## ORIGINAL COMMUNICATIONS.

# REPORT OF SOME INVESTIGATIONS INTO THE CHEMICAL NATURE AND PHYSIOLOGICAL ACTION OF COBRA POISON.

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In 1843 Prince Bonaparte separated from viper poison the active principle, to which he gave the name of '*Echidnin*'. His method was to precipitate by alcohol, collect on to a filter, wash with distilled water and ether, and dry the residue. '*Echidnin*' would therefore be a proteid body. The washing of the coagulum would no doubt dissolve off some of the albumin, which would account for the proteid of doubtful nature, which Prince Bonaparte designated 'Albumen or Mucus,' but which he did not regard as the active toxic principle.

In 1861 Weir Mitchell examined crotalus venom, and by boiling it with water to coagulation, decanting the supernatant liquid and treating by alcohol, he obtained a substance which he called '*Crotalin*.' This he described as an albuminoid body. Like Prince Bonaparte—in the case of viper venom—Dr. Weir Mitchell obtained a second "albuminoid substance coagulating by heat," but which he evidently did not regard as the active poison which was embodied in the *Crotalin*.

In 1873 Dr. Armstrong made an analysis of cobra venom. Having regard to the fact that the percentage composition of proteids is notoriously variable, and no constant formula can be assigned to albumen his results agree fairly well with what we take as the average composition of 'albumen.' Roundly speaking he found that cobra poison contained carbon, 45·76; hydrogen 6·6; nitrogen 14·3; sulphur 2·5; thus differing from 'albumen' chiefly in the smaller percentage of carbon.

From all these earlier researches the deduction is that the venom of snakes is something very like a proteid. Up to now only one active principle has been sought for in each venom, *e. g. Echidnin* for viper poison, and *Crotalin* for rattlesnake poison. The toxic property of each venom was supposed to reside in these bodies alone. We are now coming to the knowledge that there is more than one active principle in each kind of venom, and the result of my own experiments upon cobra poison leads me to agree with Weir Mitchell that the physiological effects of the different kinds of snake venoms differ in degree only.

The latest writer on snake poisons, *viz.*, Dr. Wall, states:—"It is reasonable to conclude, that as long as the poisonous agent of cobra venom is capable of recognition chemically as albumen, so long is it poisonous and no longer;"* and further, "the active agent of cobra poison is precipitated by, and is totally insoluble in, absolute alcohol; but mixtures of alcohol and water are capable of dissolving a certain amount of the poison in proportion to the quantity of water present" (p. 131); and again, "metallic salts capable of precipitating albumen render cobra poison inert." From this I infer that Dr. Wall is inclined to believe that the active venom is proteid in its nature. Dr. Wall's book is a valuable contribution to the difficult study of these snake poisons.

Weir Mitchell and Reichart in America have, for some time past, been engaged in investigating this subject, and they have examined the venom of a number of snakes, chiefly American. They are now completing their investigations, which will shortly be published by the Smithsonian Institute. One or two papers have appeared in America already from their pen. Though I have been trying for a considerable time to get these papers I have hitherto been unsuccessful, and I am consequently in ignorance of the scope and character of their investigations. I think it right to say this, before mentioning my own experiments, because it gives to my work that independent character that it properly possesses. It is only since I began my investigations into these animal poisons that I became acquainted with Weir Mitchell and Reichart's work, through a short contribution made to the *Lancet* of last year,† in which he stated some results of their joint work. This had resulted in the separation from snake venoms of three proteid poisons,—the one like a globulin, attacking respiratory centres and preventing coagulation; a second resembling albumen, and being probably innocuous; a third like peptone, and being a 'putrefactive poison.'

With some of these results, preliminarily published, I agree, but not with all. We await the further publication of details of their laborious work, for no one will deny that the work they have taken in hand is one

* Communicated through Dr. VINCENT RICHARDS, Goalundo.

* Wall. *Indian snake poison*, p. 135.
† *Lancet*, July 1883.

involving great labour, or that it will be thoroughly accomplished. The experiments I am about to describe relate entirely to cobra poison, and may be divided into two sections,—

(1) The chemistry of cobra poison.

(2) The physiological action of the venom.

I have had latterly to rely upon Dr. Vincent Richards for keeping me supplied with the dried poison, and take this opportunity of thanking him for his courtesy and for the readiness with which he, with true scientific zeal, responded to my request.

*1. The Chemistry of Cobra Poison.*

The dried poison is mostly soluble in water, occasionally leaving a little debris (epithelial).

The solution is generally cloudy or opalescent, from partial precipitation of the globulin, which is insoluble in distilled water. The reaction is neutral. The fluid has a strongly proteid reaction, yielding a copious coagulum by boiling it at 100° C, the first cloudiness beginning at about 63°-65° C. By prolonged boiling the whole of the proteids may be removed, and the filtrate is absolutely clear. This filtrate contains only salts, phosphates, chlorides and sulphates.

This filtrate is non-toxic and the interest centres round the proteids.

Saturation of the original venom solution with sulphate of magnesia in bulk, yields a copious woolly precipitate. By saturating with solid sodic chloride a copious precipitate also falls. This is soluble in weak salt solutions of less than ten per cent., and can be re-precipitated by again adding excess of sodic chloride.

The sulphate of magnesia precipitate is soluble on very much diluting with distilled water. This coagulates at a little over 70° C. We know from Hammarsten's experiments that magnesium sulphate precipitates all the globulin from a serous solution, and nothing else. This first proteid obtainable from cobra poison, is therefore a Globulin-Venom. Magnesium sulphate may be used to entirely rid the solution of all globulin. Sodic chloride is not nearly so good for this purpose. Hammarsten long ago shewed that precipitating serum with the latter salt, by no means removes all the paraglobulin. In fact this can only be done with magnesic sulphate. Cobra venom is, therefore, treated in this manner—precipitated by powdered $MgSO_4$ in excess—and agitated briskly at intervals during 24 or 36 hours. The last trace of globulin is thus removed, the precipitate is collected on a filter, washed frequently with $MgSO_4$ solution, scraped off the filter on to a porcelain dish, and dried to a powder, or suspended in water.

After removal of this globulin the filtrate still contains some proteid which yields the xanthoproteic reaction. This is precipitated by acetic acid and ferrocyanide of potash, by Millon's reagent, and by boiling. The coagulation temperature varies between 70°—80° C, cloudiness appearing below 70° C. usually; but these temperatures are much influenced by the amount of $MgSO_4$ present, being higher if the solution be dialysed for some time. This proteid appears to be Serum Albumin. It may be freed from the $MgSO_4$ by dialysis.

These two proteid bodies are always present in samples of cobra poison, though not always in the same proportion. The preponderating presence of one or the other, gives the character to the symptoms following cobra poisoning, paralytic or asphyxiating.

Though Weir Mitchell and Reichart speak, in their preliminary report, (see *Lancet*, 1883) of a proteid body resembling a peptone, I have not found such a body in cobra venom. After removal of all albumins and globulins by heat coagulation, and concentrating the residue and testing with liquor potassæ and cupric sulphate, I have not obtained any peptone or albumin reaction. I would here remark upon the extreme difficulty of detecting small traces of peptone in solution—a difficulty of which I have had considerable experience. In one sample of cobra venom I found traces of a body resembling alkali albumin, but, as before stated, the two principal proteids in the venom are globulin and serum albumin.

Morphologically the glands secreting the poison resemble parotids, and the resemblance is still more striking from the occurrence of a diastatic ferment in the secretion (which was long ago spoken of as analogous to ptyalin by Busk and others). The venom is generally thought physiologically to be modified salivary secretion, consequently we should scarcely expect to find peptone present.

My researches lead me to the conclusion that cobra venom chemically consists of—

(1). A globulin venom.

(2) A serum albumin venom.

(3) Traces of other albumins.

(4) Salts—Chlorides, phosphates, and sulphates.

(5) Water.

It is important to determine which of these is really toxic. This leads to the second part of my subject.

*II.—The Physiological Action of the Venom.*

Cobra poison deprived of its proteids is innocuous.

EXPT. I.—Freshly dissolved cobra venom was submitted to prolonged boiling at 100°C. and all the proteids thus coagulated. When filtered, ℥v. of the clear filtrate were injected under the skin of a lively and healthy rat. He never suffered the slightest inconvenience.

EXPT. II.—Some of the coagulum was suspended in a little water and ℥v. injected into another rat. Within an hour he was dead, with all the symptoms of cobra-poisoning.

It is clear from these two experiments that boiling does not destroy the toxic property of the poison, a fact which Wall has referred to in his book,* and further, that the poison is associated intimately with the coagulated proteids. The solution is toxic so long as it contains any particles of coagulated proteid, but harmless if these be previously removed.

The washings of these coagula are harmless, as will be seen from the next experiment.

EXPT. III.—Fresh venom is coagulated, the coagulum washed with distilled water, concentrated and ℥vii. injected into a rat. No effect followed.

It is therefore evident, that the venomous character of the secretion is resident in, or associated with, the albumens.

The symptoms of cobra-poisoning are so well understood in this country, that there is no necessity to recapitulate them. The colubrine poison has always been considered 'a nerve poison of great deadliness,' Fayrer and Brunton considered it to "act on the cerebro-spinal centres, especially the medulla, indicating general paralysis especially of respiration; secondly, in some cases, where the poison has been conveyed through a large vein directly to the heart, by tetanic arrest of cardiac action, probably owing to action, on the cardiac ganglia; thirdly, by a combination of these causes; fourthly, by blood-poisoning of a secondary character. The phenomena vary according to the nature of the snake, and the individual peculiarities of the creature injured, the chief difference being observed in viperine as contrasted with colubrine poison."†

The prominent clinical symptoms are two, *viz.*, paralytic, and asphyxiating—sometimes the one, sometimes the other set of symptoms appears to preponderate. The paralytic effects result probably from a gradual poisoning of the spinal cord, the asphyxiating, always from poisoning of the medullary centre. The one set of effects follows on globulin poisoning, the other on poisoning by the serum albumin venom.

*I.—The Globulin Venom.*

I have previously described how this is obtained, *viz.*, by treating cobra poison with $MgSO_4$ removing the precipitate, washing, and collecting, keeping suspended in water, or dissolved in weak salt solution.

EXPT. IV.—A small quantity of freshly-prepared globulin was dissolved in weak salt solution, and of this fluid ℥vii. were injected into a young rat. Death followed within six hours. (The animal was not under observation all the time.)

EXPT. V.—Some freshly prepared globulin was suspended in water and ℥iv. injected into a young rat at 12-45.

1 o'clock.—Running about, without distress.

1-15.—No sign of anything.

1-30.—Suddenly shews signs of dyspnœa, which increased in intensity up to 1-40, when the animal became suddenly asphyxiated, rolling over in a paroxysm; the extremities (toes, tail, nose, &c.) all blue. The heart beat for half an hour after all respiratory movement ceased.

This was a very striking case. For three-quarters of an hour the animal shewed absolutely no sign of anything, and then within ten minutes became suddenly asphyxiated. From beginning to end there was never any paralysis.

EXPT. VI.—A rat was injected in the back with ℥vi. of a *dilute* solution of globulin at 2-50.

3-20.—Breathing slightly hurried.

3-40.—Respiration embarrassed; running about.

4-15.—First marked signs of dyspnœa; head back, mouth open, tongue protruded.

4-20.—Moving, the animal induces paroxysm.

4-40.—Dyspnœa paroxysms stronger.

4-45.—Urgent dyspnœa, frequent paroxysms.

5.—Recovering: paroxysms yet less frequent and are not so strong.

* Indian Snake Poisons, p. 120.

† Sir Joseph Fayrer, *Lancet*, 1884, On the Nature of Snake Poison.

From this point the animal fell into a condition of quietude, with frequent rigors, diarrhœa, hurried respiration, cardiac action, tottering gait, and great prostration; refusing food. This continued for 24 hours, but in 48 hours the animal was quite well. From first to last there was never any muscular paralysis, the animal being able to move from place to place when stimulated.

EXPT. VII.—Freshly prepared globulin dissolved in salt solution (forming a strong solution), and ℥ ii-iii injected into a medium-sized rat at 11-45.

11-55.—Distress at place of puncture: licking it.
12-35.—Breathing rather rapid: running about.
12-40.—Dyspnœa: very uneasy: twitching of ears.
12-45.—Some muscular spasms.
12-50.—Defæcated: crouches: frequent starts.
12-52.—Great paroxysms of dyspnœa.
12-55.—Running about everywhere panting.
1.—Expiration at times stridulous: salivation.
1-10.—Lying down, but runs when pinched.
1-15.—Great dyspnœa.
1-16.—Rolled over in a paroxysm, no paralysis.
1-20.—Convulsions, great effort at inspiration.
1-30.—Asphyxiated, using all limbs.
1-31.—Dead: heart still beating.
*P.M.*—Discoloration; blood-stained froth at site of puncture; right side of heart gorged, and still beating. All organs full of blood which is fluid every where.

EXPT. VIII.—Some globulin collected off a filter from venom which had been dialysed suspended in water and ℥ vii. injected into a rat at 11-48.

12-20.—Been quiet ever since; breathing rapid.
1.—Some twitching and dyspnœa.
1-15.—Great dyspnœa: gasping; respirations very gurgling.
1-20—Powerful inspirations necessary; running about.
1-30.—Inspiration very difficult: every now and then gurgling with inspiration.
1-35.—Climbs about: inspiration difficult.
1-40.—Still running about.
1-45.—Inspiration become very difficult.
1-48.—Still walks well when stimulated.
2.—Suddenly asphyxiated.

What comes out very evidently from these five experiments just quoted, is, that death from cobra-globulin poisoning is not attended with paralysis anywhere further than that such death is brought about by embarrassment of respiration, most probably by direct implication of the respiratory centre. Death is in all cases due to asphyxia, either sudden, or preceded by dyspnœa for some time, such dyspnœa aggravated by the exudation which takes place into the bronchial tubes; globulin poisoning increasing secretions from mucous membranes. These effects produced by cobra-globulin are strikingly different from those following on poisoning by the serum albumin of cobra venom, as the following cases will shew:—

*II.—The Albumin Venom.*

After removal of the globulin by saturation with the $MgSO_4$ in the manner previously indicated, the solution is dialysed for two or three days, and evaporated down.

EXPT. IX.—A large rat was injected with about ℥ vi. of this fluid at 3-15.

3-20.—Slightly languid—not moving so actively as usual.
3-35.—Crawls, with obvious loss of power in hind limbs; slight twitchings of muscles of abdomen, head, and ears.
3-40.—Both breathing and heart accelerated, but their rhythm not disturbed. No dyspnœa.
4.—Total loss of power over hind limbs; fore-limbs unaffected.
4-5.—Cannot now crawl. If placed on its back remains motionless. Breathing and heart accelerated, but not at all embarrassed.
4-10.—Convulsions (slight) when touched. These get gradually more frequent and involve the whole body. Respiratory muscles acting well (abdominal and diaphragm); loss of power over voluntary muscles.
4-15.—Convulsions now involve head and neck, muscles of jaws, lips, tongue. Heart suddenly embarrassed and in a convulsion; the animal dies, respiration suddenly stopping. The heart goes on beating spasmodically till 4-25.
P. M.—Heart gorged. Blood everywhere fluid; no hæmorrhages at site of puncture, and no discoloration.

The toxic properties of this albumin are not destroyed by heating to 95°C, although the albumin is coagulated.

EXPT. X.—Minims vii. of boiled serum albumin were injected into a large rat at 12 o'clock.

12-15.—Sluggish,—lying on one side.

12-20.—Loss of power in hind limbs, can only crawl.

12-40.—Marked paralysis of hind limbs. Respiration and heart going on normally. Some twitching of muscles of head and neck.

12-45.—Absolute paralysis of all four limbs.

12-50.—Certain abdominal muscles paralysed, respirations 42 a minute; body surface getting continually colder.

1-5.—Respiration becoming embarrassed, inspiration being long drawn: chest muscles acting fairly well, and diaphragm.

1-10.—Respirations sink to 28, and only diaphragmatic.

1-11.—Diaphragm ceases to act.

1-12.—Animal dead. Heart still beating; all respiratory movement ceased, body surface been getting gradually colder.

P. M.—Blood fluid: heart gorged, but beating rhythmically and strongly; no sign of hæmorrhage anywhere.

EXPT. XI.—A small rat was injected at 2-55 with a mixture of ℥ ii. of globulin and ℥v. of serum albumin.

3-5.—Some loss of power in hind limbs.

3-9.—Almost complete paralysis of hind limbs, with some loss of power in fore limbs. Breathing normal.

3-12.—All four limbs completely paralysed: some muscular convulsions.

3-13.—Convulsions involving whole body. Respirations slower and deeper.

3-15,—A convulsive twitch of whole body with every expiration; chest not expanded at all in respiration, but abdominal muscles still acting.

3-16.—Abdominal muscles and diaphragm ceased acting. Animal dead, heart still beating.

The body temperature sank gradually from the first onset of the symptoms.

This albumin-venom, therefore, poisons by producing motor paralysis, beginning always in the hind limbs and speedily involving the anterior extremities. There is not from first to last any loss of consciousness, or loss of sensation, but simply a progressive motor paralysis which seems to kill finally by involving the muscles concerned in respiratory movements. It is interesting to note the gradual but constant fall in body temperature during poisoning by this venom,—an affect that might very well be brought about by obliteration of the nerve supply to the skeletal muscles. Whether the nerve endings themselves are poisoned, as by curare, a phenomenon that Fayrer and Brunton (by some very carefully conducted experiments reported to the Royal Society*) have described as the chief effect of cobra poisoning, or whether it is, as Wall maintains, that the nerve terminations suffer only '*pari passu* with the cord itself,' does not much matter, the fall of temperature in these rats was most striking, and may well be due to the cutting off of nerve influence from the muscles.

It is to be noted that there is an entire absence of the asphyxiating effects of the globulin poisoning. In the most marked case of globulin poisoning in a young rat, that I have seen, there was absolutely nothing from the time of injection until three-quarters of an hour after, the animal suddenly died asphyxiated. In globulin poisoning paralysis is uniformly absent, the troubles are those of respiration, and the fatal ending is asphyxia. There is also some increase of secretion from the mucous surfaces. This venom is destroyed by boiling at 100°C. The serum-albumin-venom is not destroyed by boiling at 95°C.

Summing up the result of these investigations, which are by no means yet to be regarded as complete, there are in Cobra poison two distinct venoms,

1. Cobra globulin-venom.
2. Cobra albumin-venom.

They exist probably in different proportions in different secretions, but these two are always present. What other albumins may be present, are not of the importance these two are.

* 1873, 1874, 1875, 1878.

The globulin venom is destroyed by high temperatures, but the albumin venom is not so affected.

The globulin venom poisons the respiratory centre, producing no paralysis of muscles; the albumin venom does not affect the respiratory centre, but produces marked and progressive motor paralysis.

I may here mention, that from the result of some investigations I have for some time been making upon the blood of many animals, I cannot consent to the generally received opinion that cobra venom exerts no influence upon the blood. My investigations, which will shortly be published, have convinced me that cobra venom decolorises, by driving out the hæmoglobin, a large proportion of the discs, and breaks up a large number of the white discs completely, filling the plasma with minute granules. The bacterial forms which are present in such large numbers, in cobra venom, I do not think have anything to do with the activity of the venom. When recovery takes place from poisoning with a dose of the poison insufficient to kill, it is not improbable that a condition of blood poisoning may supervene, secondarily, as in one of the cases I have quoted.

I may add in conclusion, that the animals experimented upon, were in all cases white rats, and the injection was made always under the skin of the back, in the dorsal region.

The globulin venom is slower in its action than the serum albumin, and a longer period often elapses after the injection before symptoms supervene, or terminate life. The globulin is very deadly, and when once the symptoms have supervened asphyxia rapidly ends the existence of the animal.

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## EXCISION OF TONGUE BY SCISSORS BY BILLROTH'S METHOD.

BY SURGEON-MAJOR J. CLEGHORN,
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Dr. O'Brien in his remarks accompanying a case of removal of the tongue by the ecraseur, published in the August number of the *Indian Medical Gazette*, claims for that method of operating a superiority over all others, more especially that of Whitehead's, on account of its simplicity and its freedom from any risk of hæmorrhage. Whitehead's method of removal by scissors is, I think, simplicity itself, but the hæmorrhage which generally accompanies it is so profuse and alarming that, in the absence of qualified assistants, it is not suitable for dispensary practice. By combining it, however, with previous ligature of the linguals, introduced by Billroth, the operation may be rendered as bloodless as in that by the ecraseur. In the case which I published in the May number of the *Indian Medical Gazette*, the bleeding proceeded chiefly from a vessel situated near the left tonsil, which had been divided in removing portions of diseased tissue in that region, and had evidently no connection with the tongue. The bleeding from the cut surface of the tongue was general, and was arrested by pressure with a sponge. In the two following cases there was practically no bleeding, and no precautions were required to keep the mouth clear.

Ligature of the linguals when once performed on the living subject, becomes in subsequent cases an easy operation. The dissection necessary to expose the artery is deep, but the line of incision avoids important parts, till the hypoglossal nerve is reached, when a few touches with the knife exposes the artery. The hyoid bone should be fixed with the tip of left forefinger pressed in the great horn; the finger thus placed serves as a guide to the position of the artery.

In extensive disease of the tongue requiring removal of the whole organ, it would, I think, be difficult to fix the wire of the ecraseur sufficiently far back as to ensure its cutting through healthy tissue. In disease limited to one side of the tongue, the organ must either be split with the knife or scissors, or removed in its whole circumference; in the one case the hæmorrhage would be equally severe as in Whitehead's operation, and in the other a healthy portion of the tongue would be unnecessarily sacrificed. For these and other reasons removal by scissors is, I think, a more surgical proceeding than by the ecraseur, but I can fancy certain cases in which the latter would be the most suitable instrument to employ.

*Case 1.*—A male Hindoo, admitted on 2nd July with a large excavated cancerous ulcer involving the middle and part of the posterior third of the tongue. The tongue was fixed to the floor of the mouth. The linguals were first ligatured, the mouth was kept open by a Mason's gag, and the whole of the tongue, with a portion of the floor of the mouth on right side, was removed with Allingham's spring scissors. There was practically no bleeding. He was fed for three days with milk injected through a catheter passed through