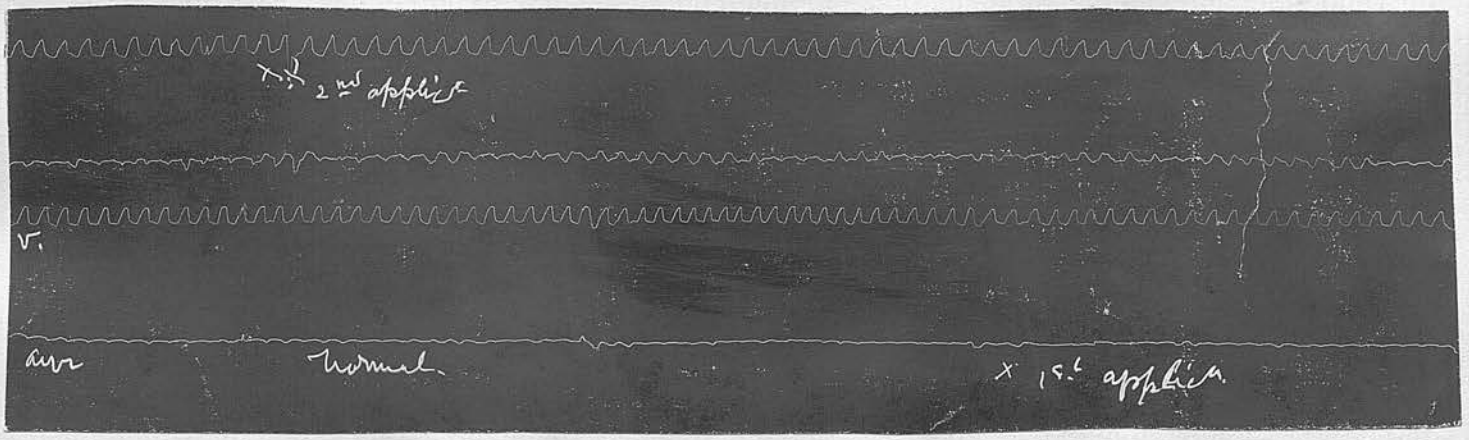



Fig 13.

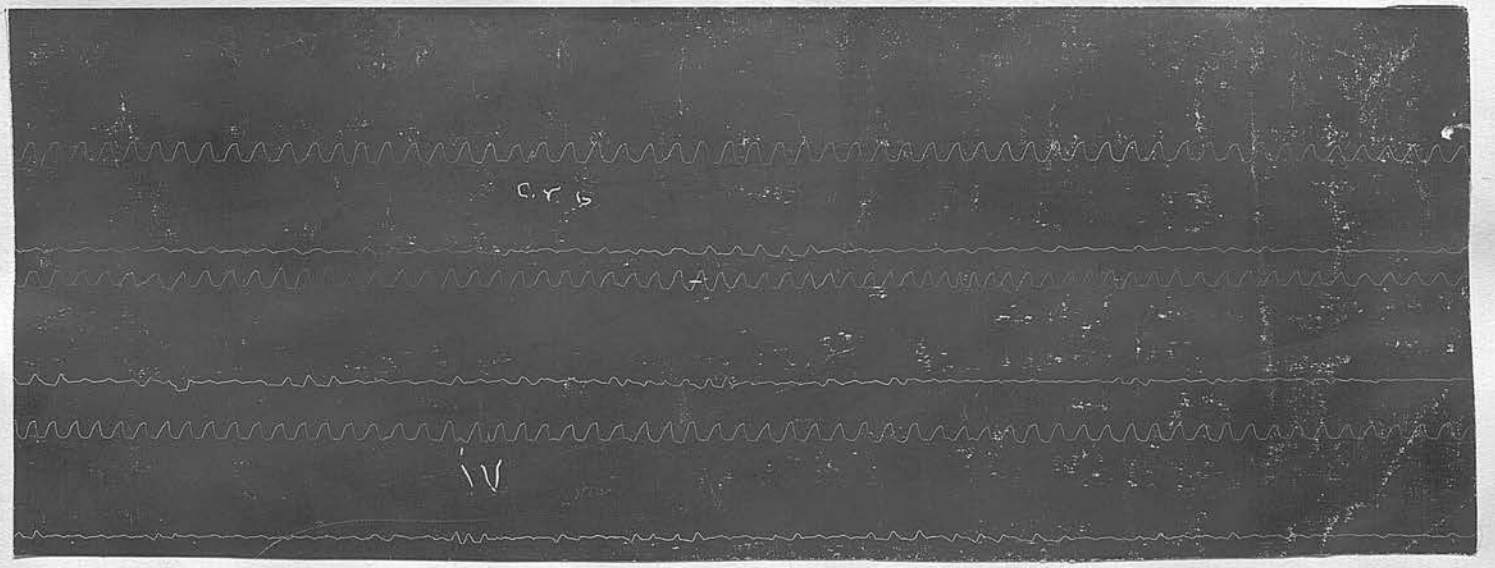



Fig. 14.

Curves illustrative of Experiment XLII page 59.

Normal = curve obtainable before the application to the exterior of the heart of a solution of African Cobra Venom in a strength of 1 in 1000.

x 1st application = first application of above noted solution.
xx 2nd application = second " " " " "
IV = fourth " " " " "
C.V. 6 = sixth " " " " "

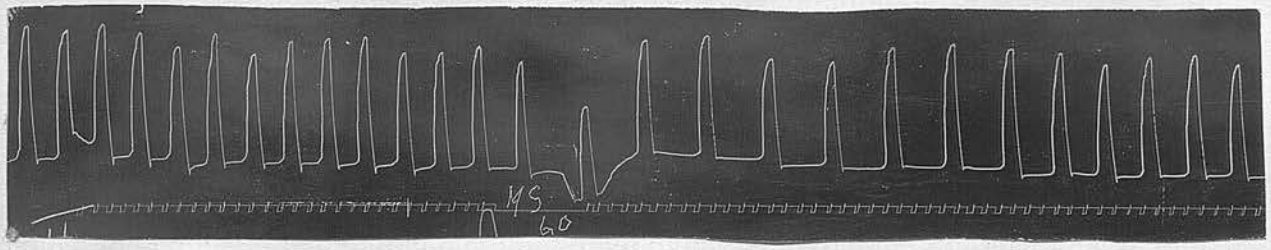



Fig. 15

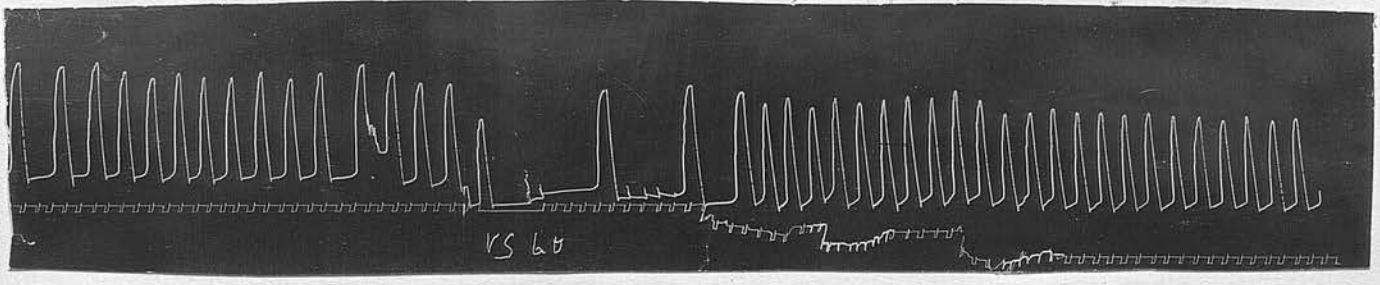



Fig. 16.

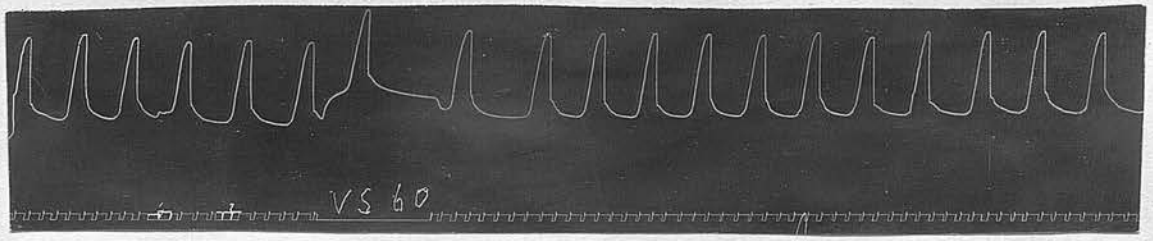



Fig. 17

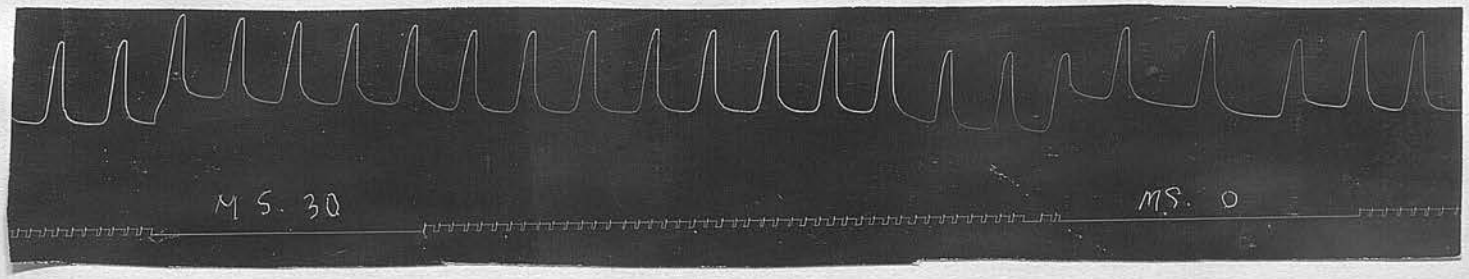



Fig. 18

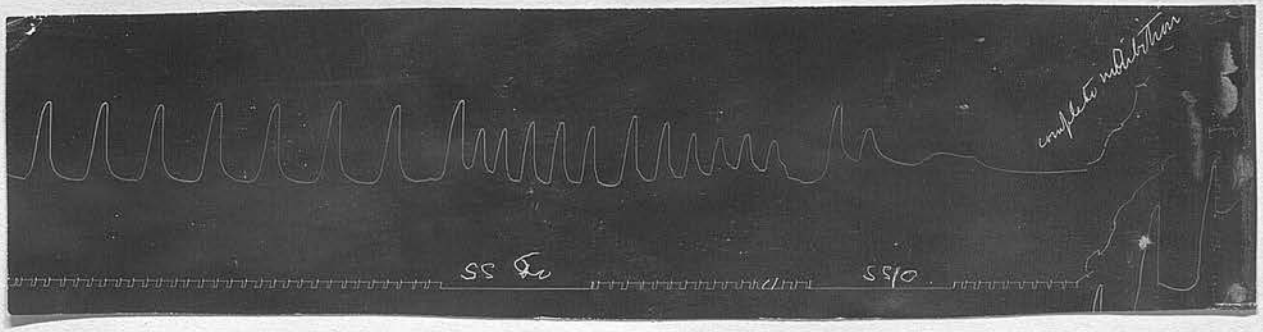



Fig. 19

Figs 15 to 19 inclusive are illustrative of Experiment XLII d:
see pages 63 and 64.

M.S. = Stimulation of vagal centre in Medulla. S.S. = stimulation of sinus.
V.S. = Stimulation of Vagus nerve. Number = distance of secondary coil from primary in MM.

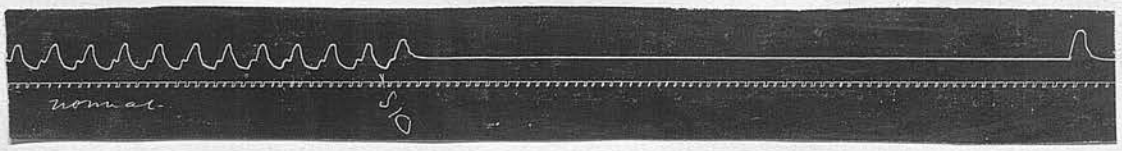



Fig. 20

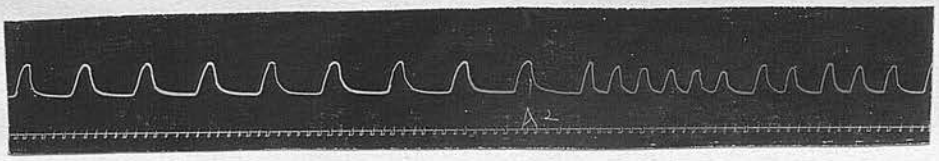


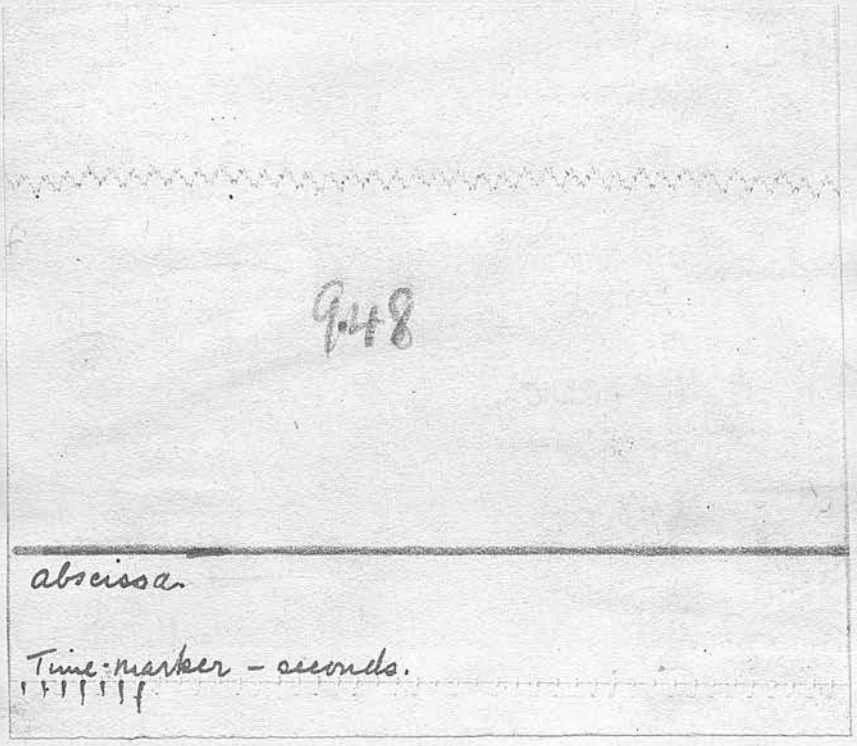

Fig. 21.

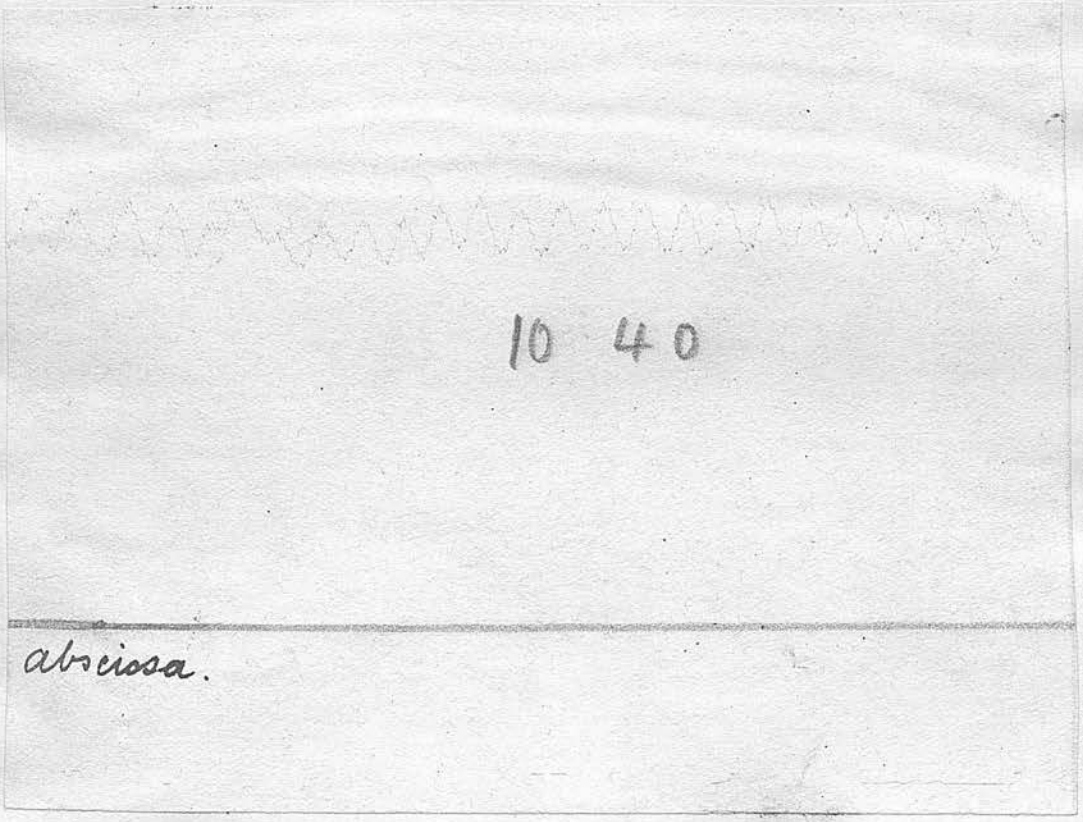
Tracings above are illustrative of Experiment XLIV.

Fig. 20, S.10 = Stimulation of the sinus of frog's heart when the animal was under the influence of African Cobra Venom.

Fig. 21. Shews the effect of the application of a solution of Atropine Sulphate to the heart in a frog under the influence of Cobra Venom.

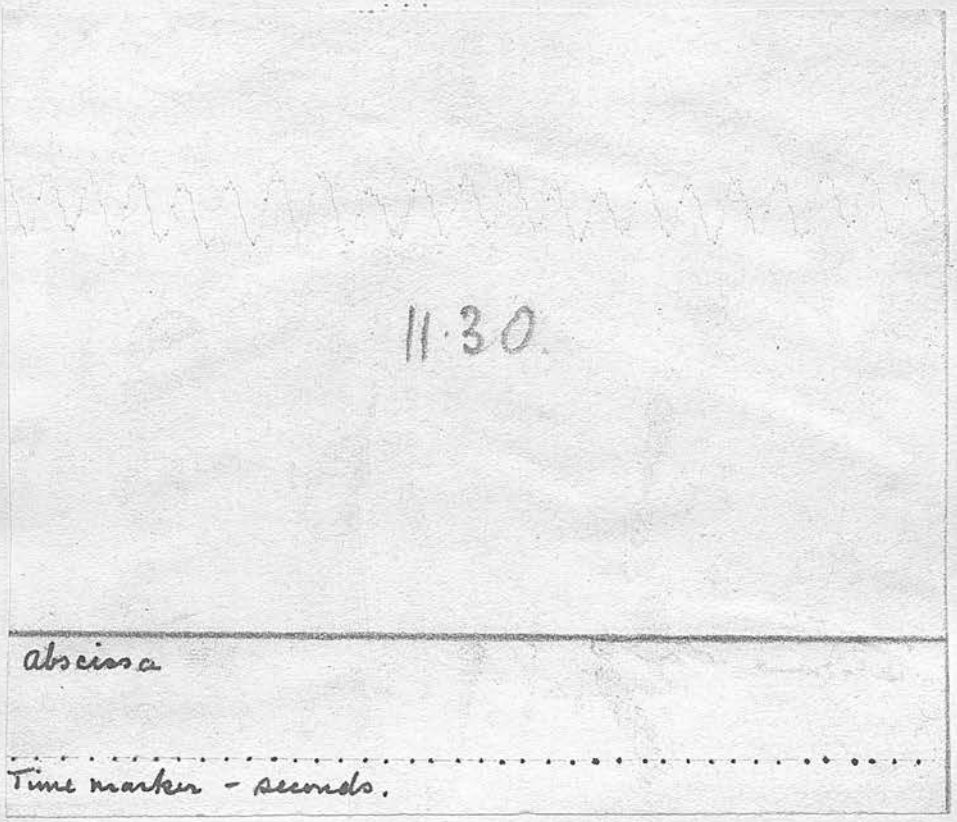
See page 65.

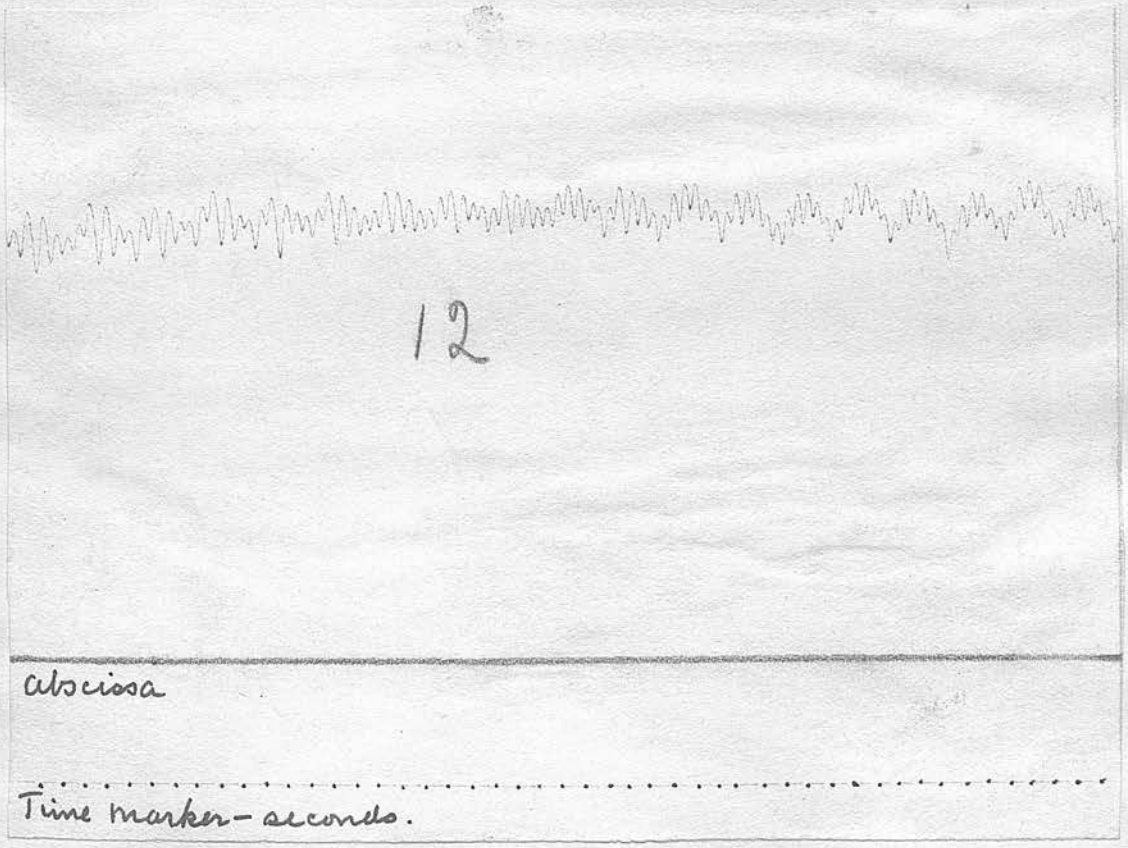



Portions of Blood Pressure tracing illustrative of Experiment LV.

See page 81.

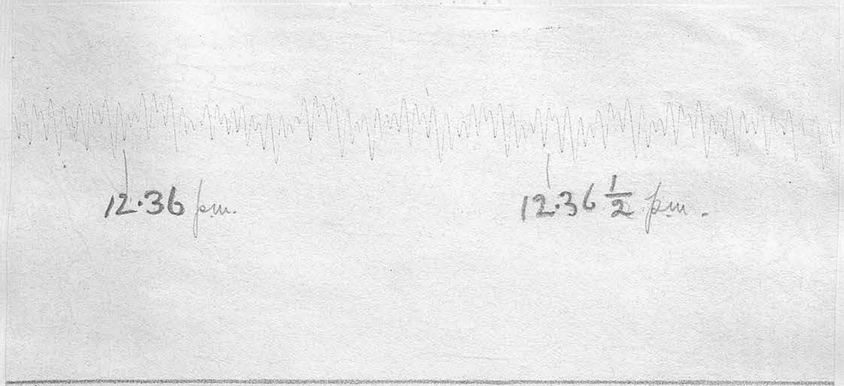


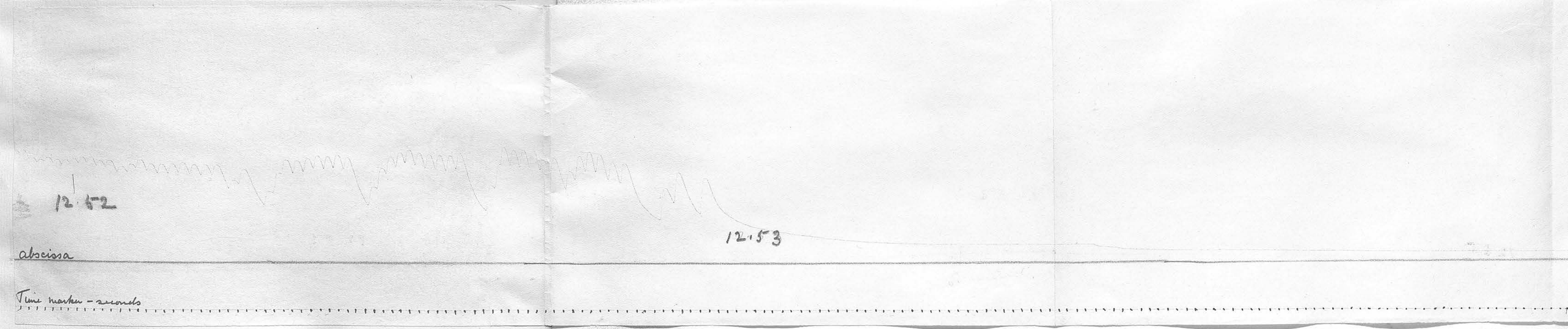




Portions of Blood Pressure tracing illustrative of experiment LV.

see page 81.

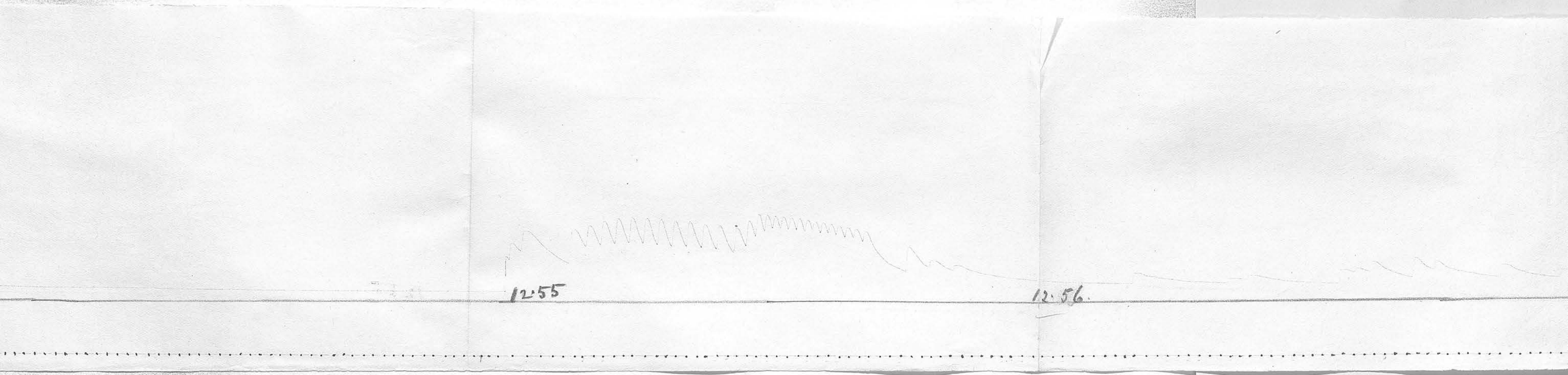
XIX.

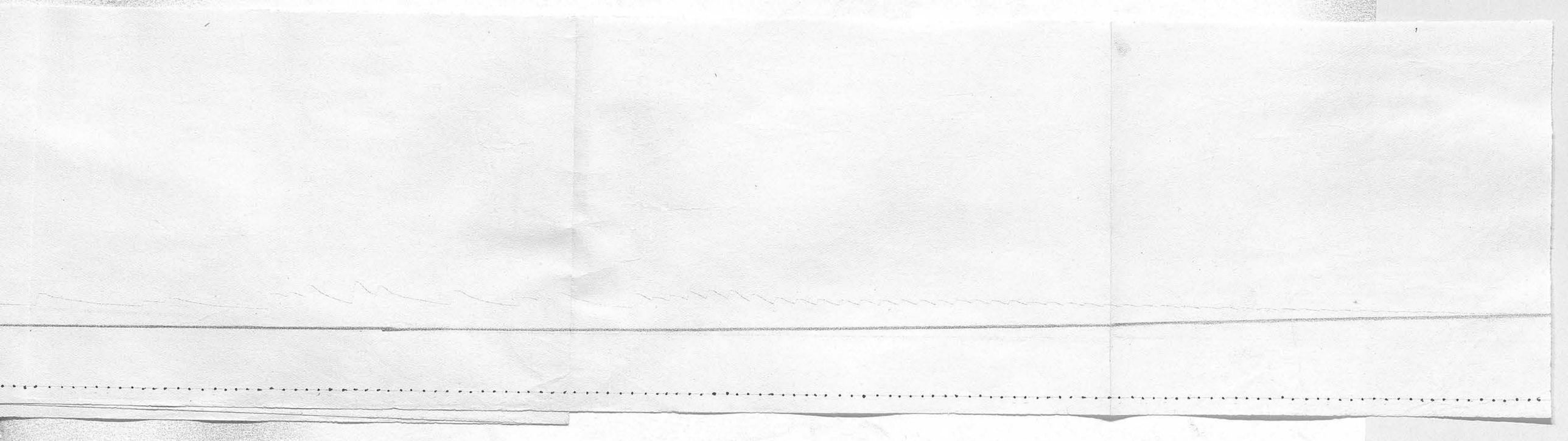


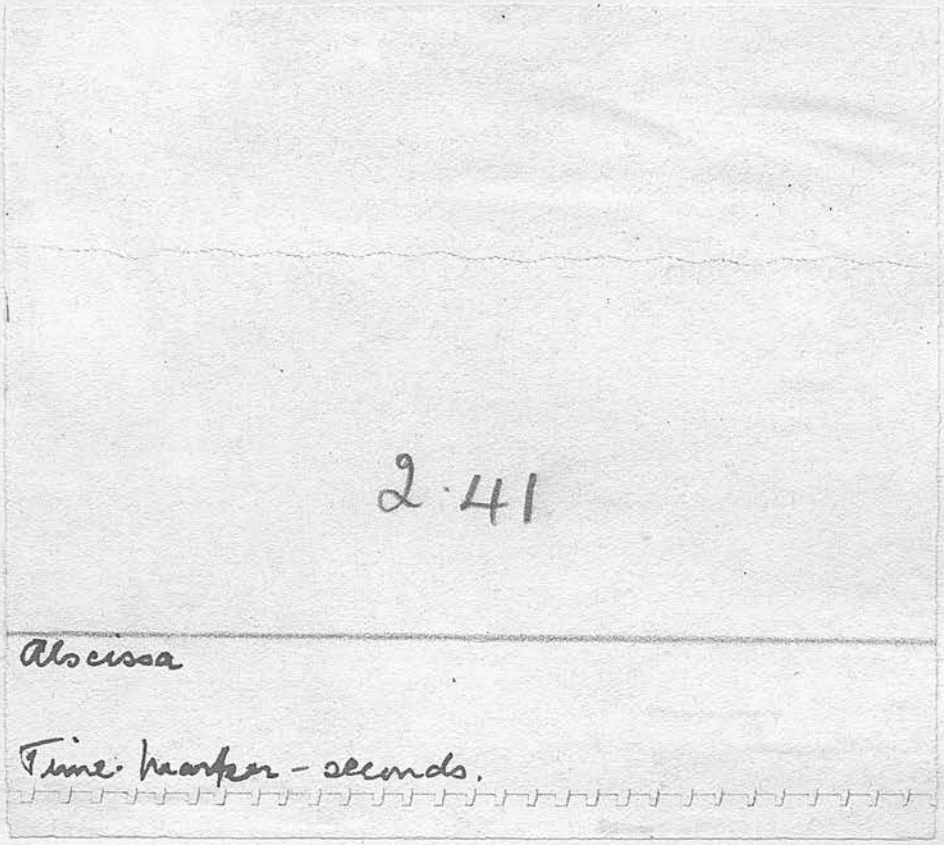



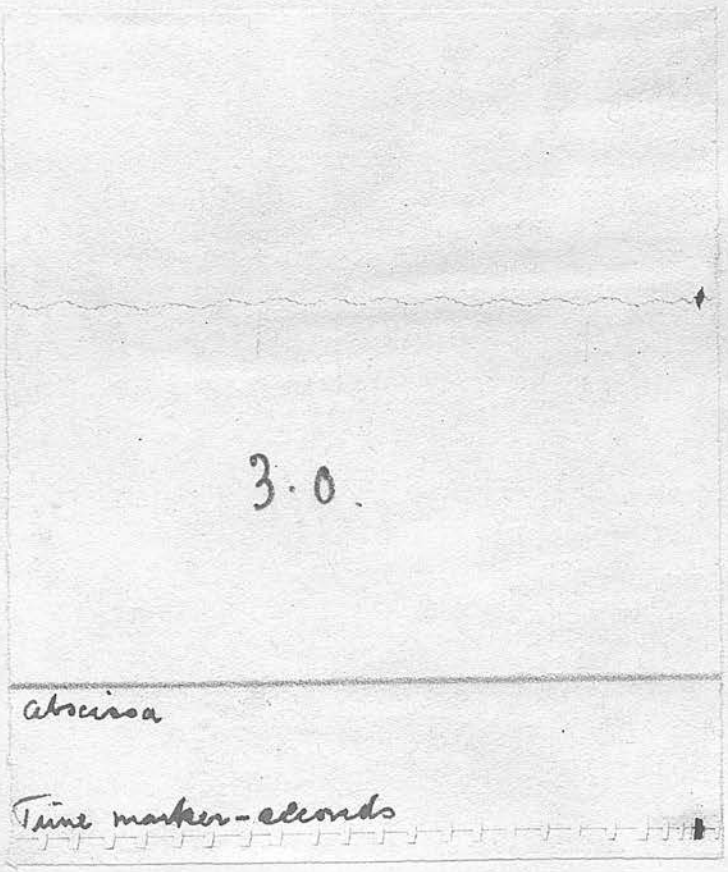

Portion of Blood Pressure tracing illustrative of Experiment LV

See page 82.


12.55
12.56.

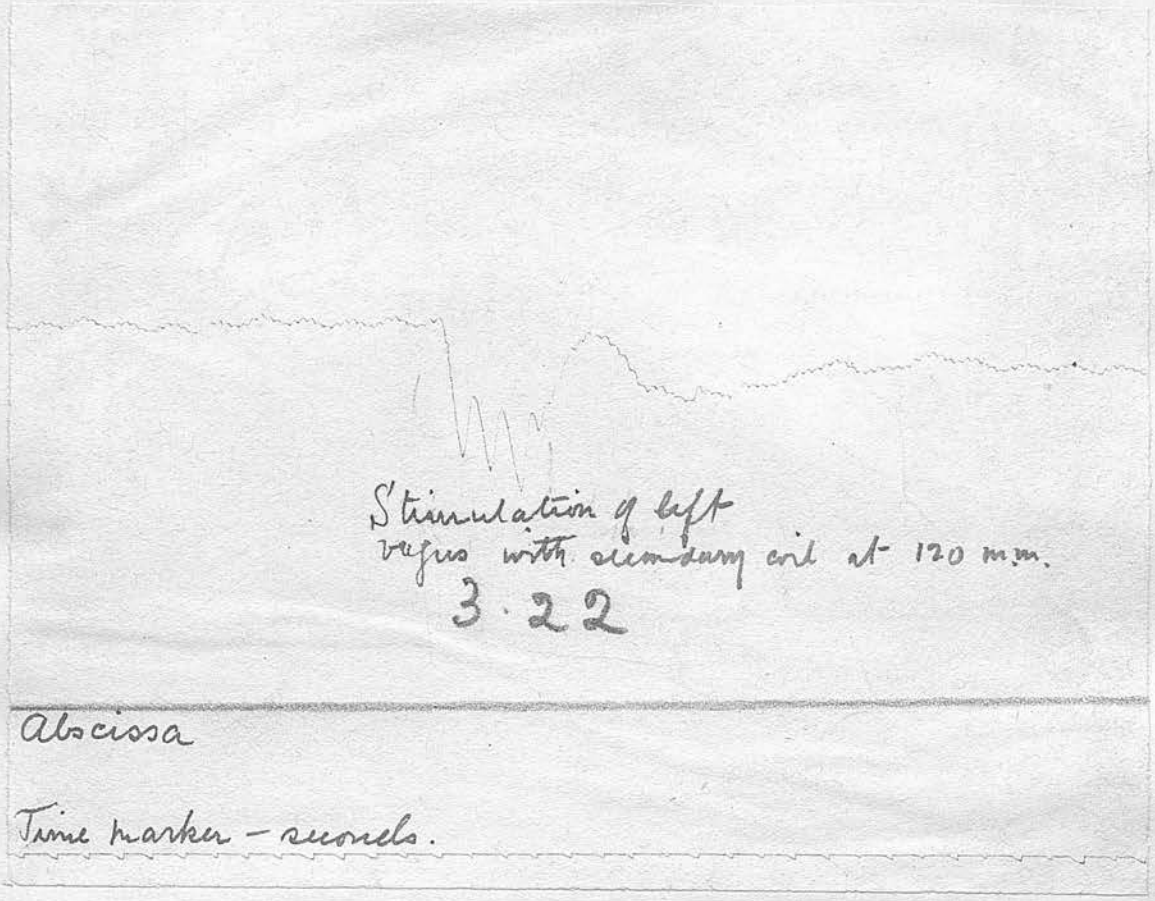


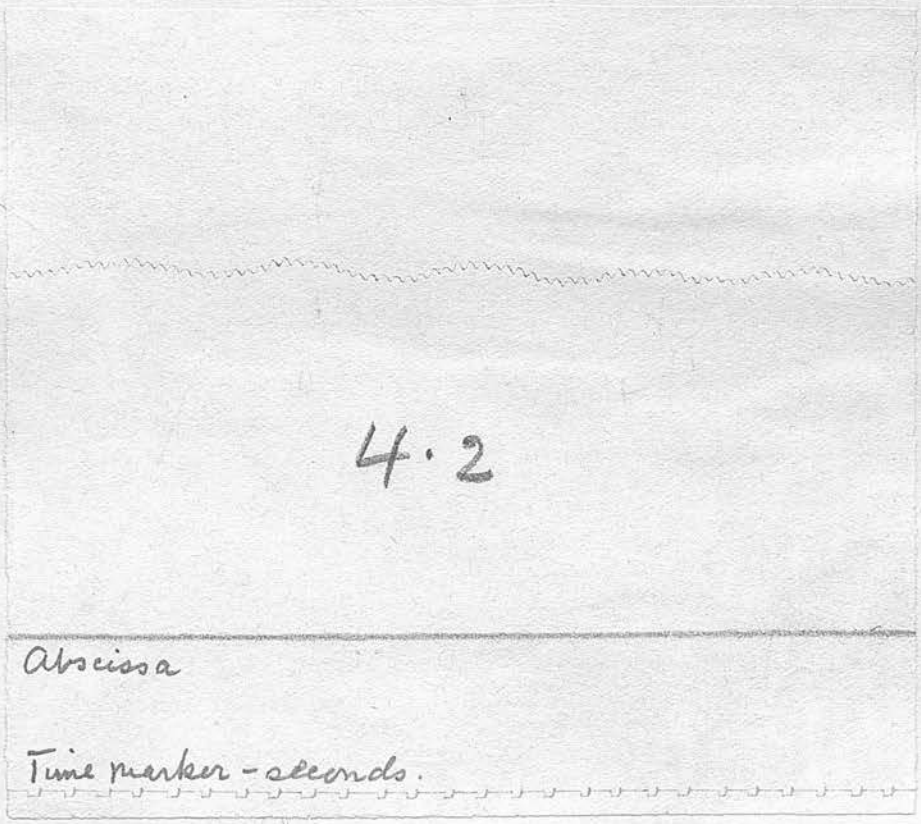



Portions of Blood Pressure tracing illustrative of experiment LVI.

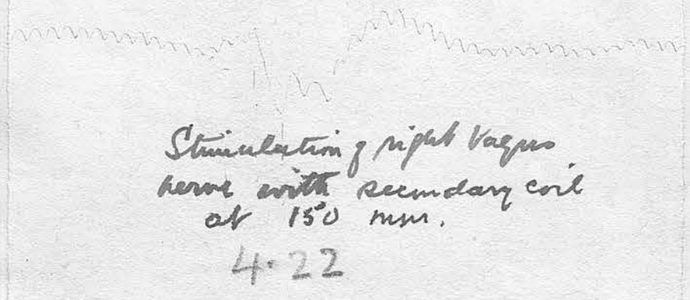
See page 84.



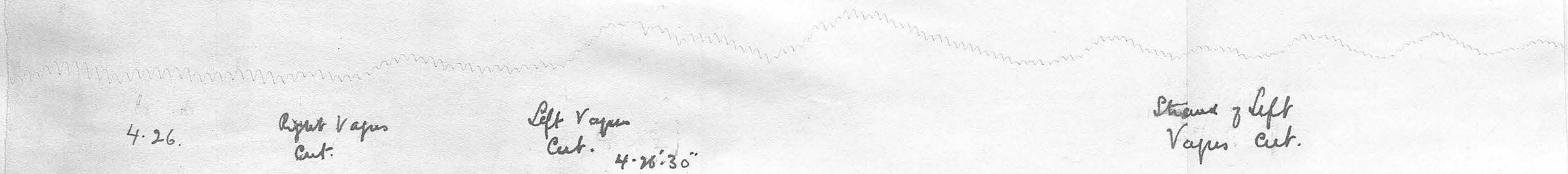

Portions of Blood Pressure tracing illustrative of Experiment LVI.

See page 84.




Abscissa

Time marker — seconds.

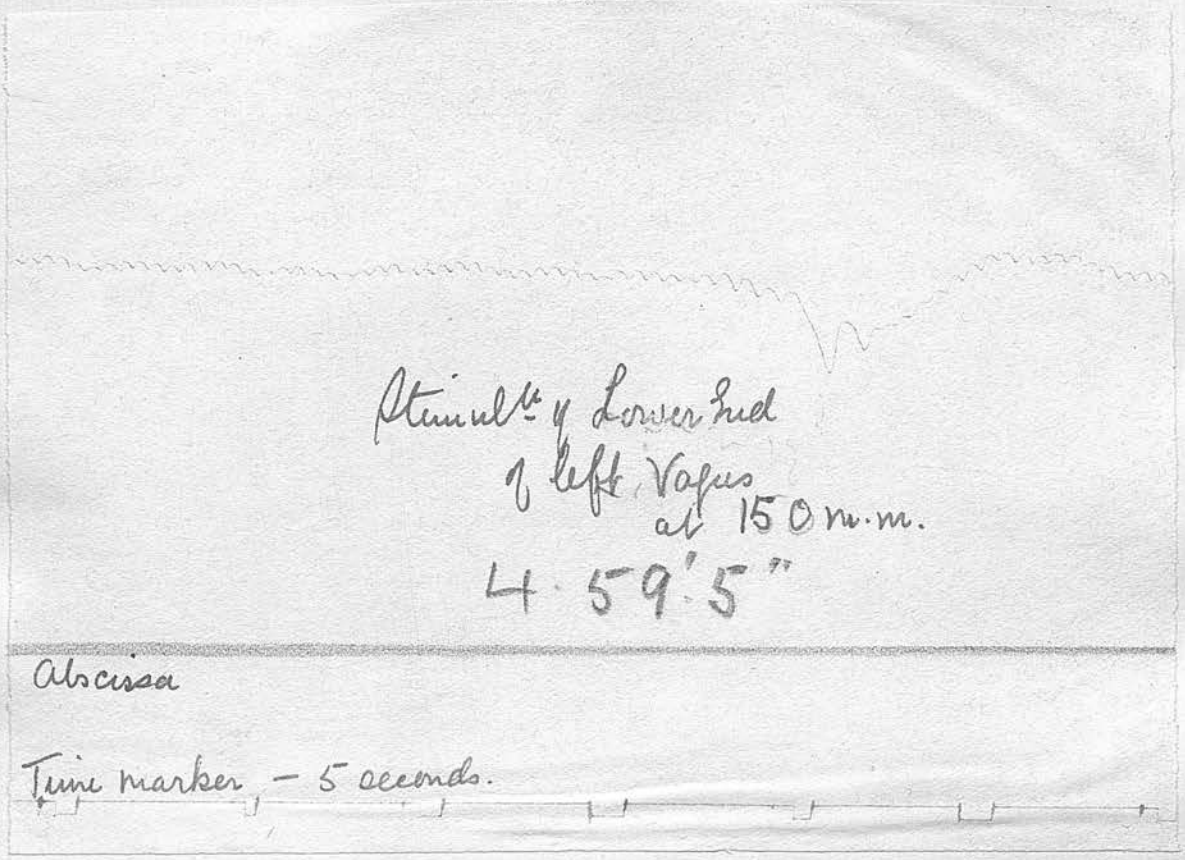


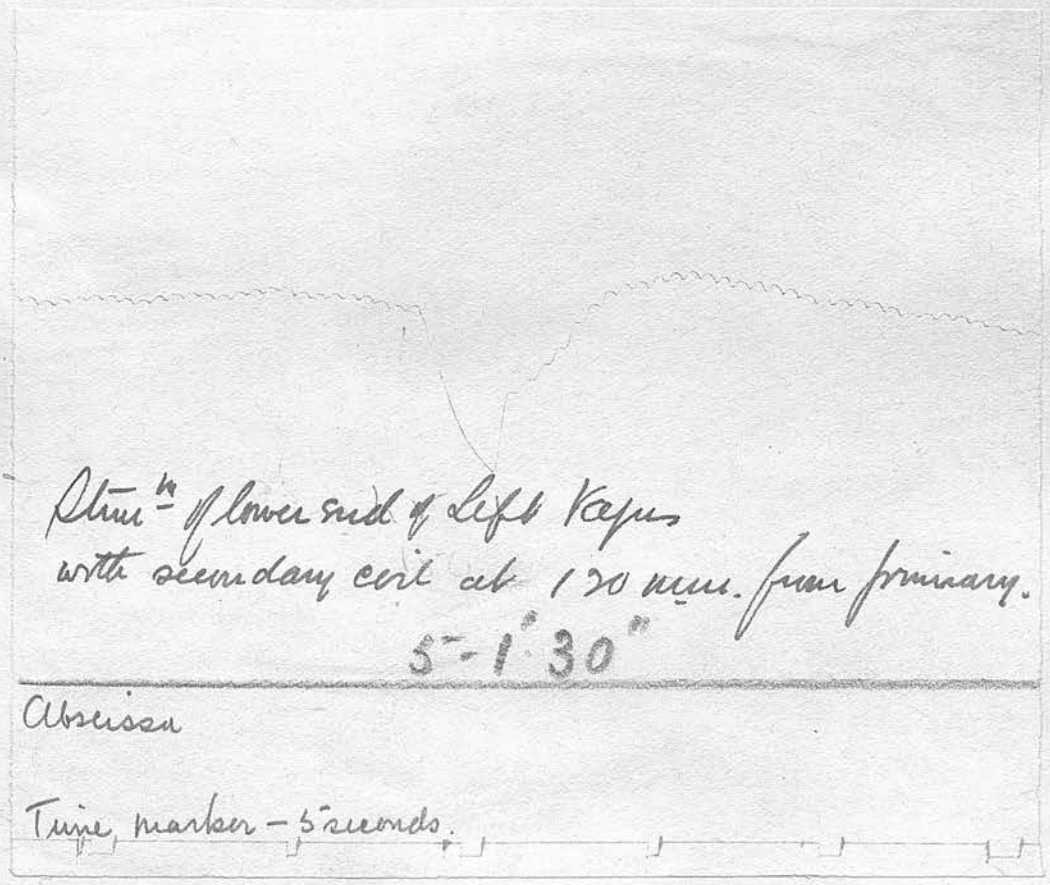

Abscissa

Time marker — seconds.

Portions of Blood Pressure tracing illustrative of Experiment LVI.

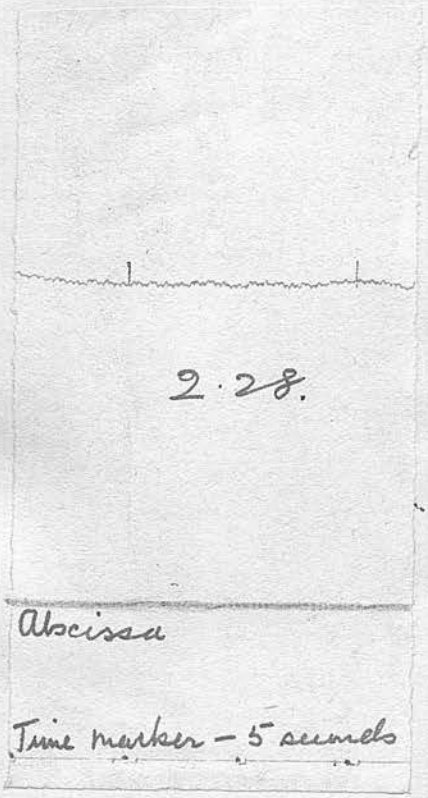
See Page 85.

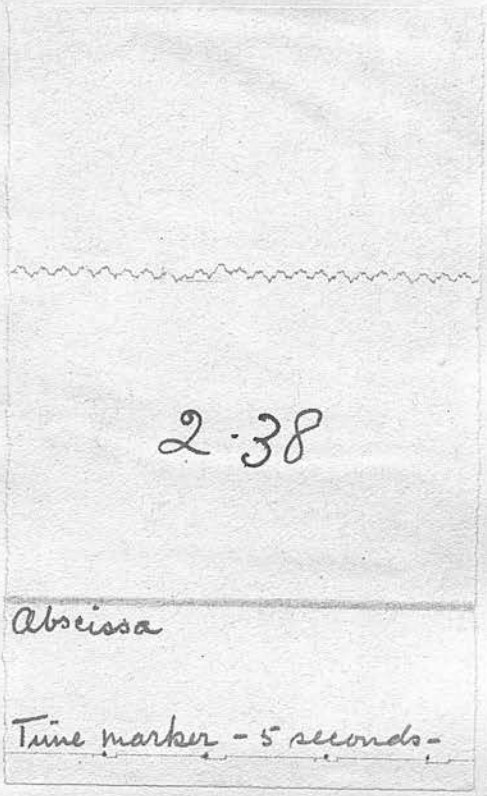


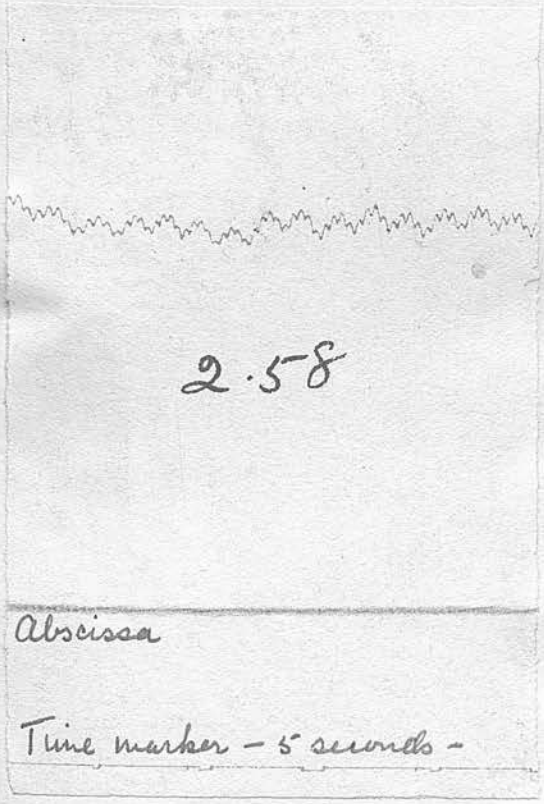



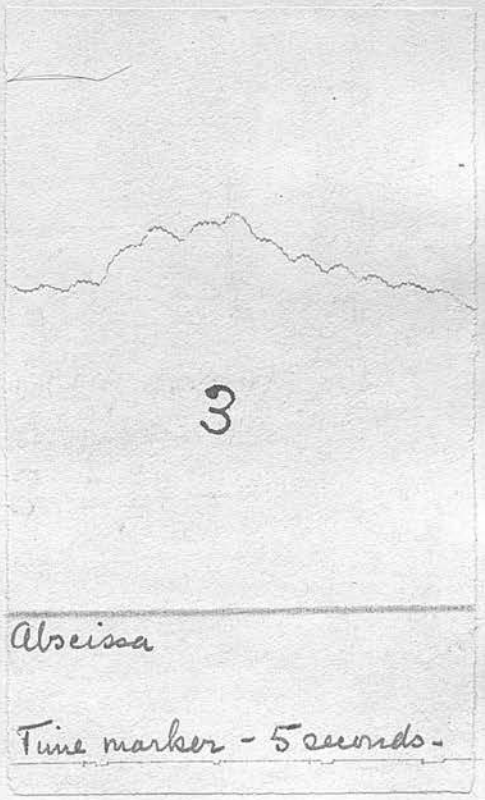

Portions of Blood Pressure tracing illustrative of Experiment LVI.

See pages 85 and 86.



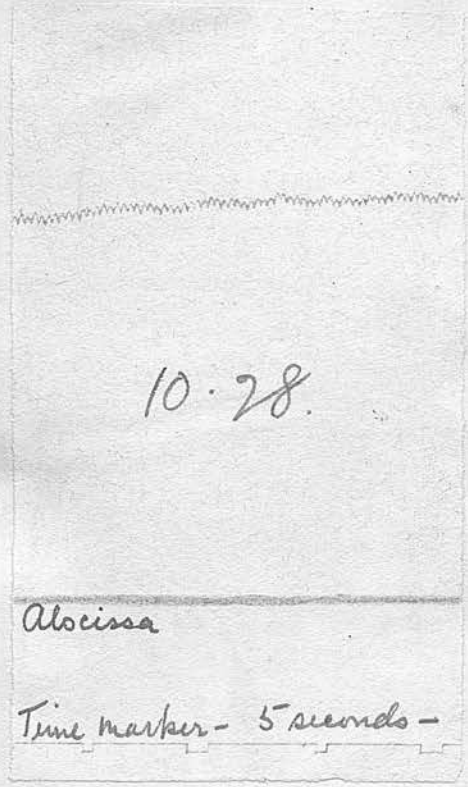



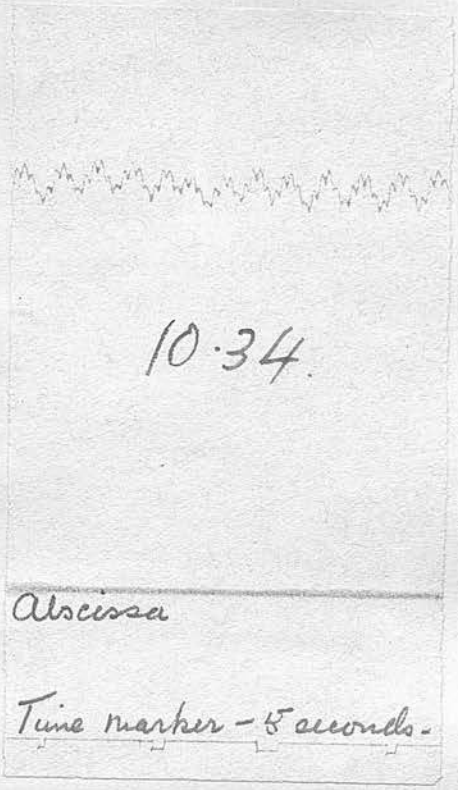



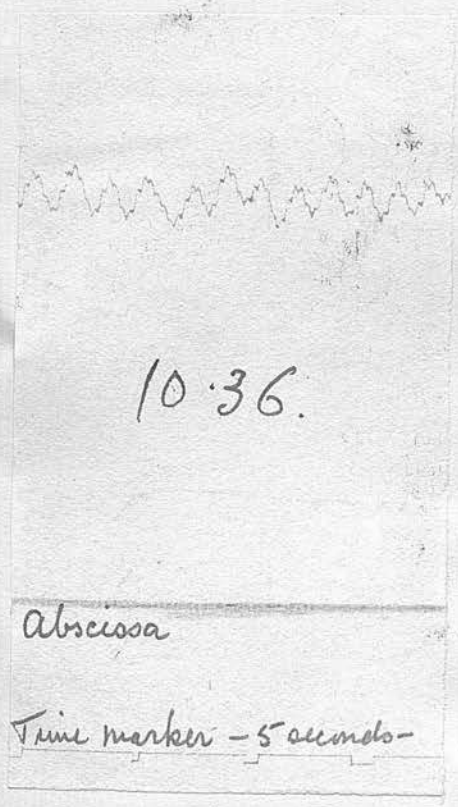



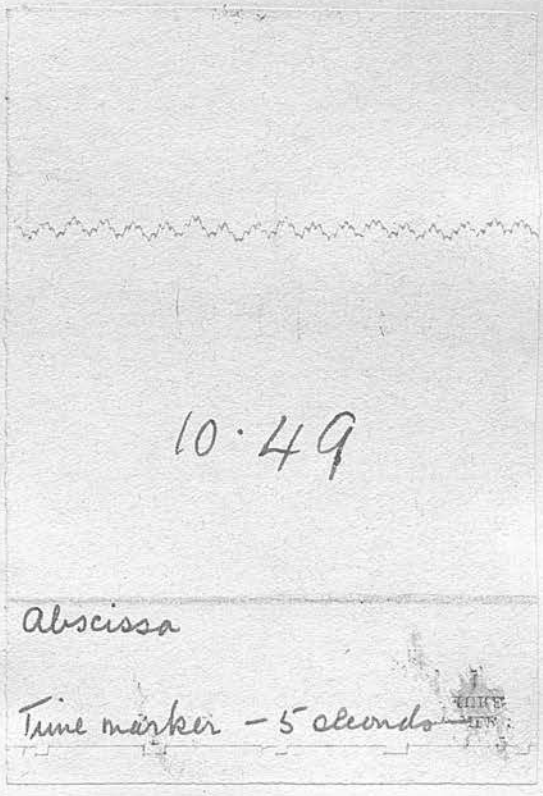

Portions of Blood Pressure tracing illustrative of Experiment LVII

See pages 88 and 89.



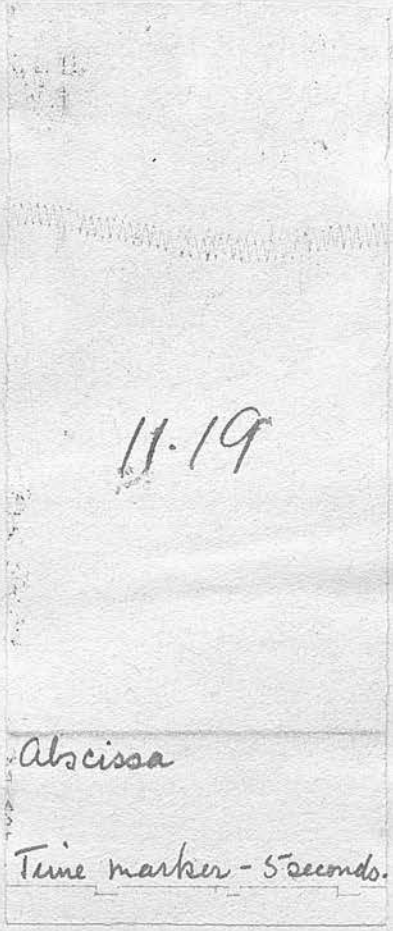



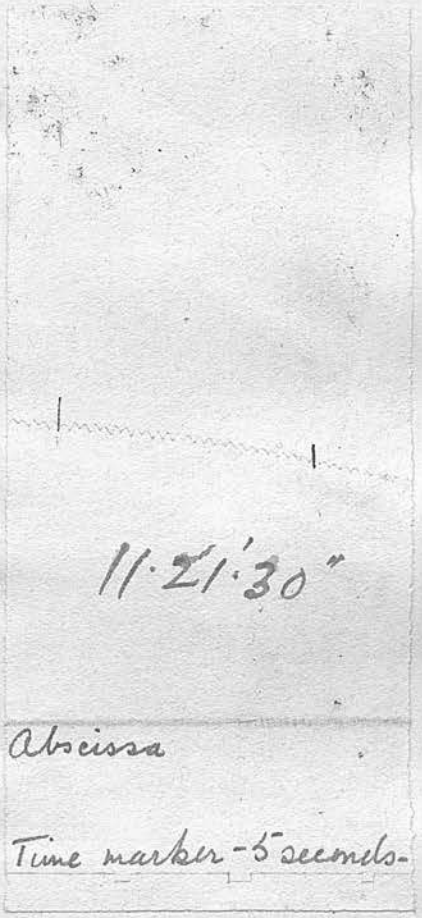




Portions of Blood Pressure tracing illustrative of Experiment LVIII.

See page 91.

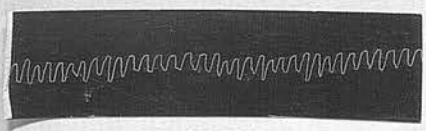




Portions of Blood Pressure tracing illustrative of Experiment LVIII.

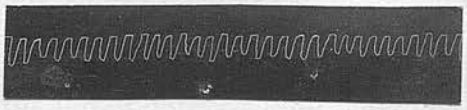
See pages 92 and 93.

On page XXVII opposite are seen portions of the tracing illustrative of Experiment LIX. See page 95. They show the variations in the number and depth of the respirations in a rabbit under the influence of African Cobra Venom, and are obtained by means of a lever writing on a revolving drum covered with smoked glazed paper - the other end of the lever being attached to a slip of the diaphragm as described on page 94. At 3.14 an extra weight was hung upon the lever as the excursus was very large.

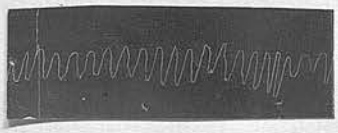


2.37

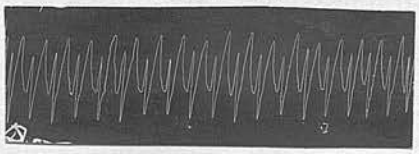
2.39.30



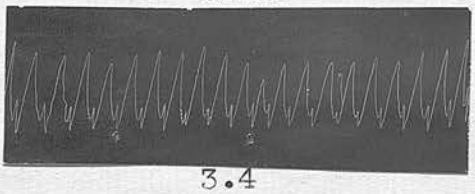
2.41.



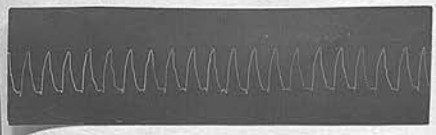
2.49



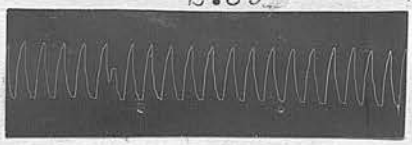
2.59



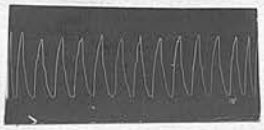
3.4



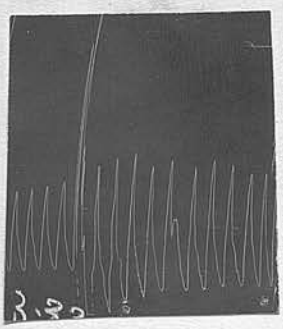
3.21



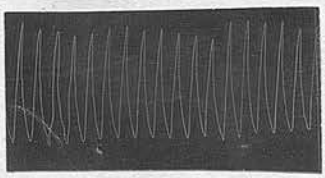
3.25



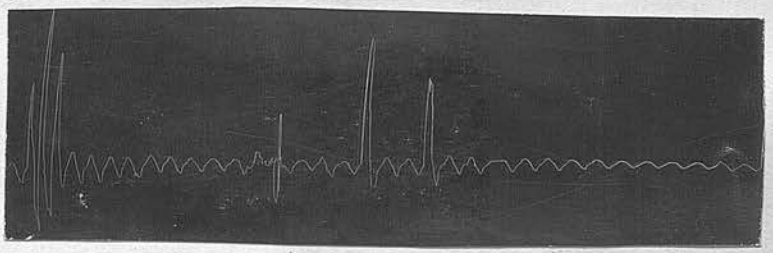
3.28



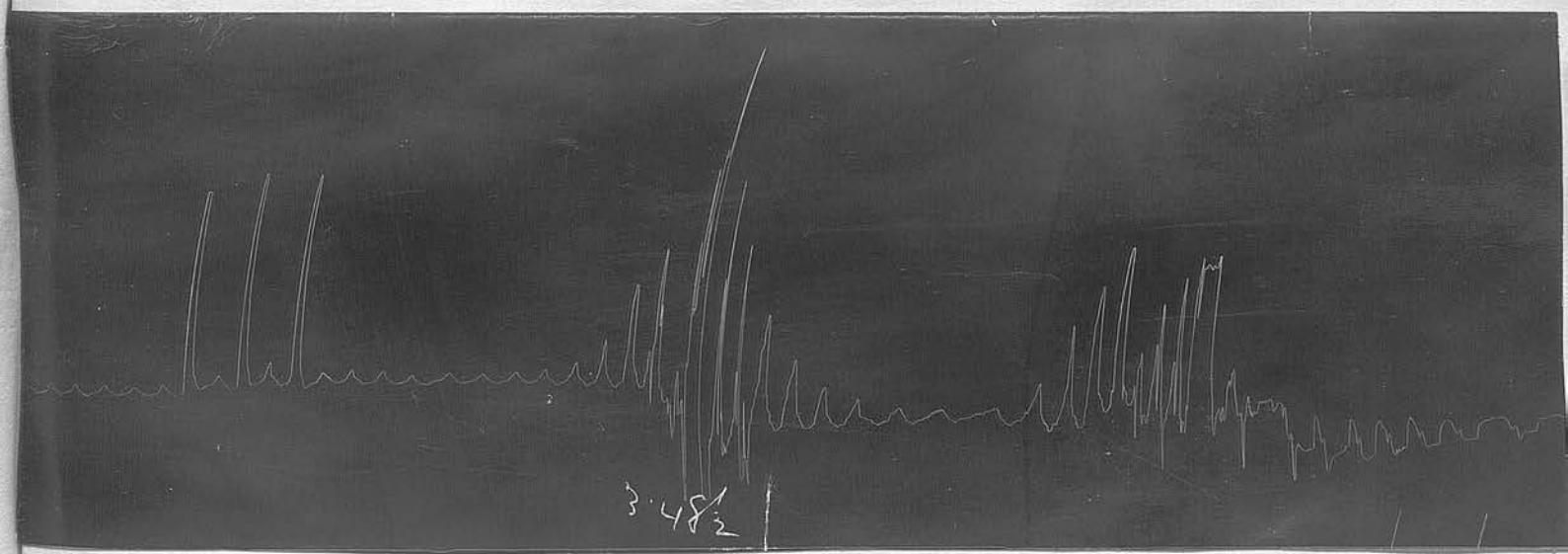
3.33



3.40

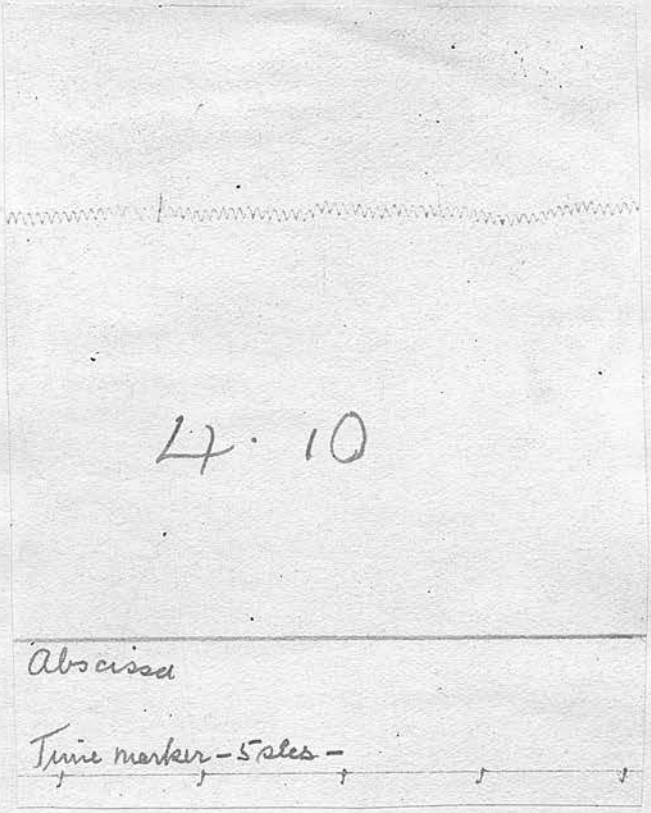


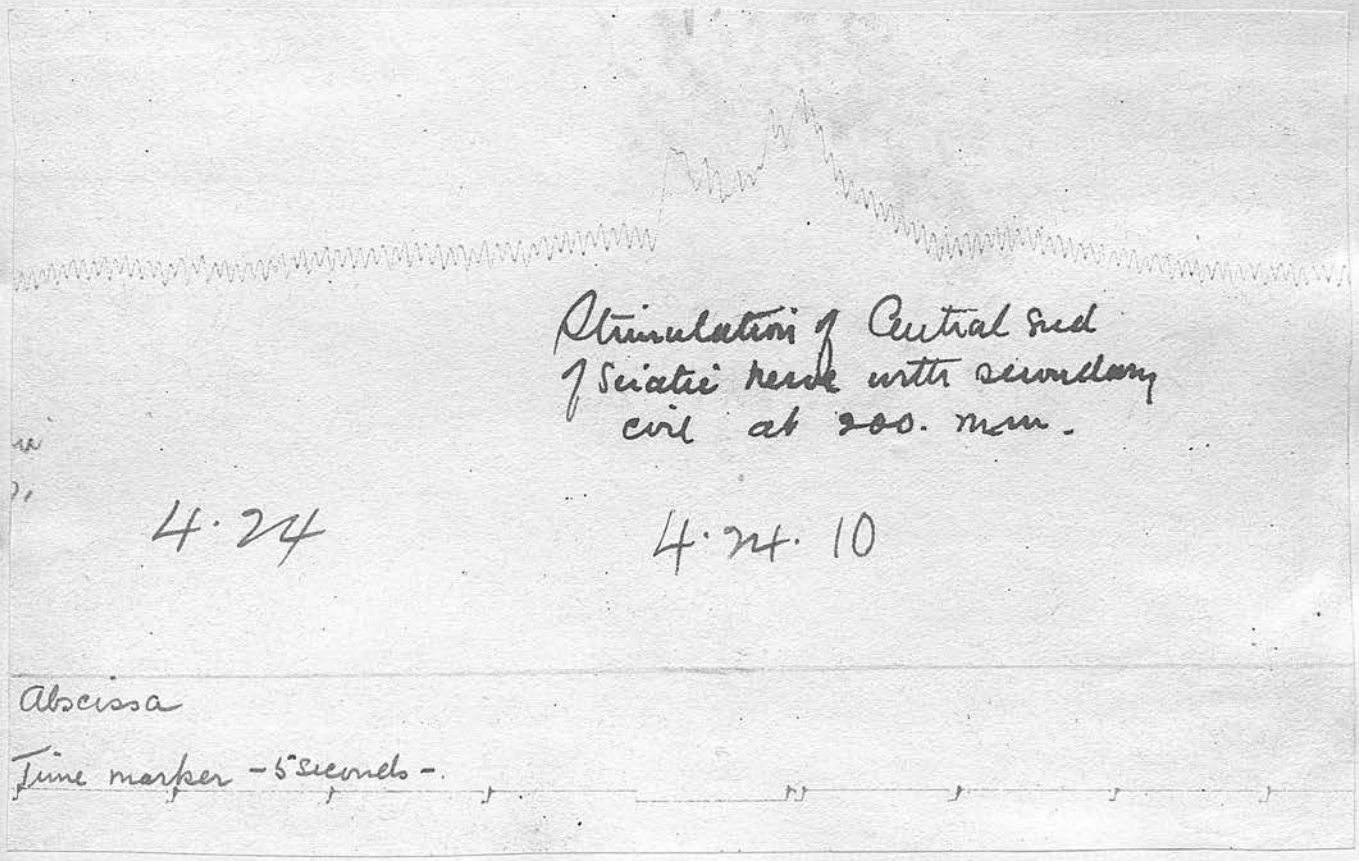
3.46




3.48.30"

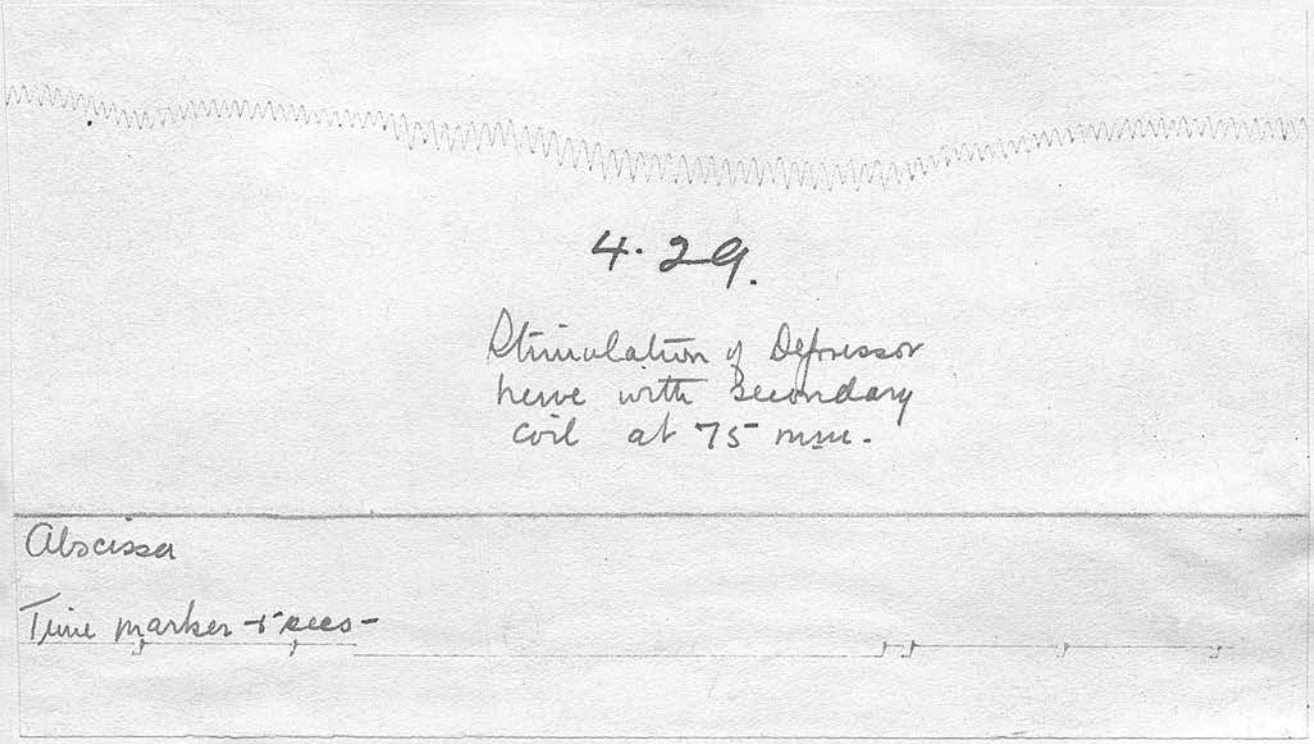

3.51.30"

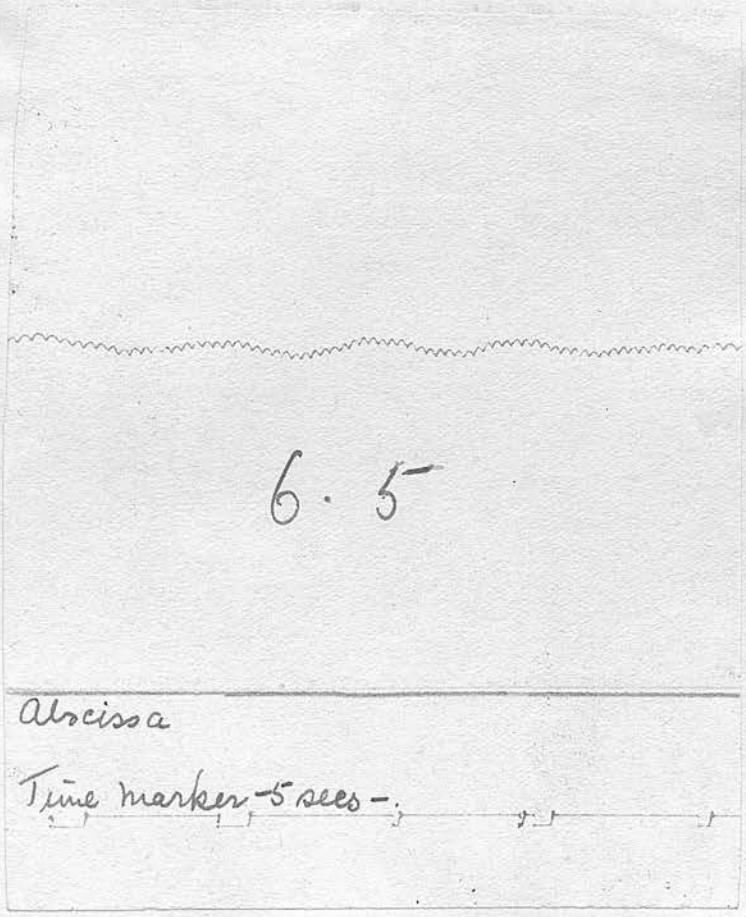



Portions of Blood Pressure tracing illustrative of Experiment LX.

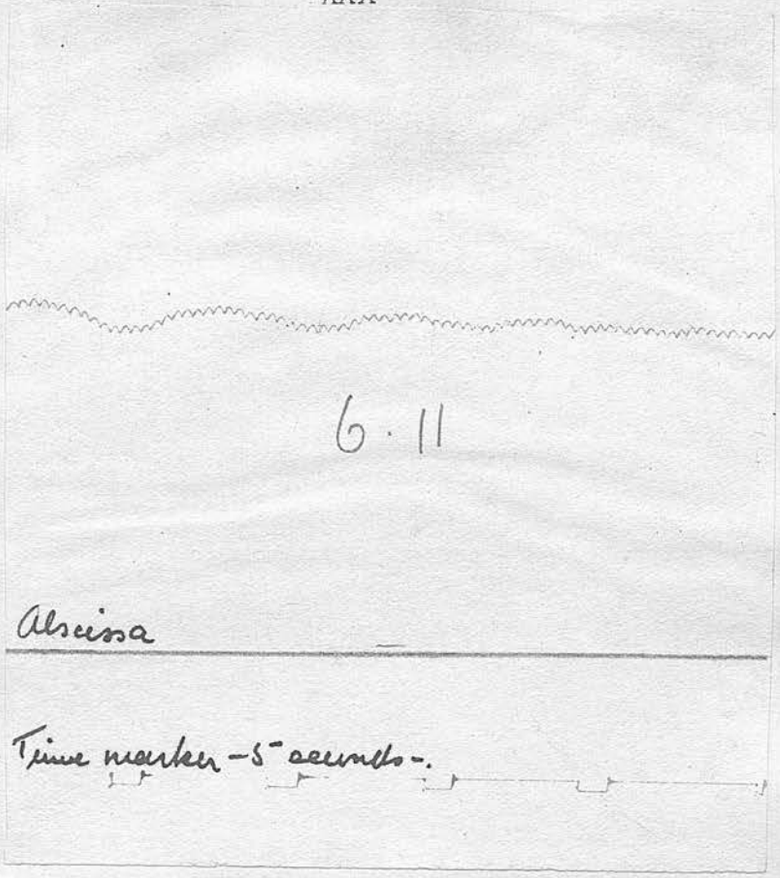
See page 98.

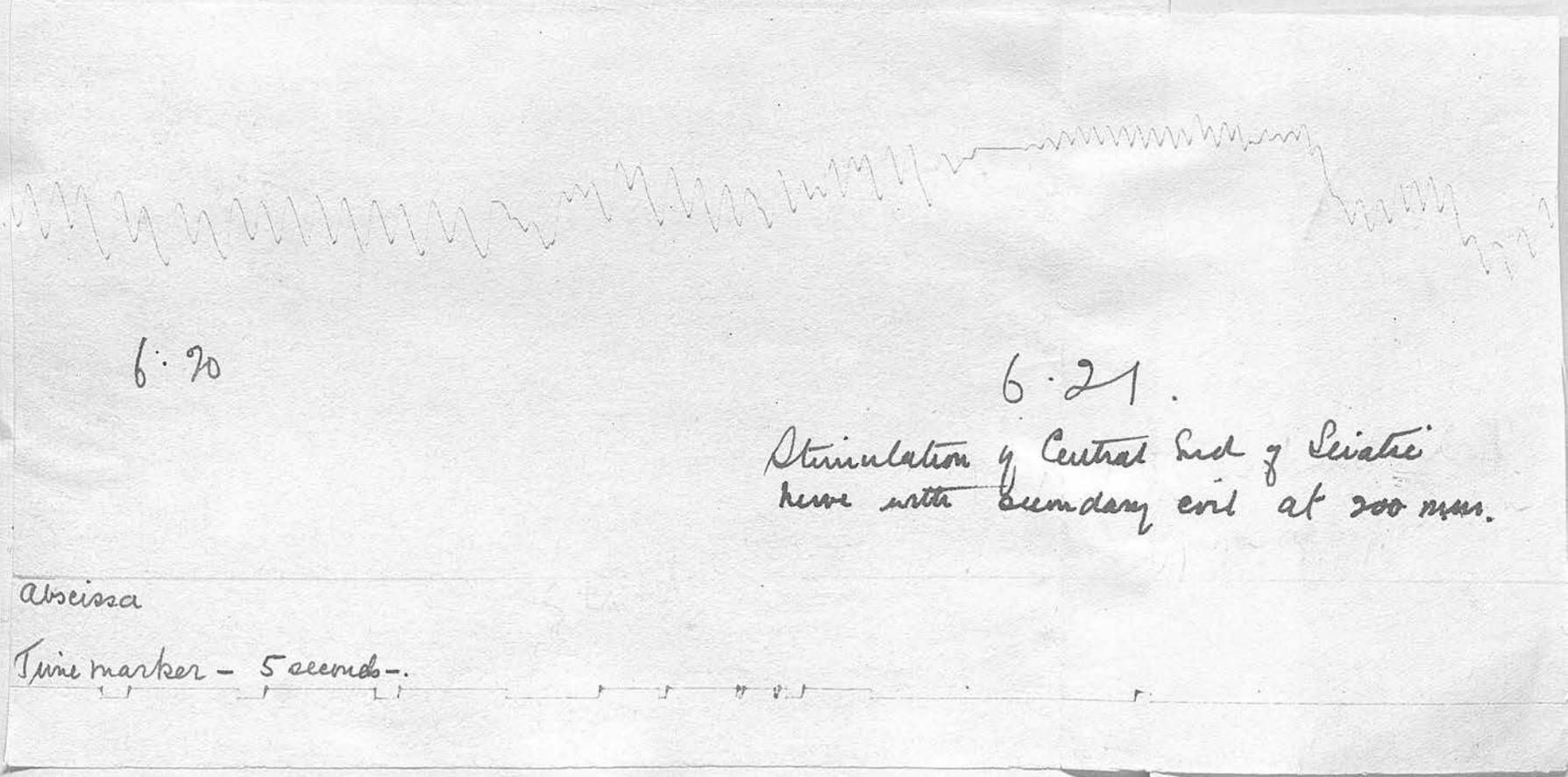



Portions of Blood Pressure tracing illustrative of Experiment LX

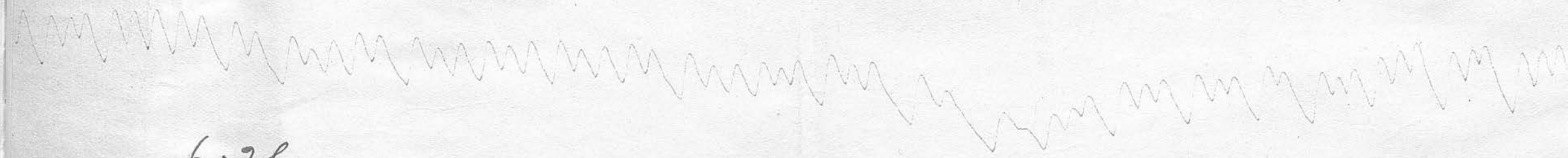
See pages 98 and 99.




Portions of Blood Pressure tracing illustrative of Experiment LX

See page 99.

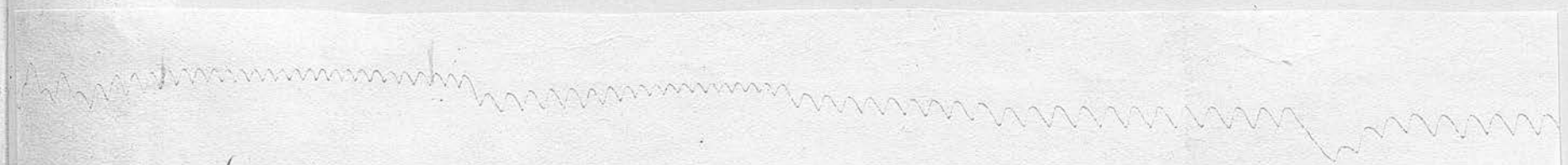


6·26

Stimulation of Depressor
Nerve with Sundary coil
at 90 mm.

Abscissa

Time marker - 5 seconds -.



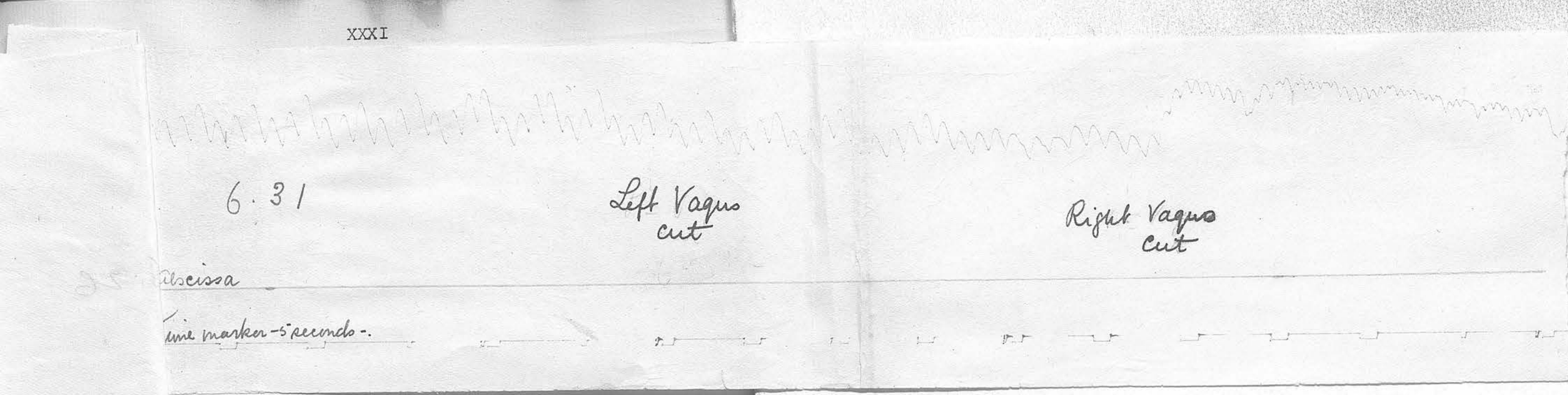
6·33

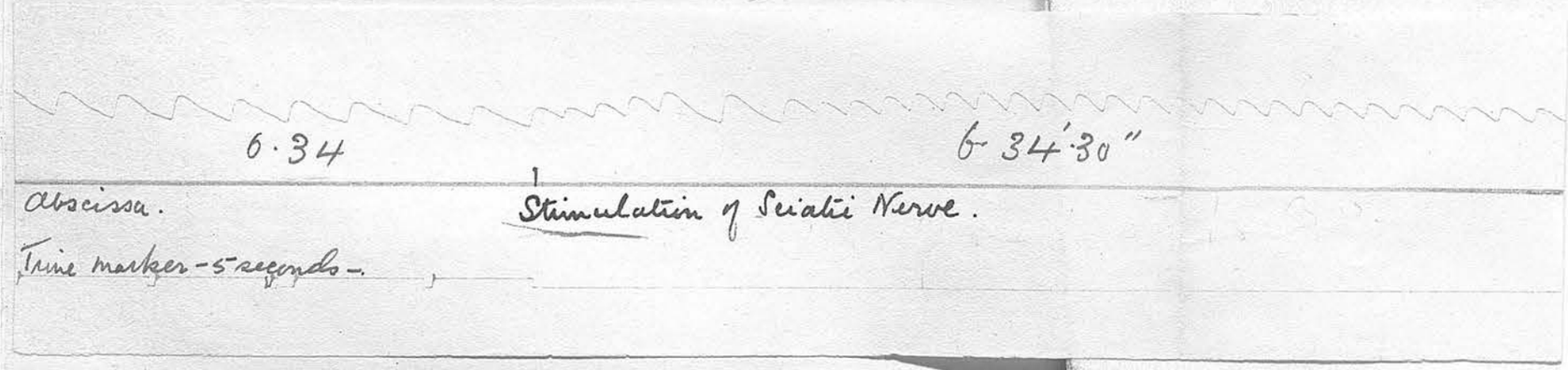
6·33·30

Abscissa

Stimulation of Depressor Nerve
with secondary coil at 40 mm.

Time marker - 5 secs -




Portions of Blood Pressure tracing illustrative of Experiment LX.

See page 99.

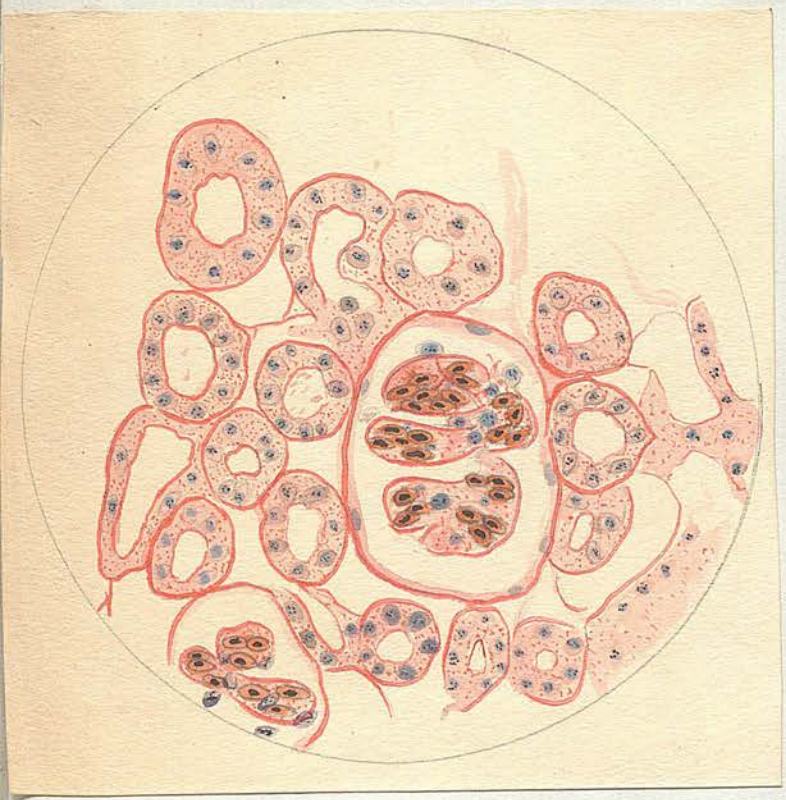


Fig. 22

Fig. 22 shows the microscopic appearance of the kidney of a healthy frog.

Stain - haematoxylin & eosin

Magnified - 600 times.

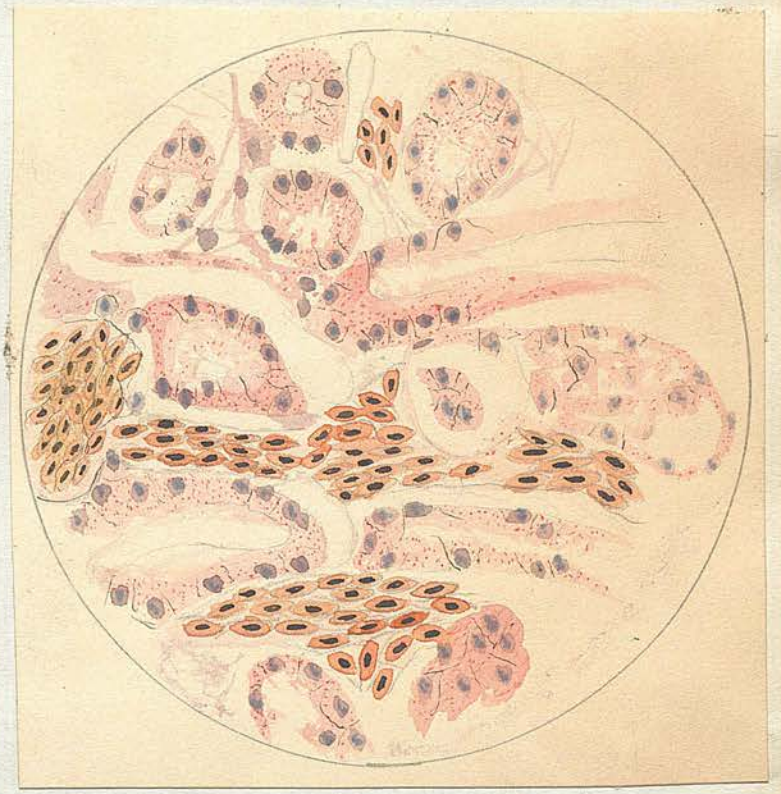


Fig.23

Fig. 23 shows the microscopic appearance of the kidney of frog No.VI, which died on the 9th day after the injection of .005 mg of African Cobra Venom to the gramme weight, subcutaneously. Note the extreme congestion and the appearances of desquamative nephritis.

Stain - haematoxylin and eosin.

Magnified - 600 times.

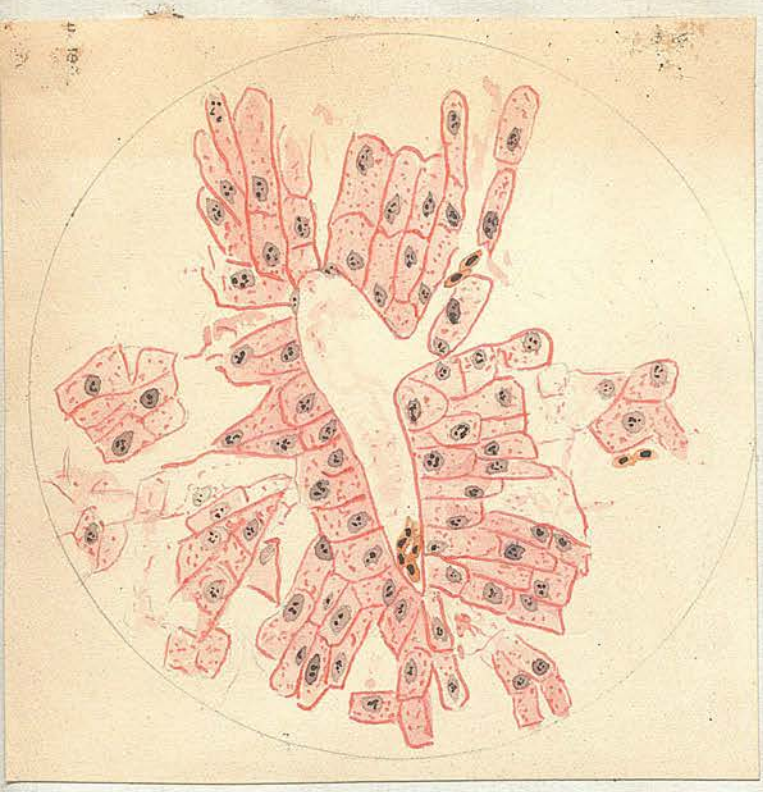


Fig. 24

Fig. 24 shows the microscopic appearance of the liver of a healthy frog.

Stain. Haematoxylin and eosin.

Magnified 600 times.

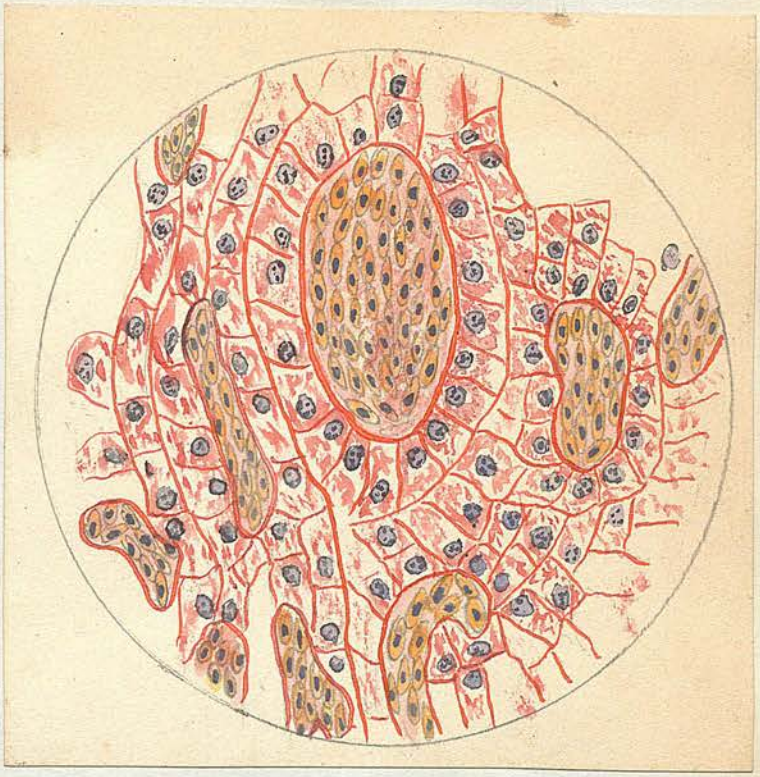


Fig. 25

Fig. 25 shows the appearances of the liver of frog No. III which died on the 8th day after the injection of .001 mg of African Cobra Venom per gramme weight, subcutaneously.

Note extreme congestion and evidence of degeneration in the liver cells.

Stain - haematoxylin and eosin.

Magnified - 600 times.