PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

On the Action of Cobra Venom. Parts I and II

Arthur R. Cushny and S. Yagi

Phil. Trans. R. Soc. Lond. B 1918 **208**, 1-36
doi: 10.1098/rstb.1918.0001

PHILOSOPHICAL TRANSACTIONS.

I. *On the Action of Cobra Venom.*—Parts I and II.

By Arthur R. Cushny, *F.R.S.*, *and* S. Yagi, M.D.

(Received January 10,—Read February 17, 1916.)

(From the Pharmacological Laboratory, University College, London.)

Part I.—The Cause of Death.

By A. R. Cushny, *F.R.S.*

The work of Fayrer (1), and afterwards of Brunton and Fayrer (2), established the fact that in cobra poisoning the failure of the respiration is the cause of death, the circulation surviving for some time after the breathing ceases, and the heart continuing to beat for hours if the aëration of the blood is maintained by artificial respiration. They satisfied themselves that a curara-like paralysis of the motor nerve ends occurs in certain conditions, but they remained in doubt whether the failure of the respiration is entirely due to this, and were inclined to believe that it is due in part to paralysis of the respiratory centre; the phrenic nerve proved insensible to the strongest stimuli in some experiments, while the sciatic remained irritable, and there was a want of co-ordination of the diaphragmatic and thoracic muscles in others, which appeared to arise from paralysis of the phrenic nerve.

In 1883 A. J. Wall (3) concluded that, while the peripheral motor nerves are weakened, this is accompanied by a similar weakening of the central nervous system, and especially of the spinal cord.

Aron (4) performed some experiments on the frog in Binz's laboratory, and stated that the peripheral nerve ends were not paralysed by cobra venom, which proved fatal by paralysis of the spinal cord. This view was combated by Ragotzi (5), who pointed out that Aron had injected very large doses of the venom, which paralysed the heart; the arrest of the circulation led to depression of the central nervous system, and at the same time prevented the action on the peripheral nerves from being developed; when smaller quantities were employed the typical curara-paralysis could be elicited beyond all question, and he drew the conclusion that the characteristic action of cobra venom lies in the paralysis of the motor nerve ends in both frogs and rabbits. Ragotzi's criticism may also be extended to Wall's experiments, for he seems to have injected no less than 0·5 cc. of fresh venom into a frog generally.

Vollmer (6) repeated Aron's experiments in Binz's laboratory, and now accepted the action on the peripheral motor nerve ends, but maintained that the central nervous system is also directly paralysed, though his grounds for this are not definitely stated.

Rogers (7) and Elliott (8), while admitting the action on the phrenic terminations in mammals, still hold that the failure of the respiratory centre is the cause of death; their grounds for this view will be examined later.

The action on the peripheral motor nerve endings is therefore now universally accepted, for the refutation of Aron's work by Ragotzi and Vollmer may be considered to eliminate his views from consideration. But, while Ragotzi regards this peripheral action as alone occurring, except when overwhelming quantities arrest the circulation, all the other investigators believe that it is accompanied by depression and paralysis of the central nervous system and, in particular, of the respiratory centre, by direct action.

I have employed two specimens of dried cobra poison, one, which I shall designate F, kindly presented to me by Sir Thomas Fraser, the other (P), obtained from Dr. J. Pinto, of Madras. These two specimens resembled each other closely in the nature of their action, but F was approximately 10 times as powerful as P. Thus, venom F killed rabbits by subcutaneous injection in quantities of 0·25 mgrm. per kilogramme within 24 hours; the minimum lethal dose was 0·15 mgrm. per kilogramme rabbit by injection into the marginal ear vein, and death occurred in 2–4 hours. Venom P was fatal in the dose of 2·3 mgrm. per kilogramme rabbit by hypodermic injection, and the minimum lethal dose intravenously was 1·5 mgrm. per kilogramme rabbit. It is of interest to compare these figures with those obtained by Faust (9) for his pure protein-free ophiotoxin, which was fatal in doses of 3 mgrm. hypodermically and 0·1 mgrm. intravenously per kilogramme rabbit. Venom F was thus 12 times as poisonous by hypodermic injection, while it was only two-thirds as poisonous intravenously as ophiotoxin; Faust explains the low toxicity of his ophiotoxin when injected subcutaneously by supposing that it is absorbed with difficulty in the pure state.

The solutions were generally made freshly each day for experiments on the minimum lethal dose; in others I found that a solution kept fairly constant for weeks, when a crystal of thymol was added to prevent putrefactive changes.

Experiments on the Frog.

There being no dispute as to the action on the peripheral nerve ends in these animals, I have directed my attention chiefly to symptoms of affection of the central nervous system. In the presence of peripheral nervous action it is notoriously difficult to determine whether the centres are affected; for example, discussion was carried on for many years as to whether atropine and coniine acted purely

peripherally or whether depression of the spinal cord was also present, and, indeed, there may still be some difference of opinion on the matter. The question is rendered more difficult in the case of cobra venom by the extreme slowness of its action, which precludes the use of the Claude Bernard method of investigation, as the ischæmia alone is sufficient to cause peripheral paralysis in the course of several hours. On the other hand, large doses of venom, which act more quickly, cannot be used to determine the question, for they arrest the heart, and thus depress the central nervous system indirectly.

Experiment 1.—A frog (*R. temporaria*) of 28 grm. weight was injected with 0·04 mgrm. of F venom (0·15 mgrm. per 100 grm.) in the abdominal lymph sac. Two hours later it was perfectly normal, except that the sac now contained more fluid and looked rather red and irritated.

5½ hr. Frog sits rather quieter than usual perhaps, but moves spontaneously at intervals; it withdraws its foot at once on being pinched, and hops away. When the dish is rotated, the frog moves round in the opposite direction. It returns immediately when it is put on its back.

6½ hr. Frog sits still, the head resting on the plate. It moves when the plate is rotated, and jumps clumsily when pinched. Afterwards crawls away in a very ungainly way. When put on its back it immediately attempts to return, but succeeds only slowly and with difficulty; it then slowly gathers its legs under it and sits with head up, but the latter slowly sinks until it rests on the plate again. Respiration is seen only when animal is disturbed. The circulation in the web is normal.

24 hr. The frog lies perfectly still and flat, and no movement can be elicited from it. The circulation in the web is normal. Brain destroyed. Stimulation of the lumbar plexus and sciatic nerve with a tetanising current causes no movement, while direct stimulation of the gastrocnemius is followed by a normal contraction.

Experiment 2.—*Temporaria* of 22 grm. weight. Injected into the abdominal lymph sac 0·2 mgrm. cobra venom F (0·9 mgrm. per 100 grm.).

1½ hr. Frog sits still, rather flat, makes spontaneous movements, turns when the plate is rotated, hops away when pinched, but rather weakly, recovers when laid on back. Respiration normal.

2 hr. Frog now lies distinctly flatter than normally. He still makes spontaneous movements, but these, as well as those from stimulation, are clumsy; otherwise no change since last observation.

2½ hr. Frog lies motionless, but still makes spontaneous movements occasionally. It raises head when the toe is pinched. When thrown on its back it makes large tremulous movements, but is unable to recover itself. The respiration has ceased, and the circulation in the web is very slow and feeble.

4½ hr. Very feeble tremulous movements when pinched or thrown on its back. Sometimes gives a feeble twitch of the hind or fore toes spontaneously.

Frog pithed and sciatic nerve exposed and stimulated with a tetanising current. The gastrocnemius gave a single twitch and then relaxed, though the stimulation was continued. On direct stimulation the gastrocnemius contracted normally, and remained contracted during the stimulation. In the web there was a very slow movement of the blood in the large veins, but none was visible in the capillaries. On exposing the heart the auricles were found beating rapidly and fairly strongly, but the ventricle beat was imperceptible, the chamber remaining in a position of half systole.

In another experiment with the same dose complete curara-paralysis was obtained in 4 hr., and the circulation in the web ceased at the same time.

The results of a series of experiments with rising doses are as follows :—

Dose per 100 grm.	Sciatic stimulation effective after	Sciatic stimulation ineffective. Direct muscle stimulation effective after	Condition of web circulation.
mgrm.	hr.	hr.	
0·07	30	—	Good after 30 hours.
0·15	7	24	,, 24 ,,
0·27	7	24	,, 24 ,,
0·55	3½	4	Ceased at 6 hours.
0·9	4½	—	,, 5 ,,
0·9	3½	4	,, 4 ,,
1·5	3	4	,, 4 ,,

In these experiments two distinct and independent effects are observed—the paralysis of the peripheral nerve ends and the failure of the circulation. The peripheral paralysis is similar to that seen under curara except in its extremely slow development. It is seen in uncomplicated form only when very small quantities are injected (0·1–0·4 mgrm. per 100 grm. weight), but is then complete only after more than seven hours. If larger quantities than 0·5 mgrm. per 100 grm. weight are injected, the curara-like action can be induced much earlier, but only at the cost of the complete failure of the circulation. Exactly the same difficulty is met with in the case of atropine in the frog (10), small doses failing to induce complete curara-paralysis, and larger doses which have this effect also affecting the heart.

But while it is impossible to elicit complete curara action by cobra-venom within a few hours, each of the features of cobra poisoning may be induced by small doses of curara. The clumsy movements, the inability to recover the normal position, the apparently imperfect co-ordination of the movements, all occur under curara given in quantities which are insufficient to interrupt completely the passage of impulses from the nerve to the muscle. On the other hand, spontaneous movements occur at a late stage of venom poisoning after the control of the muscles is obviously imperfect, and at this time efforts are made to recover the equilibrium, and to escape from painful stimuli, which indicates that the central nervous system is still capable of most of the manifestations of activity seen in the frog. The general position—the thorax sunk between the forelimbs, the head touching the plate—suggests central nervous depression, but can readily be explained by the difficulty in maintaining the muscles in continued contraction in the neck and shoulders.

The failure of the circulation may, of course, cause symptoms of central nervous depression, and this has frequently been attributed to direct action on the central nervous system in atropine, and also in cobra poisoning in the frog. When large doses are given, this indirectly induced central depression is undoubtedly present, but I have not been able to observe any symptoms pointing to affection of the central nervous system unless when the circulation was damaged. In the presence of partial peripheral

paralysis and depression of the circulation it is impossible to determine whether there is not also some direct action on the centres in the frog. But all the symptoms I have observed are such as arise from the cardiac and peripheral nervous weakness, and the view that the centres are directly affected is thus unproved and superfluous.

Two further complications of the action in the frog may be mentioned here, (1) the local irritant action, which seems less marked than when the fluid venom is used, but which may be sufficient to cause active movement in the beginning and thus to simulate nervous excitement, and (2) the local destructive action on muscle, which is often enough to paralyse the muscle of the abdominal wall when the venom is injected into the abdominal lymph sac, and may even extend to the thigh muscles when a large amount is injectod: this local muscular action was recognised by Ragotzi and others.

When one enquires after the grounds on which a central action in the frog has been supported by other authors, one finds that Brunton and Fayrer's belief in the depression of the spinal cord is chiefly based on a few (mainly five) of their many experiments, in which the reflexes disappeared, while movement could still be elicited by stimulation of the peripheral nerves or of the cord itself; in some the stimulation of the cord was ineffective, while that of the peripheral nerves caused movement. In all of these the animal had been subjected to a severe operation previously, in some the circulation is stated to have been feeble, and in others the amount of poison given may be judged as very high when the short interval before symptoms set in is considered. In view of the fact that in the great majority of their experiments no evidence of depression of the spinal cord is presented, one cannot help feeling that these authors were unduly impressed by the results of this small minority.

Wall's and Aron's experiments were all performed with large doses, as is shown by paralysis occurring within 40 minutes, and Ragotzi suggests that the final paralysis arose not from direct action on the nervous apparatus, but largely from heart failure.

Vollmer satisfied himself that the peripheral action is developed, but brings no evidence whatever in support of his view that the centres were also effected.

There is thus little experimental support for the supposed central action of cobra venom in the frog, and the symptoms when examined are seen to be those of a slowly progressing curara-like paralysis, and do not suggest central action in any way. Brunton and Fayrer, Ragotzi, and Vollmer have investigated the question by Claude Bernard's method, and with the exception of a few unconvincing results of the first mentioned authors, all concur in finding that the activity of the spinal cord remains apparently unimpaired long after the peripheral motor nerves have been paralysed.

In view of this mass of evidence I have not thought it necessary to repeat these experiments, especially as I found in my own observations every evidence that the central nervous system remains active as long as the peripheral motor nerves suffice to carry impulses to the muscles.

In natural conditions the death of the frog bitten by a cobra is beyond question due to heart action rather than to peripheral paralysis, for the latter would only develop many hours after the tragedy is complete.

Action in Mammals.

I have injected cobra venom into the rabbit (subcutaneously and intravenously) chiefly, and a few experiments were done on cats and guinea-pigs. The symptoms have been described already by several writers, and there is very little to add. Those from intravenous injection differed from those after hypodermic application only in the greater rapidity of their onset, and the intravenous dose is much nearer the hypodermic one than is usual under other poisons. Another characteristic is the slowness with which the symptoms appear and progress.

Experiment 3.—A rabbit of 1060 grm. was injected intravenously with 0·1 mgrm. per kgrm. of cobra venom F (equivalent to two-thirds of the minimum lethal dose). After 1 hr. it appeared quite normal, except that over the chest coarse râles could be heard with each respiration, and were especially marked after exertion.

After 2 hr. same condition. The animal occasionally makes a curious movement resembling vomiting or coughing, and often seems to attempt to remove something from its mouth with its fore-paws.

No further symptoms were noted, and the rabbit was quite normal the next day and remained normal.

Experiment 4.—A rabbit of 1100 grm. received 0·15 mgrm. of venom F per kgrm. intravenously (equivalent to 0·9 minimum lethal dose).

30 min. Normal except very slight rhonchus, which continued throughout the experiment.

2 hr. Rabbit sitting still, occasional chewing and swallowing movements. The eye is less widely opened than usual, giving a sleepy expression. Rhonchus is now very loud, and can be heard without auscultation.

3 hr. Rabbit sitting still; the head is supported on the floor of the cage generally, but is raised at intervals, and then slowly sinks to the floor again. The respiration is becoming progressively weaker.

3 hr. 15 min. The rabbit now remains on its side when put in this position. The corneal reflex is altered, the eyelid twitching when the cornea is touched, but not being kept closed. Feeble twitching convulsive movements at intervals. The respiration is very weak, and there seems some in-coördination of the respiratory muscles. The expiratory muscles of the abdomen are active, but only feebly contracting.

3 hr. 20 min. Very feeble respiratory movements continue, but the corneal reflex has almost entirely disappeared. A tracheotomy tube was inserted, and the breathing at once improved, the heart became quicker and stronger, and the feeble twitching response to corneal irritation returned. The animal resumed its normal position and made some spontaneous movements. The respiration continued extremely feeble for some time longer, and the animal was then killed.

Experiment 5.—Rabbit of 1240 grm. received 0·2 mgrm. per kgrm. of vemon F by intravenous injection (1·3 minimum lethal dose).

1 hr. Rabbit sits still in cage, head held rather lower than usual, eyes half closed. Respiration 52 per min. Heart 240 per min. It moves about spontaneously at intervals, and struggles when lifted up.

1 hr. 30 min. Head has now sunk to the floor, but is raised at intervals and then sinks to the floor again. The rabbit moves about when taken out of its cage, but rather clumsily, the feet slipping out along the table. Respiration 34 per min., accompanied by long rhonchi. Mucus and saliva escape from the mouth.

1 hr. 50 min. Respiration 52 per min., rather laboured.

2 hr. 10 min. Rabbit now lies still on its side, but moves when touched or at any loud sound. The conjunctival reflex is present, but is altered; when the eye is touched, there is a quick flickering movement of the lid, which is repeated at intervals if the touch is maintained, but the eye is not closed, as in the normal animal. Respiration 56 per min., partly diaphragmatic but more thoracic than previously; the expiratory abdominal muscles contract.

2 hr. 40 min. Rabbit lies in same position. Respiration 36 per min., very weak and apparently almost purely thoracic; the expiratory abdominal contractions also very weak. Heart 128 per min., irregular, dropping beats.

3 hr. Respiration 36 per min., very weak. Heart 58 per min., irregular. The foot is withdrawn when pinched, and a flickering contraction of the eyelid occurs when the conjunctiva is touched.

3 hr. 10 min. Feeble convulsive twitches of legs. Respiration 22 per min., very weak, and almost purely thoracic, in which both inspiratory and expiratory movements are just visible.

3 hr. 12 min. Respiration no longer visible. A tube was inserted in the trachea, and very feeble respiration returned; the movements are mainly thoracic, the abdominal contractions are just visible. Very feeble twitch of eyelid when cornea is touched; the foot is not withdrawn when pinched. Heart 108 per min.

3 hr. 20 min. Respiration 22; heart 100 per min. Eye reflex present.

3 hr. 33 min. Respiration 22; heart 72 per min. No abdominal respiration perceptible; the head is feebly jerked with each inspiration. Flickering eye reflex. No movement when foot is pinched. Occasionally there are feeble convulsive twitches of all four limbs.

3 hr. 37 min. Heart 90 per min., irregular.

3 hr. 42 min. The eye reflex is gone. Head movements go on simulating respiration, but no air appears to enter the chest. Heart 133 per min.

3 hr. 45 min. The heart stopped. Artificial respiration instituted at once, and the heart began beating again. The flickering eye reflex returned, but no other movement. The rabbit was rapidly decerebrated, and the thorax was opened along the middle line, artificial respiration being maintained.

3 hr. 50 min. The right phrenic nerve (uncut) was stimulated with secondary tetanising current at 15 cm. coil distance, and a weak jerk contraction of the diaphragm could be felt and seen. Left phrenic (uncut) gave the same result. Direct stimulation of the diaphragm gave a strong continuous contraction, which was maintained throughout the stimulation, while stimulation of the phrenics gave a weak jerk, the muscle relaxing immediately, even when the stimulation was continued.

3 hr. 58 min. The artificial respiration was interrupted, and very soon rapid, but very weak, rhythmical contractions of the diaphragm began; these were just sufficient to cause a visible descent of the liver, but were short jerks, and it may be questioned whether they would have caused air to enter the lungs in the intact animal. These movements, at first rapid, soon got slower and ceased. Feeble convulsive twitching of the legs. Artificial respiration resumed, as heart had almost stopped.

4 hr. 20 min. Phrenic stimulation caused feebler twitching of the diaphragm than previously. Direct stimulation of the diaphragm gave strong contraction, lasting throughout stimulation. When the artificial respiration was stopped, rhythmical contractions of the diaphragm began, weaker than before. Stimulation of the sciatic nerve causes weak twitch of gastrocnemius. Direct stimulation of the muscle causes strong tetanic contraction, apparently normal.

5 hr. 20 min. Condition unchanged. On interrupting artificial respiration the heart becomes slow, and very weak twitches of the diaphragm begin, but these are too weak to depress the liver. The artificial respiration was resumed, and it was now seen that feeble twitches of the diaphragm continued in rhythm with the artificial respiration, showing that the Hering–Breuer reflex was maintained.

6 hr. 10 min. Condition unchanged from last observation, phrenic stimulation causing a short weak twitch of the diaphragm, while direct muscle stimulation was accompanied by tetanic contraction lasting during the passage of the current.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Experiment 6.—A rabbit of 1050 grm. received by intravenous injection 0·6 mgrm. of venom F (equivalent to four times the minimum lethal dose). It hops about the floor for

10 min., when it sits still, head swaying a little. Respiration about 90 per min.

13 min. Fore-quarters sink down, but are raised repeatedly, only to sink to the floor again.

15 min. Head sinks to floor.

20 min. Respiration 40 per min., very weak movements of diaphragm and ribs and of abdominal expiratory muscles. Heart 56 per min. Weak twitch of eyelid on touching conjunctiva. Cannula inserted into trachea.

25 min. Heart 52 per min., irregular. Weak convulsive twitchings at intervals.

26 min. No respiratory movements. Heart still beating slowly and irregularly.

27 min. Artificial respiration instituted and the heart immediately accelerated to 200. Rapid decerebration and thorax opened along middle line.

35 min. On the artificial respiration being stopped, the heart became extremely slow. Very weak rhythmical twitches of the diaphragm could be made out with some difficulty; they did not suffice to move the liver. Artificial respiration was resumed.

40 min. Right phrenic (uncut) stimulated with tetanising current, and the diaphragm gave a very weak twitch and then relaxed. Direct stimulation of the diaphragm caused strong contraction, lasting as long as the current passed. Left phrenic (uncut) stimulation gave same result as right.

46 min. Stimulation of the phrenics caused no contraction of the diaphragm, whilst direct stimulation caused strong tetanic contraction.

50 min. Stimulation of the sciatic nerve caused a feeble twitch of the gastrocnemius, which relaxed again almost immediately. Direct stimulation gave a powerful tetanic contraction.

70 min. Direct stimulation of the diaphragm or of the gastrocnemius caused apparently normal tetanus. Stimulation of the phrenic caused no movement of the diaphragm. Stimulation of the sciatic caused a feeble twitch of the gastrocnemius, which then relaxed and remained relaxed. A series of tetanic stimulations, each lasting for about 15 sec., caused a series of jerks which rapidly diminished in height, and finally ceased.

These experiments are typical of the results obtained in a number in which cobra venom was injected intravenously in rabbits. Others, in which the poison was given subcutaneously, differed only in the longer interval between the injection and the first symptoms. The venom proved fatal, or would have proved fatal if artificial respiration had not been applied, in from one-half hour to three hours after the venom was injected intravenously. When given subcutaneously, death occurred after 1–24 hours, according to the dose employed. In about 10 per cent. of the fatalities from snake-bite in man, death follows within an hour, and in about 50 per cent. within seven hours of the bite (Wall).

In all my experiments, as in those of previous writers on cobra poisoning, death was obviously due to failure of the respiration, for though the heart was very slow and much dilated, it recovered immediately when artificial inflation was begun. Special attention was, therefore, directed to the respiration throughout the experiment. Several investigators have stated that in the beginning the respiration is accelerated, and have assumed that the centre is stimulated. But this is not a regular phenomenon, and is in fact exceptional in intravenous poisoning. I have seen it occasionally after subcutaneous injection, but in view of the local irritation induced by the poison and also of the increased secretion along the air passages, it is impossible

to state that it arises from direct action on the centre. Far more probably it is a reflex from the subcutaneous tissues or the result of imperfect aëration due to the partial obstruction of the trachea. In any case it is of small importance.

The first striking change in the respiration observed is the diminution in the depth. The movement of the abdomen on inspiration becomes less marked and soon is confined to the anterior half of the belly. Later the thoracic inspiratory movement becomes unusually obvious, and may in fact play a greater part than the abdominal. The expiratory movement of the belly becomes marked and the nostrils show laboured breathing. All these indications of deficient aëration are remarkably weak, so that they form a contrast to the laboured breathing of ordinary asphyxia, and the presence of various weak movements in the thorax and abdomen gives the impression of a lack of co-ordination of the respiratory muscles. On close examination, however, one can satisfy oneself that no real in-coördination is present but merely weak efforts to attain a more satisfactory inflation. Not infrequently any one respiratory movement is seen to consist of a short jerk contraction, though this is often concealed by some different movement following closely on it. Thus the quick short diaphragmatic contraction gives way to a rapid relaxation but this may be obscured by the contraction of the expiratory abdominal muscles. At this time the rate of the respiration may not depart much from the normal, or it may be somewhat quicker. As the weakness of the movements progresses, however, the rate falls considerably, until it may be only one-half or one-third of the normal. The heart becomes dangerously slow, the eye-reflex disappears, and weak twitching movements occur in the limbs. Finally the respiratory movements are no longer perceptible, and, unless artificial aid is given, death occurs.

I have attempted to record the respiratory changes graphically but with unsatisfactory results, owing to the extreme weakness of the later movements, which may not cause larger movements of the thorax than those due to the heart beat. The details of the movements can be followed satisfactorily only by the eye.

The slowness of the breathing before its failure indicates depression of the respiratory centre in the medulla, but this appears not to be due to the direct action of the poison, but to be one of the effects of the asphyxia. CO_2-excess and O_2-lack act as stimuli to the respiratory centre when they are moderate in degree, but in excess they narcotise the centre and slow its activity.* The failure of the aëration in cobra poisoning is the cause of the slowing of the respiration, as is shown by the following experiment.

Experiment 7.—A rabbit of 950 grm., anæsthetised with urethane (2 grm.).

2.35. Injected 0·5 mgrm. venom F into left jugular vein.

2.39–3.2. Respiration varied from 58 to 66 per min., gradually growing weaker.

3.5. Tracheotomy.

3.5–3.28. Spontaneous respiration at the rates of 54–42 per min., growing weak and laboured. The heart had hitherto beat at about 240 per min.

3.28. Respiration 48; heart 180.

3.32. Respiration 50; heart 144.

* For other instances, see Cushny and Lieb (11).

3.36. Respiration 38; heart 188, irregular groups of rapid beats alternating with series of slow ones.

3.40. Respiration 22; heart 162.

3.44. Respiration 19.

3.45. Respiration ceased. Heart could not be felt beating. Artificial respiration. Thorax opened along median line and heart found beating rapidly and regularly.

3.51. Artificial respiration stopped and the diaphragm began very feeble contractions, which were just visible, but did not move the liver downwards. These contractions were counted for 15 sec., and were found to be at the rate of 49, 50 and 60 per min. in successive observations. The heart then became very slow.

3.53. Artificial respiration restored the heart, and no movement of the diaphragm could be made out. The amount of air pumped into the lungs was then reduced until the feeble contractions of the diaphragm again began. These were found in repeated counts to be at the rate of 54 per min.

The respiratory centre is shown in this experiment to be capable of a faster rhythm after respiratory failure under cobra venom than that actually observed before the asphyxia occurred. When the blood had been sufficiently aërated, the centre discharged for some time at a rate which was practically that of the normal animal before the venom acted. It is obvious, therefore, that the slowness of the respiration before its cessation is not due to the venom acting directly on the centre but to the asphyxial condition of the blood, which depresses the respiratory centre with the rest of the brain. The same results were obtained in other similar experiments and in one on the decerebrated cat in which no anæsthetic was used.

A further evidence of the activity of the centre is mentioned in Experiment 5, in which it was observed during artificial respiration after the venom had caused asphyxia that each deflation of the lungs was followed by a feeble twitch of the diaphragm, showing that the vagal reflex of Hering–Breuer was still present. After cobra venom has caused asphyxia, therefore, the respiratory centre is capable of emitting impulses at the ordinary rhythm and is susceptible of stimulation by non-aëration of the blood or by reflexes; in short, its rhythmic function shows no observable aberration.

Most investigators have regarded the weakness of the respiratory movements as the result of central action, Ragotzi alone maintaining it to be peripheral. The arguments adduced by these writers will be discussed later. In my experiments the cause was obviously failure of the nerve ends to transmit the impulses to the muscles in sufficient strength to cause an efficient contraction. For in every case of failure of the respiration stimulation of the phrenics in the thorax with a tetanising series of shocks caused merely a feeble jerk of the diaphragm, which relaxed during the stimulation and which was insufficient to move down the liver, and would therefore not have caused any air to enter the thorax in the intact animal. On the other hand, direct stimulation of the diaphragm caused a strong contraction which was maintained as long as the current was closed (2–5 secs.), and which differed in no wise from the normal movement on stimulation; thus the liver was pushed down and the abdominal wall rose as in normal respiration. The characteristic feature of the intoxication is that asphyxia occurs before the phrenics are completely paralysed,

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

so that if the thorax be rapidly opened when the heart stops, electrical stimulation of the phrenics still causes movement of the diaphragm, and the conclusion is drawn that the action is not peripheral. More careful observation shows, however, that the movement is insufficient to inflate the lungs, and that the impulses can no longer reach the diaphragm in sufficient strength. Under larger doses than suffice to cause asphyxia the paralysis of the phrenic terminations becomes complete, while the muscle continues to respond to direct stimulation as before (Experiment 6).

These experiments show that when asphyxia occurs under cobra poison the terminations of the phrenic nerves are unable to transmit efficient impulses to the diaphragm. The other respiratory nerves are equally disabled. This does not negative the view that the centre is also emitting impulses which are also insufficient, but in view of the fact that the rhythm of the centre is unimpaired, and that the peripheral action affords a sufficient explanation of the weakness of the respiration, it is unnecessary to make such a hypothesis, for which there is no foundation whatever.*

It is difficult to obtain direct graphic records of the movements of the diaphragm during experiments of long duration, and, on the other hand, in more acute experiments the changes progress rapidly to complete paralysis of the phrenics. I have, however, followed the changes in another, more accessible muscle—the gastrocnemius of the cat and rabbit—in several experiments, of which I give an example in Experiment 8.

Experiment 8.—A rabbit of 1050 grm. anæsthetised with urethane. Cannulæ inserted into the trachea and jugular vein. The right sciatic nerve was isolated and divided above. The femur was clamped to a standard, and the lower part of the gastrocnemius muscle was exposed and attached to a lever writing on a drum.

2.57. Stimulated the sciatic nerve by tetanising secondary shocks for 9 sec., obtaining the tracing marked 1 in fig. 1. Stimulated muscle directly with tetanic current by electrodes hooked into the lower and upper parts of the muscle (curve not reproduced).

3.4. Injected 0·6 mgrm. venom F intravenously (four times the minimal lethal dose).

3.40. Stimulated nerve at 15 cm. coil distance as before, and obtained tracing marked 2 in figure.

3.45. Obtained tracing marked 3 in figure.

3.55. Obtained tracing marked 4 in figure.

3.57. Obtained tracing marked 5 in figure.

In these last three observations the tetanic shocks were kept on for 9 sec., long after the muscle had reached full relaxation in the last two.

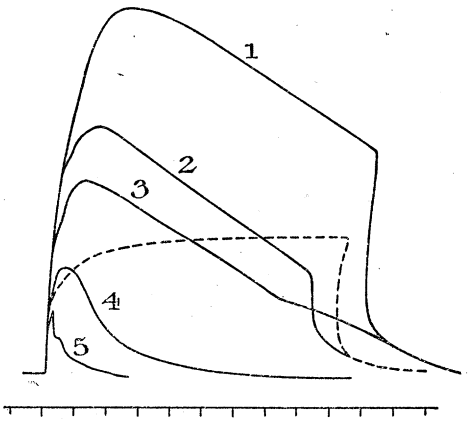

FIG. 1.

5.0. No movement of the muscle from stimulation of the sciatic at 15, 10, or 5 cm. coil distance.

5.1. Gastrocnemius stimulated directly, giving the curve which is represented in the dotted line in the figure.

* I have attempted to obtain more direct evidence of the condition of the respiratory centre by experiments with cross circulation, but find it difficult to maintain respiration in these for the time

5.5. The left sciatic nerve exposed and stimulated, but gave no contraction of the gastrocnemius. The phrenic nerves stimulated in the thorax caused no movement of the diaphragm. Direct stimulation of the left gastrocnemius or the diaphragm caused powerful contractions which lasted as long as the tetanising current passed.

In this experiment the venom is shown to shorten the duration of the muscular contraction which can be elicited by stimulation of the nerve. The contraction at first lasts as long as the current is applied, but later becomes shorter and weaker, until it is merely a weak jerk which relaxes immediately. The muscular contraction from direct stimulation is maintained as long as the shocks are applied. In other words, the venom accelerates the fatigue of the nerve ends, which finally are able to transmit only the first two or three tetanising shocks, after which the muscle relaxes. In consequence the muscle never attains its full contraction and cannot maintain a sustained effort such as is necessary for most movements. The same phenomenon has been shown to be caused by small doses of curare in the frog (Boehm, 12).

In mammals Brunton and Fayrer observed in several experiments that the terminations of the phrenic and other nerves were paralysed by cobra venom, but they were unable to determine whether the failure of the respiration depended on this or on paralysis of the medulla, and suggest that in some cases the one action preponderates, in others the other. No convincing evidence of any paralysis of the medulla is adduced, however. In some experiments, reflexes (*e.g.*, the corneal) disappeared, while electrical stimulation of the cord still caused movements of the muscles of the hind legs, and the conclusion is drawn that the grey matter of the cord was paralysed, while the white fibres still could conduct impulses. But the reflexes can often be restored by artificial respiration, as I have shown, and their disappearance is therefore not due to direct action of the venom on the central nervous system, but is the result of the asphyxia. The fact that the muscles continue to contract on electrical stimulation of the cord does not exclude the occurrence of curara action, unless it is shown that a continued contraction can be induced, and not merely the weak jerks which are often seen in incomplete curara action.

Another confusing factor which is especially liable to complicate the action in the guinea-pig and rabbit arises from the impairment of the respiration from the accumulation of fluid in the lungs and trachea. Together with the weakened movements of the respiratory muscles, this may cause complete asphyxia long before the motor nerve ends are completely paralysed in the diaphragm or elsewhere.

These two factors—the depression of the central nervous system secondary to the asphyxia and the early appearance of asphyxia from the obstruction of the air

required for the action of venom. I hope to return to these when it is possible to obtain an adequate supply of hirudin.

passages—suffice to explain the exceptional experiments noted by BRUNTON and FAYRER which led them to accept a direct action of the venom on the central nervous system. ARON and WALI seem to have made no observations on mammals to analyse the point of action. RAGOTZI found no evidence of central depression in warm-blooded animals in his numerous experiments, and attributes the whole action to paralysis of the nerve ends in the mammal as in the frog. In several experiments he excluded the poison from one limb by ligature of the vessels and found that reflexes persisted here after they ceased elsewhere, and that convulsions occurred from asphyxia in the protected limb only. He also attempted to protect the nostrils of the rabbit from the poison by pressing a ring over the muzzle, and states that after artificial respiration had been begun to prevent asphyxia, the movements of the nostrils ceased, but on interrupting the artificial inflation there was an apnoeic pause, and the nostrils then began the rhythmical movements which ordinarily occur in partial asphyxia. I have shown that the same holds true of the diaphragm and other respiratory muscles, including those of the nose, even when they are not protected from the poison, and there can be no question that RAGOTZI's measures were not sufficient to protect the nose muscles from the poison. I merely mention this experiment because it has been cited as if it were the essential part of his argument, of which it forms only a small part, and it has been suggested that the whole rests upon equally assailable methods, which is not the case.

ROGERS found that, when the respiration ceased, stimulation of the phrenic nerves was still effective, though less so than normally, and therefore concludes that the respiratory failure cannot be due to phrenic paralysis.

ELLIOT, the last writer on the subject, admits that the peripheral nerve ends are paralysed eventually, but holds that respiratory failure is ordinarily due to paralysis of the medullary centre. He bases this view upon two sets of observations. In the first he applied a large dose of cobra venom (5 mgrm.) directly to the medulla oblongata, and observed failure of the respiration very soon afterwards, while the peripheral nerves continued to react to electrical stimulation. But here the local action of the venom was induced in the medulla, and no one doubts that a large dose of cobra venom is capable of destroying any organ to which it is applied directly. Doubtless, if the same amount of venom had been applied to the phrenic terminations, there would have been immediate failure of the respiration also, but this would not have indicated that cobra venom circulating in the blood paralyses the phrenic nerve ends. The second series of observations on which he relies was made by stimulation of the phrenics when the respiration failed; in almost every case he observed twitching of the diaphragm, but he does not state whether the stimulus was a single shock or a tetanising series. In any case, I quite agree with his observations. In the great majority of experiments in which a fatal injection of cobra poison is made, the diaphragm will be found to react with twitches to phrenic stimulation if it be examined immediately. But these twitches are insufficient to

maintain the aëration of the blood, and the respiratory centre and the heart rapidly fail from CO_2 excess and O_2 lack. The failure of the centre is, however, secondary to the failure of the ends of the phrenic nerve.

The essential feature of the asphyxia is, then, the action on the nerve ends, which resembles that of curara, and, like it, affects the nerve ends in the diaphragm and other respiratory muscles somewhat earlier than those of the limb muscles; this earlier action may probably arise from the constant activity of the respiratory muscles, for the characteristic action of the curara-like poisons in small doses is to accentuate the fatigue of the nerve ends. This seemed to me especially marked in the case of cobra venom; nerve stimulation, which caused a feeble twitch in the muscle at first, failed to have any effect if soon repeated, the period required for recovery seeming longer than that observed under curara.

This action on the nerve ends leads in itself to complicating factors through the asphyxia of the nerve centres and of the heart. But, besides these, there are two features, induced by the direct action of the venom, which distort the picture. These are (1) the direct action of the venom on the heart, and (2) the obstruction of the respiratory passages by secretion.

The direct action on the heart is discussed by Dr. Yagi in Part II, and is obvious enough when large doses are injected intravenously. Under smaller doses it is less easily observed; the heart is undoubtedly gravely disturbed when the respiration fails, but this is mainly due to the asphyxia, and appears to be removed by adequate artificial respiration. But careful observation suggests that even the minimal lethal dose renders the heart more susceptible to asphyxia than the normal heart. When the artificial respiration is interrupted the heart fails more rapidly than a normal heart, and, when the inflation is resumed, the recovery of the heart is slower. These are merely impressions, and I cannot support them by actual figures, for it is useless to determine the reaction of a normal heart to asphyxia and inflation, and to compare the results with those obtained two or three hours later, when the minimal lethal dose of the venom has induced asphyxia. But these impressions are based on a considerable number of observations, in some of which it was found impossible to revive the heart at all after a brief period of asphyxia. When larger amounts of venom are injected the heart weakness becomes more obvious, and in cases in which death follows within an hour of the bite of a cobra I think there can be no doubt that the heart must be gravely affected. The weakness of the heart must, of course, intensify the effects of the mal-aëration of the blood, that is, must emphasise the apparent central action.

The obstruction of the breathing from the accumulation of secretion in the air passages has not been sufficiently emphasised by former writers, although many of them, notably Fraser (18), mention the constant rattling and gurgling which accompanies respiration. It is especially marked after small doses, and is generally absent when two or three times the minimal lethal dose is injected. When a rabbit

dies from the minimum fatal dose, the larynx or trachea is in a great majority of cases found to contain a quantity of frothy liquid like saliva. The bronchi are generally free from this, though not always, and, indeed, it often occupies only the larynx, or only a short length of the trachea. The lungs often do not collapse completely when the thorax is opened, but remain in a half expanded position, and crepitate when squeezed between the finger and thumb. When they are cut into they often appear to contain more fluid than usual. The colour is generally a slightly deeper pink than usual, and not infrequently a lobe or part of a lobe may be deep red and infarcted. Infarctions were oftener seen in the limited number of experiments done on guinea-pigs than in my usual experimental animals—the rabbits. In cats the lungs rarely showed any changes, though in the cat the bubbling respiration was also present sometimes.

The cause of this symptom I have not been able to determine as yet. The salivary secretion was found to be increased by Gunn (13), but Yagi is not able to confirm his statement. And there was no very obvious increase of the saliva, such as is seen under pilocarpine, for though the lips were often moistened, there was no actual flow from the mouth. It is possible, therefore, that the saliva reached the respiratory passages not from its being secreted in excess but from the paresis of the muscles of the pharynx and larynx, which prevented its being swallowed. This would indicate that the nerve ends in these muscles are weakened very early in the action, before any distinct change can be made out in the respiratory muscles proper, but not before there is paresis of the eyelid muscles, permitting the upper lid to fall slightly. This passage of saliva into the larynx would not be serious in normal animals, but in the weakened condition of the respiratory muscles forms a considerable obstruction to the breathing. Its import is shown by the fact that tracheotomy restores the respiration in rabbits, at any rate temporarily in some cases. At the same time, tracheotomy by shortening the respiratory passages may increase the efficiency of the feeble movements of the thorax. The unusual moisture of the lungs and the infarction which is often met with appear to arise from the saliva insufflated.

It is necessary to consider shortly how far the symptoms of cobra poisoning in man correspond with those seen in animals and whether they conform to the type of curara action. It may be premised that in man there are, in addition to the typical effects, marked symptoms from the local irritation and many symptoms from shock and fear; the importance of these has been pointed out by Fayrer, who describes a case in which the patient died of fear after being bitten by a non-poisonous snake, which he believed to be poisonous.

The most detailed account of the symptoms after cobra bite is given by Hilson (14) and cited in full by A. J. Wall (3) and by F. Wall (15). The first symptoms, apart from the pain, were loss of control over the muscles of the legs, staggering, fall of the lower jaw with frothy viscid saliva oozing from the mouth, indistinct speech (compared to that of alcoholic intoxication), moaning, and shaking of the head from side to side,

respiration increased in frequency. About 40 minutes after the bite the patient was unable to answer questions but appeared conscious, and his arms were not paralysed. The respirations were laboured but not stertorous, and gradually became slower and ceased without convulsions. The symptoms are typical of peripheral paralysis, as shown in the loss of control of the muscles of the legs, jaw, tongue, and larynx (loss of speech), while those of the arms, being unused, remained unparalysed longer. The laboured respiration, quickened in the earlier stages, then becoming slower and ceasing, corresponds closely to that of the rabbit and other animals, particularly if the observation is confined to the head, and the chest and belly are not watched. Consciousness was present after speech was lost, indicating that the motor paralysis preceded the cerebral action. The oozing of frothy viscid saliva from the mouth is characteristic of venom action in animals, in which it often leads to respiratory embarrassment by passing into the larynx, as I have said.

The essential action of cobra venom then corresponds to a slowly progressing paralysis of the peripheral ends of the motor nerves, resembling that of curara, but differing from it in the tardiness of its approach.

Experiments on Antidotes.

I have attempted to relieve the embarrassment of the respiration by the secretion in the larynx by giving atropine previously, and have succeeded in doing so and apparently in retarding the asphyxia slightly, but atropine does not appear to have any distinct effect in finally saving the life of animals under cobra venom. When the minimal lethal dose was used, a rather larger number of rabbits recovered when atropine had been given previously, but the difference was not marked. And when 1·3 times the minimal lethal dose was given, no difference could be made out in the mortality of the treated and the untreated.

A more promising line of treatment seemed to be offered by the antidotes to curara. Rothberger (16) has shown that the curara paralysis may be counteracted by physostigmine, guanidine, and other drugs which excite the nerve ends, and Langley (17) and others have in recent years investigated the antidotal action of nicotine in detail. There thus seemed every prospect that these drugs might be efficacious in cobra poisoning. I have, therefore, treated a number of rabbits under cobra venom with intravenous injections of 0·5—1·0 mgrm. of physostigmine sulphate, while in a few the physostigmine was injected before the venom. This amount of physostigmine injected intravenously in normal rabbits causes marked tremor, which lasts about an hour and then passes off, leaving the animal rather exhausted but otherwise apparently normal. When physostigmine was injected shortly after the minimal lethal dose of cobra venom and before the respiration was seriously embarrassed, the same tremor and excitement was seen as in normal animals. But the longer the interval between the injection of the venom and the physostigmine, the less was the action of the latter. During the stage of

serious impairment of the respiration from venom, physostigmine caused hardly any noticeable tremor. In some experiments the respiration was temporarily improved in depth by the physostigmine, but in others no such antagonistic effect could be observed. And more frequently the secretion of saliva was much increased, the rattling tracheal rhonchus was exaggerated, and the breathing was more embarrassed accordingly. The absence of the physostigmine tremor in the later phases was the most striking feature and indicates that the alkaloid is unable to displace the venom from its attachment in the motor nerve ends. In experiments in which the contraction of the gastrocnemius on stimulation of the sciatic nerve was recorded, it was found that after the venom had reduced the height and duration of the tetanic contraction, physostigmine injection did not improve in any way the strength of the movement.

Guanidine hydrochlorate was injected intravenously in doses of 0·25–0·3 grm. per kilogramme, which is enough to cause tremor of the head and jaw muscles in normal animals. This was absent after cobra venom, but the condition of the animal was not improved and not infrequently became very rapidly worse after the injection. I propose to continue the investigation of this antagonism further. At present there seems little prospect that either physostigmine or guanidine will be able to displace the cobra venom from its combination in the nerve ends, and on the other hand, when they are injected before the venom, they do not seem to have any prophylactic value.

Bang and Overton (19) found that the presence of calcium salts reduced the toxicity of cobra venom to tadpoles immersed in it, and I have therefore tested whether calcium lactate and chloride possess any antidotal action in mammals; these salts were injected intravenously after the cobra action was well developed. I could not find that they had any effect whatever on the progress of the poison.

Animals anæsthetised with urethane, paraldehyde or veronal generally survive under the minimal lethal dose of venom longer than normal animals, and in fact urethane is the most efficient antidote that I have met. It prolonged the intoxication very distinctly in many cases, and in some seemed to promise recovery, although the animals all died in the course of the night, probably from excessive heat loss. This action of urethane was at first puzzling, but is susceptible of a simple explanation. For any movement increases the CO_2 of the blood, and thus increases the activity of the respiratory centre, which in turn throws a greater burden upon the enfeebled nerve terminations. Now the nerve ends which are continually receiving impulses are those which fail first both under curara and under cobra venom. Anything that diminishes active movement without impairing the respiration is thus beneficial in cobra venom poisoning. And the inference is clear that in cases of cobra bite, absolute rest should be imposed, if necessary, by means of a hypnotic or bromide. Alcohol has long had a reputation in some forms of snake bite, more especially in rattlesnake poisoning, which of course differs from cobra poisoning in essential features. But the effect of urethane in my experiments again raises the question whether large amounts

of alcohol, such as are advised in those cases, may not be of benefit by promoting rest. If rattlesnake poison kills by a curara-like action, this antidotal action may very well be present, though better effects might be obtained by better hypnotics.

Summary.

1. The characteristic action of cobra venom in warm- and cold-blooded animals is a paralysis of the motor nerve terminations in muscle, resembling that induced by curara in nature, but differing from it in its very slow progress and in the difficulty with which it can be removed.

2. The central nervous system is not directly involved in the action.

3. The cause of death in the mammal is the failure of the motor nerve ends in the respiratory muscles to transmit impulses which are strong enough to maintain the movements. The nerve ends are not completely paralysed when asphyxia occurs. Contributory factors are weakness of the heart and the accumulation of secretion (saliva) in the respiratory passages.

4. The cause of death in the frog appears to be exhaustion from motor paralysis if the lowest fatal dose is employed. When larger doses are used, failure of the heart appears the chief cause of death.

5. The alkaloids which are antagonistic to curara—such as physostigmine and guanidine—are not effective in cobra poisoning, but are rendered inactive themselves. The chief principle of treatment, apart from local measures and antivenin, which I have not examined, is complete rest.

The expenses of this research were met by a grant from the Scientific Grants Committee of the Royal Society.

REFERENCES.

(1) Fayrer, 'Thanatophidia of India, being a Description of the Venomous Snakes of the Indian Peninsula,' London (1872).

(2) Brunton and Fayrer, 'Roy. Soc. Proc.,' vol. 21, p. 358; vol. 22, p. 68 (1873).

(3) Wall, A. J., 'Indian Snake Poisons, their Nature and Effects,' Allen and Co., London (1883).

(4) Aron, 'Zeit. f. klin. Med.,' vol. 6 (1883).

(5) Ragotzi, 'Virchow's Arch. f. path. Anat.,' vol. 120, p. 201 (1890).

(6) Vollmer, 'Arch. f. exp. Path. u. Pharm.,' vol. 31, p. 1 (1893).

(7) Rogers, 'Roy. Soc. Proc.,' vol. 71, p. 481 (1903); 'Phil. Trans.,' B, vol. 197, p. 123 (1905).

(8) Elliot, 'Phil. Trans.,' B, vol. 197, p. 361 (1905).

(9) Faust, 'Arch. f. exp. Path. u. Pharm.,' vol. 56, p. 236 (1906–7).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(10) Cushny, 'Journ. of Physiol.,' vol. 30, p. 176 (1903).
(11) Cushny and Lieb, 'Journ. of Pharmacol. and Exp. Therapeutics,' vol. 5, p. 451 (1914–15).
(12) Boehm, 'Arch. f. exp. Path. u. Pharm.,' vol. 35, p. 16 (1895).
(13) Gunn, 'Quart. Journ. of Exp. Physiol.,' vol. 5, p. 67 (1912).
(14) Hilson, 'Indian Med. Gazette,' October, 1873.
(15) Wall, F., "The Poisonous Terrestrial Snakes of our British Indian Dominions, and how to Recognise them," 'Bombay Natural History Society,' (1913).
(16) Rothberger, 'Pflüger's Arch.,' vol. 87, p. 117 (1901), and vol. 92, p. 398 (1902).
(17) Langley, 'Journ. of Physiol.,' vol. 33, p. 374 (1905) (Croonian Lecture, 1906).
(18) Fraser, 'Roy. Soc. Proc. Edin.,' vol. 20, p. 448 (1895).
(19) Bang and Overton, 'Biochem. Zeitschr.,' vol. 31, p. 243 (1911).

Part II.—Action on Individual Organs.

By S. Yagi.

In these experiments the venoms used were the same as in Part I (page 2). For the most part venom P was employed for the isolated organs, with the exception of the intestine; but controls were always carried out with venom F, and no difference could be made out in the character of the effects. The concentrations given refer to venom P, except when otherwise stated.

1. *Striated Muscle.**

Brunton and Fayrer observed muscular twitching, followed by paralysis, when a muscle was suspended in a solution of venom in distilled water, and thought that this was due merely to the distilled water, while Ragotzi ascribed it to the action of the venom. Brunton and Fayrer add that the venom eventually abolishes the irritability of muscle when it is directly applied to it.

The following experiments were performed on the gastrocnemius and sartorius of *Rana temporaria*; there was no essential difference in the reaction of these muscles.

The sartorius, attached to a counter-weighted lever, was suspended in Ringer's solution (frog's), containing sufficient curara to paralyse the motor nerve ending, and venom was added to form a concentration of 1 : 100,000 or 1 : 50,000. Very soon the muscle began to twitch, and this movement became gradually more intense; but sooner or later, according to the strength of the poison, it became weaker, and at last ceased. During the twitching stage the muscle contracted partially, and the

* The references to the literature of cobra venom will be found in the first part.

more intense the twitching the greater the contracture. After the twitching ceased the muscle gradually relaxed to much beyond its normal length (fig. 1). At this stage it no longer responded to the electric shock as a general rule.

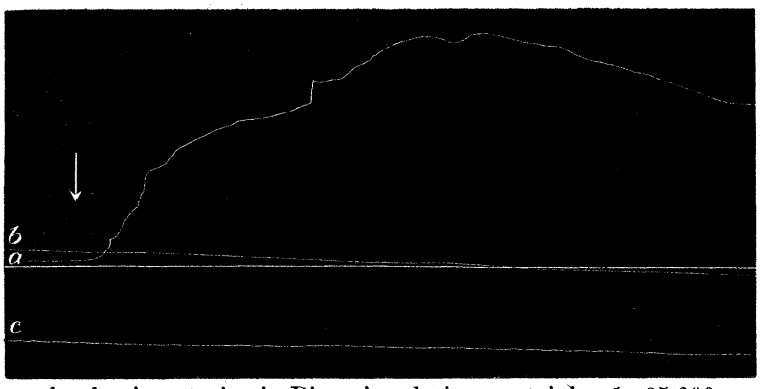

FIG. 1.—Contraction of a frog's sartorius in Ringer's solution containing 1 : 25,000 venom P. The drum moved 50 cm. per hour. The irregularities in the curve arose from the twitching of the muscles. Magnification 30 times. Intervals between *a* and *b*, *b* and *c*, 50 min.

The excitability by single electric shocks was found to undergo a gradual decrease, as is shown in Experiment 1.

Experiment 1.—The sartorius of a *Rana temporaria* in 1 : 50,000 venom solution in Ringer's solution; control in Ringer's solution.

Minutes during which the preparation had been immersed.	Minimal stimulus. Coil distance in cm.	
	Poisoned.	Control.
Before poisoning	16	16
30	15	
60	13	
90	10	
120	7	15
150	5	
180	1	
210	—	15

Similarly the response to maximal shocks was found to decrease slowly in venom solution of the concentration of 1 : 100,000, the height of the contraction falling, until, finally, no movement was recorded. The form of the curve of contraction remained practically unchanged in other respects.

The action of cobra venom upon striated muscle is therefore twofold. In the beginning it causes twitching and contraction, and later it causes relaxation and paralysis. The excitability by the electric shock decreases from the beginning.

2. *Invertebrate Muscle.*

The muscles of a large earth-worm were dissected out and suspended in oxygenated Ringer's solution (for frogs), and attached to a lever in the usual way. When the movement had become regular, venom was introduced into the bath in a concentration of 1 : 50,000. Marked augmentation of the rhythmic contractions and a distinct increase of the tone were caused, and the muscle remained in the same state for a long time without evidence of recovery. Even fairly strong solutions (1 : 10,000) did not cause in the earth-worm muscle the secondary relaxation and paralysis which were seen in the striated and unstriated muscle of vertebrates.

The addition of curara solution had no effect on these muscular movements, even when it was sufficient to arrest the contractions arising from stimulation of the nervous cord. And, on the other hand, the venom did not appear to lessen the effect of nerve stimulation on the muscle contractions, so that its action appears to be a direct one on the muscle substance.

3. *Motor Nerves and Nerve Ends.*

An isolated gastrocnemius of a *Rana temporaria*, together with the attached nerve fibre, was immersed in a bath containing venom solution. The minimal current required to produce tetanus on direct and indirect stimulation was determined at intervals.

As is seen in the following Table, when the nerve-muscle preparation was poisoned with a venom solution of 1 : 10,000 the strength of the minimal stimulus, indirect and direct, had to be increased gradually ; at first the changes ran parallel, but later the indirect stimulation altered more quickly than the direct.

Experiment 2.—Nerve-muscle preparation of a frog's gastrocnemius suspended in a venom solution of 1 : 10,000.

Minutes during which the preparation had been immersed.	Minimal stimuli required to produce tetanus of the muscle. Coil distance in cm.	
	Indirect.	Direct.
Before poisoning	31	19
10	31	19
20	30	18
30	29	18
60	26	17
90	21	16
120	14	14
150	7	10
180	—	8

The nerve fibre of a nerve-muscle preparation was immersed in a venom solution of 1 : 10,000, while the muscle was left outside on a piece of filter-paper moistened with Ringer's solution; the irritability of the immersed nerve was compared with that of a control preparation soaked in Ringer's solution. No difference in the irritability of the two preparations could be observed.

Cobra venom therefore paralyses motor nerve endings before it paralyses voluntary muscle, and the motor nerve fibres do not seem to be affected directly.

4. *The Isolated Frog's Heart.*

Brunton and Fayrer state that large quantities of venom arrest the heart in systole, "the poison seeming to act as a stimulus." Aron, Ragotzi, and Vollmer also describe systolic standstill, the latter attributing it to action on the heart-muscle. Elliot first drew attention to the similarity of the action to that of the digitalis group, while Gunn considered it more analogous to that of adrenaline.

Ringer's solution was circulated through an excised frog's heart by cannulæ inserted into the aorta and vena cava, and leading to and from a small reservoir of 5 c.c. content (Clark's method*). The contractions of the auricle and ventricle were recorded by levers.

The minimal concentration of venom required to cause noticeable change in the heart was found to be 1 : 1,000,000; more distinct effect was caused by 1 : 100,000.

The typical changes occurring when venom was added to the circulating fluid were similar to those induced by digitalis, as is stated by Elliot. The amplitude of the beats of both auricle and ventricle steadily diminished, while the level of the curves moved upwards progressively, showing that both chambers contract more completely while they do not dilate so fully as usual in diastole (fig. 2). The rate of the beat in

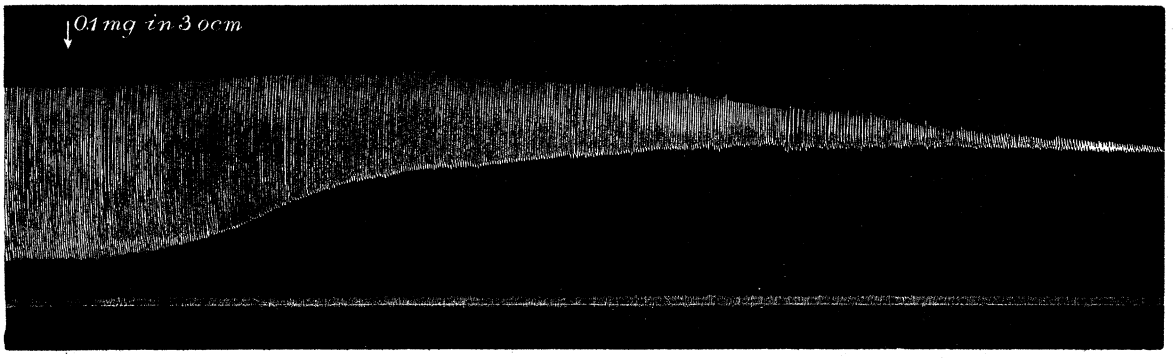

Fig. 2.—Tracing of the frog's ventricle under perfusion of venom 0·1 mgrm. in 3 c.c. of Ringer's solution by Clark's method. Reduced to 1/3.

most cases diminished gradually from the beginning, though in a few cases it increased temporarily. Elliot states that the venom slows the frog's heart *in situ*, even under atropine, while it always causes a quickening of the isolated heart. But

* Clark, 'Journ. of Pharm. and Exp. Therap.,' vol. 4, p. 399 (1913).

this difference seems to be explained by the condition of the isolated heart in his experiments, for the normal rate of all the preparations employed by him was extremely slow (7–10 per minute), while that of mine was always 28–32 per minute. Frequently there was partial A–V heart-block, the ventricle beating only once for every two or more beats of the auricle. In some cases peristaltic movements appeared in the ventricle. After the standstill this chamber did not respond to a mechanical stimulus. ELLIOT states that atropine in a concentration of 1/5000–1/10,000 causes the heart to pass into the systolic position, and that the venom reinforces the atropine action, so that when each substance is taken below the strength capable of throwing the heart into systolic tone, both together exercise stronger action than might have been expected from each separately. But in my experiments atropine (1/10,000–1/500) had no tendency to cause the heart to pass into the systolic position, and did not reinforce the action of the venom at all. The application of a Stannius ligature between sinus and auricle or auricle and ventricle did not alter the action of the venom. The separated ventricle-apex, made to beat by a series of single shocks, showed a tendency to diminish in volume, and became less responsive to the electric stimulus, thus resembling the ventricle in the intact heart under venom.

From the above experiments it is clear that under venom the amount of blood expelled at each beat must diminish. The total amount per minute was investigated by the following experiment :—

Normal Ringer was led into the inferior vena cava at a pressure which was maintained at 0–4 mm. of water by means of a manometer and screw clamps ; the fluid pumped out by the ventricle escaped from the aorta at a pressure of 150–200 mm. of water ; this was kept constant by a screw clamp altered according to the changes observed in a water manometer inserted in the other aorta. The number of drops that flowed out from the cannula was recorded by means of a lever, and when this had become constant the normal Ringer's solution was replaced by one containing venom.

It was found that the perfusion of a solution containing 1 : 10,000,000 of venom was sufficient to cause a perceptible change in the outflow. Sooner or later, according to the concentration of the poison, the amount of the outflow diminished gradually, and the aortic blood pressure became lower, and at length reached zero. The decrease of the outflow was especially marked when the rate of the heart beats increased. The venous pressure increased in inverse ratio to the arterial, and finally reached that of the perfusion tube.

5. *The Mammalian Heart.*

A rabbit was anæsthetised with urethane, and artificial respiration was maintained. Both vagi were cut, and the thorax was opened along the middle line. The movement of the left ventricle was recorded by means of CUSHNY'S* myocardiograph, and the carotid blood pressure by a mercury manometer.

* CUSHNY, 'Heart,' vol. 2, p. 1 (1910).

Shortly after the injection of the venom the rate of the beat slowly diminished; the amplitude of the ventricle contraction gradually increased, but after some time it began to diminish, and at last fell to zero. The level of the curve gradually moved upward until the ventricle stood still in a firm systolic position. In this stage the right ventricle was found in a half-systolic position. The blood pressure changed almost in direct proportion to the amplitude of the ventricle contraction.

The same phenomena were observed in the ventricle when the isolated heart of the rabbit was perfused with venom solution.

These results, except the change of rate, agree with those obtained by Elliot. Elliot states that the venom produces an acceleration of the perfused heart, while it causes a slowing of the heart *in situ*. This acceleration, however, seems to be exceptional, for all the tracings given in his paper, contrary to his statement, show a slowing of the beat, as I have also noted.

6. *The Blood Vessels.*

Perfusion of the venom solution through the frog's leg, prepared according to Trendelenburg's* method, caused a progressive decrease and final stoppage of the outflow, as has been described by Elliot.

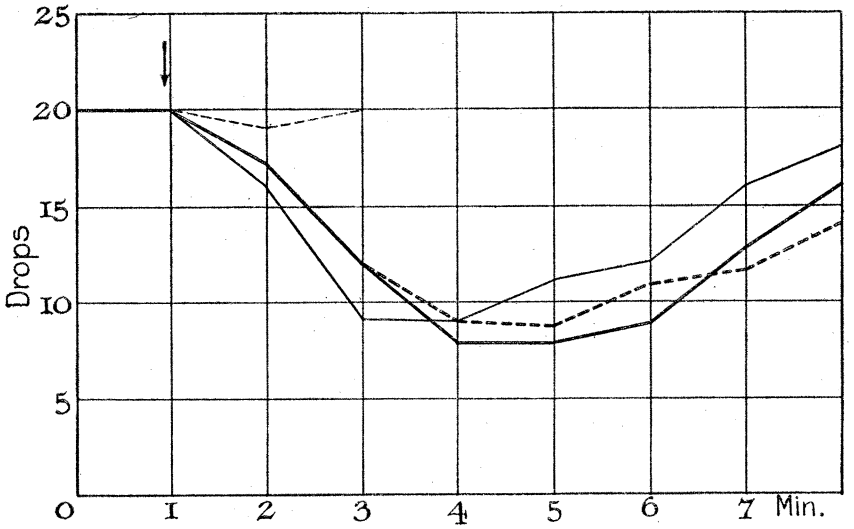

Fig. 3.—Graphs of the amount of fluid passing through the frog's hind legs perfused with Ringer. At the arrow points venom or adrenaline was injected into the tube leading to the artery.

Adrenaline 1 : 1,000,000, 0·2 c.c.
Cobra venom 1 : 10,000, 0·2 c.c.
Adrenaline 1 : 1000,000, during ergotoxine perfusion.
Cobra venom 1 : 10,000, during ergotoxine perfusion.

After the injection of 0.2 cm. of 1 : 1000 venom solution into the inflow tube, the outflow at first diminished and then returned nearly to its former velocity. This action resembles that of adrenaline. But that they are not identical was proved by

* Trendelenburg, 'Arch. f. exp. Path. u. Pharm.,' vol. 63, p. 161 (1910).

the fact that when ergotoxine (1 : 50,000) was present in the perfusion fluid, adrenaline did not act on the vessels, while the action of the venom remained unchanged.

Isolated organs of the rabbit, such as the ear, leg, and kidney, were perfused with Ringer's solution, and the outflow from the vein was measured.

When the venom was injected into the inflow tube the outflow suddenly diminished and then slowly returned nearly to its normal amount.

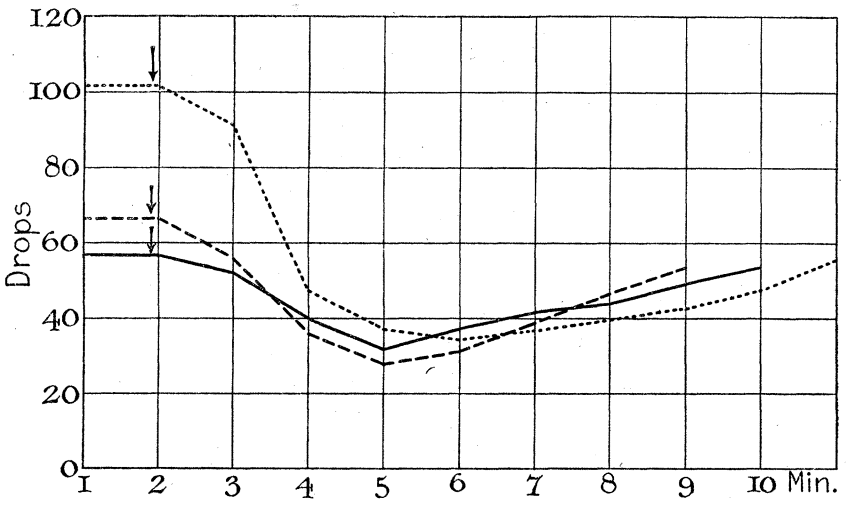

FIG. 4.—Graphs of the amount of fluid passing through the vessels of the rabbit's ear, kidney, and hind-leg perfused with Ringer. At the arrow, venom (1 c.c. of 1 : 10,000) was injected into the arterial tube.

——— = ear; - - - - = kidney; ..... = leg.

Thus all the vessels examined showed very marked contraction when venom was added to the Ringer's solution perfused through them; and the venom acts more peripherally than adrenaline, in all probability on the muscle of the vessel, so that its action is more like that of the digitalis bodies, as ELLIOT suggested.

7. *The Circulation.*

All the writers on the subject agree that when an animal, such as the cat or rabbit, is given a certain quantity of the venom intravenously, the blood pressure falls rapidly, and after a short time rises again to its former level or higher, but later begins to fall until it reaches zero.

According to the detailed analysis of ELLIOT, the preliminary fall is due to inhibition of the heart; both the central and intracardiac inhibitory mechanisms are stimulated, in part by the direct action of the venom, in part by the accompanying asphyxia. The subsequent rise is due to two factors: the force of the heart beat is increased by the direct action of the venom upon the muscular tissue, and the vessels are contracted both by the direct action of the venom upon them and by the vasomotor centre being stimulated by the steadily increasing venosity of the blood. He holds that there are two opposing factors—the inhibitory action of the venom through the vagal

centres, and the direct muscular action on the heart; when the dose is comparatively small the augmentor cardiac and vascular influence prevails and causes the secondary rise, while when the dose is a large one the inhibitory action overpowers the muscular and causes failure of the heart, so that the pressure falls without any subsequent rise.

My own experiments agree with those of the previous workers as to the general changes in the blood pressure, while they differ from them in regard to the explanation of these changes. The experiments were performed on the cat (and the rabbit) anæsthetised with urethane, choral hydrate or veronal. The carotid blood pressure was recorded by a mercury manometer and the venom was introduced into the jugular vein.

When a small quantity of venom P, such as 0·1–0·2 mgrm. per kilogramme, was injected, the blood pressure rose slightly for some time and then gradually fell to below its previous level. The rate of the heart beat diminished steadily from the beginning. No change was observed in the respiration.

When a large dose, such as 1–2 mgrm. per kilogramme, which is sufficient to arrest the heart in the course of one to two hours, was employed, the blood pressure at first fell suddenly and then rose gradually in a greater or less degree, but sooner or later it fell again slowly. In the first stage, in which the preliminary fall took place, the rate of the heart beats diminished markedly; in the second stage, in which the blood pressure rose again, the rate of the heart increased but was still slower than the normal rate; in the third stage, in which the final fall occurred, the rate gradually diminished, though it sometimes quickened at intervals. The respiration, after a temporary acceleration, became gradually slower but did not stop in the early phase of the third stage.

From these experiments it seems clear that the changes in the heart account largely for the changes in the blood pressure, as the latter falls and rises with the rate of the heart beat. In order to investigate whether changes in the calibre of the vessels are also involved in those of the blood pressure, I have recorded the blood pressure and the volume of the kidney or intestine by means of oncometers in the usual way. In most cases the volume of the organs changed almost parallel with the changes of the blood pressure, while sometimes the volume of the kidney increased in spite of the rise of the blood pressure. It is therefore possible that the contraction of the vessels plays a part in the rise of pressure, but this part seems so slight that it is masked by the changes produced by the failure of the heart.

Elliot appears to lay more emphasis on the effects of asphyxia and on the inhibitory factor than is justified by my experiments, and, on the other hand, does not recognise the capital importance of the direct action on the heart. Asphyxia is not the cause of the preliminary fall in blood pressure, the subsequent rise, or the earlier stages of the ultimate fall, for all these occur before the respiratory failure is well marked, or even when the animal is kept under artificial respiration (see Experiment 4, Fig. 5). When the respiratory embarrassment is well marked late in the

third stage, the secondary effects of the asphyxia on the heart are obvious. The beat becomes very slow and irregular, the irregularity often taking the form of partial auriculo-ventricular block. Not infrequently a run of slow contractions alternates with a series of more rapid ones. When artificial respiration is instituted at this stage, the rate of the heart quickens at once and the irregularities disappear.

Elliot observed that, when the preliminary fall took place, section of the vagi was followed by a gradual rise of pressure, and therefore concluded that the venom stimulates the inhibitory centre and so causes the preliminary fall. But this experiment is not sufficient to decide whether the rise of the pressure is due to the section of the vagi or to the natural course of the poisoning, for such a gradual rise of pressure can be observed even when the vagi remain intact. In some of my experiments a sufficient quantity of atropine was given to paralyse the vagus, but the changes in the blood pressure produced by the venom afterwards were exactly similar to those in animals not treated with atropine (see Expts. 3 and 4 and fig. 5). There can therefore be no doubt that the preliminary fall is independent of the inhibitory mechanism, whether central or peripheral, and similarly the late fall in pressure is not inhibitory in origin, as Elliot believes.

Experiment 3.—A cat weighing 3·7 kgrm. was anæsthetised with veronal and kept under artificial respiration. One hour previously 2 mgrm. of atropine was injected subcutaneously.

Time.				Blood pressure.	Pulse per 30 sec.	——
min.	sec.		min. sec.	mm. Hg.		
0	0	to	0 30	148	98	
0	30		1 0	146	96	2 mgrm. per kgrm. venom intravenously.
1	0		1 30	146–124	94	
1	30		2 0	124–40–47	67	
2	0		2 30	47–42	79	
2	30		3 0	42–39	86	
3	0		3 30	39–42	84	
3	30		4 0	42–80	85	
4	0		4 30	82–58	49	
4	30		5 0	58–56	26	
5	0		6 0	56–68	35	
6	0		7 0	68–76	44	
7	0		8 0	76–82	50	
8	0		9 0	82–80	49	
9	0		10 0	80–86	52	
10	0		18 0	86–88	83	
18	0		28 0	88–74	94	
28	0		43 0	74–64	95	
43	0		45 0	64–50	93	
45	0		55 0	50–0		

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Experiment 4.—A cat weighing 2·5 kgrm. was anæsthetised with veronal and kept under artificial respiration.

Time.				Blood pressure.	Pulse per 30 sec.	——
min.	sec.		min. sec.	mm. Hg.		
0	0	to	0 30	101	101	
0	30		1 0	101–94	100	2 mgrm. per kgrm. intravenously.
1	0		1 30	97–41	83	
1	30		2 0	41–18	37	
2	0		2 30	18–36	41	
2	30		3 0	36–47	45	
3	0		3 30	47–49	44	
3	30		4 0	48	46	
4	0		5 0	46	46	
5	0		6 0	46	46	
6	0		7 0	46–59	65	
7	0		8 0	59–64	84	
8	0		9 0	64–51	81	
9	0		10 0	51–20	92	
10	0		11 0	20–13		
11	0		12 0	13–0		

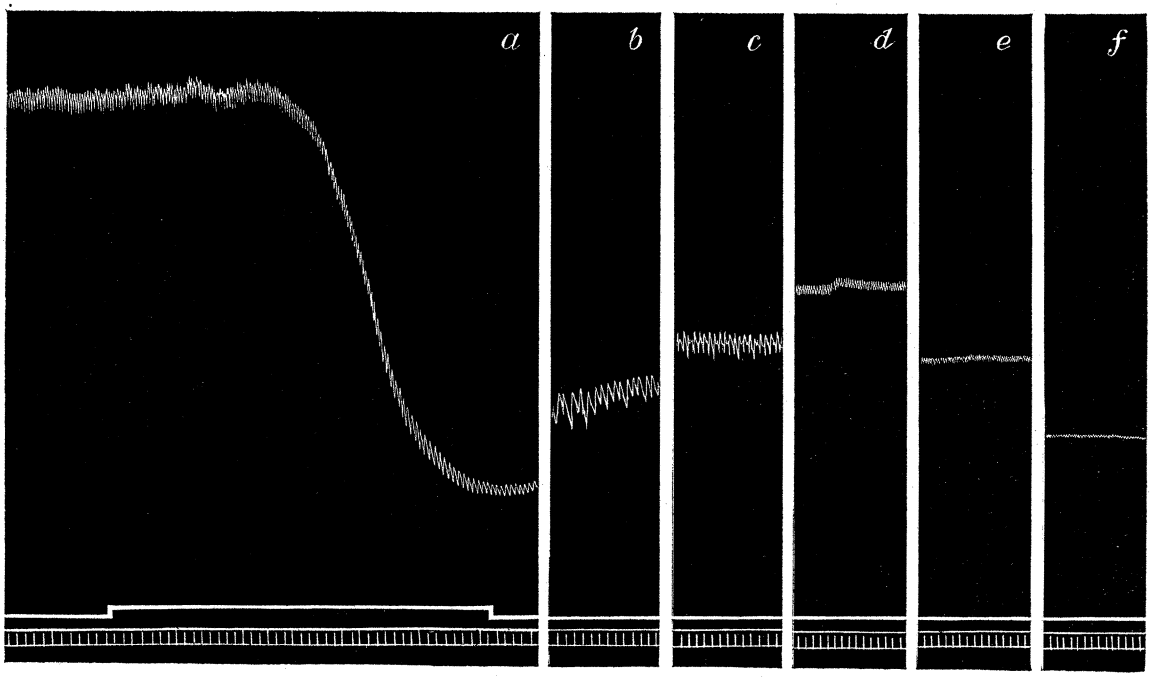

FIG. 5.—Blood pressure of an atropinised cat under artificial respiration before and after the injection of 2 mgrm. venom P per kgrm. at the point indicated on the signal line. Time in sec. Interval between *a* and *b* is 5 min.; between *b* and *c*, 3 min.; between *c* and *d*, 9 min.; between *d* and *e*, 20 min.; between *e* and *f*, 6 min.

My observations lead me to ascribe the changes in the heart and blood pressure almost entirely to direct action on the heart and vessels, the inhibitory mechanism not being markedly influenced, and the asphyxia only playing an important part in the

later stages. Direct observation of the heart *in situ* or perfused with Ringer shows that the rate of the heart is slowed and its strength and tone are increased under small quantities of venom. In a more advanced stage, however, the heart dilates less and less fully, so that the output gradually diminishes.

In the intact animal small quantities of venom at first cause a slight rise of the blood pressure through the increased strength of the heart, and perhaps through contraction of the vessels, but after some time the heart movement diminishes, and consequently the pressure falls. When a large dose is introduced into the vein and reaches the heart in a highly concentrated form, it causes a sudden failure of the heart and consequently a fall of the blood pressure. If the quantity is not large enough to arrest the heart at once, the venom is distributed throughout the entire volume of the circulating blood, so that the heart gradually recovers and the pressure rises again to the normal or above it. But the gradual accumulation of the venom in the heart leads to a progressive failure of the strength of the heart, and this failure is accelerated by the asphyxial conditions of the blood. The asphyxia may depress the heart through stimulation of the vagus, but in my experiments the heart seemed to be slowed mainly by the direct action on the heart of the CO_2 accumulation and O_2 lack.

8. *The Stomach.*

The excised stomach and intestine of a frog continued to move with a fairly regular rhythm in Ringer's solution, and the addition of P venom (1 : 10,000) increased the rhythmic movements distinctly. Rings of frog stomach suspended in oxygenated Ringer's solution and attached to a lever contracted more strongly, and in some cases more rapidly, and showed an increased tone when venom was added in a concentration of 1 : 10,000; one part in 100,000 had only a slight effect (fig. 6).

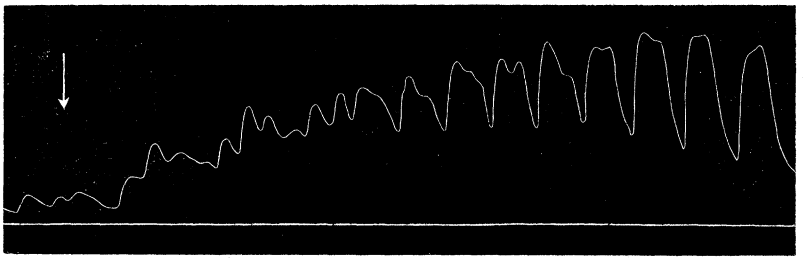

FIG. 6.—Action of venom (1 : 10,000) on strip of frog's stomach suspended in Ringer's solution.

This action was obtained both in the pyloric and in the cardiac half of the stomach, and could not be removed by the administration of atropine. After a considerable time the augmentation began to pass off, until at last the stomach ceased its movement and relaxed beyond its normal length.

Longitudinal and circular strips of a freshly isolated cat's stomach were examined in the same way, suspended in oxygenated Ringer's solution; the longitudinal strips continued to contract rhythmically, while the circular showed no movement. Both

preparations responded to the addition of venom with characteristic changes, especially marked when the concentration was over 1 : 100,000.

The longitudinal section immediately increased in tone, and the amplitude of its movement was augmented; but after this had reached its maximum, and lasted for a while, the strip gradually relaxed beyond its normal length, and its movement entirely ceased. A similar change in tone was seen in the circular strips (fig. 7), and soon after a sudden strong contraction followed; this was followed by a few further waves, and then all movement ceased.

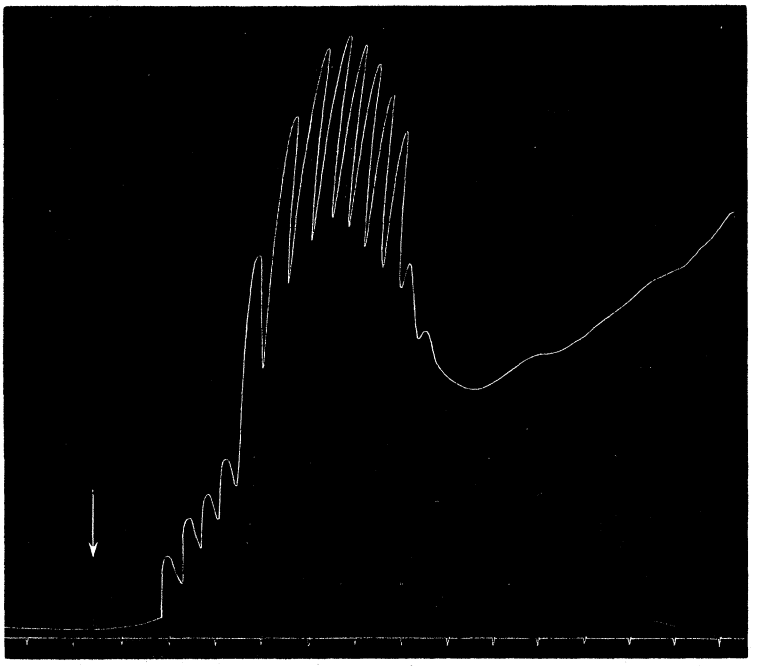

FIG. 7.—Action of venom (1 : 100,000) on strip of cat's stomach (circular fibres) suspended in Ringer's solution.

In both cases administration of atropine failed to counteract the effect of the venom, and after the strips had relaxed they did not respond to a small quantity of barium chloride, which is sufficient to produce a distinct effect on the muscle in its normal condition.

The same phenomena were elicited by the venom when the isolated stomach of the rabbit was employed.

The only previous investigation of the action of venom on the stomach was made by GUNN,* who found the tonus of rings of frog's stomach increased, as I have described, but who states that the stomach of the cat examined *in situ* is inhibited by 1 mgrm. of venom injected intravenously. This is directly opposed to my results with surviving strips of cat's stomach, and I have therefore performed five experiments in the same way as GUNN. The method consisted in tying the pylorus in etherised cats under artificial respiration, passing a catheter into the stomach through the

* GUNN, 'Quart. Journ. of Exp. Physiol.,' vol. 5, p. 67 (1912).

œsophagus, and attaching it to a reservoir of Ringer's solution, the movements of which were recorded by a tambour. The P venom was injected intravenously in doses of 0·5, 1·0, 1·5, and 2·0 mgrm. per kilogramme, but caused no movement of the fluid except in two cases, in which 2 mgrm. per kilogramme caused a small elevation of the lever, indicating a decrease in the volume (fig. 8). No such effect was obtained in

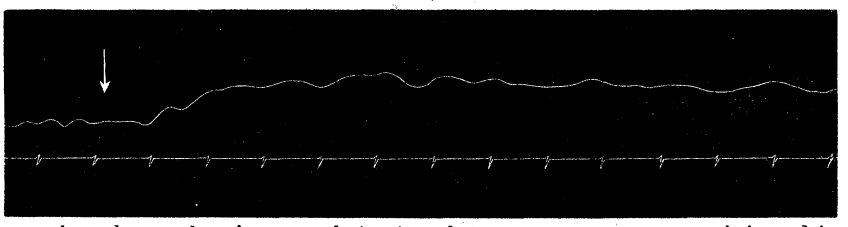

FIG. 8.—Change in volume of cat's stomach *in situ* when 2 mgrm. venom was injected intravenously.

any of my experiments as is figured by GUNN, and it seems possible that the amount of venom used by him was large enough to induce the second phase of relaxation and paralysis immediately, without the primary phase of augmentation which I have observed.

9. *The Intestine.*

A rabbit was anæsthetised with urethane, the movements of the intestine recorded by TRENDELENBURG's* method, and the respiration and blood pressure in the usual way. The injection of 1·5 mgrm. per kilogramme of P venom intravenously caused a very marked increase in the tone of the intestine, and a large augmentation in the size of the pendulum movements. This occurred before the blood pressure or respiration was affected, and is therefore independent of the changes in them.

This intestinal action was further investigated in pieces of rabbit's gut suspended in oxygenated Ringer's or Tyrode's fluid or in a mixture of these and blood. It was found that the best results were obtained in fresh intestines, though pieces that had been kept for some hours in cold Ringer's solution showed the same reaction in somewhat less degree. The addition of one part in a million of F venom to the saline bath increased the amplitude of the pendulum movements, and when the strength was increased to 1 : 100,000-20,000 the increase in the excursion was more marked, and was accompanied by higher tone, which lasted for some time, and then gave place to relaxation.

The pendulum movements became smaller and the intestine reached beyond its normal length, and afterwards did not respond to the addition of 1 : 10,000 barium chloride, which has a distinct augmentor effect on the normal intestine. Atropine had no influence on the augmentor nor on the depressor phase of the action. I found that prolonged and repeated washing of the preparation had very little effect in removing the venom action when it had once been elicited in full strength.

The same effects were produced in the intestine of the frog, guinea-pig, and cat, and also in the colon of the rabbit suspended in the same way. The action on the

* TRENDELENBURG, 'Zeitsch. f. Biol.,' vol. 61, p. 67 (1913).

intestine, like that on the stomach, thus consists of a primary phase of increased tone and increased movement, followed by one of relaxation and paralysis when large

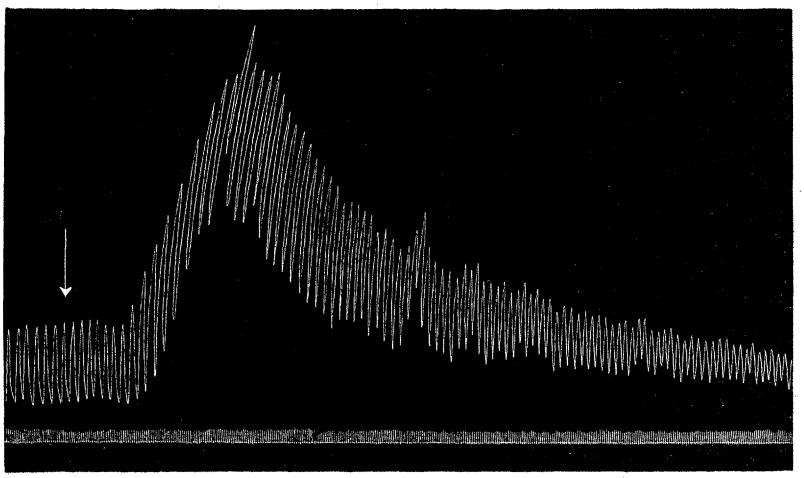

FIG. 9.—Action of venom (1 : 100,000) on surviving intestine of rabbit in Ringer's solution.

amounts of the poison are employed. In experiments on the gut of the rabbit and cat, carried out in the same way as mine, GUNN observed only a depressor action and arrest of the movements. But he seems to have generally used very much higher concentrations than I found necessary, such as 1 : 10,000–25,000. In one tracing

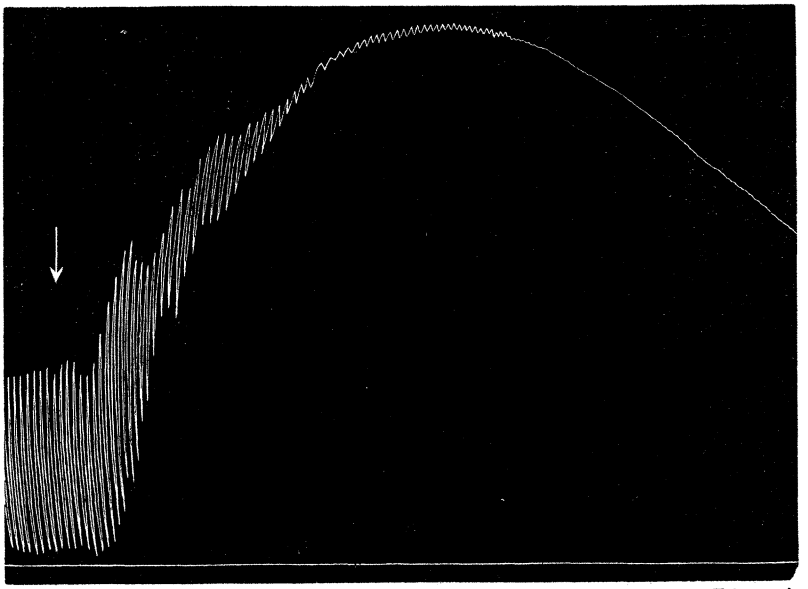

FIG. 10.—Action of venom (1 : 20,000) on surviving intestine of rabbit in Ringer's solution.

given by him of the effect of 1 in 10,000, the effect may have been inhibition, as he supposes, or depression such as I have observed; but in another tracing, in which he used 1 in 60,000, the change is clearly a slow depressant one, and quite different from the inhibition which is observed under adrenaline. I cannot agree that cobra venom exercises any inhibitory action on the intestine comparable to that of adrenaline. Its characteristic action is a primary stimulation, best seen in low concentrations, and a

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

secondary paralysis when higher concentrations are used; when strong solutions are applied to the isolated intestine this depressor action may occur alone.

10. *The Uterus.*

Experiments were performed on the isolated uterus suspended in oxygenated Ringer's solution in the usual way.

As was found by Gunn, cobra venom produces contraction of the isolated uterus of the rabbit and cat, both pregnant and non-pregnant, the effect on the pregnant uterus being very strong, while that on the non-pregnant is slight, and only elicited by a stronger solution; for example, 1 in 10,000 to 1 in 20,000 caused distinct but not very powerful movement in the non-pregnant, while 1 in 100,000 was sufficient to cause strong movement in the pregnant uterus. In each case the contraction was followed after some time by relaxation even when the venom continued present in the fluid. In the non-pregnant uterus of the cat this relaxation became even more marked when a trace of adrenaline was added to the bath, while in the pregnant uterus of the cat, and in the rabbit's uterus, whether pregnant or not, adrenaline caused a renewed contraction.

As my results on the uterus agree with those of Gunn, who has illustrated them with tracings, it is unnecessary to add curves of the uterine movements here.

11. *The Urinary Bladder.*

According to Gunn, the venom relaxes the tone of the cat's urinary bladder with some increase of the contraction subsequently. After large doses (1 mgrm. per kilogramme) the relaxation may be followed by an augmentation of tone above the normal. He performed his experiments on the cat anæsthetised with ether under artificial respiration. A catheter was inserted into the bladder through the urethra and connected to a reservoir filled with Ringer's solution, the movement of which was registered by a tambour. I have repeated his experiments, following his methods as closely as possible. In my experiments on five cats the P venom invariably produced a contraction of the bladder, followed by relaxation, but without any preliminary relaxation, and this irrespective of the doses injected (0·5–2 mgrm. per kilogramme) (fig. 11).

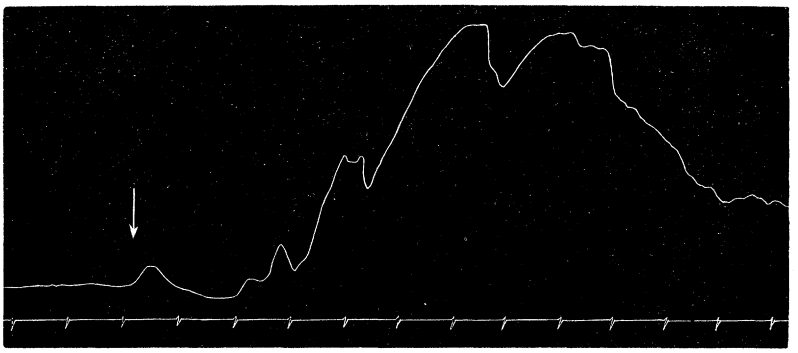

Fig. 11.—Tracing of cat's bladder *in situ* on the injection of 2 mgrm. venom P intravenously.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

In other experiments, strips of the bladder of the cat and rabbit were suspended in Ringer's solution. It was found that the venom at first increases the tone and movement of the bladder, and finally paralyses it (fig. 12).

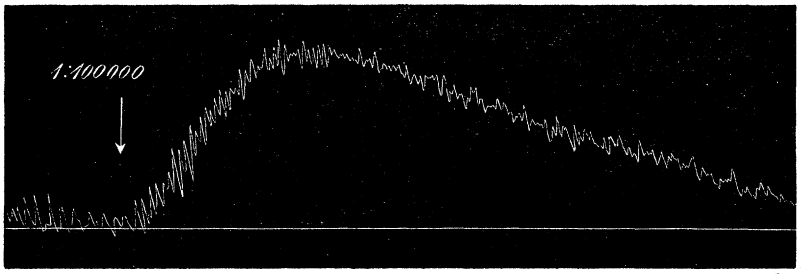

FIG. 12.—Tracing of a strip of rabbit's bladder suspended in Ringer. Venom (1 : 100,000) was added to Ringer at the point indicated by an arrow.

12. *The Iris.*

When the enucleated frog's eye was immersed in Ringer's solution containing venom in a concentration of 1 in 10,000, dilatation of the pupil was observed after two or three hours, as was found by GUNN. This dilatation, however, was very slight and remained stationary even after the application of a more concentrated solution, such as 1 : 1000 or 1 : 100. The subsequent addition of adrenaline or pilocarpine had no effect upon the pupil. The dilatation of the pupil by venom was especially marked when it had been previously constricted by pilocarpine.

This change in the diameter of the pupil was so slight that I have some hesitation in drawing any inference as to the point of action of the venom, especially as there is no possibility of estimating at what time or in what concentration it reached the iris. It is possible that, as GUNN holds, the venom stimulates the dilator mechanism in the same way as adrenaline, but it appears equally plausible that it enfeebles the circular muscle by direct action on it; but in the absence of accurate quantitative estimations this is pure speculation.

13. *The Bronchial and Tracheal Muscle.*

A piece of about 5 mm. in length was cut off from a freshly isolated bronchus or trachea of the rabbit, and was divided at the middle of the cartilage, so that it formed a rectangular strip, which was suspended in oxygenated Ringer's solution and connected to a lever. Such preparations responded readily to the addition of pilocarpine, adrenaline, or atropine. But the addition of venom (1 : 20,000) caused neither contraction nor relaxation, so that this form of muscle fails to conform to the behaviour of all the others examined.

14. *The Salivary Gland.*

When a cat or rabbit is poisoned by subcutaneous injection of venom, one of the characteristic symptoms is the occurrence of loud rales arising from the accumulation

of fluid in the larynx, trachea and bronchi. Very often some saliva escapes from the mouth, and the lips are almost always covered with a mucous fluid in the later stages. GUNN found that in cats anæsthetised with chloroform and ether, the flow of saliva along a tube inserted into Wharton's duct was distinctly accelerated by the intravenous injection of cobra venom; he ascribes this to the venom stimulating the terminations of the postganglionic sympathetic fibres in the gland, but he brings no evidence that this was the mechanism involved.

I have done four experiments on cats, following the method used by GUNN as closely as possible, but in these I did not observe any perceptible increase in the salivary secretion from the intravenous injection of 0·5–2 mgrm. of venom P per kilogramme, the rate of flow remaining fairly constant throughout the experiment. In these experiments an increased salivary secretion due to the ether narcosis might have masked the action of the venom, and I therefore repeated the experiment on cats anæsthetised by the subcutaneous injection of veronal, but again the result was negative; in each case the animal responded with profuse secretion on the injection of pilocarpine, after the venom injection had failed.

In two experiments the cat was decerebrated under ether, about half an hour before venom was injected; cannulæ were inserted in Wharton's duct on each side. 3–3·3 mgrm. per kilogramme was injected hypodermically without affecting the salivary secretion; the animals died about two hours after the injection.

Thus the venom has no action whatever in increasing the salivary secretion, at least under the conditions under which my experiments were carried out, and it seems probable that the apparent salivary flow observed in animals under cobra venom is not due to the increase of the salivary secretion, but to the lessened control over the muscles of the pharynx and larynx, which hinders the saliva from being swallowed, and thus causes its accumulation in the mouth; or there may be an actual increased secretion from some central action, which is absent in anæsthetised or decerebrated animals.

15. *The Sympathetic Ganglia.*

Many poisons which paralyse the terminations of the motor nerves also act upon the sympathetic ganglia, and I have therefore tested the influence of the cervical sympathetic on the iris and other eye-muscles in cats and rabbits poisoned with cobra venom. The result was negative in all cases, the iris and nictitating membrane continuing to move on sympathetic stimulation after quantities of venom which had paralysed the respiration; as far as could be seen the movement was as prompt and as extensive as before the poison, but no special measurements were made.

Discussion.

In my experiments on the individual organs and tissues, venom in small doses caused in its first phase activity of the muscle, which was manifested in contraction

and twitching in striated muscle, and in augmented tone and increased contractions in the heart, vessels, stomach, intestine, bladder, and uterus. The bronchial muscle and the iris are possibly exceptions to this general rule This activity was quite independent of the nature of the innervation, so that the inference may be drawn that it arises from a direct stimulation of muscle.

In the second phase depression and relaxation of the muscle followed. This may occur immediately from very high concentrations. GUNN ascribed the action to stimulation of sympathetic ends similar to that of adrenaline, and supported this by the arrest of movement and decrease of tonus of the intestine, bladder, and mammalian stomach. In these organs, however, I observed contraction from small doses, and I cannot accept his view. It is possible that, at any rate in some of his experiments, the venom concentration was high enough to cause the second phase of relaxation, and that this may account for the discrepancies in our results. He informs me that the venom which he employed was obtained by macerating the excised and dried poison-glands of the cobra, while the two samples examined by me consisted of the dried poison secretion. It seems not impossible, as he suggests, that in addition to the specific poison of the gland, his preparation may have contained some products of putrefaction, such as DALE and BARGER have shown to possess the sympathetic-mimetic action of adrenaline.

In addition to the direct action on muscular tissue, cobra venom exerts a powerful depressant action on the motor-nerve terminations of a character similar to that of curara. It does not increase the salivary secretion, and has no action on the peripheral sympathetic ganglia.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES