among whom plague was at the time prevailing. The belief was expressed that the animals had died from plague, but no positive proof of this is adduced.⁵⁶ Experimental evidence of the susceptibility of squirrels to plague is apparently lacking. These animals are extremely common in India, and the rarity of recorded instances of their contracting the disease must be taken to indicate that they are not highly susceptible to the virus. The part they take in its spread must be an extremely small one.

Plague in Guinea-pigs.

In the laboratory these animals are very susceptible to the virus of plague, and they are among the most convenient for experimental purposes. I am aware of no published evidence that these animals have contracted the disease in a natural state.

Plague in Porcupines.

The single reference that I have seen to the occurrence of plague in these animals is the following brief statement published last autumn: "It is reported at Mysore that several porcupines have died in the Zoological Gardens from bubonic plague."⁵⁷ In view of the great susceptibility of other rodents to plague, there is no *a priori* reason why porcupines should not be liable to it. Detailed observations upon the manner in which animals with so impenetrable an epidermic covering contracted the disease should prove interesting.

Plague in Marmots.

In the Transbaikal province in Eastern Siberia, a marmot known as the Tarbagan (*Arctomys bobac*) suffers at times from a disease believed to be plague, and this disease is occasionally transmitted to human beings. In an endemic plague centre in Eastern Mongolia there is also some evidence that these animals contract the disease. I have elsewhere⁵⁸ discussed in detail what is known of these two centres of disease and the part taken by the marmot in its spread.

Rabbits.

These animals, though susceptible to the disease in the laboratory, do not appear to have ever contracted it under natural conditions. Should plague, which has now for the first time in history reached Australia, spread at all widely there, it will be of great interest to observe whether these animals, which abound so in Australia, suffer at all from the disease.

NOTES AND REFERENCES.

¹ The present discussion of the subject will be confined to recent evidence. ² *Thirtieth Annual Report of the Sanitary Commissioner with the Government of the North-West Provinces*. ³ *Ibid.* ⁴ *Indian Medical Gazette*, March, 1899. ⁵ *Plague in Monkeys and Squirrels*. By Alice Corthorn, M.B. ⁶ *Report of the German Plague Commission*, p. 299. ⁷ *Report of the Russian Plague Commission*, p. 16. ⁸ In at least one instance a monkey has been artificially infected by being allowed to chew a piece of sugarcane which had been smeared with a pure culture of plague bacilli. (Wilm, *Indian Medical Gazette*, June, 1897.) ⁹ The principal families of the *Murinae*, the zoological group to which rats and mice belong, are the *Mures* and *Sigmodontes*. The former are confined to the Old World, the latter to the New World and Madagascar. The most noteworthy genus of the *Mures* is the *Neotoma*, of which the most remarkable member is the pig-rat or bandicoot (*Neotoma bandicoota*) (*Encyclopædia Britan.*). It was the last-named animal whose geographical distribution was believed to coincide to some extent with that of plague (Cantlie, *Epidem. Soc. Trans.*, N. S., vol. xvi, p. 19). The recent spread of plague to countries where the bandicoot is unknown, and the infrequency with which this animal is infected in countries where it exists have shown that this view can no longer be held. ¹⁰ The course of the rat migration in Bombay is traced in detail in the Municipal Commissioner's report for that city, 1896-7, pp. 9-10. ¹¹ *Indian Medical Gazette*, 1897, p. 307. ¹² Surgeon-Lieutenant-Colonel Henderson, on Plague in Sind. ¹³ *Ibid.* ¹⁴ Rennie, quoted by Cantlie (*loc. cit.*). ¹⁵ Baber, in *Parliamentary Papers*, 1878, China, p. 6. ¹⁶ Lowry, *Medical Reports of the Chinese Imperial Maritime Customs*, No. 24. ¹⁷ McGowan, *Ibid.* ¹⁸ *Times of India*, September 30th, 1896. ¹⁹ *Report of the Sanitary Commissioner with the Government of India*, 1897, p. 91. ²⁰ Whether a large proportion, or any, of these rats had died from plague is not stated. ²¹ Lowry (*loc. cit.*) found the lungs of rats dead from plague deeply congested; the same was observed at Lienchow, and in the districts of the southern Kiangsi in 1886, but no mention is made of pneumonic patches in the lungs. During the prevalence of plague in Yunnan nearly a century ago "it happened that in the daytime strange rats appeared in the houses, and lying down on the ground perished with blood spitting," which may have indicated a lung affection. (Mr. Kumagusu Minakata in *Nature*, February 16th, 1899.) ²² Batzaroff, *Annales de l'Institut Pasteur*, May, 1890. ²³ Cantlie (*loc. cit.*) for Hong Kong. ²⁴ A. Happer, *Chinese Customs Medical Reports*, 1880. ²⁵ *Bombay Plague Committee Report*, 1898, p. 205. ²⁶ McGowan, *loc. cit.* ²⁷ Baber, *loc. cit.* ²⁸ Compare the description of a necropsy on a rat dead from plague, by A. Rennie. (*Medical Reports of the Chinese Imperial Maritime Customs*, No. 48, p. 69.) ²⁹ *Municipal Commissioners' Report*, Bombay, 1897, p. 9. Also Rennie, *loc. cit.* ³⁰ Yersin, *Annales de l'Institut Pasteur*. ³¹ *Epidem. Soc. Trans.*, N. S., vol. xvi. ³² Kitasato, *First Report on the Cause of Bubonic Plague*. ³³ Okada, *Sei-i-Kwat Medical*

Journal, Tokyo, October 31st, 1899. ³⁴ It is beyond the scope of the present article to discuss the many other questions connected with the infection of soil with plague virus. So far as published experiments indicate it would seem that it is only the soil of rooms in which plague patients have lain, or in which rats have perhaps died of plague that becomes infected. There is nothing to support the view that the virus spreads through the soil of a district or country like a patch of ringworm spreading from a centre, as some have suggested. ³⁵ The plague bacillus has been found in the dejecta of rats. ³⁶ Government of India, *Report upon Plague in India*, 1896-7, Appendix I. ³⁷ The diagnosis in the cases of plague in human beings was beyond doubt. Unfortunately an acute attack of fever, followed by absence from Calcutta on sick leave, prevented me from obtaining any bodies of the rats for bacteriological examination, and from investigating the series of events until a week or more after their occurrence. ³⁸ *Report of the Sanitary Commissioner with the Government of India*, 1897. ³⁹ Rennie, *loc. cit.* ⁴⁰ McCabe Dallas, quoted by Weir, *Indian Medical Gazette*, 1897; this or the following is possibly the same case as that referred to by Dr. Weir at p. 55 of the *Municipal Commissioners' Report on Plague in Bombay*, 1897. ⁴¹ *Ibid.* ⁴² It can scarcely escape notice that in a large number of historical epidemics no mention is made of an affection of rats: that in some recent local prevalences of plague in India no affection of rats has been observed; and that a plague epidemic, once started, can last a long while after all the rats have apparently left the place in which it is raging. ⁴³ *Indian daily press* of March 19th, 1898. ⁴⁴ *Public Health Reports*, Marine Hospital Service, Washington, 1899, p. 1345; report of Dr. M. J. Rosenau, dated Angel Island, California, November 12th, 1898. ⁴⁵ The literature of experimental plague in rats and other animals is very extensive; among the published observations upon this subject may be mentioned those of the German and Austrian Plague Commissions; of Haffkine and Hankin in India; of Lowson, Wilm, and Yersin in China; of Roux and Simond in France; of Vysockovitch and Zabolotny and others in Russia; of Batzaroff, of Roumania, and many others. To have attempted in the text to summarise, however briefly, the results obtained by these observers would have extended the present paper far beyond its intended limits. ⁴⁶ *Annales de l'Institut Pasteur*, November, 1898. ⁴⁷ *Health Officer's Report on Plague in Bombay*, 1896-7, p. 70. ⁴⁸ Childs has recorded a doubtful instance of the kind (*Report of the Bombay Plague Research Committee*). ⁴⁹ Simond, quoted by Hankin, *loc. cit.* ⁵⁰ Hankin, *loc. cit.* ⁵¹ J. J. Matignon, *Janus*, July-August, 1898. ⁵² Noury Bey, *Annales de l'Institut Pasteur*, September, 1898, p. 604. ⁵³ La Peste à Alexandrie en 1899 (report of the Commission appointed to inquire into the origin of the epidemic), p. 19. ⁵⁴ *Bombay Gazetteer*, vol. iv, chap. xii. ⁵⁵ Hutcheson, *Transactions of the First Indian Medical Congress*. ⁵⁶ Dr. Alice Corthorn, *Indian Medical Gazette*, March, 1899. ⁵⁷ *Report of the Sanitary Commissioner with the Government of India*, 1897, Section III, p. 83. ⁵⁸ *Lancet*, September 30th, 1899, p. 910. ⁵⁹ *Journal of Tropical Medicine*, February, 1900, Plague in Siberia and Mongolia and the Tarbagan; by the author.

(To be continued.)

AN ACCOUNT OF SOME RESEARCHES INTO THE NATURE AND ACTION OF SNAKE VENOM.

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II.

ON THE SOURCE OF IMMUNITY WHEN COBRA VENOM IS TAKEN BY THE MOUTH.

In a paper published in the *BRITISH MEDICAL JOURNAL* of July 17th, 1897, Professor Fraser wrote as follows:

The failure of so highly toxic a substance (serpents' venom) to produce poisoning when it is administered by the stomach might be due to chemical changes produced upon it by the secretions of the stomach and intestines, or to non-absorbability into the blood from the stomach and intestines. It is already known that the toxicity of venom is not reduced materially by gastric digestion.....As serpents' venom introduced into the stomach is not rendered innocuous by the stomach secretions, while notwithstanding it fails to cause poisoning, it may be assumed that the stomach walls are incapable of absorbing it also. If, like other poisons, it can be absorbed from the intestines, the explanation of the failure to produce toxic symptoms when it is administered by the stomach might depend on a chemical or physiological destruction of its toxic properties by some substance or substances which it encounters soon after entering the intestinal canal, and most probably, therefore, by the bile or the pancreatic secretion. To explain the innocuousness of stomach administration fully would accordingly require that the effects on venom of the biliary and other intestinal secretions should be investigated, and also the absorbability of venom through the intestinal walls.

As a contribution to the settlement of the question I have made a number of experiments with the biliary secretion, and whatever may be the influence of the other secretions, or of intestinal absorption, that of the bile has been found to be so decided as to be in itself sufficient to account for the innocuousness of stomach administration.

The object of the present paper is to fill in some of the gaps suggested by Professor Fraser in the above very interesting sentences.

Another possibility occurred to me—namely, that the source of protection might perhaps lie in the action of intestinal epithelium during absorption. This idea was suggested by the fact that the epithelium of the serpents' venom glands has the power of so altering the fluid passing through it as to

change it from an innocuous fluid into a highly poisonous one, might not the action of intestinal epithelium during absorption be to invert this process?

In order to test the truth of this suggestion it was necessary experimentally to so introduce venom into the alimentary canal as to exclude the action of the various juices on the poison. With this end in view a series of experiments was undertaken which will be dealt with *seriatim*. It will be found that another explanation has been forthcoming, and that, therefore, the theory above stated has had to be abandoned. It is only mentioned as it formed the starting point of the series of experiments which are about to be described.

Feeding an animal with cobra venom after excluding the biliary secretion from the alimentary canal.

Two experiments were performed on dogs, thus: The abdomen was opened, the common bile duct securely tied, and an artificial biliary cutaneous fistula established; the intestines were swept clear of bile by purgatives, and the dog was so controlled that it could not reach the escaping bile with its mouth; a large dose of cobra venom was then given by the mouth, in one case in the dry state on meat, and in the other dissolved in milk; in both cases the dose of venom was repeated to make security secure; neither of the animals showed any sign of being the worse for the venom (*vide* Appendix A).

In view of the above facts it was obvious that, assuming the venom to have been absorbed, we must look farther than the action of bile for the immunity exhibited by the animals. It is obviously necessary to dispose of this assumption first. Later on in this paper I shall deal with the question of the influence on certain other animals of the ingestion of cobra venom by the mouth, and I hope there to show good cause to believe that venom taken by the mouth produces a marked constitutional effect upon the animal; this fact in itself would dispose of the question of absorption of venom through the intestinal walls, but more direct proof is not wanting. A reference to Appendix A, Experiment II, will show that the small gut was empty throughout twenty-four hours after the administration of the milk; the stomach was also empty. Final and convincing proof is, however, obtained when we come to the result of Experiment I of Appendix B; here we find that the venom introduced dissolved in milk fifty-five minutes earlier through an artificial opening in the small gut, had been so rapidly absorbed as to cause death in this short time; that the venom had been absorbed through the small gut was evident, not merely by its lethal effect, but also by the local changes produced, which were strictly comparable to those seen when venom is injected subcutaneously; there was, in fact, a gelatinous swelling of the intestinal and submucous membranes which closely resembled the gelatinous swelling we are familiar with in the subcutaneous tissues after injection of poison there. This absorption, be it noted, took place through an unbroken epithelial surface.

Having thus disposed of the question of the power of the intestine, to absorb cobra venom, we may pass on to the next series of experiments.

The injection of cobra venom through an artificial opening in the small gut, the conditions being so arranged that the contents of the gut above the artificial anus passed out through that orifice, the peptic, pancreatic, and biliary secretions being thus shut off from the lower segment of gut, into which the venom was injected.

The above conditions were fulfilled by drawing out a loop of small gut through a 2-inch abdominal incision, fastening this gut in position, and later establishing an artificial anus in it. Time was allowed for the parts to heal soundly, and then the cobra venom was injected into the lower segment of gut dissolved in milk (*vide* Appendix B).

A reference to Experiment I of Appendix B will show that the venom, under these conditions, was rapidly absorbed and powerfully lethal. It will be noted that in this experiment the dose was but little in excess of that which the two dogs dealt with in the experiment of Appendix A took with impunity; a reference to Experiment II of Appendix B will show that one-tenth of this dose injected into the bowel did not produce any marked symptoms, while half this large dose given some two days later killed the animal in an hour and a-quarter. It is to be borne in mind that even the small dose,

which when injected into the small gut proved ineffective, would have been speedily fatal had it been given subcutaneously.

From the above facts we may draw two conclusions—namely:

1. That cobra venom can be absorbed through the walls of the small gut, but not so readily as through the channels of subcutaneous tissue.

2. That some change or changes are wrought in swallowed cobra venom before it has time to reach a rapidly absorbing area.

Professor Fraser has shown in the very valuable paper I have already referred to that the bile is capable of protecting the organism from the toxic action of serpents' venom. I have repeated many of Professor Fraser's experiments, and have satisfied myself of the accuracy of his conclusions as to the antidotal powers of bile. The experiments of Appendix A point, however, clearly to the fact that bile is not the only protective agent. In the remarks already quoted Professor Fraser suggested that pancreatic juice might digest and neutralise serpents' venom. I have tested this and found that trypsin is capable of reducing very materially the lethal properties of cobra venom when the two are kept at a temperature of 100° F. over a water bath in an alkaline solution. A summary of the experiments which have led up to this conclusion will not be out of place.

First Series (October 26th, 1899).—Four rabbits were used, one being a control and the other three receiving respectively venom digested with pepsin in 0.2 per cent. solution of hydrochloric acid, venom digested with trypsin and sodium carbonate, and venom mixed with 0.07 gram of cobra bile. In the whole series 0.0007 gram of cobra venom per kilo. of the rabbit's body weight was used. The indications were not conclusive, but it appeared (1) that the pepsin had had no action on the venom, (2) that too much sodium carbonate had been given, and (3) that the bile had acted protectively.

Second Series (October 28th, 1899).—This series practically confirmed the above conclusions. They were performed before the results of the former series had had time to be known, and therefore one was unable to profit by the experience the results of the first set ultimately brought.

Third Series (November 16th, 1899).—In this series the dose was raised to 0.008 gram of cobra venom per kilo. of the rabbit's body weight. The bile experiment was omitted as it had been more exhaustively dealt with elsewhere, and an experiment was added to ascertain whether it was likely that the absorption of the products of digestion of proteins in any amount likely to be produced by the action of the digestive ferment or venom would in itself be fatal. The control venom animal died in 6 hours and 50 minutes; the pepsin experiment animal died in 7½ hours, while the trypsin experiment animal suffered but little at the time and survived 20 days; here again it seemed probable that the amount of sodium salt used was too large. In any case, however, a distinct protection had been established. The result of the control experiment on the injection of peptones was death 12 days after injection, but it is to be remembered that 25 times as much egg albumen was used for this experiment as poison in the remaining experiments, and that therefore the products of digestion produced were in great excess of what can have been produced in the venom experiments.

In all these experiments the solution of venom was at a strength of 0.001 gram per c.cm. of water, and digestion was carried on for two hours at a temperature of 100° F., the correct degree of warmth being maintained by means of a water bath.

The next series of experiments it appears worth while to give *in extenso* in Table I.:

TABLE I.—Experiments directed to Ascertain whether Pancreatic Digestion Lessens the Lethal Properties of Cobra Venom.*

Date. (Nov., 1899.)	Weight in Kilos.	Dose per Kilo.	Remarks.
21st	1.6720	0.0007 gram venom	Control; died in 34½ hours 10 minutes.
21st	1.9056	0.0007 " venom 0.0050 " trypsin 0.0050 " sod. carb.	There was no marked constitutional disturbance for some days. The rabbit gradually lost flesh, there was some sloughing at the seat of injection, and it died 17 days after the operation.
21st	1.9836	0.0008 " venom 0.0050 " trypsin 0.0050 " sod. carb.	This rabbit closely followed the same course as the previous one, but there was more local reaction. It died on the 17th day.
21st	2.0190	0.0009 " venom 0.0050 " trypsin 0.0050 " sod. carb.	Died in 32 hours.

* The venom (cobra) was dissolved at a strength of 0.0002 gram per c.cm. of boiled water. The solutions were kept at a temperature of 100° F. for a hours after the addition of the trypsin and carbonate of soda; they were then injected into the flanks of rabbits.

NOTE.—It is possible that climate had something to do with the deaths

of the second and third rabbits, as they died during a very cold night. Rabbits are very susceptible to cold and damp in the hills of India, and this will obviously work at a greater advantage in lowering conditions of nutrition such as those which follow the injection of snake venom.

It will be at once noticed that the rabbit which received the large dose of 0.0009 gram per kilo. of body weight, and which under ordinary circumstances could not have been expected to live more than two to four hours, survived thirty-two hours, living nearly as long as the control which had only received 0.0007 gram per kilo.; it will also be noted that the two other rabbits which had received trypsinised venom showed a very marked degree of protection, and indeed it is doubtful whether they would have succumbed had the weather been less severe at the time. Owing to the sensitiveness of rabbits to climatic influences, and to their slight resistance to the after-effects of venom, which has been elsewhere referred to, the next series of experiments was carried out on fowls. The facts contained in Table II leave no room for doubt as to the power of trypsin to reduce the lethal properties of snake or at least of cobra venom.

Table II may be divided into three sections, each consisting of a control venom experiment, and three trypsin experiments. In the first section the dose of venom used was 0.0015 gram, in the second 0.00175 gram, and in the third 0.002 gram per kilo. of the fowl's body weight. It had previously been ascertained by experiment that even the smallest dose mentioned was fatal in one or two days, while the highest was more rapidly fatal.

TABLE II.—Experiments designed to ascertain whether Pancreatic Digestion Destroys the Lethal Properties of Cobra Venom.*

Date. Dec., 1899.)	Weight in Kilos	Dose per Kilo.	Course of Events.
16th	0.69425	0.00166 gram venom	Dead in 23 hours 40 minutes. Control.
16th	0.92050	0.00150 " venom	Sick for 1 day; recovered.
16th	0.78630	0.00033 " trypsin	Never seriously ill; recovered.
16th	1.00590	0.00150 " venom	" " "
16th		0.00100 " trypsin	" " "
16th		0.00150 " venom	" " "
16th		0.00300 " trypsin	" " "
16th	0.80760	0.00175 " venom	Died in 60 hours. Control.
16th	1.26100	0.00175 " venom	Sick for 1 day; recovered.
16th		0.00033 " trypsin	Never seriously ill; recovered.
16th	0.86427	0.00175 " venom	" " "
16th		0.00100 " trypsin	" " "
16th	1.02000	0.00175 " venom	" " "
16th		0.00300 " trypsin	" " "
16th	0.80050	0.00200 " venom	Died in less than 40 hours. Control.
16th	0.80760	0.00200 " venom	Sick for 1 day; recovered.
16th	0.89950	0.00033 " trypsin	Never seriously ill; recovered.
16th		0.00200 " venom	" " "
16th	0.87200	0.00100 " trypsin	" " "
16th		0.00200 " venom	" " "
16th		0.00300 " trypsin	" " "

* The venom was dissolved in freshly-boiled cooled water at a strength of 0.001 gram of venom per c.cm. of water. In all but the control experiments the solution was made just alkaline by the addition of sodium carbonate, and the trypsin was then added (Merck's trypsin). All the solutions alike were then placed in a water bath at 100° F., and kept there for 3 hours. At the end of that time, they were removed and injected subcutaneously in the subcutaneous tissue under the wings of the fowls.

NOTE.—In the first experiment it was intended to give 0.0015 gram of venom per kilo as a control, but, owing to a mistake, 0.00166 gram was given.

A well-marked instance of what has above been called the "personal factor" is to be observed in the order in which these control fowls died; this does not, however, affect the deductions from the series of experiments since all the control fowls did die, while of those which received trypsinised venom every one recovered; it would appear that the smallest dose of trypsin used was rather low, for the fowls which received poison treated with this low dose of the ferment were sick in every case at first. All the fowls alike showed the usual loss of weight, so they were presumably somewhat, though not severely, affected by the venom.

This closes the case for the action of the ferment of pancreatic juice on the lethal properties of cobra venom.

Taking into account that absorption of venom is far less rapid from the gut than from the subcutaneous or muscular

tissues, the above shown properties of trypsin will readily explain why venom fails to kill when swallowed, even after the protective influence of bile has been excluded, while it kills certainly, if in large enough quantity, when both the biliary and pancreatic secretions are excluded from the area of intestine which has to deal with the venom.

There are one or two other interesting facts which may be mentioned in this place. The first is that I have been unable to produce symptoms or signs of cobraism by injecting into the rectum of a dog even large doses of cobra venom; as much as 0.5 gram was used for one injection on a 10-kilog. dog, which was carefully watched to make sure the venom was not returned; it had been given dissolved in milk and water.

Another point of interest was the readiness with which venom was taken from the peritoneal cavity; it is a very easy matter to inject a solution of venom into this cavity in rabbits, as the abdominal walls are very thin, and the needle can be felt passing into the sac by the sudden lessening of resistance to its progress; it can then be freely moved in the cavity; it was found that a dose of cobra venom which would take about seven hours to kill when injected subcutaneously, would prove lethal in less than three hours when it was delivered into the abdominal cavity; a farther point of interest was that in all intraperitoneal injections symptoms of poisoning commenced much earlier than in the case of subcutaneous injections of a similar dose of venom.

The Effects of the Ingestion of Cobra Venom in a Healthy Animal.

Professor Fraser, writing in the BRITISH MEDICAL JOURNAL of April 18th, 1896, pages 958-959, gives the result of certain experiments he conducted, which led him to the conclusions summarised in the following words:

The extraordinary result was thus obtained that serpents' venom introduced into the stomach in large quantity—in a quantity which, if injected under the skin, would be sufficient to kill 1,000 animals of the same species and weight—while it failed to produce any definite symptoms of poisoning, nevertheless produced in a few hours complete protection against the lethal effect of a dose of venom more than sufficient to kill the animal. (The italics are not in the original.)

The BRITISH MEDICAL JOURNAL of August 8th, 1896, page 346, in summarising Professor Calmette's valuable address delivered at Carlisle, makes the following noteworthy statement: "*He (Professor Calmette) had never obtained immunity by feeding animals on venom.*" (Again the italics are not in the original.)

TABLE III.—Experiments directed to Ascertain whether Feeding Rabbits on Cobra Venom affords Protection against the Effects of the same kind of Venom subsequently Injected Subcutaneously.*

Serial No.	Date.	Weight in Kilos.	Dose per Kilo.	Result.
I	27/9/99	1.559	0.1240 gram by mouth	Died in 2 hours 50 minutes.
	2/10/99		0.0210 " " "	Post mortem examination
	6/10/99		0.0416 " " "	showed that the injection
	12/10/99		0.0330 " " "	had been made into the
	13/10/99		0.5000 " " "	peritoneal cavity. This
	14/10/99		0.0007 " injected into the flank	explained the rapid death.
III	14/10/99	1.2042	0.0140 " by mouth	Died in 6 hours 14 minutes
	15/10/99		0.0154 " " "	after the injection.
	16/10/99		0.0700 " " "	
	17/10/99		0.0700 " " "	
	18/10/99		0.0890 " " "	
	19/10/99		0.7000 " " "	
	21/10/99		0.0007 " subcutaneously	
XI	21/10/99	1.3390	0.0016 gram by mouth	11/11/99. Sickened soon after
	25/10/99		0.0070 " " "	injection; at 10 P.M. was
	26/10/99		0.0140 " " "	breathing jerkily and with
	27/10/99		0.0560 " " "	difficulty. 2/11/99. Lying
	28/10/99		0.0700 " " "	prone and nearly paralysed
	29/10/99		0.0700 " " "	at 8 A.M.; died at
	30/10/99		0.1400 " " "	10.10 A.M.—that is, 19 hours
	31/10/99		0.7000 " " "	40 minutes after injection.
	1/11/99	1.530	0.0007 " subcutaneously	

* The poison in all these experiments was dissolved in a small quantity of water and then mixed with chopped cabbage leaves; the animals ate the mixture readily.

I accordingly instituted a number of experiments to ascertain, if possible, what the cause of the discrepancy in evi-

dence was. A review of Tables III, IV, and V given below will show the results obtained.

In the earlier experiments (Table III) no control animal was used, as it was expected that the protection would be too well marked to need control. In view of the conclusions eventually arrived at, we may neglect the first experiment, on the ground that, as the injection was made accidentally into the peritoneum, the test was unfairly severe. Experiments III and XI were free from this objection; it will be noted that a dose of venom was given nearly every day for a week in III, and for a slightly longer period in XI; the earlier doses were comparatively small, while the last dose in each case was one which, if given subcutaneously, would have been fatal to 1,000 animals of the same weight and species. There was no evidence of protection in either of these cases; on the contrary, the animals seemed to have been markedly influenced unfavourably by the swallowed venom, so far as their power of resisting the lethal effects of the venom went.

Two series of experiments were therefore undertaken; the first of these is comprised by Experiments LV-LIX (Table IV). The most noteworthy point here was that during the few days immediately following the injection the control rabbit was decidedly less ill than any of those which had beforehand received venom by the mouth. The control rabbit died in 24 days, while the other 3 died respectively in 7 hours, 11 days, and 37 days after injection. It is evident that no case for protection can here be established.

TABLE IV.—*A Controlled Series of Experiments directed to Ascertain whether Feeding Rabbits on Cobra Venom affords them Protection against the Effects of the same kind of Venom subsequently Injected Subcutaneously.**

Serial No.	Date.	Weight in Kilos.	Dose of Venom per Kilo.	Result.
LV	28/10/99	0.9070	0.0007 gram injected into flank as control	28/10/99. Not seriously unwell. 29/10/99. Unwell, but eating, and decidedly better than rabbits of next three experiments. 21/11/99. Was ill for several days, then improved, but emaciated, and died 24 days after injection.
LVII	27/10/99 28/10/99	0.9068	0.4500 gram by mouth 0.0007 " subcutaneously	28/10/99. Very sick to-night. 29/10/99. Very sick, not eating. 3/12/99. Was very sick for several days, then improved, but emaciated, and died on the 36th day.
LVIII	27/10/99 28/10/99	0.8501	0.7000 gram by mouth 0.0007 " subcutaneously	This rabbit followed much the same course as the previous one, and died on the 11th day with a sloughy wound at the seat of injection.
LIX	27/10/99 28/10/99	0.8500	0.7000 gram by mouth 0.0007 " subcutaneously	Died 7 hours after injection.

* The venom was administered in the same way as in the previous experiments.

NOTE.—The hypodermic injections of venom on the 28th were all made in the flank.

Another series of experiments was undertaken on November 21st and 22nd, and here the indication seemed more clear than ever, for the control lived 24 days, while the two rabbits, which on the previous day had each received by the mouth 500 times the subcutaneous lethal dose, died respectively in 24 hours 50 minutes and 33 hours 40 minutes.

Throughout all these experiments the writer could not help observing that though feeding an animal on snake poison did not appear at the time to affect it injuriously, yet when a dose of poison subcutaneously given was superadded, the symptoms of cobraism were more rapidly developed and more markedly pronounced than would have been the case in an animal which had only the subcutaneous dose of poison to contend with.

From this it is surmised that cobra venom when taken by the mouth passes in some form, probably an altered one, into the general circulation. It is likely that if time is given for

the disposal of these products their effect will be found to be in the direction of immunisation, but in the meantime they appear to affect the animal's resistance to the venom prejudicially.

TABLE V.—*Farther Experiments on the Acquisition of Immunity by Feeding Rabbits.**

Serial No.	Date.	Weight in Kilos.	Dose per Kilo.	Remarks.	Result.
85	21/11/99 22/11/99	1.22 1.22	0.3500 gram 0.0007 "	By mouth Subcutaneously	Nil. Sickened in a few hours, and was dead 24 hours 50 minutes after injection.
86	21/11/99 22/11/99	1.785 1.785	0.3500 " 0.0007 "	By mouth Subcutaneously	Nil. Sickened rapidly, and died in 33 hours 40 minutes.
87	21/11/99	1.813	0.0007 "	Subcutaneously	This rabbit was less ill than either of the preceding ones, and, though sick for a few days, gradually got better. It died 24 days after the injection, greatly emaciated.

* The poison was dissolved at a strength of 0.001 gram. per c.cm. of water.

I have noticed a very similar phenomenon with regard to sublethal doses of venom subcutaneously administered; if sufficient time is allowed to elapse between the injections, the animal acquires a protection against the influence of the venom, but if the doses are given at too short intervals, death will result from what otherwise would have been a sublethal dose. These facts appear to point to the inference that it takes the organism some little time to deal with venom, however administered, and that in the earlier stages of this complex and little-understood process the animal is more susceptible to poison than it ordinarily would be. There is no want of analogy in the action of other similar protective processes.

This is not, as it may at first sight appear to be, a question of purely theoretical interest, for on it hinges the correctness or otherwise of Professor Fraser's views as to the source of immunity among snake charmers all the world over. I thoroughly believe those views to be correct so far as Indian snake-charmers are concerned, but I am disposed to disagree with Professor Fraser as to the rapidity with which the immunity can be acquired by animals. It is always to be remembered, however, that it is a common practice of Indian snake charmers to rub the poison of cobra on to the back of one hand with the palm of the other; this habit, which one frequently sees the snakeman practise, must often lead to the absorption of venom through abrasions, even if this is not deliberately aimed at. Possibly it is to this practice that Professor Fraser refers when he speaks of "inoculation of venom" being "apparently sometimes practised" by snake charmers.

It is more than likely that the mongoose owes its large share of immunity against snake venom to its practice of devouring the head of its victim as soon as the victory has been won. I shall have more to say on this subject on a subsequent occasion when dealing with the acquired resistance of the mongoose. For the present it only remains for me to express the regret I feel in differing, even in a minor matter, from one to whom all workers in the field of snake poison research owe so much as they do to Professor Fraser.

There are many to whom I would like to express my acknowledgements did space allow, but I should be indeed remiss did I forget to mention Lieutenant-Colonel Price, I.M.S. (retired), Captain Samman, R.A.M.C., Mr. C. Grey, and Mr. Balavendaram, all of whom have given valuable assistance in carrying out my experiments. Last, though far from least, I desire to acknowledge the kind and ready assistance given by Mr. C. H. Mounsey, Collector of Coimbatore, and Mr. C. Weir, Collector of the Nilgiri District, in obtaining snakes, animals for experimental purposes, etc.

APPENDIX A.

Experiments Directed to Ascertain whether the Influence of Bile is the Sole Protective Factor when Cobra Venom is administered by the Mouth to Animals.

EXPERIMENT I.

- Date.
2/10/99 A black pariah bitch, weight 13.6 kilos., was chloroformed, the abdomen opened with antiseptic precautions, and the common bile duct tied close to the duodenum; the gall bladder was opened, emptied, and sutured to the peritoneum (parietal) at the upper part of the wound, a tube inserted, and the rest of the wound carefully closed, a biliary fistula being thus left; the dog recovered well from the anaesthetic.
- 3/10/99 No signs of peritonitis; dog doing well.
- 4/10/99 Dog well and brisk. At 8.13 A.M. a dose of cobra venom (dry), weighing 0.30 gram, was given by the mouth on meat, and a similar dose repeated at 11.45 A.M. The dog thus took an amount of cobra venom corresponding to a dose of about 0.044 gram per kilo. of body weight.
- 5/10/99 The dog has not been any the worse for the venom. No sign of cobraism appeared. A dose of castor oil was given and the dog was freely purged thereby.
- 6/10/99 Passed two stools during the night; the first was well bile-stained, the second quite white; bile escapes freely from wound; fresh dose of oil given.
- 7/10/99 The dog had got free from its bandages during the night, and had been licking the bile from the wound; a fresh dose of oil was given, and the dog was securely tied, so that it could not reach the fistula with its mouth; the dose of oil was repeated in the evening to clear out any bile that had been licked up.
- 8/10/99 Dog passed quite white stools this morning, and so at 7.30 A.M. she received by the mouth a dose of 0.62 gram of cobra venom, that is, a dose of 0.045 per kilo.; at 10.30 A.M. she was brisk and wagging her tail.
- 9/10/99 The dog has not been at all sick, and is wagging her tail; seems in excellent health.
- 10/10/99 The dog is in excellent health; she passed white stools last night; at 7 A.M. a dose of 0.13 gram of cobra venom (0.01 gram nearly per kilo.) was given hypodermically. The dog quickly sickened, and was dead at 3.30 P.M., that is, in eight and a half hours.

Post-mortem Examination at 6 P.M.—Following down the common duct from the external opening of the biliary fistula, it was found that the ligature had been applied to the duct beyond all branches; the contents of the intestines were tested for bile, but the result was quite negative: there was no appearance of bile staining anywhere on the surface of the intestines. The stomach was full of food.

EXPERIMENT II.

- Date.
10/11/99 Under chloroform the abdomen of a pariah bitch (weight, 10 kilos.) was opened, the gall bladder drawn out, packed with sponges, and then emptied; the common bile duct was found, pulled out from the gut for the space of $\frac{1}{2}$ inch, and ligatured as close to the gut as possible; the external wound was then closed, an artificial biliary fistula being established externally in the same manner as in the last operation. The gall bladder was very full, as the dog had been fasting over night. To avoid all danger of the animal licking up bile from the wound, it was so fastened that it could not reach any part of its body with the mouth.
- 11/11/99 Dog doing well; no motion passed.
- 12/11/99 7.30 A.M.—2 ounces of castor oil given by mouth. 6 P.M.—Bowels acted; the first part of the motion was slightly bile-stained, the last part quite white.
- 13/11/99 Bowels acted early this morning, colour quite white; 0.5 gram of cobra venom was given by the mouth dissolved in milk at 10 A.M. The dog evidently disliked the taste, and the milk was poured down its throat; all the milk was retained. The dog seemed none the worse for the dose, and was quite brisk in the evening.
- 18/11/99 The animal did not at any time seem at all the worse for the venom; bile continued to escape freely from the fistula, and daily motions destitute of bile were passed. At 8 A.M. another dose of 0.50 gram of cobra venom was given dissolved in milk; the poison again appeared to burn her mouth, but she was easier after taking some broth; the whole quantity of milk was retained; she made no effort to vomit any of it. On each occasion the dose of poison corresponded to 0.05 gram per kilo. of her body weight.
- 19/11/99 The dog was in good health, and had shown no signs whatever of cobraism; she was given an overdose of chloroform, and a post-mortem examination made at once.

Post-mortem Examination.—The adhesive process around the sutured gall bladder was firm and healthy; there was no other evidence of peritonitis; the common duct was traced down from the fistulous opening in the gall bladder, and was slit up till it joined the duodenum; the silk ligature used was found retracted into the walls of the gut, and the duct was quite impervious till the ligature was cut; the whole length of the duct was then slit up into the gut, and a very marked contrast was noticed in the colour of the mucous lining of the duct above and below the site of the ligature; above it was stained a deep brown by bile, while below the surface was white and glistening. The small gut was empty throughout, and was destitute of any trace of bile; the large gut contained faecal masses also destitute of bile. The stomach was empty, and natural in appearance.

APPENDIX B.

Experiments to Ascertain whether Cobra Venom introduced into the Alimentary Canal of a Dog under such circumstances that the Peptic, Pancreatic, and Biliary Secretions cannot act upon it, retains its Lethal Properties.

EXPERIMENT I.

- Date.
23/11/99 The abdomen of a pariah bitch was opened, under chloroform, a coil of small gut was drawn out, and fixed outside the abdominal wall by means of a silk suture passed through the whole thickness of both lips of the incision and the mesentery of the coil; the suture, which was a double one, was fastened by tying its ends over two buttons, one at either side; a small horsehair suture at each end of the incision including the peritoneal coat of the gut made all secure. The dog had been prepared for operation by a dose of oil overnight and an enema in the morning.
- 25/11/99 The portion of gut above the skin surface was cut away with scissors; the mesenteric or posterior wall of the gut thus projected, forming a spur which effectually shut off the upper and lower openings of the artificial anus from each other.
- 27/11/99 The edges of the incision in the gut have healed, and the contents of the upper segment are voided freely, and show no tendency to pass into the lower segment's artificial orifice.
- 4/12/99 The dog has made a good recovery, and is gaining flesh. Its weight is a little over 9 kilos. 0.5 gram of dried cobra venom was weighed out, dissolved in 1 ounce of milk and water, and injected into the lower opening in the gut through a soft rubber catheter passed in about 3 inches; the catheter was withdrawn, and the lips of the orifice were kept closed by the pressure of the finger and thumb, so that no fluid could regurgitate. Within fifteen minutes the dog sickened, and was dead in fifty-five minutes.

Post-mortem Examination.—A post-mortem examination was performed at once. There was a healthy adhesion of the gut to the abdominal wall, and no other evidence of peritonitis; the artificial anus was found to be in the small gut, 12 inches above its junction with the large intestine; the mesenteric vessels of the area were turgid with blood, and the glands were swollen; the small gut above the artificial anus was flaccid and flattened, while that below stood out as if full of fluid. On slitting up the small gut above and below the artificial opening it was found that the mucous membrane of the lower segment was three times as thick as that of the upper. It presented an appearance closely resembling the gelatinous exudation one sees in subcutaneous tissue after the injection there of cobra venom, and the swelling was obviously of the same origin. This swelling accounted for the turgid state of the gut before it was slit up, for nearly all the fluid injected had disappeared and the gut was empty. The poison solution had evidently been absorbed through the walls of the gut, and in so doing had set up an inflammatory process, the outcome of which was the exudation referred to. There was no infiltration of the subcutaneous tissue around the attachment of the gut to the abdominal walls.

EXPERIMENT II.

- 23/11/99 Experiment I of this appendix was repeated, with slight variations. The intestine was opened at the time of operation, and the gut edges united at once to the abdominal walls by horsehair sutures; the posterior or mesenteric wall of the gut was drawn well forward into the wound, so as to separate the portion of gut above from that below the opening.
- 27/11/99 The cut edges of the intestine are healed. The dog has done well in every way.
- 6/12/99 The dog being in good health, and the artificial anus being in a healthy condition, an injection of cobra venom dissolved in milk and water was made into the lower orifice of the artificial anus. Weight of dog was just under 10 kilos. Total dose of poison given was 0.05 gram; therefore dose per kilo. was 0.005 gram. The dog showed no signs of suffering from this dose.
- 8/12/99 A dose of 0.25 gram of cobra venom dissolved in a few drachms of milk was administered, as before, by injection into the lower opening of the artificial anus. This represents a dose of 0.025 gram per kilo. of body weight. The dog was dead in an hour and a quarter.

Post-mortem Examination Four Hours after Death.—The opening of the artificial anus was situated 7 inches above the junction of the small gut with the large. As in the previous case a marked contrast was noticed between the thickness of the mucous membrane of the small gut above and below the artificial anus: above the membrane was natural; below it was much thickened, and was gelatinous in appearance; this gelatinous appearance also extended down some 5 or 6 inches of the first part of the large gut; the portions of both large and small gut which were affected in this way also had the mucous surface covered with a thick film of mucous exudation; the remainder of the intestine was normal in appearance.

In future communications it is proposed to deal with the protective properties of the serums of the cobra and Russell's viper, with the cause of the immunity possessed by the mongoose, with the nature of the protection conferred by bile, and with certain other topics.

THE Sanitary Council of Tangier has obtained from the Sultan of Morocco a concession of the island of Mogador as the site of a lazaretto.