THE VENOM OF THE AUSTRALIAN BLACK SNAKE,
(Pseudechis porphyriacus).

By C. J. Martin, M.B., B.Sc. Lond., Demonstrator of Physiology in
the University of Sydney, and J. McGarvie Smith.

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The literature of the chemistry of Australian snake poison is
very scanty, and with the exception of a few stray observations,
which will be mentioned later, there has been no investigation
from a chemical standpoint.

This paucity of literature is not surprising, when one considers
that those who have interested themselves in the subject of snake
poison, have almost exclusively, been gentlemen engaged in the
practice of medicine, having neither time nor opportunity for
chemical work, but who have used their best endeavours to find a
successful method of treatment in cases of snake bite.

A complete investigation into the subject of snake poison must
attempt to answer three questions:

1. What is the poison?
2. What is its exact physiological action?
3. How can one best prevent or counteract this action?

We venture to suggest that the majority of previous workers
have begun at the wrong end, for out of about four hundred
references which we have consulted on the subject of snake poison
over three hundred are to papers in which the author answers to
his own satisfaction this third question, and describes the bene-
ificial results following the administration of some such potent
drug as ash-tea, or human saliva, and the utter and entire futility
of the whiskey or any other treatment.

A chemical investigation into the poison of Australian snakes
is beset with even greater difficulties than is the case elsewhere,
which no doubt accounts for the fact that whereas the venom of American and Indian species have received the attention of many observers the chemistry of Australian snake poison is almost an unopened book.

There is the initial difficulty of obtaining the snakes in sufficient number and of sufficient size. Further one is unable here to command the services of a professional snake catcher to manipulate the creatures, so that it is necessary to overcome that dislike and dread of the serpent which is instilled into the youthful intelligence at an early age in every Christian land.

Again, the quantity voided at one time by the Black snake is insignificant compared with that obtained from a decent-sized Cobra or Rattle-snake, which latter discharges at a single bite five to ten times the amount of venom that we have been able to procure at any one time, under the most favourable circumstances, from the largest of our specimens, a snake six feet long.

But although the yield of poison is so small, we find that the virulence of our Black snake compares very favourably with that of the Cobra. That is to say, the minimal fatal dose per pound weight is in our hands considerably less than that given for the Cobra by the Indian Snake Commission, and not very considerably greater as has been previously stated.* Some idea of this virulence may be gathered from the fact that \( \frac{1}{100} \) grain invariably kills a rabbit of 5 lbs. weight, when injected into a vein, in about one hundred seconds.

The following experiments illustrate the toxic power of the poison:

\[ \frac{1}{10} \text{ grain of the dried Black snake poison was dissolved in 5 c. cm. of 1\% Na Cl solution, 0.05 c. cm. would accordingly equal } \frac{1}{1000} \text{ of a grain.} \]

Four rabbits, each weighing 5 lbs., were taken and the poison injected into the median vein of the ear. The first two received


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0.1 c. cm. of the solution (= $\frac{3}{300}$ grain) and died in 90 seconds and 91 seconds after the withdrawal of the syringe.

Two others received 0.05 c. cm. of the solution (= $\frac{1}{600}$ grain) and succumbed in 97 and 98 seconds respectively.

Similar experiments were performed with a solution of the venom from the Tiger snake (*Hoplocephalus curtis*) prepared in a similar manner, so that 0.05 c. cm. equalled $\frac{1}{600}$ grain.

The two rabbits which received 0.1 c. cm. (= $\frac{3}{500}$ grain) died in 92 and 101 seconds, and those which had 0.05 c. cm. (= $\frac{1}{600}$ grain) in 104 and 105 seconds respectively. All four rabbits weighed over 5 lbs.

The above experiments show a marked uniformity between the toxic power of these two venoms.

In the Proceedings of the Royal Society, London, for 1889, Dr. Sidney Martin gives a table purporting to represent the relative toxicity of the venoms from snakes of different species and from different countries which appears to us to be extremely untrustworthy, as there is no guarantee that the poison was procured in an equally pure state, nor that the method of introduction into the system was the same in all cases. When a snake bites, often twice as much saliva escapes from the mouth as venom, and unless this be prevented from passing into the receptacle by some such arrangement as was used by us, it would be dried and estimated as poison. Again, whether the poison be introduced intravenously or subcutaneously makes a difference of at least a decimal place in the lethal dose.

And further, the kind of animal experimented on, influences the result to a marked degree, rabbits being seven times as sensitive to Black snake poison as guinea-pigs, weight for weight. It should be noted too that the quotation made by Dr. Sidney Martin from the Victorian Medical Society's Proceedings, refers to fresh, that is wet poison, whereas that from the late Mr. Vincent Richard's "Landmarks of Snake-poison Literature," obviously refers to poison in a dried condition. Lastly, we have consulted the data
given in this little book just referred to, and fear that Dr. Martin has made a mistake of one decimal place in favour of the Cobra, in his calculation, and that the venom of this snake is really less powerful than he would lead us to believe, but in a matter of arithmetic we are anxious not to insist too strongly.

A short historical resumé of the chemical work on the poisons of snakes from other countries is necessary, in order that the point from which we start may be appreciated and our results compared with those obtained by the various observers working with other species.

In this resumé we shall not include the historically interesting but otherwise unimportant views as to the composition of snake venom, held by those pioneers in the subject, Francesco Redi, Dr. Mead, Charas, and the Abbé Fontana. Some account of these early workers and their contentions is given by Mr. Vincent Richards, Chairman of the late Indian Snake Commission, in the Landmarks of Snake-poison Literature."*

The first investigation into the chemistry of snake poisons of any importance was by Prince Lucien Bonaparte on the poison of an adder (Pelias berus) in 1843. An interesting account of this is given by Sir Joseph Fayrer in a paper in the Proc. Med. Soc. Lond. 1884. Bonaparte found that the activity of the poison was associated with that portion precipitated by alcohol, and he gave the name of echidnine or viperine to this precipitate. The result of his analysis is stated in the table below—†

1. Echidnine or viperine (the active principle)
2. A yellow colouring matter
3. A substance soluble in alcohol
4. Albumen or mucus
5. Fat
6. Chlorides and phosphates

* Landmarks of Snake-poison Literature, Madras, 1886.
† For this table we are indebted to a paper by Badaloni—Lancet, 1883, Vol. 1.
After Bonaparte the subject appears to have had no investigation until about 1860, when Dr. Weir Mitchell turned his attention to the subject. This accomplished author is essentially the founder of our present knowledge on the subject, though conclusions drawn at the time when animal chemistry was in its infancy, have very naturally had to be modified as knowledge of the chemistry of proteids has advanced.

The following analysis of the venom of the Rattle-snake was published by Mitchell in 1861:*  

1. An albuminoid active principle (Crotalin)  
2. An albumen (coagulable by heat)  
3. Colouring matter  
4. A trace of fat  
5. Chlorides and phosphates

The parallelism between this and the analysis of Bonaparte is striking. The great advance made by Mitchell was the determination of the proteid nature of the active principle. These researches left the study of venoms in as satisfactory a position as could be gained with the laboratory facilities of 1843–1861.

In 1861, this author published some more observations on the subject, and then in 1883, in conjunction with Reichert published a preliminary report of some very extensive researches they had been carrying on for some years, on the chemistry and physiological action of the poisons of the North American snakes; a complete account of which was published by the Smithsonian Institute in 1886.†

In these papers the authors establish the proteid nature of the venom of the American snakes, and ascribe poisonous properties

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‡ "Researches upon the Venoms of Poisonous Serpents."—Smithsonian Contributions to Knowledge, 1886. This paper contains an exhaustive bibliography on the subject of Snake Poison up to 1885.
to a peptone, and three varieties of globulins which they separate by "appropriate processes." The reactions given by their so-called peptone are characteristic of those bodies which we know now as albumoses, and a mixture of the primary albumoses is capable of behaving in many respects as their "globulins."

In India the subject has received the attention of many observers. About 1871 Brunton and Fayrer* began an extensive series of experiments with the venoms of the Cobra, Kraits, and Indian viper (Daboia russellii). Their valuable work however hardly deals with the chemical aspect of the question.

In the first volume of the Analyst, Winter Blyth† published a paper, the contents of which, coming from an analyst of such acknowledged experience, attracted considerable attention. In this paper Blyth stated that he had discovered in Cobra venom a highly poisonous crystalline body, to which he gave the name "cobric acid," and that this cobric acid was the sole poisonous constituent.

We have consulted his methods and are convinced that he is not justified in coming to any such conclusion. Blyth's conclusions were criticised by Wolfenden,‡ who maintained that the crystals figured by him were sulphate of lime (!) derived from the water in which the poison was dissolved.

In 1878 Professor Pedler§ of Calcutta published an account of his investigations. He made an ultimate analysis of the dried poison, and showed that in percentage composition it corresponded fairly with that of albuminous bodies generally. He also claimed to have separated a "semi-crystalline" body of an alkaloidal nature to which he ascribed the potency of Cobra venom.


† "Analyst," 1876, Vol. i.


Armstrong, and still earlier Dumas, had previously made ultimate analyses of the poison with somewhat similar results. Dr. Armstrong's analysis was appended to the Snake Commission Report, 1874.

In 1883 Wall* published his interesting book "Indian Snake-poisons their nature and effect," which contains two facts of especial importance from a chemical point of view, viz.,

(1) That the whole of the poisonous properties reside in the coagulum by absolute alcohol, and that if the alcohol be absolute, the filtrate is quite innocuous.

(2) That the poisonous principle is taken up by distilled water from the precipitate by alcohol, and that the solution so obtained possesses all the toxic properties of Cobra poison.

Dr. Wolfenden's† valuable contributions to the subject appeared in 1886. In these papers the author establishes the proteid nature of the poison, and excludes the possibility of alkaloids, ptomaines, germs, and any body of the nature of Blyth's "Cobric acid." He claims to have separated an albumen, an albuminate, and a globulin, to all of which he ascribes poisonous properties. This author does not appear to have been aware of Wall's results, for all of these bodies, would be rendered insoluble by absolute alcohol.

When Wolfenden's work is read by the light of recent developments in proteid chemistry his conclusions do not by any means necessarily follow.

In the last number of the Journal of Physiology for May of this year, is a paper by Kanthack,‡ in which he shows that Cobra poison contains a proto-albumose which is capable of producing all the symptoms of the fresh venom. This paper is especially interesting as the methods used were similar to those employed by us, and his results as far as they go point to a close analogy between the composition of the venom of the Cobra and that of Australian snakes.

* "Indian Snake Poisons, their nature and effect."
In this paper we have endeavoured to give as complete an account of the composition of the venom of our Black snake, as our supply of material would allow. The particular species was chosen because we had the largest supply of this kind of poison.

Method of obtaining the poison.

Our method is a modification of that adopted by the Indian snake-men, who employ a mussel-shell covered with a plantain leaf to catch the poison. The snake is firmly grasped immediately behind the head and as a result of this and previous manipulation he generally opens his mouth. The shell is thrust between the jaws so that when the creature bites, the fangs penetrate the leaf and the poison trickles into the receptacle below. We follow a like procedure, only instead of a mussel-shell and plantain leaf, we use a hollow framework of wood in the shape of an inclined plane, and in both planes of which holes are cut near the sharp angle A. (See figure.)

The upper hole is covered with thin rubber sheeting such as is used by dentists in the stopping of teeth, and the edges of the lower are bevelled so as to hold a small watch-glass, which is kept in position by a pair of clips. The ingenious idea of using rubber is due to Mr. Bray a naturalist of this city.

By this device we obtain the poison free from all secretion from the mouth. It flows direct from the fang into a watch-glass the weight of which is known. The glass is removed and reweighed with contents, then placed in a desiccator over calcium chloride, and when dry, again weighed. From these data it is easy to calculate the percentage of solids in the sample of poison obtained.
This has been done in every case, so that we have a long series of observations showing how this percentage varies under different circumstances, such as previous discharge of poison, feeding, time of year &c.

The variation in the percentage of solids (which means variation in the toxic strength of the venom) is very extensive. We have obtained poison containing as much as 67% of solids and as little as 12%. These results which we hope to communicate in detail at some future period are interesting and important, as they show that one is not justified in dealing with the fresh (i.e. wet) snake poison as a constant factor.

This error vitiates all the experiments of the committee appointed to enquire into the subject of snake poison by the Medical Society of Victoria in 1875-6.

Nicholson* notes variations in the proportion of solids in cobra venom of nearly as great a range as ours.

The poison thus obtained is a clear straw coloured fluid of varying viscosity and strongly acid reaction. On drying at about 16° C. in a desiccator it forms clear glistening scales, behaving just in the manner of white of egg under similar circumstances. It will keep for six months (probably any length of time) in this condition, and retains its virulence unimpaired. At the end of this time it dissolves up completely in a small quantity of distilled water or weak salt solution (1 to 10%) forming a perfectly clear solution. The poison though strongly acid when received direct from the fangs, loses its acidity by drying, so that the solution formed afterwards is neutral or very faintly acid. The acid is volatile and by drying except at low temperatures is driven off.

In recent times the effects following the injection of snake poison, have been ascribed by various authors to five different classes of bodies, which according to these authors are present in venom, viz.:

(1) Germs.

* Indian Snakes, Madras, 1874.
Some body of the nature of a digestive ferment.
Alkaloids or ptomaines.
A crystalline acid.
Proteids.

Although grave doubt has been thrown even upon the existence in venom of the majority of the above list, and although it has further been shown that the active properties of the Indian and North American snakes are resident in the proteid constituents of these venoms, facts which afford a basis a priori, for inferring that the poisonous qualities of Australian snakes are due to similar substances, we yet thought it advisable to exclude the possibility of the presence of any poison of another nature. The results of our observations and experiments in reference to each of these alleged poisonous bodies may now be discussed seriatim.

(1.) In 1878 Dr. Jean Baptist de Lacerda* of Rio, announced his discovery that the effects of snake poison were due to germs contained in the venom. To test this statement, snake poison was allowed to drop from the fang directly upon a slide, means being taken to prevent contamination by saliva. The drop of poison was then dried and stained with methyl violet and examined for micro-organisms, but with negative result.

We have also inoculated tubes of nutrient beef broth and gelatine, and of nutrient snake broth and gelatine, with the poison and have kept them at 20° C., again with negative result.

Lacerda does not say how he collected his poison, most probably it was contaminated with saliva, which in snakes under some circumstances teems with bacteria. When a snake swallows another animal, frequently almost as large as itself, the creature swallowed undergoes putrefaction as well as digestion, and judging from the appearance and smell of the gastric contents in snakes we have opened some few days after a meal, one would say that the former process was in excess. With such a decomposing mass in the stomach it is not surprising that the mouth should be swarming.

with bacteria, and from the negative results of our observations 
we are in a position to state—that snake poison as discharged 
from the fangs does not under normal circumstances contain any 
micro-organisms.

(2.) The second suggestion that the poison contains a ferment 
analogous to a digestive ferment has suggested itself to many, owing 
no doubt to the fact that the venom gland is the homologue of the 
parotid salivary gland. Prof. Halford* stated as his theory of 
the action of snake poison that it digested or dissolved the 
fibrinous elements in the blood and so prevented it from clotting, 
and he considered all other symptoms as secondary to this primary 
effect on the blood.

In 1881 we find Lacerda, who had in the mean time wearied of 
his former hypothesis, advancing a similar view. He states† that 
the poison is a digestive juice comparable to pancreatic secretion 
but much stronger.

Recently too, some observations appeared in one of our daily 
papers to the effect that snake poison was capable of digesting 
pieces of flesh, and hard boiled egg. To ascertain whether our 
snake poison really possessed any such digestive activity, we per-
formed the following experiments:—

Eight test-tubes were taken and divided into two groups. Four 
were half filled with 0·2% H.Cl., and the remaining four with 1% 
Na₂ CO₃ solution, in order to test whether the poison might be 
capable of digesting in an acid or alkaline medium. To two tubes 
each group was added a small piece of clean, fresh fibrin, and 
to the other two in each group an addition of 1 c.c. of dog’s serum 
was made. Thus we had four pairs of tubes, the pairs being 
exactly alike in every respect. To one tube in each pair a small 
quantity of snake poison was added and the whole eight tubes 
were placed in an incubator at 40° C. for forty-eight hours, a 
small crystal of thymol having been added to those containing

\( \text{Na}_2\text{CO}_3 \) to prevent putrefaction. In none of the tubes was the fibrin dissolved nor the serum affected. We may therefore conclude that there is no reason to ascribe the effects of Black snake poison to any digestive action.

(3.) Gautier stated* that he had extracted two ptomaines from the venom of the Cobra. Wolfenden† however was unable to confirm this, and Prof. Walcott Gibbs‡ who examined the venom of the Rattlesnake at the request of Dr. Weir Mitchell, was unable to discover the presence of any body of this nature.

The possibility of the presence of ptomaines adherent to the proteid constituents of snake poison demands careful attention. In the breaking up of proteids under the digesting influence of micro-organisms (e.g. Anthrax bacilli) it has been shown by Dr. Sidney Martin,§ that alkaloids or ptomaines are frequently found as part-end-products in addition to albumoses. Similar results were obtained with tubercle bacilli by Crookshank and Herroun,|| Hunter,** and Hankin.†† Brieger ‡‡ also in his monograph on Animal Alkaloids, has shown that a ptomaine “pepto-toxine” is formed during artificial gastric digestion.

Accordingly we carefully examined a small quantity of poison by Brieger’s§§ modification of the Stas-Otto process for the separation of alkaloids. The extract which should contain any body of this nature, was quite harmless when injected into the peritoneal cavity of a small guinea-pig.

We therefore conclude that such bodies are absent from Black snake poison.

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† Loc. cit.
‡ Smithsonian Contribution to Science, 1886.
(4.) Blyth* is the only author who claims to have derived a crystalline acid body from snake venom and his statements have, to say the least not been confirmed. As we mentioned earlier the acid present in Black snake venom, at any rate in the quantities which we have been able to experiment with, produces when injected into an animal, no obvious physiological effect.

(5.) As it has been conclusively shown that the albuminous bodies in the venom of Cobra and Rattle-snake do actually possess toxic qualities, we naturally look to the proteids of Black snake poison, as the active agents. This venom we find to be a strong solution of proteids in which we can discover nought else but the volatile acid and a small amount of inorganic salts, of which sodium chloride forms the major portion. All reagents which precipitate the proteids from a solution of venom deprive it of its toxic powers and submission to those conditions which are capable of decomposing or altering proteids in solution—e.g. prolonged boiling, either alone or with diluted acids or alkalies, convert this virulent solution into an innocent liquid. Reagents such as Ag NO₃ which precipitate proteids in an insoluble form, render both the precipitate and filtrate perfectly harmless. We therefore conclude that there is no poisonous body present in the venom other than proteids.

The proteids present in the venom.

A two per cent. solution of Black snake poison in one per cent. NaCl behaves in the following manner with the undermentioned reagents:

(1) Warming with nitric acid and the subsequent addition of ammonia—orange colouration (Xantho-proteic).
(2) Millon's reagent—usual proteid reaction.
(3) Heating—turbidity between 80 – 86° C., which settles as a precipitate.

The filtrate from this solution still contains proteid and gives the following reactions:

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* Loc. cit.
(4) Caustic potash and trace of CuSO₄—brilliant biuret reaction.
(5) Nitric acid—a precipitate soluble on heating but returning on cooling. (This precipitate is increased in presence of more NaCl).
(6) Picric acid—precipitate disappearing on heating, returning on cooling.
(7) Saturated with NaCl—precipitate.
(8) Saturated with MgSO₄—precipitate.
(9) Saturated with Am₂SO₄—precipitate.
(10) Dropping the fresh solution into distilled water produces a cloud.
(11) CuSO₄ 5% solution—precipitate.
(12) Alcohol—precipitate.

From the above reactions it is obvious that the poison contains:

(a) A proteid coagulable by heat, viz. albumin or globulin.
(b) Some proteid or proteids not thrown out of solution by this treatment, which might include albuminates (acid albumin), albumoses, or peptone.

A solution of the fresh poison gives no precipitate when neutralized by 1 in 5,000 KHO, thus excluding the presence of acid albumin. Peptone also is absent, for the solution was acidulated with a drop of 5% H₂SO₄* and shaken with Am₂SO₄ crystals for twenty-four hours. After this thorough saturation the filtrate was proteid-free.

The albumoses present are hetero-albumose, proto-albumose, and possibly deutero-albumose in small quantity, these were separated in the following manner:

A solution of the original poison was heated to 90° C. and filtered. The filtrate was saturated with MgSO₄ and shaken for twelve hours. By this means a flocculent precipitate was produced. The whole was then thrown on to a filter and thoroughly washed with a saturated solution of MgSO₄.

The filtrate was dialysed for twenty-four hours in running water and two days in distilled water, and then concentrated by dialysis against absolute alcohol. We thus obtained a few c.c. of fluid, containing a small quantity of proteid in solution.

The proteids which alone could be present in this solution after the above manipulations are, proto-albumose (traces), deutero-albumose and peptone. The last we have previously shown is not present in the venom.

Neumeister has shown that it is impossible by saturation with neutral salts, to absolutely precipitate all proto-albumose from solution,* and as the filtrate became cloudy on the addition of a few drops of 5% Cu SO₄, some proto-albumose was present in the filtrate. Whether deutero-albumose was also present we were unable to determine, as we are not acquainted with any method by means of which traces of this proteid may be determined in the presence of proto-albumose.

The contents of the filter (i.e. the precipitate thrown down by saturation with Mg SO₄) were all washed through by means of distilled water, and the solution freed from Mg SO₄ by dialysis as in the previous case.

At the end of three days there was a considerable precipitate on the dialyser. The contents of the dialyser were emptied into a test-tube and centrifugalised. In a few minutes the precipitate was deposited at the bottom of the tube.

The supernatant fluid was pipetted off and concentrated by dialysis against absolute alcohol and evaporated to dryness at a gentle heat (40° C.). The precipitate was thoroughly washed with a large volume of distilled water, by means of the centrifuge, and then dried over chloride of calcium.

In this way we obtained two albumoses, both precipitable by saturation with Mg SO₄ and therefore belonging to the class of primary albumoses, one of which was soluble in distilled water, the other insoluble, but readily soluble in dilute solutions of

* Neumeister, Zeitschrift f. Biologie, Bd. xxiii.
neutral salts. The names given to similar bodies formed in the gastric digestion of proteids* are respectively, proto-albumose and hetero-albumose.

We next allowed some venom to remain under a large volume of absolute alcohol for several weeks, by which means proteids other than albumoses or peptones are rendered permanently insoluble in water or dilute saline solutions, whereas proto-albumose is unaltered and hetero-albumose only to a certain extent, some portion of it being changed into dys-albumose and therefore no longer capable of being dissolved by these media. The results obtained by this treatment were confirmatory of those detailed above.

After the sojourn under alcohol the precipitate was extracted with 5% Na Cl solution. The extract so obtained was very virulent and contained proto- and a small amount of hetero-albumose, which were separated in the following manner. The precipitate was digested with the salt solution for twenty-four hours and filtered. The filtrate was dialysed against distilled water, which was frequently changed, for forty-eight hours. At the end of this time there was a slight flocculent precipitate on the membrane. The whole contents of the dialyser were then decanted into a test-tube, the deposition of the precipitate hastened by means of the centrifuge and the supernatant fluid carefully pipetted off.

The small precipitate was then thoroughly washed with an abundance of distilled water and then the tube again placed in the centrifuge, and in a few moments the precipitate was deposited at the bottom of the tube, and the excess of fluid could now be easily removed. The sediment was dried at a gentle heat. The fluid contents of the dialyser were concentrated by a further dialysis against absolute alcohol and evaporated to dryness at 40°C.

In this way we again obtained two bodies (albumoses) the one insoluble in distilled water and therefore precipitated by dialysis,

the other soluble. These two albumoses gave the same chemical and physiological reaction as those separated by our first method.

We now turned our attention to the proteid coagulated by heating.

On dialysing the fresh poison, after some days there is a precipitate on the membrane. This might be either a globulin or hetero-albumose, but from the fact that dilute solutions of this precipitate in 1% NaCl gave no obvious precipitate on heating, we are of the opinion that the former of these bodies (globulin) is absent.

The venom of this snake contains a small and variable amount of albumen. The temperatures of coagulation were determined by an arrangement similar to that suggested by Prof. Schäfer and described by Professor Halliburton.* This apparatus was so modified as to enable us to work with very small quantities of fluid (viz., 2 c.c.), and the tube was viewed by reflected light against a black back-ground in order that the slightest turbidity should be visible. The solutions used contained 2% of the dry venom. We noticed an extraordinary sensitiveness on the part of the albumen to the acidity of the solution. The slightest excess of acetic acid prevented coagulation, the albumen being converted into acid albumen, as on neutralizing the boiled solution carefully with 1 in 10,000 KHO it was precipitated.

Some idea of this sensitiveness can be drawn from the fact that if a glass rod 2 m.m. in diameter moistened with 0.05% acetic acid were used to stir the 2 c.c. of solution, too much was added, and unless the rod had been previously shaken to remove all excess, coagulation was prevented. If, on the other hand, the fluid were alkaline or even sometimes neutral, no coagulation occurred until it was acidified. Having ascertained the necessary degree of acidity, we found that there were two temperatures at which definite flocculi were formed, separated from each other by about 4° C. That is to say, if the first formed coagulum were filtered off, and the filtrate reacidified, on further heating, the solution

* Journ. of Physiol., Vol. v.
again became turbid and a second precipitate was formed. This occurred whether the fluid were reacidified or not, but under the latter circumstances happened at a few degrees lower than if the second acidification had not taken place. The mean temperatures at which these two coagulations took place in eight experiments were 82° C., and on raising the temperature to 95° C. no further precipitate occurred. After saturation with Mg. SO₄ and ridding the filtrate from excess of the salt by dialysis, in the one experiment in which this was done, flocculi appeared between 83° C. and 84° C., and again at 90° C.

The virulence or innocence of these albumins is a point which is very difficult to determine. Albumin in a soluble condition and unaltered is very hard to separate from a solution containing proto- and deutero-albumose. We have tried various neutral salts and saturation with combination of salts, but in every case in which albumin was thereby precipitated, deutero-albumose came down also.

It is not only impossible to separate the albumin from deutero-albumose, but also from traces of proto-albumose by the saturation methods. For though it is true Mg. SO₄ solution precipitates proto-albumose and does not affect albumin—it is impossible to precipitate the whole of the proto-albumose by this method, unless acetic acid be added at the same time.* Unfortunately this addition of acetic acid to a solution saturated with Mg. SO₄ brings down at the same time any albumin.

Owing to the virulence of the albumoses present in the venom it is essential that any albumin separated, be absolutely free from these former bodies, in order to determine whether the albumin itself may possess any toxic powers.

To separate the albumen in an uncoagulated condition we were obliged to resort to dialysis. 0.5 gramme of dried venom was dissolved in 25 c. cm. of distilled water, and placed in a specially prepared dialysing tube made from the muscular coat of a rabbit's

* Neumeister, loc. cit.

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intestine. This membrane was much thinner than the sausage skins made of sheep's-gut or vegetable parchment, and hence allowed diffusion to proceed at a more rapid rate.

The skin containing the solution of venom and a crystal of thymol was suspended in a vessel through which a gentle stream of distilled water passed. After twenty-four hours the contents of the skin had become turbid, and at the end of forty-eight hours a sticky precipitate adhered to the inside of the tube. The contents were then turned into a test-tube and centrifugalized, the supernatant clear fluid pipetted off and placed in a new dialysing tube of the same material, together with a crystal of thymol. The contents of this second tube were dialysed in distilled water for between three and four weeks. At the end of this time, a few drops gave no cloudiness when allowed to stand over Mg. SO₄ crystals for twenty-four hours. The dialyser, however, contained a small quantity of proteid in solution, which coagulated on heating.

The whole of the remaining solution was concentrated to 3 c.c. by warming at 40° C., 2.5 c.c. of which was injected into the jugular vein of a small guinea-pig, and the remainder soaked up by a small piece of sponge which was placed in the abdominal wall, in the manner described below. The solution produced neither local nor general effect. These experiments point to the innocence of the contained albumens.

To sum up, we find Black snake venom contains the following proteids—albumin in small quantity: hetero-albumose and proto-albumose in considerable amount: with perhaps a trace of deutero-albumose.

*Note.*—In every case of dialysis a crystal of thymol was added to the fluid to prevent decomposition. The various filtrations were much facilitated by the adoption of the method of Fessenden* by whose arrangement of folding the paper, not only does one dispense with a funnel, but in no part is the paper double or triple, so that the whole surface of the filter paper is utilised.

We now come to the consideration of the question as to which of these separated proteids possess toxic properties?

The results of the introduction of any poison into the system can be divided into (1) local, (2) general effects. To test the former it is necessary to have an extremely small dose of the poison acting continuously. For a very excellent method of accomplishing this we are indebted to the suggestion of Mr. C. J. Pound, who also performed and took charge of the majority of the experiments to test the local effect of our various solutions.

A guinea-pig was taken and the belly shaved, and washed with a solution of $\text{HgCl}_2$, 1 in 1,000, the rectal temperature having been previously taken. Then two small pieces of sterilized sponge (2 m.m. cube) one of which had been soaked in the proteid solution concerning the local action of which we desired information, were aseptically introduced into the inter-muscular planes of the abdominal wall, one on each side. The one not previously treated with the supposed poison acted as a control. The small incision which was one inch distant from the situation of the sponge, was brought together with a horse-hair suture, and covered with collodion. In this way we obtained the maximum of local with the minimum of general symptoms.

Dilute solutions of our albumoses inserted in this manner, produced edema spreading over the whole of the same side of the abdomen in from six to eight hours. In no case was there any exudation around the control sponges.

To test the general effect, the solutions were introduced either into a vein or into the peritoneal cavity. The proto- and hetero-albumose killed in a few hours. We therefore conclude that the active principles of this venom are, proto-albumose, and hetero-albumose.

Action of heat on a solution of the venom.

Boiling does not destroy the activity of Black snake poison, unless this be continued for some hours, and momentary heating to 100° C. did not appear to cause any diminution in the virulence.
of the solution, until we repeated our experiments under circumstances in which much greater accuracy was possible, viz., by using the minimal fatal dose and direct introduction into the venous circulation.

The following experiments illustrate this diminution in toxic power:—Six guinea-pigs of nearly equal weight were taken and the minimal fatal dose of the poison was ascertained by injecting into the jugular vein of the first \(\frac{3}{2}\) grain, into the second \(\frac{1}{2}\) grain. Both these animals suffered from convulsive movements which gradually decreased in severity and they recovered in four to five hours. The third guinea pig received \(\frac{1}{2}\) grain, which caused death in sixty seconds.

The three remaining pigs each received an intra-venous injection of a solution of the poison which had been momentarily raised to 100° C. One of these latter received an amount of the solution = \(\frac{3}{2}\) grain, and two others = \(\frac{1}{2}\) grain. All three suffered from some twitching, but none died, in fact in three hours one could not detect anything wrong with them.

We have been unable to detect any chemical change in the solution on heating to 100° C. with the exception of the coagulation of the albumin, which we have reason to believe is not possessed of poisonous properties, and cannot therefore attribute the diminution in toxic strength of the solution to this cause. The boiled solution even when boiled for several days, by placing in a small flask with a little vertical condenser attached to it, and surrounding the flask by a water bath, still contains proteids in solution which possess the same chemical characters as before, though it has lost its physiological importance. This effect of heat on Black snake venom, is similar to the results obtained by authors for the venoms of American and Indian snakes.

Mitchell and Reichert found that heating the solution produced a continued impairment in its toxic power even after the separation of the "globulin" by coagulation, which occurred below 75° C., and attributed this to the action of heat on the
solution of their "venom-peptone" (albumose). Wall showed
that heating to 100° C. lessened the virulence of Cobra venom.
Kanthack denies that boiling ordinary Cobra poison diluted with
water momentarily diminishes its toxic power and gives three
experiments to prove this, and remarks "These three examples
may suffice." As three experiments performed in the same manner
with Black snake venom gave a similar result, but on repeating
with the more accurate method previously mentioned, showed a
marked deterioration in power, we cannot help thinking that if
Dr. Kanthack repeated his experiments with similar precautions
he might obtain the same result as Dr. Wall. The effect of rais-
ing the temperature of the venom of the Indian viper to 100° C.
entirely does away with the direct effect of that poison on the
nervous system, as Wall has shown.

Dr. Sidney Martin's results with abrus-albumose and the albu-
moses produced by "Anthrax-digestion" are exactly analogous,
the former being especially sensitive, so that raising the solution
to 85° C. rendered a lethal dose harmless, while at the same time
no chemical change could be detected.

The action of caustic potash appears to be very much the same
on Black snake venom as that observed by Weir Mitchell, Wall,
and Vincent Richards with Crotalus, and Cobra venoms. When
the poison is mixed with twice its weight of caustic potash dissolved
in a few drops of distilled water, and then injected subcutaneously,
a fatal dose fails to produce any effect. If however the mixture
be introduced into the vascular system a fatal result follows, but
the time which elapses before death is immensely increased. A
few experiments illustrate these points.

\( \frac{1}{16} \) grain dried Black snake poison was dissolved in \( \frac{5}{1} \) c. cm. of
a 1% solution of Na. Cl. and injected subcutaneously into the thigh
of a rabbit. This animal died in four hours, twelve minutes.

\( \frac{1}{16} \) grain Black snake poison and \( \frac{1}{16} \) grain KHO dissolved in \( \frac{5}{1} \)
c. cm. 1% Na. Cl. solution injected into thigh of a rabbit as in
previous experiment. No effects followed.
\frac{1}{10} \text{ grain of Black snake poison dissolved in } 0.5 \text{ cm. } 1\% \text{ NaCl. introduced into the marginal vein of the ear of a rabbit. Dead in ten seconds.}

\frac{1}{10} \text{ grain Black snake poison and } \frac{1}{10} \text{ grain KHO dissolved in } 0.5 \text{ cm. } 1\% \text{ NaCl. introduced into a vein of the ear of a rabbit. Dead in one hour fifteen minutes.}

Our conclusions that albumoses are the active agents in Black snake poison are interesting when compared with similar results obtained by Sidney Martin,\textsuperscript{*} Hunter,\textsuperscript{*} Hankin, and Crookshank and Herroun,\textsuperscript{*} with the poisons produced by the bacilli of anthrax diphtheria, and tubercle. Albumoses are the products of the hydration of albumins, and this hydration can be accomplished in many ways, for instance by boiling solutions of albumin at high pressure, by gastric or pancreatic digestion, or by the vital influence direct or indirect of cells. In the case of gastric or pancreatic digestion the result is brought about in an indirect manner, i.e., the gland cell manufactures a ferment, pepsin or trypsin, which, under suitable conditions, hydrate the albumins, forming albumoses &c. In the case of digestion by the diphtheria bacillus, Martin\textsuperscript{*} has shown that the bacilli, grown either in the juices of the body or in culture-media outside it, produce by their activity a ferment which is capable of forming albumoses from the proteids. These albumoses formed by such bacilli are highly poisonous, much more so than those produced during gastric digestion, and give rise to the pathological conditions which we recognise as the disease diphtheria. Anthrax bacilli do not give rise to any ferment but are capable of directly digesting albumins, producing poisonous, but different albumoses. The same is the case with tubercle bacilli.

In all these processes of hydration some of the albumin is further broken up, giving rise to some nitrogenous body of the ammonia type. In the case of gastric digestion we have a ptomaine peptotoxin.\textsuperscript{†} In pancreatic digestion we have leucin and tyrosin, in

\textsuperscript{*} Loc. cit.

\textsuperscript{†} Brieger, "Ueber Ptomaine," loc. cit.
anthrax and tubercle digestion we have an alkaloid. In diphtheria, no alkaloid is formed, but an organic acid, and the question whether this be nitrogenous, is as yet undetermined.

The following table taken, and somewhat altered from Sidney Martin's Goulstonian Lectures to the Royal College of Physicians, London, illustrates this analogy, to which we have added our results with snake poison for comparison:

Table illustrating the analogy between various processes of hydration due to vital activity.

<table>
<thead>
<tr>
<th>Primary Agent</th>
<th>Ferment</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial cell of gland (Stomach)</td>
<td>pepsin</td>
<td>hetero, proto, and deuter-albumoses, peptone.</td>
</tr>
<tr>
<td>Epithelial cell of gland (pancreas)</td>
<td>trypsin</td>
<td>globulin like body peptone</td>
</tr>
<tr>
<td>Bacillus anthracis</td>
<td>(none)</td>
<td>hetero, proto, and deuter-albumoses, peptone.</td>
</tr>
<tr>
<td>Bacillus diphtheriae</td>
<td>ferment</td>
<td>proto and deuter-albumose.</td>
</tr>
<tr>
<td>Epithelial cell of gland (venom-gland snake)</td>
<td>(none)</td>
<td>hetero-albumoses...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>homo-albumoses...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>proto-albumose...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hetero-albumose...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>deutero-albumose...</td>
</tr>
</tbody>
</table>

Our conception of the formation of these albumoses in the venom-gland of the snake is the following:—The cell, by a vital process directly exercises an hydrating influence on the albumins supplied to it by the blood, the results of which influence are the albumoses, which we find in venom. The differences between this process and the digestion by pepsin or by anthrax bacilli, is that in the case of the gland cells of the venom gland the hydration stops short at the albumose stage, and is not continued so as to form peptone, as is the case with the others mentioned. That the protoplasm of gland epithelium is capable of exercising such an hydrating influence, we will instance the conversion of glycogen into sugar by the liver.

It must be borne in mind that although the proto-albumose, hetero-albumose, and deuter-albumose which are formed by these various agencies, have so far not been chemically differentiated,
they are not identical. And when submitted to that infinitely more sensitive test, the physiological one—produce vastly different results.

In view however of the essential chemical identity which does nevertheless underlie such physiological differences, we may be permitted to express our conviction that the discovery of a method of antagonising those effects which follow the administration of snake poison, would be a highly important contribution towards the solution of the problem of dealing with the effects of the virulent products in zymotic diseases.

In conclusion we wish to express our gratitude to Mr. C. J. Pound for invaluable help with the various experiments, the results of which are recorded in this paper.

SOME FOLK-SONGS AND MYTHS FROM SAMOA.

Translated by the Rev. G. Pratt.

With Introduction and Notes by John Fraser, LL.D.

[Read before the Royal Society, N.S. Wales, November 2, 1892.]

XXXI.—Losi and Malae-Lā—A ‘Tala.’

The war of the gods and the giants.

(Three Versions, Nos. xxxi., xxxii., xxxiii.)

Introduction.—The classical scholars who are here to-night will be interested to learn that the Sāmoans have a myth which is a local version of the Grecian story of the war of the gods and the giants. I was myself surprised when, on turning over a bundle of old manuscripts, I found one written by a Samoan hand and headed with the words ‘ia le malaga na alu i le lagī,’ about the expedition which went up to the heavens.' When translated, it was found to be the description of a contest between

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