

The Action of the Venom of *Echis carinatus*

Thomas R. Fraser and James A. Gunn

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PHILOSOPHICAL TRANSACTIONS.

I. *The Action of the Venom of Echis carinatus.*

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

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Echis carinatus is classified in the sub-family Viperineæ of the family Vipirideæ. It has been described, also, under the names of *E. carinata*, *E. arenicola*, *E. superciliosa*, *Vipera carinata*, *V. echis* and several other names. It is popularly known as Afæ, Kuppur, and Phoorsa, in Hindustan, and Efa in Egypt. It occurs abundantly in many parts of India, and its distribution extends over South Asia and Northern Equatorial Africa. It has an average length of rather less than 2 feet, and occasionally exceeds the length of 2 feet by 1 or 2 inches; and it is reputed to be one of the most active and aggressive of the venomous serpents.

The venom used by us was obtained in 1904 from Lieutenant-Colonel BANNERMAN, I.M.S., the Director of the Research Institute at Bombay. It was contained in a hermetically sealed glass tube, and was in the form of dry, glistening, and translucent scales of an amber colour, which consisted of the liquid venom that had been rapidly dried, immediately after it had been taken from living serpents, by the process of "milking." In this thoroughly dry state no deterioration in activity or change in action is likely to occur, for at any rate many years, as one of us has shown in the case of cobra and other venoms (FRASER).

In this communication a description is given of our examination of the lethality and the general action of the venom in several species of animals, and also of its effects on the blood and on each of the more important organs and systems of the body. In regard to certain points of importance also, *Echis* venom is contrasted with *Sepedon* venom, the action of which has recently been described by us,* as these venoms illustrate very definitely the characteristic actions of Viperine and Colubrine venoms respectively.

The work previously done with *Echis* venom appears to have been limited to observations on the general symptoms it produces and on its effects on the blood. The former observations were made by FAYRER,† whose experiments dealt mainly with the effects of rapidly lethal doses on fowls and pigeons. Observations on the blood changes produced by *Echis* venom were made by MARTIN, LAMB, and MELLANBY, and to their investigations reference will be made subsequently in the section dealing with the action of *Echis* venom on the circulation.

A.—LETHALITY OF *ECHIS* VENOM.

The lethality of the venom was determined for frogs, rabbits, guinea-pigs, rats and pigeons, with the following results:—

(a) *In Cold-blooded Animals.*

TABLE I.—Minimum Lethal Dose by Subcutaneous Injection for Frogs.

No. of experiment.	Weight of frog in grammes.	Dose per kilogramme in grammes.	Actual dose in grammes.	Result.
1	10	0·007	0·00007	Recovery.
2	12	0·008	0·000096	"
3	10	0·009	0·00009	Death in 8 days.
4	14	0·01	0·00014	" 11 "
5	13	0·02	0·00026	" 6 "
6	18	0·1	0·0018	" 1½ "

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

* FRASER and GUNN, 'Phil. Trans.,' B, vol. 200, p. 241.

† FAYRER, 'The Thanatophidia of India,' 1874, pp. 136—142.

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(b) *In Warm-blooded Animals.*

TABLE II.—Minimum Lethal Dose by Subcutaneous Injection for Rabbits.

No. of expt.	Weight of rabbit in grms.	Dose per kilo. in grms.	Actual dose in grms.	Result.
7	2400	0·00075	0·0018	Recovery.
8	1850	0·0009	0·0017	Death in 2 days.
9	2500	0·002	0·005	„ 30½ hrs.
10	2100	0·005	0·0105	„ 2 days.

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

TABLE III.—Minimum Lethal Dose by Subcutaneous Injection for Guinea-pigs.

No. of expt.	Weight of guinea-pig in grms.	Dose per kilo. in grms.	Actual dose in grms.	Result.
11	450	0·0009	0·0004	Recovery.
12	1100	0·001	0·0011	Death in 3 days.
13	680	0·002	0·0014	„ 2 „

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

TABLE IV.—Minimum Lethal Dose by Subcutaneous Injection for Rats.

No. of expt.	Weight of rat in grms.	Dose per kilo. in grms.	Actual dose in grms.	Result.
14	140	0·0006	0·000084	Recovery.
15	200	0·00075	0·00015	Death in 3 days.
16	190	0·003	0·00057	„ 3 „
17	130	0·006	0·0008	„ 18 hrs.

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

TABLE V.—Minimum Lethal Dose by Subcutaneous Injection for Cats.

No. of expt.	Weight of cat in grms.	Dose per kilo. in grms.	Actual dose in grms.	Result.
18	3200	0·006	0·019	Recovery.
19	2600	0·008	0·02	Death in 3 days.
20	1700	0·015	0·025	„ 30–40 hrs.

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

TABLE VI.—Minimum Lethal Dose by Subcutaneous Injection for Pigeons.

No. of expt.	Weight of pigeon in grms.	Dose per kilo. in grms.	Actual dose in grms.	Result.
21	305	0·002	0·0006	Recovery.
22	310	0·003	0·0009	„
23	320	0·004	0·0013	Death in 20 hrs.

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

TABLE VII.—Minimum Lethal Dose by Intramuscular Injection for Pigeons.

No. of expt.	Weight of pigeon in grms.	Dose per kilo. in grms.	Actual dose in grms.	Result.
24	310	0·00075	0·00023	Recovery.
25	300	0·001	0·0003	Death in 3 days.
26	295	0·003	0·0009	„ 2½ hrs.

The injections were made into the right pectoral muscle.

Minimum Lethal Dose: Subcutaneous.—From these tables it is seen that the minimum lethal dose by subcutaneous injection per kilogramme was found to be: for the frog, 0·009 gm.; for the rabbit, 0·0009 gm.; for the guinea-pig, 0·001 gm.; for the rat, 0·00075 gm.; for the cat, 0·008 gm.; for the pigeon, 0·004 gm.

Intramuscular.—Table VII shows the minimum lethal dose by intramuscular injection to be 0·001 gm. per kilogramme for the pigeon.

Intravenous.—An attempt was made to determine the minimum lethal dose by intravenous injection for rabbits, the injections being made into the marginal vein of the right ear. It was found, however, that the minimum lethal dose depended so largely upon the rate of injection of the venom and the concentration of the solution that no exact point could be fixed. It may be given, however, as an indication that 0·0001 gm. per kilogramme always proved rapidly fatal if injected at the same rate and in the same dilution as the subcutaneous injections.

The comparison of Echis and Sepedon venoms in reference to their lethality for different animals is of some interest. In the following table such a comparison is made, the minimum lethal dose for the rabbit being taken as unit in the case of each venom:—

TABLE VIII.—Comparison of Lethality for different Animals.

	Frog.	Rabbit.	Rat.	Cat.	Pigeon.
Comparative lethality of Echis venom.....	10	1	0·8	9	4·4
„ „ Sepedon „	1·2	1	1·6	15	3·3

It is noteworthy that, as compared with the rabbit, the frog shows a high resistance to *Echis* venom, but not to *Sepedon* venom; that the rat shows less resistance to *Echis*, but more to *Sepedon* venom; that the cat is less susceptible to either venom, but especially to *Sepedon* venom; and that the pigeon shows a diminished susceptibility which is proportionally about the same in the case of both venoms.

B.—SYMPTOMS PRODUCED BY ECHIS VENOM.

The following experiments will serve to illustrate the general symptoms of poisoning by the venom :—

(a) *In Cold-blooded Animals.*

Frogs. Experiment 6. May 31, 1910.—*Rana temporaria*, male, weight 18 gm.

At 9.15 A.M., the respirations were 25, and the cardiac impacts 6, in 10 seconds. At 9.30, 0.0018 gm. dissolved in 0.4 c.c. of saline solution was injected into the dorsal lymph sac. This was equivalent to 0.1 gm. per kilogramme of frog-weight, and, therefore, about 10 times the minimum lethal dose.

At 11.30 A.M., the respirations were 23, and the cardiac impacts 7, in 10 seconds. There was a reddish discoloration of the skin over the dorsal lymph sac.

Up to 5 P.M., there was no change in the frog's condition apart from a slight increase in the rate of the respirations and heart-beats, the respirations being now 28, and the cardiac impacts 8, in 10 seconds; also the reddish area over the dorsal lymph sac was somewhat œdematous, and the skin of the rest of the body showed distinct pallor.

At 9.30 P.M., the respirations were 20 in 10 seconds, otherwise there was no apparent change.

At 9 A.M. on the following morning, the respirations were somewhat irregular and at the rate of 15 in 10 seconds. The cardiac impacts were 7 in 10 seconds, and could be seen with difficulty. There was no impairment of voluntary or reflex movements.

At 2.30 P.M., the respirations were 14 in 10 seconds and somewhat irregular, both in rate and depth. The throat was swollen.

At 6 P.M., the respirations were feeble and irregular, occasional pauses of a few seconds occurring, during which no respirations took place; in one interval during which respirations were continuous they were at the rate of 14 in 10 seconds. The cardiac impacts were 6 in 10 seconds. The conjunctival and nose reflexes were active. The frog jumped vigorously when disturbed, but when laid on its back it made no tilting movements until pinched, whereupon it rapidly resumed the usual posture. There was no rise of the lower eyelids.

Blood-stained fluid was now dropping from the mouth, and the mucous membrane of the mouth and gullet was found to be stained with blood. The web of the foot was examined under the microscope and only the larger blood vessels could be seen, the capillaries, presumably, being empty, and the circulation in the former was very sluggish. The weight of the frog was now 21 gm.

At 7.30 P.M., the respirations were 15 in 10 seconds, and were limited to very feeble undulations of the floor of the mouth. The cardiac impacts could not be seen. There was no external hæmorrhage. At 9.30 P.M., the respirations had ceased, and reflex movements could not be elicited.

The cerebrum was pithed and the left sciatic nerve exposed. Faradic stimulation of it with the secondary coil at 400 mm. induced tetanus of the left gastrocnemius muscle, but no reflex movements were obtained when the nerve was stimulated even with the coil at 50 mm.

The thorax was now opened. The heart was found to be beating very feebly at the rate of four in 10 seconds. The ventricle was very pale, and appeared to be almost empty of blood, for it showed no reddening even at the end of diastole. It ceased to beat at 9.40, and when it was opened no blood exuded, and there were no clots in the heart chambers. A drop of pale fluid was obtained from the interior of the heart, and when examined microscopically it was found to contain very few red corpuscles, they being indeed fewer in number than the white corpuscles. The heart was arrested in the diastolic condition, the muscle being flaccid.

There were small localised hæmorrhages in the pericardium and walls of the auricles. The mucous membranes of the mouth and gullet, and of parts of the small and large intestines, were injected with blood. The stomach and intestines throughout contained much bright red fluid, microscopic examination of which showed the presence of numerous unaltered red blood corpuscles. The bladder was distended with blood-coloured fluid containing numerous red corpuscles. The muscles of the back and flanks were infiltrated with hæmorrhages; the muscles of the limbs were quite pale and their blood vessels almost empty. No hæmorrhages were seen in other organs.

(b) *In Warm-blooded Animals.*

Rabbits. Experiment 9. July 20, 1910.—Grey buck rabbit, weight 2500 gm. At 9.30 A.M., the cardiac impacts were 44 in 10 seconds, and the respirations, when the animal was sitting quietly, were 14 in 10 seconds. The rectal temperature was 38°·5 C.

At 10 A.M., 0·005 gm. of Echi venom dissolved in 2 c.c. Ringer's solution was injected under the skin of the right flank. This dose was equivalent to 0·002 gm. per kilogramme. {For a few minutes after injection the animal was restless, but thereafter remained very quiet.

At 1 P.M., the cardiac impacts were 48, and the respirations 12, in 10 seconds. The rectal temperature was 39° C. There was an œdematous swelling and tenderness on pressure at the site of injection.

At 10 P.M., the cardiac impacts were 50, and the respirations 12, in 10 seconds. The rectal temperature was 40°·1 C. The local swelling was more marked, and there was now a bag of fluid also under the skin of the lower part of the abdomen. The

animal was disinclined to move, but when disturbed showed normal power of progression apart from some stiffness of the right hind leg.

July 21, 1910. At 10 A.M., the cardiac impacts were 32 in 10 seconds, feeble and irregular. The respirations were somewhat laboured, and at the rate of 12 in 10 seconds. The rectal temperature was 35° C. The skin over the lower part of the abdomen to the extent of about a square inch had sloughed, and a hæmorrhagic discharge oozed from it. This discharge had evidently been going on for some time, as there was a considerable amount of blood-stained fluid on the tray. Some of the fresh discharge was collected and found to consist largely of hæmolysed and incoagulable blood. When examined under the microscope no formed red corpuscles could be found in it. The rabbit was crouching with its head and ears down; when made to move, it showed impaired mobility of the hind limbs, but otherwise normal progression and co-ordination.

At 2 P.M., the respirations were 8 in 10 seconds. The cardiac impacts could not be felt. The rectal temperature was 33° C., and the skin felt very cold. The animal was lying with the abdomen and thorax on the tray and the hind limbs stretched out anteriorly, and the eyes were partly closed.

At 3.50 P.M., the rabbit was lying on its side, with its eyes almost closed, and the conjunctival reflex was active. The respirations were 12 in 10 seconds, but the heart-beats could not be felt.

At 4.15, convulsive movements took place involving the head and limbs. They occurred again and in a more violent form at 4.25. In the interval, the animal lay on its side and the respirations were 9 in 10 seconds.

At 4.27, there were a few gasping respirations at irregular intervals. They ceased in about one minute, and the pupils, which had been medium throughout the experiment, dilated widely. The cornea was insensitive. The rectal temperature was 31° C.

At 4.30 the chest was opened, and the heart was found to be beating feebly and irregularly at the rate of 12 in 10 seconds. It continued beating at a diminishing rate for three minutes more.

At 4.33, stimulation of the phrenic nerve with the secondary coil at 490 mm. induced a powerful contraction of the diaphragm; and stimulation of the sciatic at 300 mm. induced a vigorous contraction of the gastrocnemius.

Post-mortem examination showed extensive hæmorrhagic infiltration and a pulpy condition of the muscles of the right flank and abdomen, but no hæmorrhage in other muscles. There was a considerable amount of colourless serous fluid in the pericardium. No clots were found in the heart or vessels. Blood taken from the right ventricle coagulated feebly in 24 hours and showed no hæmolysis. There was a large amount of blood in the lumen of the large intestine, showing unaltered red corpuscles. No hæmorrhages were found elsewhere, and no blood in the urine. The lungs were very pale.

(c) *Summary of Symptoms.*

From the experiment on the frog, which has been described, and which is typical of *Echis* poisoning in frogs, it is seen that the cardinal symptoms, in order of time and of prominence, are hæmorrhages occurring locally and distally, feebleness of the circulation and anæmia, arrest of respiration and loss of reflex excitability, and arrest of the heart in diastole. Rise of the lower eyelid, which is a conspicuous symptom of *Sepedon* poisoning, does not occur. Even in frogs which die after several days there is no marked increase in weight due to œdema, such as we have shown to occur with *Sepedon* venom.

In the rabbit the same cardinal symptoms occur, and in the same order. In addition there is observed a diminished coagulability of the blood, an initial rise, followed by a fall, of temperature, and asphyxial convulsions occurring for a short time before death.

The symptoms observed in the other warm-blooded animals which were investigated were so similar to those described in the rabbit that a detailed description of the symptoms in the former is unnecessary. Such slight differences as do occur will be mentioned in succeeding sections.

It is of interest to compare with the results obtained by experimental poisoning in animals the symptoms described by FAYRER* as occurring in a non-fatal case of *Echis* bite. This observer states that death from *Echis* bite is extremely rare, and he does not record any case of it. A native woman, who had been bitten on the left index finger by a "kuppur" (*Echis carinatus*), showed the following symptoms ten hours afterwards:—"Much exhausted; extremities cold; blood exuded from the eyes, gums, tongue, nose, and vagina, and from under the nails of both great toes and thumbs; she suffered from much thirst and gastric sinking; had vomited before admission. Breathing diaphragmatic, and with a sensation as if the interior of the chest were fixed to the spine in an immovable manner. Voice choleraic, almost to huskiness. Heart-beats weak and impulse imperceptible. Pulse hæmorrhagic, and, as if the arteries were not full, was obliterated by the slightest pressure. The left arm was much swollen, bluish, œdematous, and wholly devoid of sensibility"

FAYRER† describes another case of a Hindoo woman who was bitten above the heel by a snake which was probably *Echis carinatus*. She showed the following symptoms:—"The whole of the bitten limb was much swollen. There was salivation; blood flowed freely from the nose and mouth, and was also discharged from the rectum. So great was the hæmorrhagic tendency, that an old cut on the sound leg bled for two days." MARTIN and LAMB‡ have recorded a fatal case, in which a man died 25 hours after being bitten on the temple by an *Echis carinatus*.

* FAYRER, 'The Thanatophidia of India,' 1872, p. 59.

† FAYRER, *ibid.*, p. 56.

‡ MARTIN and LAMB, 'ALLBUTT'S System of Medicine,' 1907, vol. 2, pt. II, p. 808.

The symptoms observed were very similar to those described by FAYRER. The symptoms in cases of Echis bite in man, therefore, resemble very closely those which we have observed in the lower animals. It is noteworthy that distal hæmorrhages are described as occurring from the tongue and gums, nose, eyes, and rectum. We have found hæmorrhage from the tongue and gums especially in frogs, from the nose and eyes almost invariably in rats, and in the rectum in nearly every case of Echis poisoning in mammals. It is also of interest to note that stress is laid on circulatory rather than respiratory embarrassment, and that the heart-beats are recorded as weak and the pulse hæmorrhagic and imperceptible.

C. EFFECTS ON THE CEREBRO-SPINAL NERVOUS SYSTEM.

(a) *Brain and Spinal Cord.*

The symptoms produced in frogs by poisoning with Echis venom are so widely different from those produced by Colubrine venoms that it is probable that if the earlier investigators of the action of snake venoms had conducted a larger number of experiments on frogs, the fallacy could not have arisen of considering the actions of Colubrine and Viperine venoms substantially alike. From the experiment described by us (Experiment 6), it is seen that even with a dose so large as ten times the minimum lethal, there is little or no impairment of voluntary or reflex movements or of the respirations until shortly before death, indeed until a time when the circulation in the blood-vessels is so inadequate on account of hæmorrhage that the inexcitability of the nerve centres can be satisfactorily explained by insufficiency of blood supply. The absence of a direct action on the central nervous system was borne out also by experiments in which smaller doses were administered. For example, in Experiment 5, the frog received about twice the minimum lethal dose. The animal lived for six days, and on the fifth day there was no impairment of respiration and a scarcely perceptible diminution of reflex excitability. In this case, also, *post-mortem* examination showed that the heart and blood-vessels were almost empty of blood owing to extensive local and remote hæmorrhages.

As in frogs, so in mammals and pigeons no symptoms are to be observed which necessarily point to a direct action of this venom on the central nervous system. Thus, the power of progression and of co-ordination, the rate and vigour of the respirations, and the excitability of the conjunctival and spinal reflexes are not impaired until there has occurred so great deterioration of the quality of the blood and such feebleness of the circulation as to satisfactorily account for the interference with the functions of the central nervous system. The convulsions that occur shortly before death are due to asphyxia.

Other indirect evidence can be obtained of the absence of a central acting neurotoxin. If the venom contained such a neurotoxin in sufficient quantity to be of importance in accounting for the lethality of the venom, it would be expected that an increase of the dose to three or four times the minimum lethal would markedly

accelerate the time of death, as is the case with *Sepedon* venom. We have not, however, found this to be true of *Echis* venom. Further, it is well known that the neurotoxins of snake venoms are much less easily destroyed by heat than the hæmotoxins. If the neurotoxin is present only in small amount, one would expect to find that heating the venom to such an extent as to impair or destroy only the hæmotoxins would markedly diminish the toxicity of the venom. We performed a series of experiments on rats to determine the lethality of heated *Echis* venom and found that the minimum lethal dose by subcutaneous injection of venom which had been kept in solution at a temperature of 78 to 80° C. for 30 minutes was 0·008 grm. per kilogramme—that is to say, the heated venom possessed only one-tenth the lethality of the unheated venom. It might be inferred that one-tenth of the toxic power of the venom was due to neurotoxin, but against this assumption is the fact that the rat which died of the heated venom showed hæmorrhages, both local and remote. It is more probable, therefore, that heating to this extent does not completely destroy the hæmotoxins of the venom.

(b) *Nerves.*

Not only is there an absence of evidence pointing to a direct action of this venom on the central nervous system, but there is likewise no evidence of an action on the nerves or nerve terminals of voluntary muscle. Thus in the frog (Experiment 6), faradic stimulation of the sciatic nerve with the secondary coil at 400 mm. induced tetanus of the gastrocnemius muscle, after failure of the circulation and respiration. It is interesting to note in this connection that elevation of the lower eyelids of the frog, which is a conspicuous symptom of curare-like poisons and which is invariably seen in *Cobra* and *Sepedon* poisoning, is never seen in *Echis* poisoning. Similarly, in the rabbit (Experiment 9), stimulation of the sciatic nerve with the coil at 300 mm. induced a vigorous contraction of the gastrocnemius muscle six minutes after arrest of respiration, showing that there was no paralysis of the nerve or nerve ends.

That the venom contains no toxin acting on the peripheral nerve ends was also shown by experiments on isolated nerve-muscle preparations of the frog. Thus it was found that when a gastrocnemius-sciatic preparation of the frog was completely immersed for 24 hours in a solution of venom of 1 in 2000 of Ringer's solution, the muscle, when stimulated either directly or indirectly, showed only a slight loss of excitability, this diminution in excitability being not greater than that which occurred in a control preparation kept for the same time in Ringer's solution only.

D. EFFECTS ON SKELETAL MUSCLE.

This experiment also shows that a solution of venom of 1 in 2000 does not affect the excitability of the muscle in 24 hours. In other experiments, graphic records were taken of single twitches of a muscle immersed in a solution of venom, but no alteration in the character of the muscle contraction was produced by the venom. The venom has, therefore, no appreciable effect on skeletal muscle.

E. EFFECTS ON THE CIRCULATION.

(a) *Heart.*

As has been already pointed out, in death from *Echis* poisoning in the frog, the heart continues to beat after cessation of respiration and after paralysis of reflex excitability. Though at this time the rate and amplitude of the heart's contractions are diminished, it is impossible to say whether this effect is due to a direct action of the venom on the heart or is merely a result of the great diminution in the amount of blood in the heart chambers.

Further experiments accordingly were made to determine what effects, if any, the venom produces on the isolated frog-heart, Schäfer's plethysmograph being used. The bulb of the plethysmograph which contained the ventricle was filled with Ringer's solution, and the contractions of the ventricle were recorded on a vertical drum, by means of an air-piston recorder attached by a rubber tube to the brass cylinder. A mixture of defibrinated ox blood (one part) and Ringer's solution (two parts) was used as the nutrient solution and as the solvent for the venom.

Experiment 27.—June 1, 1908. Figs. 1 to 5 inclusive. Strength of solution 1 in 1000. The effects produced by this solution are stated in the following table:—

TABLE IX.—Experiment 27.

Time.		Amplitude of excursus.	Rate per 60 secs.	Notes.
hrs.	min.			
3	29	6	11	
3	37	6.5	9	
3	38	—	—	<i>Echis</i> venom (1 in 1000) turned on.
3	41	6	15	Diastolic pause shortening. Pin-point red spots on surface of ventricle.
3	45	6	28	Blood solution leaking through wall of ventricle. No diastolic pause. Greater part of ventricular surface bright red, even at end of systole.
3	47	—	—	Blood solution pouring through ventricle wall.
3	51	4.5	36	Systole occupies a longer part of the cardiac cycle than diastole.
4	3	2	43	Incomplete relaxation of ventricle.
4	10	1.5	42	
4	22	?	24	Contractions reduced to mere undulations.
4	30	—	—	Arrest of heart in almost complete systole.

This strength of venom solution therefore modifies the heart's contractions in the following ways:—There is produced, within a few minutes after turning on the venom solution, a great increase in the rate of beat, this being due to shortening of the diastolic pause. At a later period the amplitude of the excursions diminishes progressively, owing to incomplete relaxation of the ventricle, leading ultimately to arrest of the heart in systole. In these respects, the action of a strong solution of *Echis*

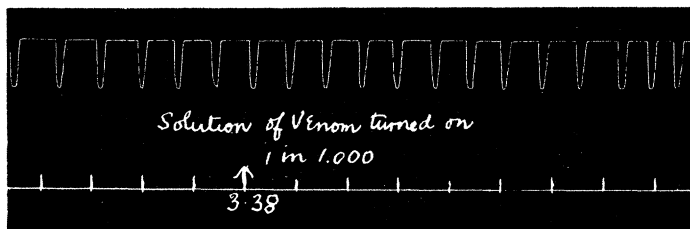


FIG. 1.

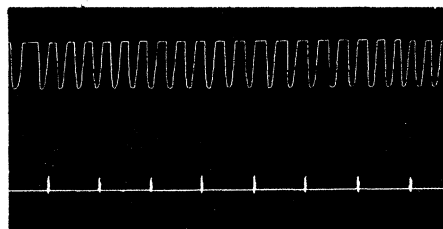


FIG. 2.

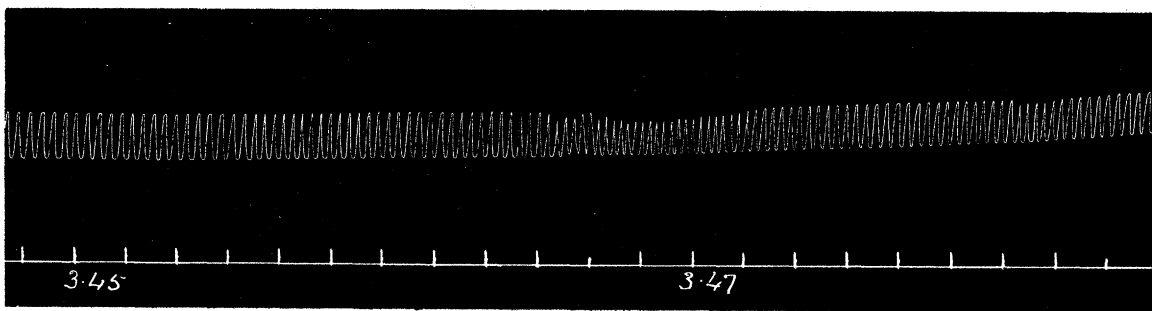


FIG. 3.

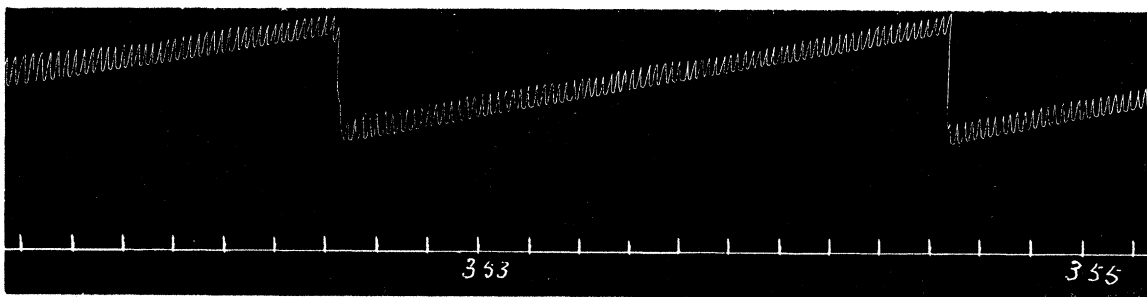


FIG. 4.

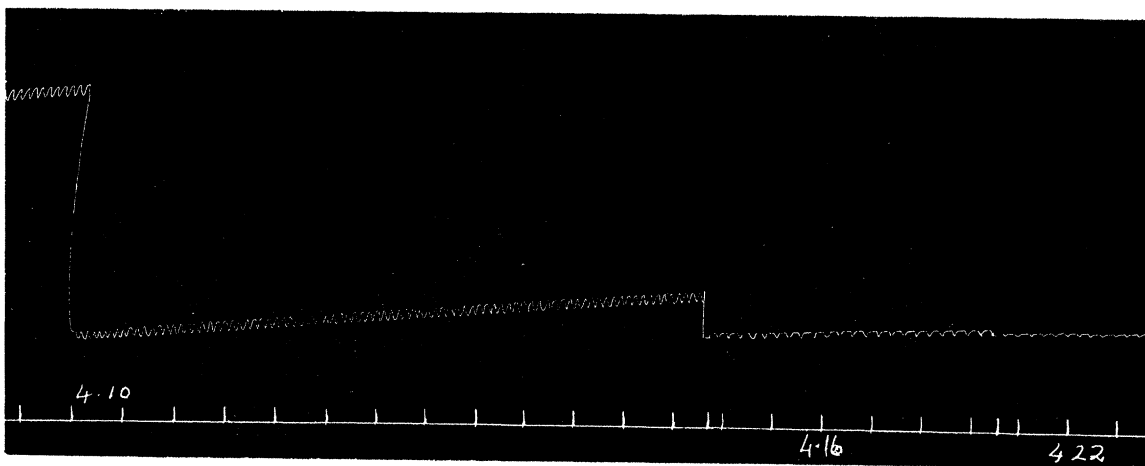


FIG. 5.

venom resembles that of Sepedon or Cobra venom, the systolic action being, however, less powerful.

The most interesting effect of Echis venom on the heart, and one not observed with Sepedon venom, is that by which the wall of the ventricle is rendered pervious to the contained blood solution. This action is produced on the ventricle by all solutions of Echis venom not less dilute than 1 in 40,000, the rapidity of its occurrence increasing with the strength of solution. At first, punctate red spots are seen on the surface of the ventricle, and are especially distinct at the end of systole, at which time the rest of the ventricle is pale. The reddish spots increase in size until eventually the whole surface is uniformly red, even at the end of systole, and the blood solution now passes freely from the interior of the heart into the bulb of the plethysmograph. The escape of the solution from the heart gradually pushes out the piston, and explains the deviations of the heart tracing from the abscissa (*vide* figs. 3 to 5), which necessitated frequent lowering of the lever.

In order to determine the nature of this phenomenon, a frog's ventricle, which had been perfused with venom solution until the wall was freely permeable, was removed from the plethysmograph, hardened and cut into serial sections, which were stained and examined. It was comparatively easy to determine the course of the blood solution by staining in such a way as to display the ox-blood corpuscles, which served the purpose of an artificial injection. It was found that the blood solution had passed freely between the muscle fibres of the heart, apparently by dissolving some material which causes the fibres to adhere together. No pathological change was observed in the muscle fibres themselves. The accompanying drawing, kindly made for us by Mr. RICHARD MUIR, shows the appearances presented (fig. 6).

This action of the venom in so greatly increasing the permeability of the heart wall appears to us to be due to the hæmorrhagin contained in the venom, the separation of the muscle fibres being produced by a process similar to that which also causes separation of the cells of the capillaries, to which reference will be made in the succeeding section.

Experiment 28.—Strength of solution 1 in 20,000. This weaker solution also produced increased permeability of the heart wall, as in the previous experiment, but after a longer interval. It resembled the stronger solution, also, in producing a marked increase in the heart's rate, but it differed in that the diminution in the amplitude of excursus was due to less complete contractions of the ventricle, leading to ultimate arrest in diastole.

We found that the systolic type of effect was produced by solutions of from 1 in 1000 to 1 in 5000, and the diastolic type by solutions of from 1 in 10,000 to 1 in 40,000. A solution of 1 in 100,000 had no effect on the permeability or contractions of the heart wall within two hours.

Echis venom therefore resembles Sepedon venom in its effects on the heart of the frog, inasmuch as it produces systolic arrest with concentrated solutions, and

diastolic arrest with weaker solutions, but it differs in so far that the phenomenon of increased permeability is not exhibited by the latter venom.

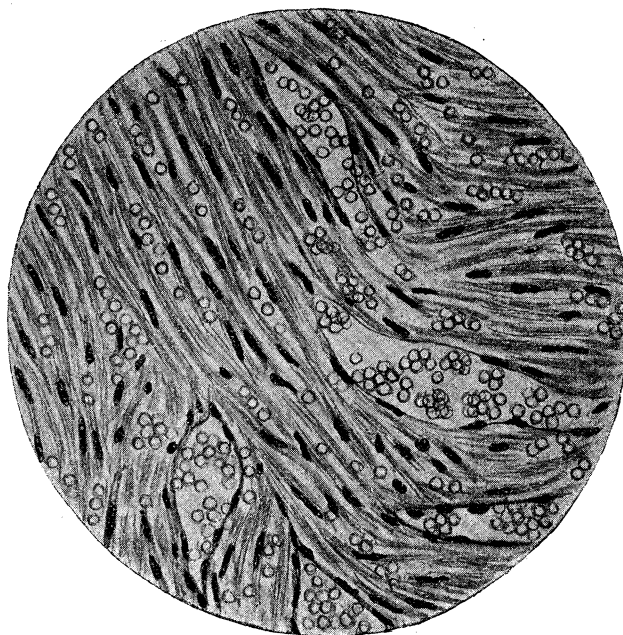


FIG. 6.

(b) *Blood-vessels.*

Two factors require to be differentiated in regard to the action of *Echis* venom on the blood-vessels: firstly, the property it possesses of destroying the integrity of the vessel walls; and, secondly, the alterations, if any, that it may produce on the calibre of the blood-vessels apart from this.

It has been shown by many observers that a hæmorrhagic toxin constitutes one of the most important factors in explaining the cause of death from Viperine venoms especially. In all the animals with which we have experimented, we have found that the production of hæmorrhages, both at the site of injection and distally, after the absorption of the venom into the blood stream, is a characteristic feature of *Echis* poisoning, and it would seem that, apart from intravenous injections, in which rapid death may be produced by intravascular clotting, death from *Echis* venom is mainly due to hæmorrhage. It has been shown by WEIR MITCHELL and REICHERT* that in the case of *Crotalus* venom the blood extravasations take place not by diapedesis, but through actual rents in the vessel walls, the dissolution of the walls of the vessels being limited to the capillaries and small veins.

In its general effects, the hæmorrhagin of *Echis* venom resembles those of the hæmorrhagins of other venoms, and there are therefore only the following points which we desire to mention specially:—

* WEIR MITCHELL and REICHERT, 'Smithsonian Contributions to Knowledge,' 1886, vol. 26.

The statement generally made that the frog is only slightly susceptible to the action of venom hæmorrhagins is not true of the hæmorrhagin of Echis venom. We have shown that the local and distal hæmorrhages in the frog are very severe.

Although the blood-vessels of almost no organ of the body seem to be entirely immune to the action of the hæmorrhagin, this toxin shows a conspicuous predilection for the blood-vessels of the alimentary canal of all animals. From the uniformity and preponderance of hæmorrhages into this area, it appears probable that the hæmorrhagin may, after absorption into the blood, be excreted through the intestinal mucosa, as is known to be the case with many poisons.

Lastly, different animals show peculiarities in regard to the commonest sites of hæmorrhages. Thus, in the rat, hæmorrhages almost invariably occur on the conjunctival and nasal mucous membranes, but this is a rare site of hæmorrhage in other animals; in the rat, also, blood is found in the urine more frequently than in other animals. In the rabbit, hæmorrhages occur with greater frequency into the intestines and stomach; and, in the cat, we have noted hæmorrhages in the lungs more commonly than in the other animals. In guinea-pigs, hæmorrhages are very often found in the mesentery and large omentum. In pigeons, the local hæmorrhages seem to be more severe than in any of the above animals.

To ascertain whether Echis venom produces any effects on the calibre of the blood-vessels of the frog, the following method was used: After exposure of the heart of a pithed frog, the *venæ cavæ* were cut across, and a fine canula was tied into the left aorta, the right aorta being ligatured. This canula was attached by glass tubes to a series of Mariotte's flasks, the fluids in each of which were maintained at the same level. The amount of fluid exuding per minute from the cut *venæ cavæ* was accurately measured. The normal calibre of the vessels was ascertained by allowing Ringer's solution to flow through them for at least 20 minutes, and until the flow was uniform. The venom was also dissolved in Ringer's solution.

It was found that a solution of Echis venom of 1 in 10,000 reduced the flow through the vessels from 3·4 c.c. per minute to 1·5 c.c. per minute in 15 minutes; a solution of 1 in 25,000 reduced the flow from 3·4 c.c. to 1·5 c.c. in 35 minutes; a solution of 1 in 50,000 reduced the flow from 2 c.c. to 1 c.c. in 50 minutes; while a solution of 1 in 100,000 only slightly reduced the flow in 60 minutes.

It was very clearly manifested in all these experiments that the diminution in the flow through the blood-vessels was at least mainly due, not to the more usual cause, viz., contraction of the arterioles, but to the occurrence, during the perfusion of the venom solution, of an intense œdema of the tissues of the frog. To such an extent, indeed, did the venom solution escape from the blood-vessels into the surrounding tissues that it was usual to find that the weight of the frog was more than doubled after perfusion with a concentration of venom of 1 in 25,000 for half an hour.

Perfusion with saline solution alone may produce a small amount of œdema in the frog, and perfusion with certain poisons produces a still greater œdema, but in

regard to this effect no poison is known to us which is equal in potency or rapidity to *Echis* venom.

This phenomenon would seem to be of importance from its being an interesting and uncomplicated demonstration of the action of the hæmorrhagin of *Echis* venom, for we have here to deal with an independent action on the blood-vessels apart from any effect on blood by a hæmolysin or anticoagulin present in the venom, as the perfusing fluid contained no blood, but was Ringer's solution only. The escape of the perfusing solution from the vessels into the surrounding tissues is due to the rupture of the capillary wall by the hæmorrhagin. The same action of the hæmorrhagin after its entrance into the blood stream gives rise to the hæmorrhages which form the most conspicuous feature of *Echis* poisoning.

Our experiments seem to show that *Echis* venom has no direct constricting effect on the arterioles, such as is produced by Cobra or *Sepedon* venom. The reasons for ascribing the diminished outflow through the vessels solely to the œdema are that the diminution comes on only when the œdema becomes marked, and also that a solution which is of insufficient strength to produce œdema fails also to produce diminished flow through the vessels.

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

Kymographic experiments were made on rabbits and cats with a view to determining the effects of the venom on the blood-pressure and the respiration. In these experiments we were confronted with the difficulty that other investigators have experienced with Viperine venoms, namely, that the introduction into the circulation of doses of *Echis* venom sufficiently large to have any pronounced effect on blood-pressure causes intravascular clotting, unless special precautions are taken to prevent it.

It was, however, possible to determine the uncomplicated action of the venom on the circulation by taking advantage of the fact, first investigated in regard to snake venoms by MARTIN,* that a condition of incoagulability of the blood can be produced by the preliminary slow injection of a dose of *Echis* venom which, if injected rapidly, would produce intravascular clotting. This was the method adopted in the following experiment :—

Experiment 29.—December 28, 1909. Table X. Figs. 7–16 inclusive. A cat, weighing 2000 grm., was anæsthetised with chloroform. The trachea was then opened and a canula tied into it through which the animal respired. Ether was thereafter inhaled through this canula and chloroform discontinued. The blood-pressure was taken in a carotid artery, the respirations were recorded by means of a double stethograph, and the venom was administered by a canula tied into the external jugular vein.

* C. J. MARTIN, 'Roy. Soc. N.S.W. Proc.,' July, 1895.

TABLE X.

Time.	Average blood-pressure in mm. Hg.	Pulse rate per 10 secs.	Respirations rate per 10 secs.	Respirations excursus in mm.	Notes.
hrs. mins. secs.					
3 40	130	33	7	1.5	
3 41	—	—	—	—	
to					
3 44 30					0.00025 gm. per kilo. injected intravenously (fig. 7).
3 46	124	31	8	1.5	
3 54	102	31	7	1.5	
4 3	104	30	6	2.0	
4 4	—	—	—	—	
to					
4 9					0.001 gm. per kilo. injected intravenously (fig. 8).
4 10	89	29	7	1.5	
4 32	91	28	6	2.0	
4 35	100	29	6	2.0	
4 36	—	—	—	—	
					0.0025 gm. per kilo. injected intravenously (fig. 9).
4 37	80	27	6	2.0	
4 39	61	27	6	2.0	
4 47	60	27	6	2.0	
4 47 30	—	—	—	—	
					0.0025 gm. per kilo. injected intravenously (fig. 10).
4 49	58	25	6	2.0	
5 15	44	25	5	2.0	(Fig. 11.)
5 25	38	25	5	2.0	(Fig. 12.)
5 26	—	—	—	—	0.005 gm. per kilo. injected intravenously (fig. 13).
5 27	27	25	4	2.0	
5 30	20	25	0	0.0	(Fig. 15.)
5 33	9	9	—	—	Few respiratory gasps (fig. 16).

In this experiment five injections were made, and they illustrate the effects of small and large doses of Echis venom on the blood-pressure. The first injection of 0.00025 gm., the second of 0.001 gm., and the third of 0.0025 gm. per kilogramme were each dissolved in 10 c.c. of Ringer's solution and injected slowly, this method of combining dilution with slowness of injection having been shown by MARTIN to be the best way of producing the negative phase of coagulability of the blood with venoms. The blood was then examined and found to be quite incoagulable, and it thus became possible to determine the effects produced by injecting relatively large quantities of venom rapidly and in concentration into the circulation. Two further doses were therefore given of 0.0025 and 0.005 gm. per kilogramme, both being dissolved in 2 c.c. Ringer's solution and injected rapidly.

The effects on blood-pressure and respiration are stated in Table X, and they can be summarised briefly. Each injection produced a fall of blood-pressure. This fall was always accompanied by a diminution in the rate of the heart-beats, but this reduction in rate was slight, except in the case of the last dose, which was a very

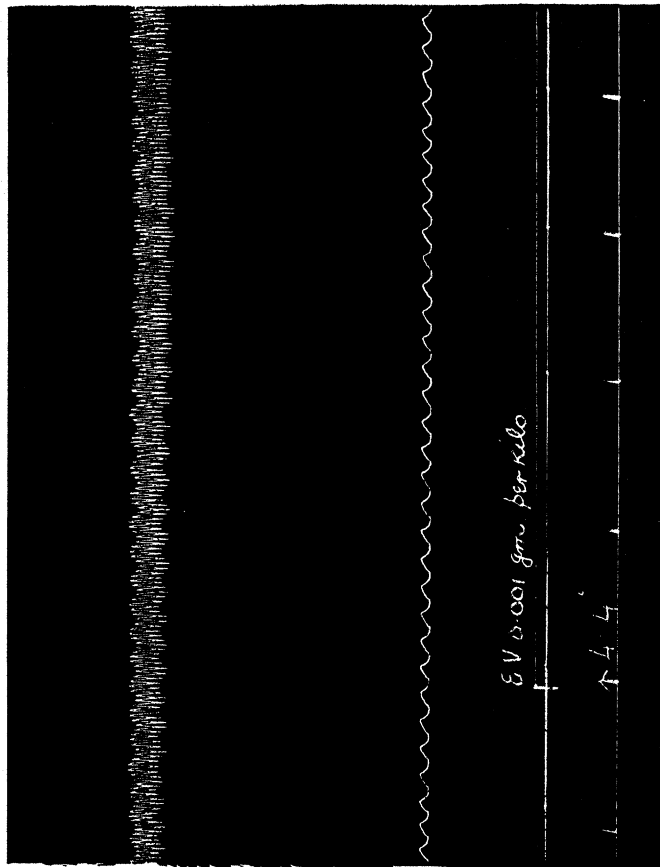


FIG. 8.

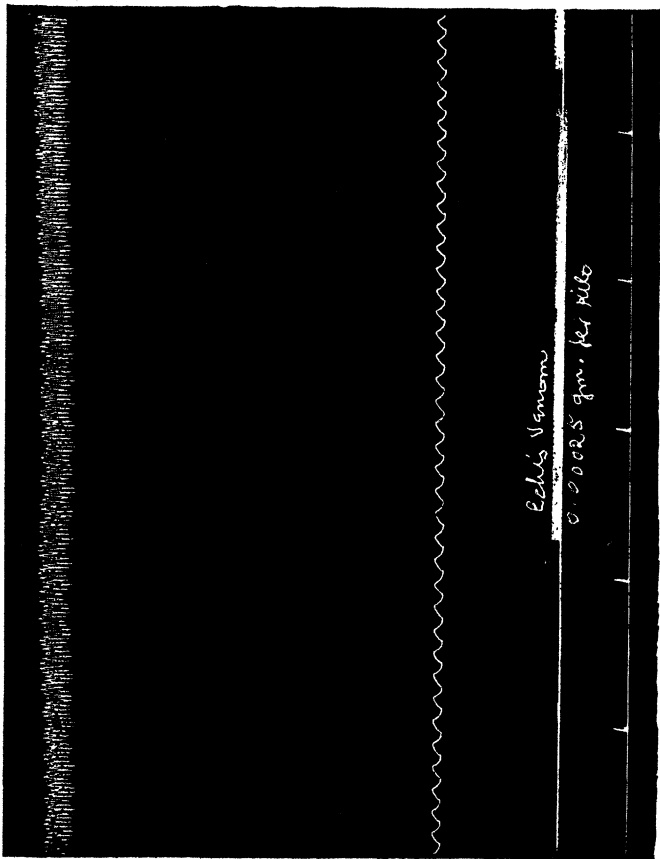


FIG. 7.

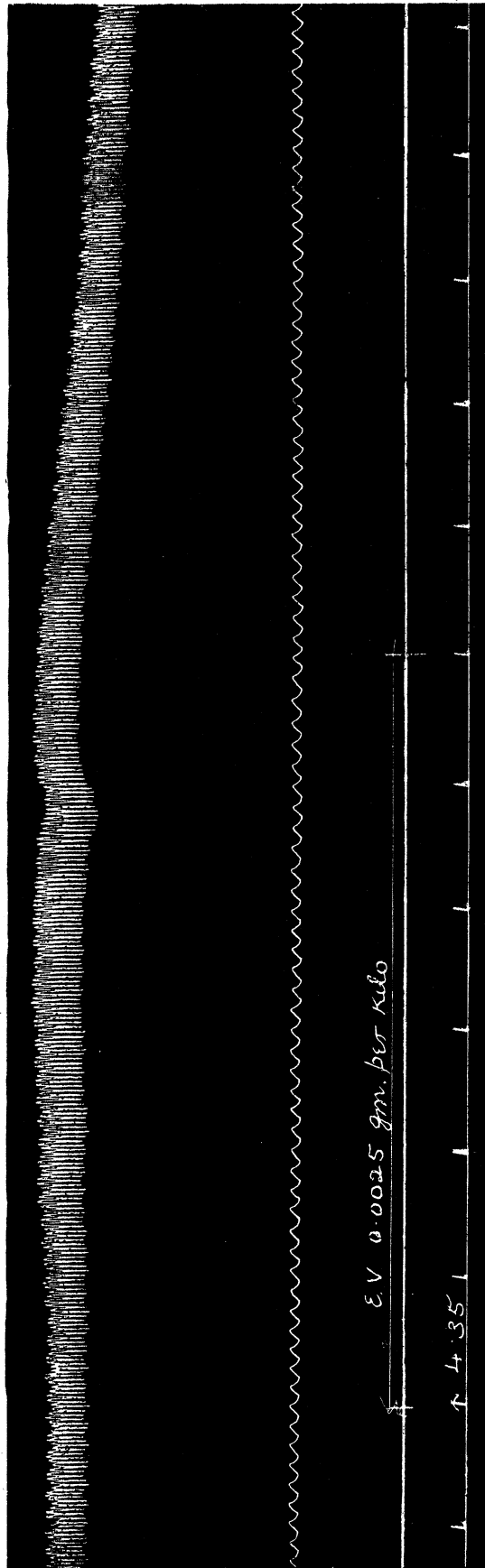


FIG. 9.

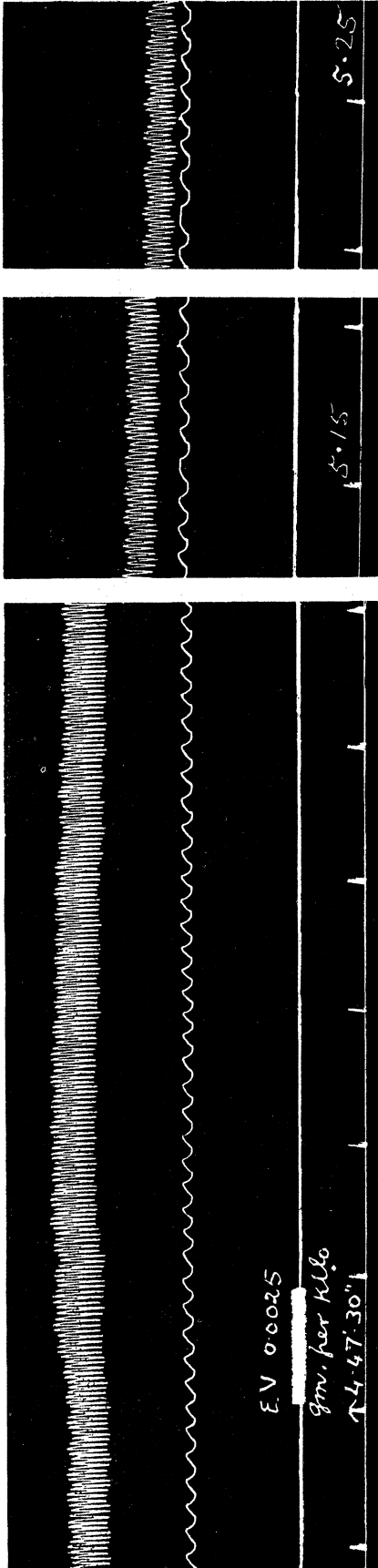


FIG. 10.

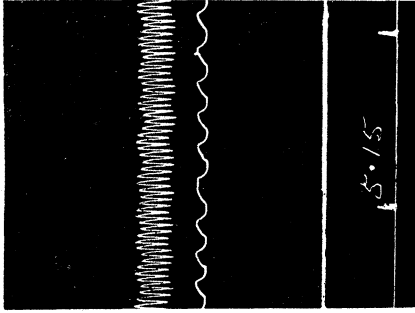


FIG. 11.

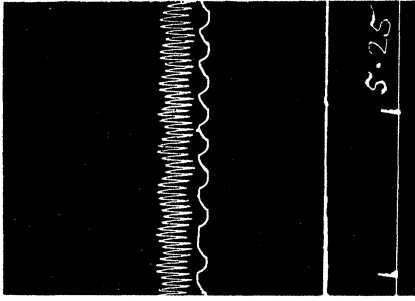


FIG. 12.

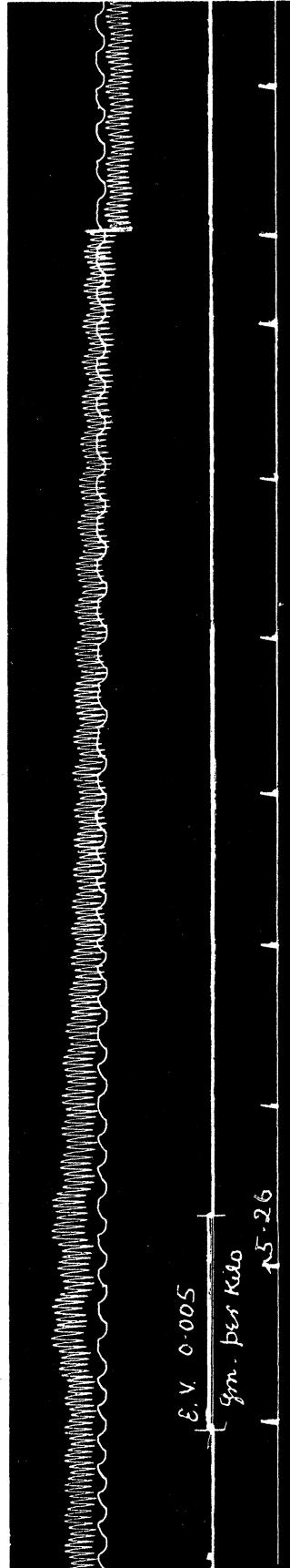


FIG. 13.

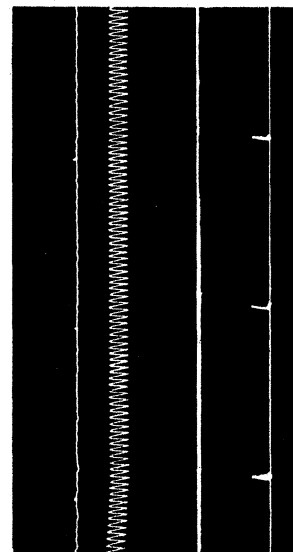


FIG. 14.

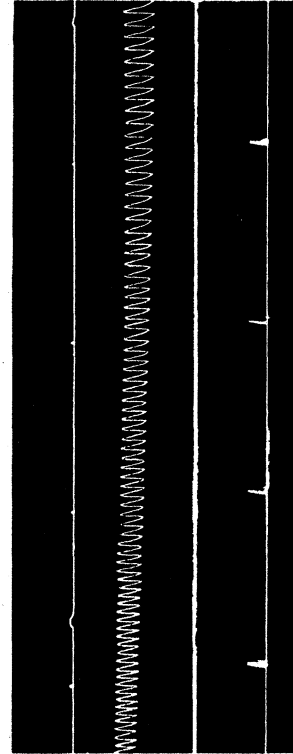


FIG. 15.

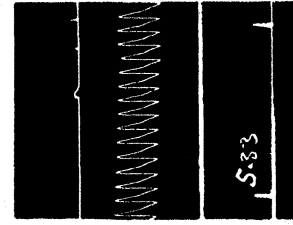


FIG. 16.

large one. It may be said at once that in many other experiments we have found the above to be the invariable effect of *Echis* venom on blood-pressure. This experiment also illustrates a phenomenon which we have observed in many experiments, namely, that a first dose produces a relatively greater effect on blood-pressure and pulse rate than is produced by even a considerably larger second or subsequent dose. A similar result was obtained by MARTIN in his experiments with *Pseudechis* venom.

A point of great interest is the effect, or rather the absence of effect, on the respirations. The first four injections, involving a total of over 0·006 grm. per kilogramme, produced practically no effect on the respirations. Failure of respiration did not occur until after injection of the last dose, when the blood-pressure had fallen to below 30 mm. of mercury, at which time impairment of respiration was adequately explained by the great fall of blood-pressure. This is a corroboration of the fact which we have previously emphasised, namely, that there is no evidence that *Echis* venom contains a neurotoxin acting on the central nervous system.

The effects of this venom on blood-pressure offer a striking contrast to the effects of Cobra and *Sepedon* venoms. In the case of the latter venoms the effects on respiration are of primary importance, failure of the circulation being largely consequent upon respiratory failure. In the case of *Echis* venom the reverse is true. It follows from this, as we have found in several experiments, that artificial respiration is quite ineffective in restoring the circulation in poisoning by *Echis* venom, whereas in *Sepedon* venom poisoning* we have shown that a dose which will arrest the respiration within half an hour has practically no effect on the blood-pressure in three and a-half hours if artificial respiration be maintained.

Another point which may be mentioned is the small part played by any direct effect on blood-pressure as compared with intravascular clotting, in the production of death from intravenous injection of *Echis* venom. In the experiment just described the intravenous injection of over 0·006 grm. per kilogramme did not produce rapid death when intravascular clotting was prevented, whereas in another experiment the injection of the much smaller dose of 0·0002 grm. per kilogramme, also in the cat, produced death within a few seconds by intravascular clotting.

One point of some interest requires further elucidation, namely, the cause of the fall of blood-pressure. If the dose administered be small, recovery of blood-pressure may occur to nearly the original level. If the dose be sufficiently large, the decline is progressive until death. Though the effects on blood-pressure produced by different Viperine venoms are substantially alike, they have received different interpretations by different investigators. Thus WEIR MITCHELL and REICHERT† considered that the primary fall of pressure produced by *Crotalus* venom was due to an action chiefly on the vasomotor centre and slightly on the heart, but that the final fall of pressure was

* FRASER and GUNN, 'Phil. Trans.,' B, vol. 200, p. 241.

† WEIR MITCHELL and REICHERT, *loc. cit.*

cardiac in origin. ROGERS* considered that the fall of pressure produced by Viperine venoms was due to a specific action on the vasomotor centre. On the other hand, MARTIN, as the result of many careful experiments, believed that the fall of pressure produced by Pseudechis venom, which in so many respects resembles the Viperine venoms, was due to impairment of the heart. Our experiments with Echis venom support MARTIN'S view. We have made many experiments, under varying conditions and with varying doses, in which simultaneous records were taken of the blood-pressure and of the volume either of the kidney or a loop of intestine. In all of these experiments, which were made on cats, the results were the same in so far as the fall of blood-pressure was in no case accompanied by an increase in the volume of the kidney or intestine, that is to say, that it was not due to dilatation of the blood-vessels. The following experiment illustrates this:—

Experiment 30.—January 27, 1910. Fig. 17. Cat, 3000 grm. The conditions of the experiment were the same as those of Experiment 26, except that no record was taken of respiration, but a record was taken of the volume of the right kidney by an oncometer attached to an air-piston recorder. An intravenous injection was given of 0·002 grm. per kilogramme, which had been heated at 80° C. for half an hour to prevent intravascular coagulation. This injection reduced blood-pressure from 120 to 104, and the pulse rate from 32 to 27 per 10 seconds, but no increase of volume of the kidney occurred. It would seem, therefore, that the fall of blood-pressure produced by Echis venom is due to slowing and probably in greater measure to weakening of the heart. This result corresponds with that obtained in perfusing the frog's heart, when it was found that dilute solutions of Echis venom cause weakening and diastolic arrest of the heart. The absence of any effect on the vasomotor centre is also in accord with the absence of neurotoxins from this venom.

(d) *Lymph Hearts.*

In frogs poisoned with even many times the minimum lethal dose, the lymph hearts continue to beat very nearly as long as the blood heart. Echis venom apparently has no direct action on the lymph hearts, which are therefore less affected by this venom than by Sepsedon venom.

(e) *Blood.*

Alterations in the quality of the blood constitute the most important effects produced by this venom, and these alterations are of such a nature as can be explained by the presence in the venom of (1) a hæmorrhagin, (2) a hæmolysin, and (3) a substance, or substances, which modifies the coagulability of the blood. The effects of each of these may be discussed separately, but as the blood-changes produced both by Echis venom itself and by other venoms which act like it have already received much attention, these effects will only briefly be discussed.

* ROGERS, 'Phil. Trans.,' B, vol. 197, p. 188.

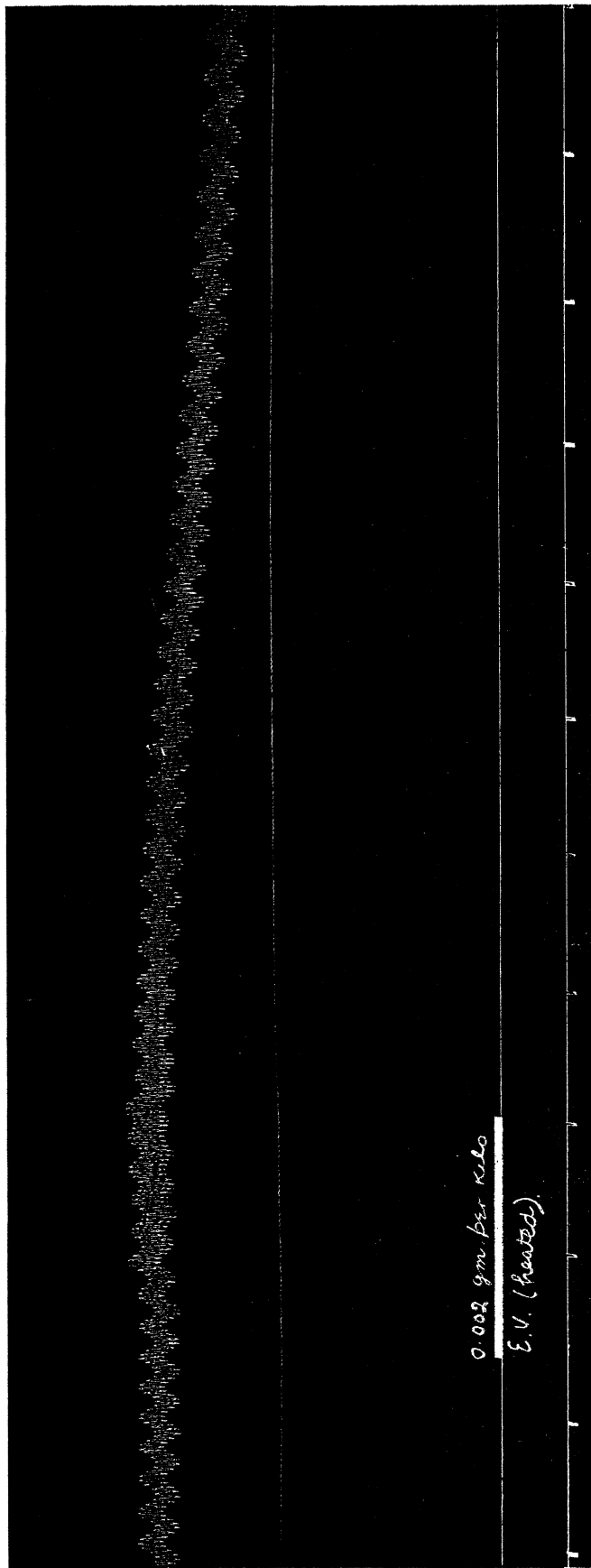


FIG. 17.

1. The direct effects of the hæmorrhagin having already been considered, it remains only to mention the indirect effects of the hæmorrhages on the composition of the blood. We have investigated these changes after subcutaneous injection in frogs and rabbits. It was found that, after the administration of lethal doses, there occurred a great reduction in the number of the red blood corpuscles in the circulating blood, as in some cases they were reduced to about half the original number. After non-lethal doses, these corpuscles were considerably diminished in number for several days, after which they gradually increased to the normal number. In regard to the leucocytes, their number was generally considerably increased for some days after subcutaneous injection. These results correspond with those obtained by MARTIN with *Pseudechis* venom.

2. The hæmolytic action of *Echis* venom has already been carefully investigated by LAMB.* LAMB first clearly demonstrated the divergence in the activity of the hæmolytic actions of Colubrine and Viperine venoms *in vivo* and *in vitro*, and showed, in regard to *Daboia* venom in contrast with *Cobra* venom, that, *in vivo*, hæmolysis is a more prominent effect of the former venom, whereas, *in vitro*, *Cobra* venom is more actively hæmolytic than *Daboia* venom. He found later, in the course of experiments with many venoms, that *Echis* venom in large amounts (*e.g.*, 5 mgrm. to 1 c.c. of a 5-per-cent. suspension) can hæmolyse washed dog's corpuscles *in vitro*, being much feebler in this respect than either *Cobra* or *Daboia* venom. A more powerfully hæmolytic action was obtained by activation with dog's serum or lecithin, for 0.05 mgrm. of *Echis* venom then completely hæmolyse the same amount of blood.

Our experiments *in vitro*, which have been made with washed and unwashed corpuscles of the dog, rabbit, and man, have been confirmatory of LAMB'S work. We found that *Echis* venom in strong concentrations hæmolyse washed corpuscles, that weaker solutions caused hæmolysis in the presence of serum, and that the corpuscles of the dog were more sensitive to the hæmolytic action of this venom than those of the rabbit or of man.

In regard to *in vivo* hæmolysis, the following points are of interest:—*Echis* venom, unless when injected intravenously in large quantity, produces only an inconspicuous amount of hæmolysis. With the minimum lethal dose, or even with twice that amount, there may be no hæmolysis of the corpuscles in the circulating blood, though with much smaller doses there is usually complete hæmolysis of the extravasated blood at the site of injection. LAMB suggests that the hæmorrhagic discharges from the alimentary canal and in the urine, in cases of bite and experimental poisoning by *Daboia* venom, consist largely of free hæmoglobin. In the case of *Echis* venom, however, we have found that serious hæmorrhages may occur without any hæmolysis, the intestines and bladder being filled with unhæmolyse corpuscles. This indicates that the hæmorrhagin of *Echis* venom is relatively much

* LAMB, "Snake Venoms in relation to Hæmolysis," 'Scientific Memoirs,' 1905, No. 17.

more powerful than that of Daboia venom, whereas its hæmolysin is relatively weaker than that of the latter venom.

3. MARTIN* first set on a sure foundation the knowledge of the action of snake venoms on blood coagulability by his experiments with Pseudechis venom. He showed that, when administered intravenously, this venom produces intravascular clotting if injected rapidly and in sufficient amount, whereas if injected slowly it may produce a condition in which the blood cannot be made to clot by further additions of venom, however great.

MELLANBY† demonstrated the production of the positive and negative phases of coagulability in regard to Echis venom, and as the result of a long series of experiments, he came to the conclusion that the active coagulating principle of this venom consists of pure kinase. When injected intravenously, or when added to blood *in vitro*, this kinase produces a positive phase of coagulability. His explanation of the negative phase is ingenious and well explains the known facts. He considers that when the venom is injected very slowly the formation of fibrin is not sufficiently rapid or complete to cause intravascular clotting, but that the fibrin is formed in such minute quantities that it can be removed by the tissue cells; and thus, if a sufficient amount of venom be slowly injected, all the fibrinogen is removed from the blood and, therefore, further coagulation is impossible until such a time as the fibrinogen is regenerated in the blood.

With regard to our experiments with Echis venom, it is noteworthy that, although in a rabbit so small an amount as 0·0001 gm. per kilogramme produces immediate death from intravascular clotting if injected intravenously, a dose of 0·005 gm. per kilogramme does not produce intravascular clotting if injected subcutaneously. On MELLANBY'S explanation, it seems necessary to assume from these data that the kinase of Echis venom is only very slowly absorbed from the subcutaneous tissues. Other evidence, of the following nature, may be adduced for assuming that the toxins of Echis venom are only slowly absorbed from the subcutaneous tissues. Hæmorrhages in various tissues are produced rapidly by intravenous injection, and hæmorrhage comes on rapidly at the site of injection. Therefore, the injury to the vessel-wall produced by the venom is not in the nature of a degeneration which requires any considerable time for its production. In spite of this, after subcutaneous injection of five times the minimum lethal dose in a rabbit, the distal hæmorrhages are usually not severe until 24 hours subsequently. We have also observed that the greatest prolongation in the coagulation time of the blood after subcutaneous injection of Echis venom is not reached till at least 24 hours. The fact that five times the minimum lethal dose does not kill much more rapidly than a single minimum lethal dose subcutaneously administered may be explained by slowness of absorption.

* C. J. MARTIN, *loc. cit.*

† MELLANBY, 'Journ. Physiol.,' 1905, vol. 32, p. 207.

In order further to investigate this point we compared the minimum lethal dose subcutaneously and intramuscularly in pigeons (see Tables VI and VII). It was found that a dose of 0·003 gm. per kilogramme injected intramuscularly killed in one hour and a half, whereas this dose was not fatal when subcutaneously injected; and a dose of 0·004 gm. per kilogramme subcutaneously administered killed only in 20 hours. These tables (pp. 2 to 4) show that intramuscular injection is, both quantitatively and especially in regard to rapidity, much more fatal than subcutaneous injection. The slowness of absorption of Echis venom from the subcutaneous tissues suggests doubts as to the value of the experiments of BRUNTON, FAYRER, and ROGERS* to determine the practical antidotal value of potassium permanganate, at least in regard to Viperine venoms. They administered the venom subcutaneously, whereas cases of snake bite practically always correspond with intramuscular injection. In the latter case, it is not only more difficult to ensure contact of the permanganate with the venom, but, as our experiments show, a much more rapid absorption of the venom has to be dealt with.

F. EFFECTS ON RESPIRATION.

It has already been shown that Echis venom, when injected subcutaneously, exerts little if any direct action on the respiratory centre. The absence of any pronounced effect on respiration, even after intravenous injection of large doses, has also been discussed in connection with Experiment 29. Echis venom thus offers a striking contrast to Sepedon venom, which owes its lethality almost solely to a neurotoxin acting directly on the respiratory centre.

G. EFFECTS ON TEMPERATURE.

The effects of the venom on body temperature were investigated mainly in rabbits. It was found that non-lethal doses, if large enough to affect the temperature, produce a rise of temperature. In the case of a large sub-lethal dose the temperature may remain 2° C. above the normal for over a week. The rise of temperature is probably due largely to septic and other changes produced round the site of injection.

Lethal doses of Echis venom produce a rise, followed by a fall, of temperature. The fall of temperature does not come on until there are symptoms of great feebleness of the circulation, and it is probably an indirect result of a low blood-pressure and of blood loss. The fall of temperature produced by Sepedon venom, on the other hand, is a concomitant of symptoms of paralysis of the central nervous system, and is probably due to a direct action of the venom on the central nervous system. This affords another of the many instances showing the preponderance of the neurotoxic effects of Sepedon venom and the insignificance of these effects in the case of Echis venom.

* BRUNTON, FAYRER, and ROGERS, 'Roy. Soc. Proc.', 1904, vol. 73.

H. EFFECTS ON THE URINARY SYSTEM.

In the experiments on rats special observations were made of the effects of Echis venom on the urine. It was found that lethal doses produce a diminution in the total quantity of urine secreted, also albuminuria, and, at a later period, frequently hæmaturia.

Microscope examination of the kidneys in a case of hæmaturia showed a condition of acute hyperæmia, especially of the superficial arteries and veins of the cortex. The capillaries of the tufts and the interlobular capillaries were greatly engorged, and there was some evidence, which was not conclusive, of hæmorrhages occurring from the tufts.

GENERAL SUMMARY.

A. The venom used consisted of the liquid venom which had been rapidly dried immediately after it had been taken from living serpents in India, by the process of "milking." Its minimum lethal dose by subcutaneous injection per kilogramme was found to be for the frog, 0·009 grm. ; for the rabbit, 0·0009 grm. ; for the guinea-pig, 0·001 grm. ; for the rat, 0·00075 grm. ; for the cat, 0·008 grm. ; for the pigeon, 0·004 grm. ; and, by intramuscular injection, for the pigeon, 0·001 grm.

B. In the case of all these animals, the cardinal symptoms, in order of time and of prominence, are hæmorrhages, occurring locally and distally, feebleness of the circulation and anæmia, arrest of respiration and loss of reflex excitability, and arrest of the heart in diastole.

C. No evidence could be obtained, either in cold- or warm-blooded animals, of a direct action of the venom on the brain or spinal cord. At a time when there occurs definite impairment of the functions of the central nervous system, this can adequately be accounted for by the coincident or pre-existent deterioration of the quality of the blood and the feebleness of the circulation. The venom has, moreover, no effect on the nerves or nerve terminals of voluntary muscle.

D. The venom has no appreciable effect on skeletal muscle.

E. The effects on the circulation are produced partly directly and partly indirectly. From the point of view of lethality, the indirect effects, due to blood changes, are the more important.

When perfused through the frog's heart strong solutions bring about an increase in the rate, followed by arrest of the heart in systole, and weaker solutions also quicken the heart, but arrest it in diastole. The venom also renders the heart-wall permeable to the contained blood solution, an effect apparently due to separation of the cardiac muscle fibres.

Solutions of the venom diminish the flow through the frog's blood-vessels, when perfused through them, but this effect is due, not to a direct constricting action on the vessels, but to the production, by the hæmorrhagin contained in the venom, of an intense œdema of the tissues of the frog.

In mammals, even very small doses of the venom injected intravenously may rapidly arrest the circulation by the production of intravascular clotting. If intravascular clotting be prevented, the venom produces a fall of blood-pressure, due partly to slowing, but mainly to weakening, of the heart's contractions.

Echis venom has no appreciable effect on the lymph hearts of the frog.

The toxic effects of the venom are due chiefly to the production of alterations in the quality of the blood, of which the following are the most important:— Especially with lethal doses, there occurs a great reduction in the number of the red blood corpuscles in the circulating blood, which is due chiefly to hæmorrhages, but, in the case of large intravenous doses, is due also to a small amount of hæmolysis. The number of the leucocytes is usually considerably increased for some days after subcutaneous injection. The hæmolytic action of the venom is unimportant in producing its lethal effects, and death frequently occurs without any hæmolysis. The venom has a moderate hæmolytic action on the blood *in vitro*. Positive and negative phases of blood coagulability are produced by Echis venom in a manner similar to that which has already been described in the case of several venoms.

F. Echis venom exerts little, if any, direct action on the respiratory centre or on the terminations of the phrenic nerves.

G. Non-lethal doses of the venom produce a rise of temperature; lethal doses, a rise, followed by a fall, of temperature.

H. Lethal doses cause a diminution in the total quantity of urine secreted, and produce albuminuria and, frequently, hæmaturia.

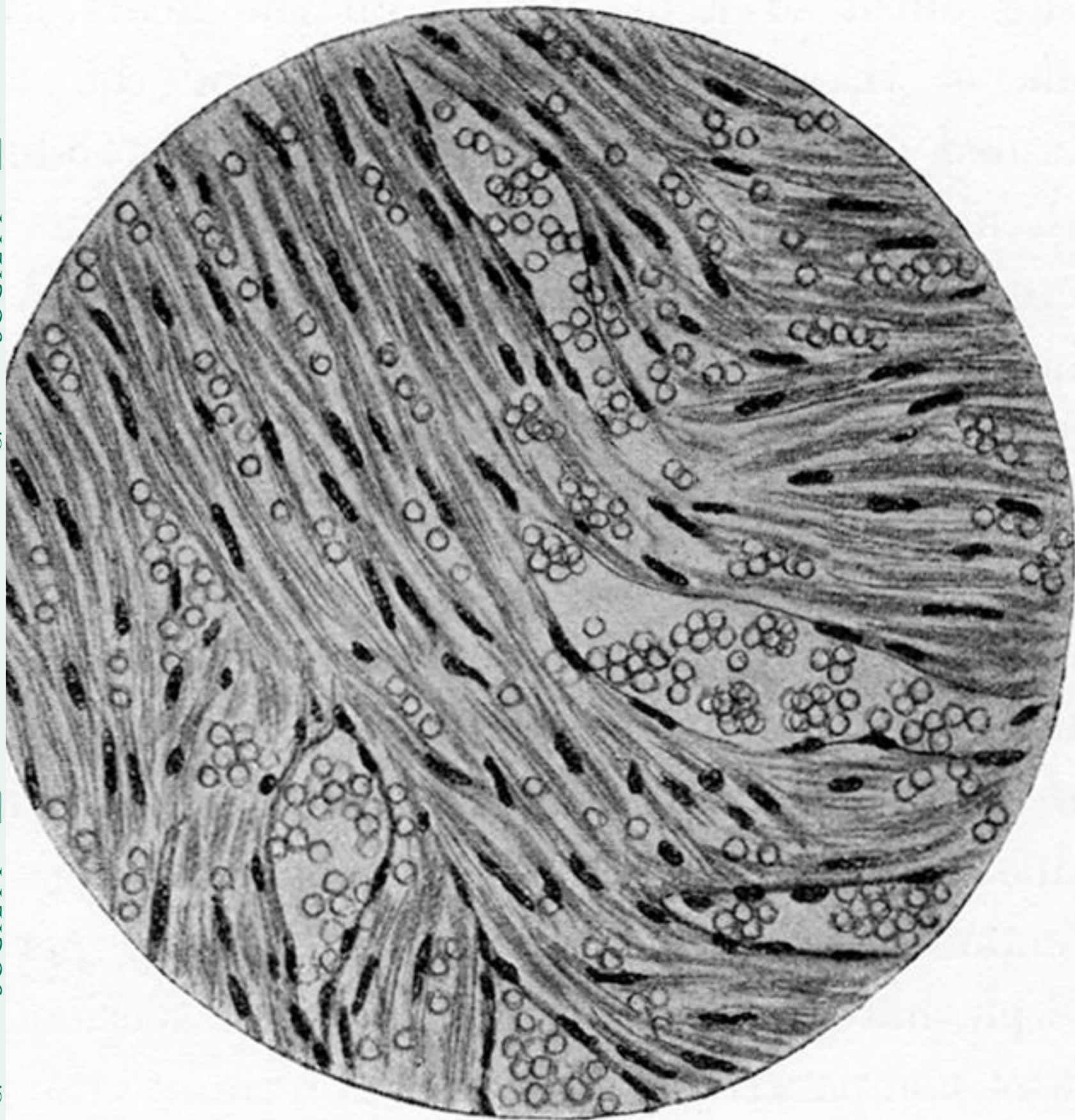


FIG. 6.