(*NEW SERIES.*)

No. 7.

SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA.

SOME OBSERVATIONS ON THE POISON OF THE BANDED KRAIT (BUNGARUS FASCIATUS).

BY

CAPTAIN GEORGE LAMB, M.D. (GLAG.), I.M.S.

ISSUED UNDER THE AUTHORITY OF THE GOVERNMENT OF INDIA
BY THE SANITARY COMMISSIONER WITH THE GOVERNMENT
OF INDIA, SIMLA.

CALCUTTA:
OFFICE OF THE SUPERINTENDENT OF GOVERNMENT PRINTING, INDIA.
1904.

Price 8 Annas or 9d.

(*NEW SERIES.*) No. 7.

SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA.

SOME OBSERVATIONS ON THE POISON OF THE BANDED KRAIT (BUNGARUS FASCIATUS).

BY

CAPTAIN GEORGE LAMB, M.D. (GLAG.), I.M.S.

ISSUED UNDER THE AUTHORITY OF THE GOVERNMENT OF INDIA
BY THE SANITARY COMMISSIONER WITH THE GOVERNMENT
OF INDIA, SIMLA.

CALCUTTA:
OFFICE OF THE SUPERINTENDENT OF GOVERNMENT PRINTING, INDIA.

1904

Agents for the Sale of Books published by the Superintendent of Government Printing, India, Calcutta.

IN ENGLAND.

E. A. ARNOLD, 37, Bedford Street, Strand, London, W.C.

CONSTABLE & CO., 2, Whitehall Gardens, London, S.W.

SAMPSON LOW, MARSTON & CO., St. Dunstan's House, Fetter Lane, London, E.C.

P. S. KING & SON, 2 & 4, Great Smith Street, Westminster, London, S.W.

LUZAC & CO., 46, Great Russell Street, London, W.C.

KEGAN PAUL, TRENCH, TRÜBNER & CO., Charing Cross Road, London, W.C.

BERNARD QUARITCH, 15, Piccadilly, London, W.

WILLIAMS and NORGATE, Oxford.

DEIGHTON BELL & CO., Cambridge.

ON THE CONTINENT.

R. FRIEDLANDER & SOHN, 11, Carlstrasse, Berlin, N.W.

OTTO HARRASSOWITZ, Leipzig.

KARL W. HIERSEMANN, Leipzig.

ERNEST LEROUX, 28, Rue Bonaparte, Paris.

MARTINUS NIJHOFF, The Hague.

IN INDIA.

THACKER, SPINK & CO., Calcutta and Simla.

NEWMAN & CO., Calcutta.

R. CAMBRAY & CO., Calcutta.

S. K. LAHIRI & CO., Calcutta.

THACKER & CO., LD., Bombay.

D. B. TARAPOREVALA, SONS & CO., Bombay.

A. J. COMBRIDGE & CO., Bombay.

RADHABAI ATMARAM SAGOON, Bombay.

HIGGINBOTHAM & CO., Madras.

V. KALYANARAMA IYER & CO., Madras.

G. A. NATESAN & CO., Madras.

RAI SAHIB M. GULAB SINGH AND SONS, Mufid-i-Am Press, Lahore.

N. B. MATHUR, Superintendent, Nazair Kanun Hind Press, Allahabad.

Superintendent, American Baptist Mission Press, Rangoon.

SOME OBSERVATIONS ON THE POISON OF THE BANDED KRAIT (BUNGARUS FASCIATUS).

IN recent papers [1] on the problems surrounding the physiological actions of the venoms of the different species of snakes and the antitoxic sera prepared with these venoms, I have brought forward facts, which show that no conclusions can be drawn regarding the action of the poison of one species of poisonous snake from the observations made on the action of the venom of another species, no matter how closely allied to each other the two species may be; facts, which show that the venom of each species must be studied separately. Let us take, for example, the case of the poisons of Naia tripudians (cobra) and of the snake known as Bungarus fasciatus. The former of these snakes is a species of the genus Naia and the latter of the genus Bungarus. Both these genera belong to the same sub-family, *viz.*, Elapinæ, of Colubridæ, and are very closely allied. Now, I have shown that an immune serum prepared with the venom of Naia tripudians causes a well-marked *precipitum* with this poison but has no such action when tested with the venom of Bungarus fasciatus. We can, therefore, conclude that the proteids of cobra venom are of a different nature, or at any rate do not possess the same 'haptophoric groups' as the proteids of the venom of Bungarus fasciatus. Further, I have shown that a serum antitoxic to cobra venom, *e.g.*, Calmette's serum, has no power to neutralise the venom of Bungarus fasciatus. Similar phenomena can be demonstrated in the case of the venoms of the cobra and of the Australian tiger-snake (Hoplocephalus curtus), which also belongs to the same sub-family, *viz.*, Elapinæ, of Colubridæ, as the cobra. Thus, a serum prepared with cobra poison, which causes a *precipitum* with this venom, has no precipitating action for the venom of Hoplocephalus curtus, and a serum which is highly antitoxic for the venom of Hoplocephalus curtus has no neutralising power for cobra poison. It is, therefore, evident that snake venoms, even the poisons of allied species, will probably differ markedly, if not entirely, from one another in physiological action, and that it is necessary to study more thoroughly than has hitherto been done the action of each venom separately. With this end in view I have taken up the study of the poisons of the various species of the poisonous Indian snakes. Regarding the venoms of the cobra and of the daboia some observations have already been published.[2] This work had special reference to the action of these poisons on the nervous system, on the red blood corpuscles and on the blood plasma. I have shown that those two venoms differ from one another in every detail of their physiological action.

In the present communication we shall consider some observations which were made with the venom of Bungarus fasciatus, and compare, as far as can be compared, the action of this poison with the actions of the two venoms mentioned above. When opportunity and material permit, I propose to do the same with the venoms of Bungarus caeruleus, Naia bungarus and Echis carinata.

Up to the present time very few observations have been made with the poison of any species of the genus Bungarus. As a result of a few experiments with the venom of Bungarus fasciatus, Wall [3] has come to the conclusion that this poison differs markedly in physiological action from the venom of the cobra. He has pointed out that experimental cases of intoxication with the venom of Bungarus fasciatus may be divided into two classes; (1) a class of acute cases, and (2) a class of chronic cases. In the acute cases death takes place within 48 or 72 hours after the injection of the poison. There is sometimes slight swelling at the site of inoculation: profuse salivation, accompanied sometimes by vomiting, is a common symptom: ultimately paralysis with twitching of the muscles developes: death rapidly follows from paralysis of respiration. Wall states that these cases present symptoms which are indistinguishable from the symptoms seen in the case of cobra venom intoxication; there is, however, one difference, namely, that the local reaction is not nearly so severe as is the case in cobra venom poisoning. At the *post-mortem* examination he found a slight effusion of a pale pinkish serum in the areolar tissue round the site of inoculation: there was no extravasation of blood and apparently little or no breaking up of the red cells. The blood was found to clot solidly on withdrawal from the vessels.

Wall has stated that in his experience in cases of cobra venom intoxication, if the animal survives 49 hours, ultimate recovery takes place. While not limiting myself to this exact interval of time, I can say from observations on a very great number of these cases, that if an experimental case of cobra venom poisoning is going to end fatally it does so within the first two or three days. Wall's second class of cases of intoxication with the venom of Bungarus fasciatus shows that the same statement cannot be made as regards this poison. For, in the class of chronic cases described by him death is long delayed, perhaps for ten or twelve days. After the injection of the poison there is an interval varying from two to six days, during which period no symptoms are observed, the animal being apparently in perfect health. After this interval of time a more or less chronic disease begins, which, Wall states, invariably ends fatally. The symptoms observed during this late period of intoxication are not in the least like the symptoms seen in the acute form of poisoning either with cobra venom or with the venom of Bungarus fasciatus itself. There are loss of appetite and great depression: there is a marked diminution in the urinary secretion.

Then follow a slight failure of the respiratory function and irregular elevations of temperature, accompanied by great muscular weakness. Purulent discharges from the eyes, nose and rectum are also seen. There is no tendency to hæmorrhages. Death closes this scene after a few days' illness.

As a result of the observation of the symptoms in these two groups of cases, Wall arrived at the conclusion that the venom of Bungarus fasciatus acts mainly on the central nervous system, causing paralysis in the acute cases and great weakness and depression in the chronic cases. On the coagulability of the blood he considered it had little or no action, although in the chronic cases he noticed that the coagulum was of a loose and imperfect character. Further, he thought that it had a slight destructive action on the red blood corpuscles, as shown by the pale pinkish exudate at the site of inoculation. This short description completes the summary of Wall's researches.

The observations which I shall bring forward in this communication, while confirming Wall's statements in almost every detail, further extend his results and also demonstrate some new facts as regards the action of this particular venom.

The material which was used in all my experiments was pure, dry, fresh venom of Bungarus fasciatus. This was collected, under my personal supervision, during a period of a month or two from two full-sized living snakes. The poison was obtained by allowing the snakes to bite through a sheet of American cloth tightly stretched over the mouth of a strong wine-glass. When the poison had been thoroughly dried over lime, it was added to the general stock, which was preserved in an air-tight tube kept from the light.

Before the venom was weighed, it was reduced to an impalpable powder and again dried over lime. In this way it was found possible to obtain a poison of uniform strength for all experiments at different intervals of time, thereby ensuring observations comparable amongst themselves.

Amount of coagulable proteid in the venom of Bungarus fasciatus.

The temperature at which the coagulable proteids were thrown down was found to be between 70° and 75° C. An experiment was made with the view of estimating the amount of proteid which was coagulated at this latter temperature. A reference to the protocols (series No. 1) will show that this amount was about 20 *per cent.* of the total venom.

I have already[4] detailed some similar experiments which were made with cobra and daboia venoms. As the samples of the venoms which were used for these estimations had been procured by squeezing the glands of the snakes, it was suggested to me by Professor Martin of Melbourne, that this rather rough treatment of the glands might cause a certain amount of serous exudation into them, and that in this way the coagulable proteids of the venom

might be augmented. To get rid of this possible fallacy I made another experiment with cobra venom, using the same *technique* as before, with the exception that the sample of venom was obtained from three snakes by allowing them to bite through a sheet of rubber stretched over a strong wine-glass, no pressure being made over the glands. The amount of coagulable proteid obtained in this experiment was found to be 24·6 *per cent.*, practically the same quantity as in the first observation, the details of which have already been published. We may, therefore, take it that squeezing the glands causes no increase in the amount of coagulable proteid contained in the venom.

For the sake of comparison the results of the estimation of the coagulable proteids in the three poisons with which I have worked are tabulated as follows :—

	Cobra venom.	Daboia venom.	Venom of Bungarus fasciatus.
Amount of proteid coagulable by heat.	24 *per cent.*	25 *per cent.*	20 *per cent.*

Rapid death, the result of the intravenous injection of the venom of Bungarus fasciatus.

I have mentioned above that Wall has divided cases of intoxication with this venom into two groups, and that he has accurately described the symptoms which are observed in those two classes of cases. There is, however, still another class of case, which would probably only be met with in experimental work.

When a comparatively large quantity of this poison is injected directly into the blood stream of an animal, death takes place within a few minutes after the injection. On *post-mortem* examination a more or less extensive intravascular thrombosis is found. The following two experiments will serve as illustrations of this class of case.

RABBIT 3.—Weight 1,700 grammes. Received intravenously 17 milligrammes of venom, *viz.*, 10 milligrammes per kilo. Death, preceded by general convulsions, opisthotonus and dyspnœa took place 2¾ minutes after the injection. At the *post-mortem* examination, made immediately after death, the blood in the portal vein and inferior vena cava was found to be liquid. The pulmonary arteries were full of solid clot. Thrombi were also found in the right auricle. There were no clots in either ventricle. The blood collected in a test-tube from the inferior vena cava clotted solidly soon after withdrawal. The serum which exuded from this clot was perfectly clear and free from staining with hæmoglobin.

RABBIT 18.—Weight 1,570 grammes. Received intravenously 6·28 milligrammes of venom, *viz.*, 4 milligrammes per kilo., along with 2 c.c. of Calmette's serum. General convulsions soon set in and ended in death, which took place 2½ minutes after the injection of the poison. At the *post-mortem* examination, made immediately after death, the blood in the portal vein and in the inferior vena cava was found to be liquid. Clots were found in the right heart and in the pulmonary arteries. Blood collected in a test tube from the inferior vena cava clotted solidly. Three hours after withdrawal no serum had exuded from this clot.

It would appear from these observations that, when death rapidly follows an injection of the venom of Bungarus fasciatus directly into the blood stream, a more or less extensive intravascular clotting is found on careful *post-mortem* examination performed immediately after death. Further, there can be no doubt that this thrombosis is the direct cause of the symptoms observed and of death in these cases.

I have already shown in another communication [5] that a similar result follows the injection of daboia venom, either intravenously or subcutaneously, if the quantity of venom be sufficient. But, while in the case of daboia venom a comparatively small amount, namely, about 0·1 milligramme per kilo., injected intravenously into a rabbit is sufficient to bring about this remarkable phenomenon, it requires a much larger quantity of the venom of Bungarus fasciatus to produce a like effect. For, while thrombosis and death may possibly result from the intravenous injection of 4 or 5 milligrammes per kilo. of this latter venom, it is necessary to inject as much as 10 milligrammes per kilo. to be certain of killing a rabbit in this way. In the same paper I showed that, when cobra venom, even in very large quantity, is injected directly into the blood stream of an animal, and when death results almost immediately afterwards, the most careful *post-mortem* examination does not reveal the least trace of intravascular clotting. The heart is still beating and the blood throughout is quite liquid. In these cases the fatal result is probably due to a direct action of the poison on the central nervous system. This question, however, is still under investigation and will form the subject of another communication.

Acute cases of intoxication with the venom of Bungarus fasciatus.

As a result of many experiments on different species of animals I have been able to confirm and extend the observations made by Wall, a summary of which has been given above. Let us first consider the cases he classifies as acute cases.

The symptoms which Wall has described in this group of cases are well

exemplified in the following experiments, which are typical of the results I have obtained :—

RABBIT 4.—Weight 1,640 grammes.

12-53 P.M.—Received intravenously 8·2 milligrammes of venom, *i.e.*, 5 milligrammes per kilo.

1-1 P.M.—Appears ill : the respiration much quickened : paresis of the legs.

1-4 P.M.—Paralysis of legs well marked.

1-6 P.M.—Completely paralysed in all legs.

1-8 P.M.—Dyspnœa with inspiratory effort.

1-9 P.M.—Slight convulsions : breathing slow and laboured.

1-10 P.M.—General convulsions.

1-11 P.M.—Died, *viz.*, 18 minutes after the injection of the poison.

Post-mortem examination, made immediately after death. The heart was beating vigorously. No clots were found in the portal vein, inferior vena cava, pulmonary arteries or right heart. Blood collected from the inferior vena cava in a test-tube clotted almost immediately.

RABBIT 22.—Weight 1,490 grammes.

1-35 P.M.—Received intravenously 5·96 milligrammes of venom, *i.e.*, 4 milligrammes per kilo.

1-55 P.M.—Appears ill : slight paresis.

2 P.M.—Completely paralysed : dyspnœa with inspiratory effort : general convulsions at intervals.

2-5 P.M.—Died, *viz.*, half an hour after the injection of the venom.

Post-mortem examination, made immediately after death. The heart was beating vigorously. No clots were found anywhere. Blood collected from the heart clotted solidly almost immediately.

RABBIT 5.—Weight 1,560 grammes.

1-30 P.M.—Received intravenously 5·5 milligrammes of venom, *i.e.*, 3·5 milligrammes per kilo.

2-30 P.M.—Slight paralysis : breathing rapid.

3-30 P.M.—Paralysis almost complete : dyspnœa.

4-35 P.M.—Paralysis complete : respiration slow, with inspiratory effort.

4-44 P.M.—General convulsions and tremors.

4-45 P.M.—Died, *viz.*, 3¼ hours after the injection of the venom.

Post-mortem examination, made immediately after death. Heart still beating, although feebly. No intravascular thrombosis was found. Blood collected from the inferior vena cava and the heart clotted immediately. No lesion of any organ was discovered.

RABBIT 6.—Weight 1,600 grammes.

3 P.M.—Received intravenously 4·8 milligrammes of venom, *i.e.*, 3 milligrammes per kilo.

3-40 P.M.—Legs appear to be paralysed.

3-45 P.M.—Paralysis of legs complete.

3-52 P.M.—Marked dyspnœa.

3-55 P.M.—Slight convulsive movements, repeated at short intervals: respiration slow, with inspiratory effort.

3-59 P.M.—Died, *viz.*, 59 minutes after the injection of the venom.

Post-mortem examination, made immediately after death. Heart still beating, although feebly. No clots were found in any of the vessels. Blood collected from the heart and the inferior vena cava clotted solidly immediately on withdrawal. The serum which exuded from the clots was quite clear and free from hæmoglobin staining.

RABBIT 10.—Weight 1,280 grammes.

20th January, *1 P.M.*—Received intravenously 0·96 milligramme of venom, *i.e.*, 0·75 milligramme per kilo.

21st January.—Is ill and refuses food: slight paralysis of legs.

22nd January.—Paralysis almost complete.

1 P.M.—Died, from paralysis of respiration.

Post-mortem examination.—*Rigor mortis* well marked. The upper and lower lobes of the right lung as well as the upper lobe of the left lung were infiltrated with blood, which was quite liquid. No clots were found anywhere. Blood collected in test tubes from the heart and the inferior vena cava clotted slowly, namely, in about 11 minutes: the clots were loose and not at all firm.

RABBIT 14.—Weight 1,530 grammes.

20th January, *1 P.M.*—Received subcutaneously 3·8 milligrammes of venom, *i.e.*, 2·5 milligrammes per kilo.

21st January, *1 P.M.*—Is very ill: paralysis nearly complete: considerable swelling around the site of inoculation.

3 P.M.—Died.

Post-mortem examination.—Slight serous exudation at the site of inoculation but no ecchymosis. All the organs appear healthy. Blood collected in test tubes from the heart and the inferior vena cava clotted only after some time: the clots were very loose and soft.

These six cases, examples of many I have observed, confirm in a striking manner the accurate observations made by Wall, the results of which have been summarised above. It is, therefore, unnecessary to again detail the symptoms which one meets with in these cases. It is sufficient to draw attention to the fact that no difference can be seen between the symptoms observed in these cases and the symptoms seen in cases of cobra venom intoxication. It is probable that death is due in both instances to the action of the poison on the central nervous system, paralysis of respiration being the primary cause of death. I shall

return later on to the consideration of the action which the venom of Bungarus fasciatus has on the blood plasma and on the red blood corpuscles.

Chronic cases of intoxication with the venom of Bungarus fasciatus.

The observations which I have made in this class of cases confirm Wall's statement, namely, that there is a chronic form of intoxication following the injection of this venom, a condition which is never met with in cases of cobra venom poisoning.

The following experiments will serve as examples of this disease, which has been studied both in monkeys and in rabbits :—

MONKEY 5.—Weight 1,950 grammes.

4th February 1902.—Received subcutaneously in the front of the chest 4·87 milligrammes of venom, *i.e.*, 2·5 milligrammes per kilo.

5th February.—No swelling or extravasation of blood at the site of injection. Is not eating well, but appears to be otherwise in good health.

6th February.—Is eating well and seems to be quite well.

7th February.—Weight 1,845 grammes. Is very ill to-day : is not eating and is much depressed. There is great muscular weakness, and apparently slight paralysis.

8th February.—Weight 1,771 grammes. There is much emaciation and muscular weakness : paralysis is almost complete. Died at 2 P.M. to-day.

MONKEY 2.—Weight 1,640 grammes.

27th February 1902.—Received subcutaneously in the front of the chest 6·2 milligrammes of venom, *i.e.*, 3·7 milligrammes per kilo.

28th February.—Very slight swelling at the site of injection. Œdematous swelling of the abdominal wall between the pubes and umbilicus. The animal otherwise is in good health.

1st March.—Weight 1,500 grammes. Appears ill and is not eating. There is no paralysis.

2nd March.—Is apparently in good health.

3rd March.—Weight 1,330 grammes. Is very ill and depressed, and has become very emaciated. It is unable to walk, and when laid down on its side is unable to rise up. In addition to this great muscular weakness there appears to be slight paralysis, affecting principally the hind legs. There is considerable muscular atrophy.

4th March.—Died this morning.

Post-mortem examination.—The animal was much emaciated. No lesion of any kind was found at the site of inoculation. All the internal organs appeared normal. The medulla, cord and motor area of the cortex were removed for special microscopical examination, the results of which will be detailed later.

MONKEY 6.—Weight 1,260 grammes.

29th April 1902.—Received subcutaneously in the front of the chest 3·15 milligrammes of venom, *i.e.*, 2·5 milligrammes per kilo.

30th April.—Weight 1,200 grammes. Is eating and appears quite well. There is no swelling at the site of inoculation.

1st May.—Weight 1,170 grammes. Is ill and appears depressed. There is no paralysis.

2nd May.—Weight 1,190 grammes. Is better than it was yesterday, but is still depressed.

3rd May—7th May.—Continued dull and ill, but without any marked symptoms.

8th May.—Weight 940 grammes. Is eating nothing: has become much emaciated: there is great muscular atrophy and weakness: appears to be completely paralysed.

9th May.— Died this morning.

Post-mortem examination.—The body was much emaciated. No lesion of any kind could be found. Pieces of the central nervous system were removed for special histological examination, the results of which will be detailed later.

RABBIT 9.—Weight 1,590 grammes.

20th January 1902.—Received intravenously 1·27 milligrammes of venom, *i.e.*, 0·8 milligramme per kilo.

21st January.—Is eating: on being stimulated there is slight general twitching of the muscles: there is no paralysis.

22nd January.—Weight 1,380 grammes. Is eating and appears quite well.

23rd January.—Weight 1,360 grammes. Is very ill and depressed, and is not eating well. Has become considerably emaciated.

24th January.—Weight 1,180 grammes. Is very ill, greatly depressed and emaciated: muscular atrophy marked: there does not appear to be any paralysis.

25th January.—Moribund: was killed.

Post-mortem examination.—No lesion of any kind was found in the internal organs.

RABBIT 12.—Weight 1,380 grammes.

20th January 1902.—Received intravenously 0·82 milligramme of venom, *i.e.*, 0·6 milligramme per kilo.

21st January—27th January.—During this time the animal appeared to be in good health, although there was a progressive loss of weight. The weight on 27th January was 1,130 grammes.

28th January.—Weight 1,151 grammes. Is very ill and depressed: muscular atrophy and weakness marked: there is no paralysis: a thick purulent discharge from the eyes is present.

29th January and 30th January.—Still very ill and depressed with purulent discharge from the eyes.

Weight on 30th January was 1,170 grammes.

31st January.—The animal is much better to-day and is eating well. After this date it gradually improved: the weight increased rapidly, and the animal ultimately recovered.

RABBIT 15.—Weight 1,610 grammes.

20th January 1902.—Received subcutaneously 4·8 milligrammes of venom, *i.e.*, 3 milligrammes per kilo.

21st January.—There is slight swelling at the site of inoculation: appears slightly ill: there are, however, no marked symptoms.

22nd January.—Weight 1,430 grammes. Is not eating much, but otherwise appears to be in good health.

23rd January.—Weight 1,450 grammes. Depressed, but no marked symptoms.

24th January.—Weight 1,390 grammes. Is very ill to-day: much emaciated; great muscular weakness.

25th January.—Weight 1,410 grammes. Has improved since yesterday.

26th January.—Is depressed and weak.

27th January.—Weight 1,140 grammes. Extreme emaciation and atrophy: is very depressed: much muscular weakness, but no apparent paralysis.

28th January.—Found dead this morning.

Post-mortem examination revealed no lesion of any of the internal organs.

As the above six experiments are typical examples of the chronic form of intoxication with this venom, it is unnecessary to detail any more cases of this class. It will be seen from the above data that this chronic condition of poisoning follows either an intravenous or a subcutaneons injection of the venom, if the amount injected is not large enough to cause the acute form of intoxication. Further, the symptoms of this chronic intoxication are well marked and distinctive. Such a condition is never met with in experiments with cobra venom nor, in fact, with the venom of any other species of snake with which I have worked.

These experiments, while confirming in the main points Wall's observations, show that his description can be somewhat modified. Thus, he states that no symptoms are observed during the first few days after the injection of the poison. In my experience I have generally found that the animals are depressed and refuse their food during this interval. Further, a careful weighment of the animals shows that there is a progressive loss of weight even during this early period, (*vide* monkey 6, and rabbits 9, 12, and 15). Again, Wall does not mention the occurrence of paralysis in these chronic cases. He looks upon the condition as being only one of great muscular weakness. I have not been able to satisfy myself that there is no paralysis. There is, no doubt,

great muscular atrophy and, as a result, great weakness. In some of my cases, however, I am inclined to think that paralysis accompanied this muscular atrophy. Lastly, Wall has mentioned that no case under his observation had recovered, if once these late symptoms had begun. I have observed one such case which recovered, *i.e.*, rabbit 12. This animal, as we have seen, showed well marked symptoms, namely, loss of appetite and depression, great loss of weight, muscular atrophy and a purulent discharge from the eyes. It ultimately made a good recovery. This condition, even when well marked, can be recovered from. In the great majority of cases, however, if the typical symptoms have once appeared, a fatal result follows.

It is important and interesting to be able to put on record the histological appearances which were found in the central nervous tissues of two of these chronic cases, namely, monkeys 2 and 6. I am greatly indebted to Dr. Walter K. Hunter of Glasgow for cutting, examining and describing the sections of the tissues with which I provided him.

The following are Dr. Hunter's reports:—

MONKEY No. 2.—The tissues were fixed in perchloride of mercury, cut in paraffin and stained according to Nissl's method by thionin and toluidin blue. The parts examined were cortex, medulla and spinal cord. Sections were taken from four levels of the medulla, from three levels of the cervical cord, from three levels of the dorsal cord and from two levels of the lumbo-sacral enlargement.

In all of these there is evidence of the existence of diffuse chromatolysis (primary degeneration), affecting a very considerable portion of the ganglion cells. The change is most marked in the cortex, where one has difficulty in finding a single normal cell. The vast majority of the cells have a rather deeply stained plasma, and in this are scattered dust-like granules, the remnants of the Nissl bodies. Many cells, too, show vacuolation of their plasma. This vacuolation may probably be regarded as a fatty change. A few cells have their Nissl bodies not yet entirely fragmented, but these are few in number, and it is doubtful if one perfectly normal cell is to be found. In the medulla the changes are not nearly so marked as in the cortex, but nevertheless throughout the whole of the sections at the different levels in the medulla a very considerable proportion of degenerated cells is to be seen. These are most numerous in the VIIIth nucleus, less so in the XIIth and less still in the Xth motor. In the XIIth nucleus nearly all the cells show a certain breaking up of the Nissl granules, but only in a small number of cells is there the typical dust-like fragmentation affecting the whole cell as seen in the cells of the cortex.

The degenerative process is more marked in the cord than in the medulla, though less so than in the cortex. It is about equally marked in the cervical, dorsal and lumbo-sacral regions. In many of the sections through the cervical

and lumbo-sacral enlargements not more than 3 or 4 normal cells are to be seen. Many cells have an almost clear plasma with a few dust-like granules scattered throughout, but most of the cells have a darker plasma with more of these dust-like granules. The degenerative process seems to affect the whole of the cell uniformly, though in a few cells the fragmentation is more marked at the periphery than round the nucleus. The nucleus in almost all the cells still remains central.

Considering the appearances generally it may be said that the chromatolysis above described does not present the typical picture such as one sees it, *e.g.*, in a case of alcoholic neuritis. In the latter case the dust-like fragments are better defined and more closely and orderly packed in the cell plasma. In the case before us the fragmented granules are more scattered and, as has been noted in the cortical cells, there is the vacuolation of the plasma. The process here seems as if it were more acute than in alcoholic neuritis, more as if the granules were being dissolved out, rather than definitely broken up.

MONKEY No. 6.—The parts of the nervous system examined were cortex, pons, medulla and cord. Sections from these were stained by Nissl's method. All the preparations showed at least some degeneration of cells, and in some of the sections the degeneration was extremely well marked. Generally it was more marked in the cells of the cord and cortex than in those of the nuclei in the medulla and pons.

In the cervical cord not a single normal cell was to be found. Many cells were simply outlines, without any granulation whatever ('ghost cells'). Some cells stained more deeply and more diffusely than normal and the Nissl granules, when present, were always fragmented. A good many cells were definitely vacuolated. There seemed, too, a considerable diminution in the number of the cells in each anterior horn. It was difficult to estimate this accurately, but judging by comparison with monkey No. 2 the loss was undoubtedly almost two-thirds of the normal number. In this cervical region some of the sections included the posterior root ganglia. But the change in the cells of these ganglia was much less marked than in the ganglion cells of the anterior horns. Indeed, many of the posterior root ganglion cells seemed quite normal, and few showed any marked degeneration. The contrast between the condition of the anterior horn cells and the posterior root ganglion cells is a point to which one would draw special attention. The appearances at the dorsal, lumbar and sacral levels were much the same as were found in the cervical cord.

Of the ganglion cells in the medulla and pons a considerable proportion showed well marked chromatolysis, but the changes were by no means so extreme as those just described in the cord. This doubtless explains why movements of the heart and respiration continued some time after paralysis of the other muscles of the body.

In the cortex the degenerative change was about as marked as in the cord, there being many 'ghost cells' and many more deeply stained cells, some with fragmented granules and some with no granules at all. A considerable proportion of the cells was vacuolated. It is difficult to give a reliable opinion as to whether or not there was any considerable loss in the number of the cells of the cortex.

The importance of these observations is at once evident. We have now direct evidence of the action of the venom of Bungarus fasciatus on the nervous system, an action which results in marked degenerative changes in the ganglion cells of the cortex, cord, medulla and pons. These degenerative changes afford also an explanation of the symptoms which are observed in these chronic cases of intoxication which I have described above. The depression, muscular atrophy and paralysis are no doubt dependent on these pathological changes which are so striking and well marked.

With these preliminary observations we have opened up a fruitful line of research, the results of which should set at rest many disputed points as regards the physiological actions of the venoms of the different species of poisonous snakes. They should also, no doubt, throw some light on neuro-pathological problems in general.

The action of the venom of Bungarus fasciatus on the blood plasma *in vivo* and *in vitro*.

We have already seen (*vide* page 4) that, when a sufficiently large quantity of the venom of Bungarus fasciatus is injected directly into the blood stream of a rabbit, death takes place very rapidly, and that on *post-mortem* examination made immediately after death a more or less extensive intravascular thrombosis is found. This clotting is confined to the pulmonary arteries and right heart and does not appear to be present in the portal system of veins. The quantity of poison which is necessary to produce this result is very much greater than is the case with daboia venom. Further, the thrombosis is not nearly so marked or so widespread as one finds with this latter venom. There can be no doubt, however, that we are also dealing in this instance with the phenomenon of an increase of blood coagulability, brought about by the action of the poison on the plasma, so great as to lead to an intravascular clotting in those vessels in which it is more liable to occur, and that death in these cases is due to thrombosis of the pulmonary arteries.

We have, now, to consider what is the condition of the blood coagulability in those cases of intoxication, in which this temporary increase does not result in a fatal thrombosis. When death takes place within a few hours after the

injection of the poison, as we have seen occurs in the more rapidly fatal of the acute class of nervous cases, the blood taken from the heart and from the inferior vena cava is found to clot almost immediately after withdrawal, and the clots which form are firm and solid. This condition of blood coagulability was found in four rabbits. The details of the experiments on these rabbits have already been described, *viz.*, rabbits 4, 5, 6 and 22. It is evident, then, that in this short interval of time, *viz.*, up to three or four hours, and with the quantities of venom used, no appreciable diminution of the blood coagulability had developed.

When, however, death is delayed for a day or two after the injection of the venom, there is evidence to show that an appreciable deficiency of blood coagulability has developed. Thus, in the case of two rabbits, namely, Nos. 10 and 14, the experiments on which have been recorded above, it was found that the blood collected from the heart and from the inferior vena cava at the *post-mortem* examinations made immediately after death clotted much more slowly than normally, and that the clot which formed was loose and imperfect. These animals died 48 and 26 hours, respectively, after the injection of the venom. In addition to these observations the blood coagulation time has been estimated at intervals in the case of five animals, namely, two rabbits and three monkeys, which received an amount of venom that did not produce any acute nervous symptoms, and which later on developed nervous symptoms of the chronic type, to which I have already drawn attention. The details of these observations are given in the protocols, series No. II. A reference to this table will show that in every instance a considerable deficiency in the blood coagulability was present. This deficiency was observed on the first or second day after the injection of the poison and persisted, as a rule, till the disease was ended by death or by recovery.

In previous papers[6] I have recorded similiar series of observations made with both cobra and daboia venoms. In the case of the former poison I was able to show that a slight diminution of blood coagulability takes place after the subcutaneous injection of non-lethal doses, and that this diminution of blood coagulability was more marked the nearer the quantity of venom injected approached to the minimum lethal dose. Further, in cases of cobra venom poisoning one seldom sees any symptoms which might be due to this deficiency of blood coagulability. Lastly, this poison causes no primary phase of increased coagulability.

With daboia venom the lengthening of the blood coagulation time is much more marked, amounting in some instances to a complete loss of coagulability, the blood when drawn from the vessels remaining permanently liquid. Further, in the case of intoxication with daboia venom I pointed out that symptoms, such as widespread œdema and hæmorrhages, were observed, and that the marked defi-

ciency of blood coagulability might be an ætiological factor in the production of these symptoms.

In the case before us now, *i.e.*, that of intoxication with the poison of Bungarus fasciatus, the diminution of the blood coagulability is by no means excessive and cannot be said to be an important factor in the production of any general symptoms observed. For, with the exception of an insignificant local swelling at the site of injection no œdema is produced nor have any hæmorrhages been observed to occur. The symptoms referable to the action of the poison on the nervous system are the predominant symptoms. The action of this venom on the blood plasma in these chronic cases would appear to be of very secondary importance.

We can now pass on to consider if the venom of Bungarus fasciatus has any action on the coagulability of blood plasma *in vitro*. In one of the papers [7] above referred to I have put forward a method of testing *in vitro* the action of snake venoms on whole blood and on plasma freed from red cells. This method consists in adding different amounts of venom to blood, which has been kept liquid by the addition of citrate of soda, and to citrate plasma which has been completely freed from the red corpuscles by allowing them to sediment and then syphoning off the clear supernatant fluid. Lime, in the form of a solution of chloride of calcium, is added in the quantity required. Working in this way I showed that cobra venom completely prevents the clotting of citrate plasma, which results normally from the addition of a small quantity of a soluble salt of lime, and that daboia venom has a marked effect in increasing the coagulability of citrate plasma. Even in small quantities, however, this latter venom does not produce *in vitro* any diminution of blood coagulability, which is such a prominent action *in vivo*.

This method was employed to study the action of the venom of Bungarus fasciatus on citrate plasma *in vitro* (*vide* protocols, series Nos. III and IV).

In the first series of observations varying amounts of the venom were added to 2 c.c. of citrate plasma, the plasma both of the donkey and of the horse being employed. It was found that even 5 milligrammes of venom did not produce after 24 hours a trace of clot in this amount of plasma. Now, I have already shown that daboia venom in small quantities causes a firm clotting of citrate horse plasma. It was found that any quantity of this poison between 3 and 5 milligrammes caused a firm clot in 2 c.c. of citrate horse plasma in less than three hours; that with an amount between 0·4 and 1 milligramme clotting occurred but was delayed for about 20 hours; and that 0·2 milligramme caused only a slight clot after 20 hours. The explanation of the failure in the experiments with the venom of Bungarus fasciatus to cause clotting of citrate plasma *in vitro* is probably to be found in the fact, that the venom was not added to the

plasma in sufficient quantity. We have seen above that 8 to 10 milligrammes per kilo. of this venom injected rapidly into the blood stream of a rabbit are required to be certain of so increasing the blood coagulability as to lead to a fatal intravascular thrombosis. Now, I have stated that a fatal intravascular clotting can be brought about by 0·1 milligramme per kilo. of daboia venom, when injected intravenously into a rabbit, a quantity 80 to 100 times less than the amount of the venom of Bungarus fasciatus required to produce the like effect. I have also mentioned that 0·4 milligramme of daboia venom causes a firm clot to form after 20 hours in 2 c.c. of citrate plasma. Therefore, if there is any analogy between the actions of these two venoms on the blood plasma, an analogy which is almost certain in consideration of their actions on the blood coagulability *in vivo*, we should expect that it would require between 30 and 40 milligrammes of the venom of Bungarus fasciatus to produce any clot in 2 c.c. of citrate plasma, provided always, as was the case in my experiments, that the amount of citrate in the samples of plasma used was the same. It is to be regretted that the quantity of venom at my disposal did not allow of such a large amount as this being used. Therefore, until experiments are carried out with much larger quantities of poison than I used, it is impossible to affirm that the action of the venom of Bungarus fasciatus on the plasma is in any way different from the action of daboia venom. It can, however, be said that daboia venom is in this respect of much greater potency than the poison of Bungarus fasciatus.

We have seen above that in the more or less chronic cases of intoxication with the venom of Bungarus fasciatus there is a certain amount of deficiency in the blood coagulability, but that there are few or no symptoms which can be said to depend on this deficiency. A second series of experiments was undertaken with the view of ascertaining if this poison had the power of preventing the clotting of citrate plasma which results from the addition of a soluble salt of lime. To each tube containing 2 c.c. of plasma varying amounts of venom were added. From one to two hours afterwards there was added to each tube a quantity of calcium chloride solution which clotted the control in less than 15 minutes. A reference to the protocols (series No. IV) will show that in every instance, both in the case of donkey plasma and in the case of horse plasma, solid clotting took place after the addition of the lime solution.

In the paper to which I have referred above I showed that cobra venom tested in the same manner, prevented the clotting of citrate plasma. The action, therefore, of cobra venom on the blood plasma is evidently of a different nature from that of the venom of Bungarus fasciatus. I showed, further, that daboia venom, even in infinitesimal quantities, did not prevent this clotting.

From the consideration of all these experiments and observations it would appear that the action of the venom of Bungarus fasciatus on the coagulability

of the blood plasma is analogous to that of daboia venom. The positive phase of increased coagulability, which is seen *in vivo* when large quantities of this venom are injected directly into the blood stream, is comparable to the similar phenomenon brought about by small amounts of daboia venom. Again, both these poisons produce *in vivo* a secondary negative phase of diminished coagulability. This condition, however, is much better marked in cases of daboia venom intoxication than in cases of poisoning with the venom of Bungarus fasciatus, and is no doubt a factor in the ætiology of several of the symptoms which are seen in cases of poisoning with the former venom. Further, when tested *in vitro* with citrate plasma both these poisons failed to produce any diminution in the normal coagulability of this plasma. The only discrepancy in the analogy between these two venoms, as far as their action on the coagulability of plasma is concerned, is the failure of the venom of Bungarus fasciatus to produce clotting of citrate plasma. I have already explained the reason of this failure in my experiments, namely, a deficiency in the amount of venom added to each preparation. While, therefore, it is certain that the actions of these two poisons on the blood plasma are similar, we have seen that daboia venom is, as regards this particular action, of much greater potency than the poison of Bungarus fasciatus, the action of which is mainly on the nervous system. In this connection of analogous action of the venoms of Daboia Russellii and Bungarus fasciatus it is interesting to remember that the former snake is classified among the Viperidæ, and the latter among the Colubridæ.

The action of the venom of Bungarus fasciatus on the red blood corpuscles *in vivo* and *in vitro*.

All experiments go to show that the venom of Bungarus fasciatus has only a slight destructive action *in vivo* on the red blood corpuscles. In his description of the physiological action of this poison Wall draws attention to the fact that in a few fatal experimental cases he found an effusion of a pale pinkish serum into the areolar tissue around the site of inoculation. In no instance does he mention any symptoms or *post-mortem* appearances which might be dependent on any great destruction of the red cells. The experience I have had with this venom points to the same conclusion. I have never seen any hæmoglobin-stained exudations, nor any red-stained discharge from any orifice. Further, at many *post-mortem* examinations, I have collected blood from the heart and from the larger vessels, have allowed this blood to clot and have noted that the serum which exuded from the clot was perfectly clear and free from hæmoglobin. The venom of Bungarus fasciatus, therefore, *in vivo* has only a slight if any destructive action on the red blood corpuscles. Experiments were next undertaken to ascertain if this poison had any

destructive action *in vitro* on the red blood corpuscles of the rabbit. The method which was used in these experiments was the following. The venom was dissolved in a solution of common salt which was isotonic[8] for the red cells of the rabbit. The strength of this solution was 1 c.c.=2 milligrammes, namely, 0·2 per cent. From this original solution a succession of two-fold dilutions, made with salt solution of the same strength as used for the original solution of venom, was prepared in a series of test tubes, so that each dilution contained half the amount of the poison of the one just above it in the series. A convenient quantity, namely, 0·5 c.c., of each dilution was then measured into a series of small test-tubes. To each tube a measured quantity, namely, 0·005 c.c., of fresh blood was added by means of a graduated capillary pipette. A control tube of salt solution and blood alone was also prepared. The preparations were allowed to stand at laboratory temperature (about 25° C.) for 20 hours, when the results were recorded. At the same time observations were made in an exactly similar manner with a heated solution of venom. A 0·2 *per cent.* solution was heated in a water bath for half an hour at 75° C. and then filtered. The filtrate was treated in the same way as the original unheated solution. The results of these observations are detailed in the protocols, series No. V. A glance at this table enables us to come to the conclusion that the venom of Bungarus fasciatus has only a slight destructive action on the red corpuscles *in vitro* and, further, that the heated venom has the same hæmolysing power as the unheated venom. In consideration of this result, and also in consideration of the observations *in vivo* to which attention has been already drawn, we can conclude that the action which the venom of Bungarus fasciatus has on the red blood corpuscles is slight and of quite minor importance compared with its action on the nervous system.

Toxicity of the venom of Bungarus fasciatus.

In order to compare the toxicity of this poison with the toxicity of the venoms of other species of snakes with which I have worked, several series of experiments were undertaken. The results of these observations have now to be put on record.

In the first place, the minimum lethal dose for rabbits of the venom under consideration was determined both by intravenous and subcutaneous injection. A reference to the protocols (series Nos. VI and VII) will show that it was found that 0·7 milligramme per kilo. was the smallest quantity which killed a rabbit when the injection was made directly into the blood stream, but that 2·5 to 3 milligrammes per kilo. were required to cause death when the injection was made under the skin.

In the second place, a series of experiments was undertaken to determine the

minimum lethal dose of this venom for rats of about 118 grammes in weight. A glance at the protocols, series No. VIII, will show that the results following the injection of small quantities of venom into rats were variable. Thus, it was found that in two instances 0·5 milligramme caused a fatal result, while in another case 1·5 milligrammes brought on very urgent symptoms but did not kill. Again, in one instance one milligramme caused death, while in another experiment this amount failed to kill. It appears, therefore, that in order to be certain of producing a fatal result a greater amount than 1·5 milligrammes would have to be given.

In the third place, the minimum lethal dose of the venom of Bungarus fasciatus for monkeys, when the poison is injected subcutaneously, was determined. A reference to the protocols, series No. IX, will show that this amount was ascertained to be about 3 milligrammes per kilo.

With a view of comparing the toxicity of this poison with that of cobra venom, two series of experiments were made to determine the minimum lethal dose of this latter venom for rabbits and monkeys, respectively, when the injections are made subcutaneously. It was found (*vide* protocols, series Nos. X and XI) that 0·35 milligramme per kilo. for rabbits and 0·25 milligramme per kilo. for monkeys were the smallest amounts which were able with certainty to produce death. In previous series of experiments,[9] which have been already published, I demonstrated that the minimum lethal dose of cobra venom for rats of about 118 grammes in weight was about 0·05 milligramme: it was found to vary between 0·04 and 0·07 milligramme according to the sample of the venom employed. From these data we can construct the following table, showing the minimum lethal doses of the venoms of the cobra and Bungarus fasciatus for three species of animals, when the poison is injected subcutaneously :—

	Rabbits.	Rats.	Monkeys.
Cobra venom	0·35 milligramme per kilo.	0·04 to 0·07 milligramme.	0·25 milligramme per kilo.
Venom of Bungarus fasciatus .	2·5 to 3 milligrammes per kilo.	1·5 milligrammes .	3 milligrammes per kilo.

From this table it will be seen that cobra venom is at least ten times as toxic as the venom of Bungarus fasciatus.

It is more difficult to compare the degree of toxicity of the venom of Bungarus fasciatus with that of the venom of Daboia Russellii. We have seen, however, that it requires about 10 milligrammes per kilo. of the venom of Bungarus fasciatus, injected intravenously into a rabbit, to cause intravascular clotting, while as small a quantity as 0·1 milligramme per kilo. of daboia poison injected in the same way kills a rabbit from thrombosis. As regards this

particular physiological action daboia venom is about 100 times more potent than the poison of Bungarus fasciatus. Injected subcutaneously into a rabbit daboia venom varies greatly as regards the smallest amount which can cause death. As a result of a great number of experiments on rabbits I have found that a dose of from 1 to 2 milligrammes per kilo is required in most cases. Thus, when given subcutaneously the venom of the daboia is still somewhat more toxic than the poison of Bungarus fasciatus, for we have seen that from 2·5 to 3 milligrammes per kilo of this latter venom is required to kill a rabbit.

The neutralising power of two antivenomous sera for the venom of Bungarus fasciatus.

I have now to place on record some observations which were made to test the neutralising power of two antivenomous sera for the poison of Bungarus fasciatus. The details of these observations have been already incorporated in a paper on the 'Specificity of Antivenomous Sera'.[10] It is, however, thought that the present observations would not be complete unless some reference was made to the results obtained.

In the first place, two series of observations were made with Calmette's serum, an antivenomous serum which this observer claims to be efficacious against the venom of all species of snakes.[11] In both series of experiments rabbits were the animals employed, and the injections were made directly into the blood stream. We have already seen (*vide* protocols, series No. VI) that the minimum lethal dose of Bungarus fasciatus venom for rabbits by intravenous injection is 0·7 milligramme per kilo. In the first series of experiments (*vide* protocols, series No. XII A) four milligrammes, *i.e.*, about six lethal doses, were mixed *in vitro* with different quantities of serum. The mixture was allowed to stand at laboratory temperature (about 25° C.) for half an hour. It was then injected into the marginal vein of the rabbit's ear. A glance at the table appended will show that even 5 c.c. of serum were of no avail to save the life of the animal, the rabbit which received this amount of serum along with the test dose dying after practically the same interval of time as the control animal. In the second series of experiments with Calmette's serum and the venom of Bungarus fasciatus (*vide* protocols, series No. XII B) two milligrammes of venom, *i.e.*, about three lethal doses, were used as the test dose. With this exception the same *technique* was employed as in the previous series of experiments. It will be seen by reference to the protocols that even with this test dose, small multiple as it was of the minimum lethal dose, 5 c.c. of serum were not able to preserve the life of the animal. The rabbits, however, which received 4 c.c. and 5 c.c. of serum, respec-

tively, lived somewhat longer than the control animal. Inasmuch as the test dose in these cases was such a small multiple of the minimum lethal dose, this result does not appear to me to influence the conclusion to which we are led by these two series of observations, *viz.*, that Calmette's serum has practically no neutralising power for the venom of Bungarus fasciatus.

In the second place, a series of experiments was made with a serum prepared by Dr. Tidswell of Sydney with the venom of the Australian tiger-snake (Hoplocephalus curtus). This snake belongs to the same sub-family, *viz.*, Elapinæ, of Colubridæ, as Bungarus fasciatus. The details of the preparation of this venom and the results which he obtained with it are to be found in a paper by Dr. Tidswell in the Australasian Medical Gazette.[12] It need only be mentioned here, that Tidswell found this serum to be strongly antitoxic to the venom with which it was prepared, but to be quite inactive towards the venom of three other Australian snakes. For this series of experiments rabbits again were the animals employed. The test dose of venom used was 4 milligrammes per kilo., namely, about six lethal doses. This test dose and different quantities of serum were mixed *in vitro*. The mixture was allowed to stand at laboratory temperature for half an hour. It was then injected into the marginal vein of the ear of a rabbit. It will be seen from the protocols that the rabbit which received 5 c.c. of serum along with the test dose died in even a shorter time than the control which received no serum. We have, therefore, to conclude that the serum had failed to neutralise even a modicum of the venom. The bearing of the results of these observations has already been entered into in full in the paper from which they are now summarised. It need not further be discussed here.

We may summarise the main conclusions to be drawn from the above observations as follows:—

(1) Cases of intoxication with the venom of Bungarus fasciatus can be divided into three classes:—

(*a*) Cases in which rapid death due to intravascular thrombosis follows intravenous injection of large quantities of venom.

(*b*) Cases which present acute nervous symptoms and which terminate fatally within 2 or 3 days after the injection of the poison. These cases are indistinguishable, as far as symptoms are concerned, from cases of cobra venom intoxication.

(*c*) Cases which run a chronic course and end fatally between the 6th and 12th day after the injection of the poison. Such cases are peculiar to intoxication with this venom and present marked special symptoms. A histological examination of the nervous system in these cases shows a well marked primary degeneration of the cells of the central nervous system.

(2) The venom of Bungarus fasciatus has an action on the blood plasma *in vivo*. Injected directly into the blood stream in considerable quantity it produces an increase of the blood coagulability which may end in a fatal intravascular thrombosis. In the more or less chronic cases of intoxication there is a deficiency in the blood coagulability, but this deficiency cannot be said to be a factor in the ætiology of any symptoms observed. No definite results were obtained with citrate plasma *in vitro*.

(3) The venom of Bungarus fasciatus has only a slight destructive action on the red blood corpuscles. This action is of little or no account as far as the production of symptoms is concerned.

(4) The venom of Bungarus fasciatus is much less toxic than the venom either of the cobra or of the daboia.

(5) The venom of Bungarus fasciatus is not neutralised either by the serum prepared by Calmette of Lille or by a serum prepared by Dr. Tidswell of Sydney with the poison of another colubrine snake, namely, Hoplocephalus curtus.

Protocols.

SERIES NO. I.—Experiment to estimate the amount of coagulable proteid contained in the venom of Bungarus fasciatus.

A considerable quantity of the dried venom, *viz.*, 93 milligrammes, having been carefully weighed, was dissolved in a measured quantity of doubly-distilled water. The strength of this solution was 1 *per cent.* The solution was then heated in a water bath at 75° C. for half an hour. It was then thrown on to a weighed filter paper, which had been previously dried in a hot water oven at 100° C. till it reached constant weight. The clear filtrate was caught in a thoroughly cleaned and weighed platinum basin.

The filter was well washed through with small quantities of distilled water. It was then, along with the coagulated proteid, again dried in a water oven at 100° C. until a constant weight was recorded. The filtrate in the platinum basin was evaporated to dryness in a water bath at 60° C. After thorough desiccation over lime the basin with the residue was again weighed.

The following were the results obtained :—

Amount of venom	= 0·093 gramme.
Strength of solution	= 1 *per cent.*
(*a*) Weight of filter paper and coagulated proteid	= 0·2982 gramme.
Weight of filter paper alone	= 0·2803 „

∴ Weight of coagulated proteid = 0·0179 gramme.
(*b*) Weight of platinum basin and dried residue =56·7133 „
Weight of platinum basin alone =56·6389 „
∴ Weight of dried residue = 0·0744 „

Thus, there were recovered from 93 milligrammes of venom 17·9 milligrammes of coagulated proteid and 74·4 milligrammes of incoagulable residue, a total of 92·3 milligrammes. We arrive, therefore, at the following percentage composition :—

Coagulable proteids	20 *per cent.*
Incoagulable residue	80 „ „

SERIES NO. II.—Experiments to ascertain the blood coagulation time at intervals in chronic cases of intoxication with the venom of Bungarus fasciatus.

The method which was adopted to estimate the coagulation time of the blood in these observations was the method which has been elaborated by Professor A. E. Wright. As the observations were always made at a uniform temperature, namely, the day temperature of the laboratory in the months of January and February, which in Bombay varies very little from day to day, it was found unnecessary to make use of the device, described by the inventor, for maintaining the tubes at a standard temperature.

The results obtained can therefore be considered as comparable amongst themselves as is possible. Two rabbits and three monkeys were the animals used. With one exception the venom was injected subcutaneously.

The following were the results obtained :—

Animal.	Weight in grammes.	Amount of venom per kilo. in milligrammes.	Blood coagulation time in minutes.							Remarks.
			Before injection.	2nd day.	3rd day.	4th day.	5th day.	6th day.	7th day.	
Rabbit 9	1,590	0·8 (Intrav.)	2	...	6	9	5½	...	...	Found dead on the morning of 6th day.
„ 15	1,610	·3 (Subcut.)	2½	...	10	10	3¾	4⅛	...	Found dead on the morning of 8th day.
Monkey 3	2,760	3 (Subcut.)	2½	3¼	8	7⅛	7½	...	8½	Recovered.
„ 5	1,950	2·5 (Subcut.)	3	7	8½	5¾	4½	...	...	Died on 5th day.
„ 2	1,640	3·7 (Subcut.)	5	6	12	...	7⅛	...	...	Died on morning of 6th day.

Series No. III.—Experiments to ascertain if the venom of Bungarus fasciatus has the power of clotting citrate plasma in vitro, *no soluble salt of lime being added.*

Two series of experiments were made, one with citrate donkey plasma and the other with citrate horse plasma. In each instance the citrate of soda was present in the strength of 1 in 100 of plasma. The plasma was completely free from red cells.

Two cubic centimetres of plasma were measured into each tube, to each of which there was then added a different amount of the venom of Bungarus fasciatus. The strength of the venom solution used was 1 *per cent.* It was dissolved in normal saline solution.

The following were the results obtained :—

(*a*) *Donkey plasma* (1 per cent. *citrate*).

Plasma.	Bungarus fasciatus venom in milligrammes.	Result.
2 c.c.	5	Liquid after 24 hours.
2 „	4	„ „ 24 „
2 „	3	„ „ 24 „
2 „	2	„ „ 24 „
2 „	1	„ „ 24 „

(*b*) *Horse plasma* (1 per cent. *citrate*).

Plasma.	Bungarus fasciatus venom in milligrammes.	Result.
2 c.c.	5	Liquid after 24 hours.
2 „	4	„ „ 24 „
2 „	3	„ „ 24 „
2 „	2	„ „ 24 „
2 „	1	„ „ 24 „

Series No. IV.—Experiments to ascertain if the venom of Bungarus fasciatus can prevent the clotting of citrate plasma, which normally results from the addition of a soluble salt of lime.

Two series of experiments were made, one with citrate donkey plasma and the other with citrate horse plasma. In each case the citrate of soda was present in the strength of 1 in 100 of plasma.

Two cubic centimetres of plasma were measured into each tube, to each of

which there was then added a different amount of the venom of Bungarus fasciatus. The strengths of the venom solutions used were 0·1 *per cent.* and 1 *per cent.* From one to two hours afterwards there was added to each tube a quantity of calcium chloride solution which clotted the control in less than 15 minutes.

The following were the results obtained :—

(*a*) *Donkey plasma* (*1* per cent. *citrate*).

Plasma.	Bungarus fasciatus venom in milligrammes.	Calcium Chloride solution, 2%	Result.
2 c.c.	0·5	0·5 c.c.	Clotted solid in 4 minutes.
2 „	0·4	0·5 „	„ „ 4 „
2 „	0·3	0·5 „	„ „ 4 „
2 „	0·2	0·5 „	„ „ 4 „
2 „	0·1	0·5 „	„ „ 4 „

(*b*) *Horse plasma* (*1* per cent. *citrate*).

Plasma.	Bungarus fasciatus venom in milligrammes.	Calcium Chloride solution, 1%	Result.
2 c.c.	5	0·5 c.c.	Clotted solid in 12 minutes.
2 „	4	0·5 „	„ „ 12 „
2 „	3	0·5 „	„ „ 12 „
2 „	2	0·5 „	„ „ 12 „
2 „	1	0·5 „	„ „ 12 „

SERIES *No. V.—Experiments to demonstrate the result of the hæmolytic action of the venom of Bungarus fasciatus, unheated and heated,* in vitro *on the blood of rabbits.*

A sample of the venom of Bungarus fasciatus was dissolved in sterile salt solution (0·75 *per cent.*). The strength of this solution was 0·2 *per cent.* A portion of this solution was heated in a water bath at 75° C. for half an hour and then filtered to remove the coagulated proteids.

An estimation of the hæmolytic power of the original unheated venom and of the heated solution was made. Each tube in the series contained 0·5 c.c. of venom solution, which was half the strength of the solution in the tube next above it in the series.

The dilutions were made with salt solution (0·75 *per cent.*). To each tube was then added 0·005 c.c. of fresh rabbit's blood drawn from the artery of the ear. The preparations were kept at laboratory temperature (about 25° C.). The observations were recorded 20 hours after the preparations were made.

The following were the results obtained:—

Strength of venom solutions.	Unheated.	Heated.
0·5 c.c. = 1 milligramme	Slight H.	Slight H.
0·5 „ = 0·5 „	„	Trace H.
0·5 „ = 0·25 „	No H.	No H.
0·5 „ = 0·125 „	„	„
0·5 „ = 0·0625 „	„	„
0·5 „ = 0·03125 „	„	Trace H.
0·5 „ = 0·0156 „	Trace H.	Slight H.
0·5 „ = 0·0078 „	Slight H.	„
0·5 „ = 0·0039 „	„	„
Salt Solution	No H.	...

Series No. VI.—Experiments to determine the minimum lethal dose for rabbits by intravenous injection of the pure venom of Bungarus fasciatus.

The poison was carefully dried fresh venom of Bungarus fasciatus. A 1 *per cent.* solution in sterile salt solution was made. Five-fold and ten-fold dilutions of this original solution were prepared when necessary. All the solutions were kept unheated. The injections were made into the marginal vein of the ear of the rabbit.

The following were the results obtained:—

Animal.	Weight.	Amount of venom per kilo.	Result.
Rabbit 1	1,700 grammes	1 milligramme	Died in 2¼ minutes.
„ 2	1,640 „	5 „	„ 18 „
„ 3	1,500 „	3·5 „	„ 3½ hours.
„ 4	1,600 „	3 „	„ 1 hour.
„ 5	1,370 „	2·5 „	Found dead after 20 hours.
„ 6	1,520 „	1 „	Died in 24 hours.
„ 7	1,470 „	1 „	„ 30 „
„ 8	1,590 „	0·8 „	„ 120 „
„ 9	1,760 „	0·75 „	Found dead after 66 hours.
„ 10	1,280 „	0·75 „	Died in 48 hours.
„ 11	1,680 „	0·7 „	Found dead after 48 hours.
„ 12	1,380 „	0·6 „	Ill: lost weight; recovered.
„ 13	1,830 „	0·5 „	„ „ „
„ 14	1,340 „	0·25 „	Slightly ill; recovered.

From the above table it appears that about 0·7 milligramme per kilo. of the venom of Bungarus fasciatus is the minimum lethal dose for a rabbit when the injection is made intravenously.

Series No. VII.—Experiments to determine the minimum lethal dose for rabbits by subcutaneous injection of the pure venom of Bungarus fasciatus.

The poison was the same as used in the previous series of experiments. The strengths of the solutions employed were 0·2 *per cent.* and 0·1 *per cent.* These were made with normal salt solution. All injections were made in the inner side of the thigh.

The following were the results obtained :—

Animal.	Weight.	Amount of venom per kilo.	Result.
Rabbit 1 . .	1,610 grammes .	3 milligrammes .	Died in 8 days.
2 . .	1,530 „ .	2·5 „ .	„ 26 hours.
„ 3 . .	1,560 „ .	2 „ .	Lost weight ; recovered.
„ 4 . .	1,300 „ .	1·5 „ .	No symptoms.
„ 5 . .	1,460 „ .	1 „ .	Lost weight; recovered.
„ 6 . .	1,430 „ .	0·75 „ .	No symptoms.
„ 7 . .	1,270 „ .	0·5 „ .	„ „

From the above table it appears that 2·5 to 3 milligrammes per kilo. of weight is the minimum lethal dose for a rabbit when the injection is made subcutaneously.

Series No. VIII.—Experiments to determine the minimum lethal dose for rats by subcutaneous injection of the pure venom of Bungarus fasciatus.

The poison was the same as used in the experiments of series Nos. VI and VII. The strengths of solutions employed were 0·2 *per cent.* and 0·1 *per cent.* These were made with normal salt solution. Care was taken in choosing rats of about 118 grammes in weight. The injections were made subcutaneously in the inner side of the thigh.

The following were the results obtained:—

Animal.	Amount of venom in milligrammes.	Result.
Rat 1	4	Found dead after 18 hours.
„ 2	2	„ „ 18 „
„ 3	1·5	Very ill: recovered.
„ 4	1·5	Found dead after 11 days.
„ 5	1	„ „ 36 hours.
„ 6	1	Very ill: recovered.
„ 7	0·5	Found dead after 36 hours.
„ 8	0·5	„ „ 48 „
„ 9	0·25	Ill: recovered.
„ 10	0·1	Slightly ill: recovered.
„ 11	0·075	No symptoms.
„ 12	0·05	„ „

From the above series of experiments it is difficult to determine exactly the amount of poison which can be depended on to kill a rat. Thus 0·5 milligramme killed in two cases, while three times this amount, *viz.*, 1·5 milligrammes, failed to kill, although grave symptoms followed the injection. We must therefore, take it that to produce a certain lethal effect an amount greater than 1·5 milligrammes must be given.

Series No. IX.—Experiments to determine the minimum lethal dose for monkeys by subcutaneous injection of the pure venom of Bungarus fasciatus.

The poison was the same as used in the experiments of series Nos. VI, VII, and VIII. The venom was dissolved in normal saline solution. The strength

of the solution was 0·2 *per cent.* The injections were made subcutaneously in front of the chest. The following were the results obtained:—

Animal.	Weight.	Amount of venom per kilo.	Result.
Monkey 1 . .	2,480 grammes .	6 milligrammes .	Died in 44 hours.
„ 2 . .	1,640 „ .	3·7 „ .	Found dead after 120 hours.
„ 3 . .	2,760 „ .	3 „ .	Very ill: lost weight: paralysed: recovered.
„ 4 . .	2,150 „ .	3 „ .	Died after 144 hours.
„ 5 . .	1,950 „ .	2·5 „ .	Died after 96 hours.
„ 6 . .	1,260 „ .	2·5 „ .	„ 220 „
„ 7 . .	1,540 „ .	2 „ .	Recovered.

It will be seen from the above table that a certain minimum lethal dose is over 3 milligrammes per kilo., although 2·5 milligrammes per kilo. killed in two cases.

Series No. X.—Experiments to determine the minimum lethal dose for rabbits by subcutaneous injection of pure cobra venom.

The venom used was pure fresh cobra poison, got from five recently captured cobras by squeezing the glands. The strength of the solution employed was 0·1 *per cent.* This was made with normal salt solution and was left unheated. All injections were made subcutaneously in the inner side of the thigh.

The following were the results obtained:—

Animal.	Weight.	Amount of venom per kilo.	Result.
Rabbit 1 . .	1,470 grammes .	0·8 milligrammes .	Died in 2½ hours.
„ 2 . .	1,550 „ .	0·7 „ .	„ 2½ „
„ 3 . .	1,740 „ .	0·6 „ .	„ 2½ „
„ 4 . .	1,570 „ .	0·5 „ .	Found dead after 24 hours.
„ 5 . .	1,430 „ .	0·4 „ .	„ „ 24 „
„ 6 . .	1,500 „ .	0·35 „ .	„ „ 24 „
„ 7 . .	1,520 „ .	0·3 „ .	„ „ 24 „
„ 8 . .	1,370 „ .	0·3 „ .	Very ill: recovered.
„ 9 . .	1,820 „ .	0·25 „ .	Ill at first; recovered; died suddenly on 4th day.
„ 10 . .	1,360 „ .	0·2 „ .	Lost weight: recovered.
„ 11 . .	1,620 „ .	0·2 „ .	„ „
„ 12 . .	1,360 „ .	0·15 „ .	„ „
„ 13 . .	1,590 „ .	0·1 „ .	No symptoms.

From the above series of experiments we can conclude that 0·35 milligramme per kilo. is a certain lethal dose for a rabbit.

SERIES No. XI.—Experiments to determine the minimum lethal dose for monkeys by subcutaneous injection of pure cobra venom.

The venom used was pure cobra venom, got by allowing the snakes to bite through a sheet of water-proof cloth stretched over a stout wine-glass. The strength of the solution employed was 0·1 *per cent.* All injections were made subcutaneously in the front of the chest.

The following were the results obtained :—

Animal.	Weight.	Amount of venom per kilo.	Result.
Monkey 1 . .	2,200 grammes .	3 milligrammes .	Died in 1 hour.
„ 2 . .	1,260 „ .	0·6 „ .	„ 3 hours.
„ 3 . .	1,480 „ .	0·4 „ .	„ 6 „
„ 4 . .	1,830 „ .	0·25 „ .	„ 6 „
„ 5 . .	1,170 „ .	0·2 „ .	Ill : recovered.

From the above series of experiments we can conclude that 0·25 milligramme per kilo. is the minimum amount which can kill a monkey.

SERIES No. XII.—Experiments to ascertain if Calmette's serum has any neutralising power for the venom of Bungarus fasciatus.

The serum used in these series of experiments was perfectly fresh serum. It had been received direct from Lille a few days before the observations were made. Rabbits were the animals used.

All injections were made intravenously. Two series of experiments were performed.

(A) In the first series the test dose of venom used was 4 milligrammes per kilo., *viz.*, about 6 lethal doses. This test dose of venom in solution (1 *per cent.*) in normal salt solution and the serum in different quantities were mixed *in vitro*. The mixture was allowed to stand at laboratory temperature (about 25° C.) for half an hour. It was then injected into the marginal vein of the ear of the rabbit.

The following were the results obtained :—

Animal.	Weight.	Amount of venom per kilo.	Amount of serum.	Result.
Rabbit 1 .	1,180 grammes .	4 milligrammes .	0·5 c.c. . .	Died in 27 minutes.
„ 2 .	1,890 „ .	4 „ .	1 „ . .	„ 34 „
„ 3 .	1,570 „ .	4 „ .	2 „ . .	„ 2½ „
„ 4 .	1,810 „ .	4 „ .	5 „ . .	Found dead after 50 minutes.
„ 5 (control).	1,490 „ .	4 „ .	*Nil* . .	Died in 30 minutes.

From this series of observations it will be seen that 5 c.c. of serum failed to neutralise 7·24—1·08 milligrammes =6·16 milligrammes of venom.

(B) In the second series of experiments the test dose of venom employed was 2 milligrammes per kilo., *viz.*, about 3 lethal doses. With this exception, the same *technique* was used as in the first series of observations with this poison.

The following results were obtained :—

Animal.	Weight.	Amount of venom per kilo.	Amount of serum.	Result.
Rabbit 1 .	1,300 grammes.	2 milligrammes.	2 c.c. . .	Died in 18 hours.
„ 2 .	1,460 „ .	2 „ .	3 „ . .	Found dead after 17 hours.
„ 3 .	1,370 „ .	2 „ .	4 „ . .	„ „ 60 „
„ 4 .	1,200 „ .	2 „ .	5 „ . .	„ „ 40 „
„ 5 (control).	1,320 „ .	2 „ .	*Nil* . .	„ „ 18 „

From this series of experiments it is evident that 5 c.c. of serum failed to neutralise 2·4—0·72 milligrammes =1·68 milligrammes of this venom.

These two series of observations show conclusively that Calmette's serum is inactive against the venom of Bungarus fasciatus.

Series No. XIII.—Experiments to ascertain if the serum of a horse immunised with the venom of Hoplocephalus curtus has the power to neutralise ehe venom of Bungarus fasciatus.

The serum which was used in this series of experiments was prepared by Dr. Tidswell of Sydney by immunising a horse with the pure venom of Hoplocephalus curtus. It was very active for this poison but was quite inactive against the venoms of three other Australian snakes. The observations were made with rabbits. All injections were made intravenously.

The test dose of venom used was 4 milligrammes per kilo., *viz.*, about 6 lethal doses. In each instance this test dose (1 *per cent.* solution) and the serum were mixed *in vitro*. The mixture was allowed to stand at laboratory temperature for half an hour. It was then injected into the marginal vein of the ear of the rabbit.

The following results were obtained :—

Animal.	Weight.	Amount of venom per kilo.	Amount of serum.	Result.
Rabbit 1 .	1,450 grammes.	4 milligrammes.	0·5 c.c. .	Died in 32 minutes.
„ 2 .	1,780 „ .	4 „ .	1 „ . .	„ 12 „
„ 3 .	1,460 „	4 „ .	2 „ . .	„ 22 „
„ 4 .	1,680 „ .	4 „ .	5 „ . .	„ 10 „
„ 5 (control).	1,490 „ .	4 „ .	*Nil* . .	„ 30 „

From this series of experiments it is at once evident that the serum of a horse immunised with the venom of Hoplocephalus curtus has no power to neutralise the venom of Bungarus fasciatus.

Notes and References.

(1) (*a*) "Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India." New series, 1903, No. 3.

(*b*) Ditto: New series, 1903, No. 4.

(*c*) Ditto: New series, 1903, No. 5.

(*d*) Lancet: August 16th, 1902.

(2) Loc. cit.

(3) "Indian snake poisons, their nature and effect." London, W. H. Allen and Co., 1883.

(4) "Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India." New series, 1903, No. 3.

(5) Loc. cit., *vide* reference 4.

(6) Loc. cit.

(7) "Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India." New series, 1903, No. 4.

(8) The term 'isotonic' is not used in the strictly physiological sense. I have employed it to mean the weakest strength of salt solution in which no 'laking' of the blood was observed; in other words, 'laking' and not swelling of the corpuscles was taken as a standard.

(9) "Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India." New series, 1902, No. 1.

(10) "Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India." New series, 1903, No. 5.

(11) "Notice sur le sèrum antivenimeux et sur le traitment des morsures des serpents." Lille 1901.

(12) "Australasian Medical Gazette." April 21st, 1902, p. 177.

(NEW SERIES.) No. 7.

SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA.

SOME OBSERVATIONS ON THE POISON OF THE BANDED KRAIT BUNGARUS FASCIATUS).

BY

CAPTAIN GEORGE LAMB, M.D. (GLAG.), I.M.S.

ISSUED UNDER THE AUTHORITY OF THE GOVERNMENT OF INDIA
BY THE SANITARY COMMISSIONER WITH THE GOVERNMENT
OF INDIA, SIMLA.

CALCUTTA:
OFFICE OF THE SUPERINTENDENT OF GOVERNMENT PRINTING, INDIA.
1904.

Price 8 Annas or 9d.